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Stem Cell

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Stem Cell Research Literatures (4)

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
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Key words: stem cell; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells,[5] i.e. cells derived from any of the three germ layers.

The following introduces recent reports as references in the related studies.

Abbasalizadeh, S. and H. Baharvand (2013). "Technological progress and challenges towards cGMP manufacturing of human pluripotent stem cells based therapeutic products for allogeneic and autologous cell therapies." Biotechnol Adv **31**(8): 1600-1623.

Recent technological advances in the generation, characterization, and bioprocessing of human pluripotent stem cells (hPSCs) have created new hope for their use as a source for production of cell-based therapeutic products. To date, a few clinical trials that have used therapeutic cells derived from hESCs have been approved by the Food and Drug Administration (FDA), but numerous new hPSC-based cell therapy products are under various stages of development in cell therapy-specialized companies and their future market is estimated to be very promising. However, the multitude of critical challenges regarding different aspects of hPSC-based therapeutic product manufacturing and their therapies have made progress for the introduction of new products and clinical applications very slow. These challenges include scientific, technological, clinical, policy, and financial aspects. The technological aspects of manufacturing hPSC-based therapeutic products for allogeneic and autologous cell therapies according to good manufacturing practice (cGMP) quality requirements is one of the most important challenging and emerging topics in the development of new hPSCs for clinical use. In this review, we describe main critical challenges and highlight a series of technological advances in all aspects of hPSC-based therapeutic product manufacturing including clinical grade cell line development, large-scale banking, upstream processing, downstream processing, and quality assessment of final cell therapeutic products that have brought hPSCs closer to clinical application and commercial cGMP manufacturing.

Abdelalim, E. M., et al. (2014). "Pluripotent stem cells as a potential tool for disease modelling and cell therapy in diabetes." Stem Cell Rev **10**(3): 327-337.

Diabetes mellitus is the most prevailing disease with progressive incidence worldwide. To date, the pathogenesis of diabetes is far to be understood, and there is no permanent treatment available for diabetes. One of the promising approaches to understand and cure diabetes is to use pluripotent stem

cells (PSCs), including embryonic stem cells (ESCs) and induced PCSs (iPSCs). ESCs and iPSCs have a great potential to differentiate into all cell types, and they have a high ability to differentiate into insulin-secreting beta cells. Obtaining PSCs genetically identical to the patient presenting with diabetes has been a longstanding dream for the in vitro modeling of disease and ultimately cell therapy. For several years, somatic cell nuclear transfer (SCNT) was the method of choice to generate patient-specific ESC lines. However, this technology faces ethical and practical concerns. Interestingly, the recently established iPSC technology overcomes the major problems of other stem cell types including the lack of ethical concern and no risk of immune rejection. Several iPSC lines have been recently generated from patients with different types of diabetes, and most of these cell lines are able to differentiate into insulin-secreting beta cells. In this review, we summarize recent advances in the differentiation of pancreatic beta cells from PSCs, and describe the challenges for their clinical use in diabetes cell therapy. Furthermore, we discuss the potential use of patient-specific PSCs as an in vitro model, providing new insights into the pathophysiology of diabetes.

Aberdam, E., et al. (2017). "Induced pluripotent stem cell-derived limbal epithelial cells (LiPSC) as a cellular alternative for in vitro ocular toxicity testing." PLoS One **12**(6): e0179913.

Induced pluripotent stem cells hold great potential to produce unlimited amount of differentiated cells as cellular source for regenerative medicine but also for in vitro drug screening and cytotoxicity tests. Ocular toxicity testing is mandatory to evaluate the risks of drugs and cosmetic products before their application to human patients by preventing eye irritation or insult. Since the global ban to use animals, many human-derived alternatives have been proposed, from ex-vivo enucleated postmortem cornea, primary corneal cell culture and immortalized corneal epithelial cell lines. All of them share limitations for their routine use. Using an improved protocol, we derived limbal epithelial cells from human induced pluripotent stem cells, named LiPSC, that are able to be passaged and differentiate further into corneal epithelial cells. Comparative RT-qPCR, immunofluorescence staining, flow cytometry analysis and zymography assays demonstrate that LiPSC are morphologically and molecularly similar to the adult stem cells. Moreover, contrary to HCE, LiPSC and primary limbal cells display similarly sensitive to cytotoxicity treatment among passages. Our data strongly suggest that LiPSC could become a powerful alternative cellular model for cosmetic and drug tests.

Abruzzese, R. V. and R. A. Fekete (2013). "Single cell gene expression analysis of pluripotent stem cells." Methods Mol Biol **997**: 217-224.

Analyzing gene expression profiles from cells en masse provides an average profile for the population which may obscure differences in individual cells. Using an optimized workflow for qRT-PCR, gene expression profiles of undifferentiated pluripotent stem cells reveal distinct gene expression profiles for individual cells, and a large expression level range of almost every gene. Importantly, this technique allows for the identification and characterization of small subpopulations.

Abu-Hassan, D. W., et al. (2015). "Induced pluripotent stem cells restore function in a human cell loss model of open-angle glaucoma." Stem Cells **33**(3): 751-761.

Normally, trabecular meshwork (TM) and Schlemm's canal inner wall endothelial cells within the aqueous humor outflow pathway maintain intraocular pressure within a narrow safe range. Elevation in intraocular pressure, because of the loss of homeostatic regulation by these outflow pathway cells, is the primary risk factor for vision loss due to glaucomatous optic neuropathy. A notable feature associated with glaucoma is outflow pathway cell loss. Using controlled cell loss in ex vivo perfused human outflow pathway organ culture, we developed compelling experimental evidence that this level of cell loss compromises intraocular pressure homeostatic function. This function was restored by repopulation of the model with fresh TM cells. We then differentiated induced pluripotent stem cells (iPSCs) and used them to repopulate this cell depletion model. These differentiated cells (TM-like iPSCs) became similar to TM cells in both morphology and expression patterns. When transplanted, they were able to fully restore intraocular pressure homeostatic function. This successful transplantation of TM-like iPSCs establishes the conceptual feasibility of using autologous stem cells to restore intraocular pressure regulatory function in open-angle glaucoma patients, providing a novel alternative treatment option.

Acharya, M. M., et al. (2015). "Defining the optimal window for cranial transplantation of human induced pluripotent stem cell-derived cells to ameliorate radiation-induced cognitive impairment." Stem Cells Transl Med **4**(1): 74-83.

Past preclinical studies have demonstrated the capability of using human stem cell transplantation in the irradiated brain to ameliorate radiation-induced cognitive dysfunction. Intrahippocampal transplantation of human embryonic stem cells and human neural stem cells (hNSCs) was found to functionally restore cognition in rats 1 and 4 months

after cranial irradiation. To optimize the potential therapeutic benefits of human stem cell transplantation, we have further defined optimal transplantation windows for maximizing cognitive benefits after irradiation and used induced pluripotent stem cell-derived hNSCs (iPSC-hNSCs) that may eventually help minimize graft rejection in the host brain. For these studies, animals given an acute head-only dose of 10 Gy were grafted with iPSC-hNSCs at 2 days, 2 weeks, or 4 weeks following irradiation. Animals receiving stem cell grafts showed improved hippocampal spatial memory and contextual fear-conditioning performance compared with irradiated sham-surgery controls when analyzed 1 month after transplantation surgery. Importantly, superior performance was evident when stem cell grafting was delayed by 4 weeks following irradiation compared with animals grafted at earlier times. Analysis of the 4-week cohort showed that the surviving grafted cells migrated throughout the CA1 and CA3 subfields of the host hippocampus and differentiated into neuronal (approximately 39%) and astroglial (approximately 14%) subtypes. Furthermore, radiation-induced inflammation was significantly attenuated across multiple hippocampal subfields in animals receiving iPSC-hNSCs at 4 weeks after irradiation. These studies expand our prior findings to demonstrate that protracted stem cell grafting provides improved cognitive benefits following irradiation that are associated with reduced neuroinflammation.

Alberio, R. and A. R. Perez (2012). "Recent advances in stem and germ cell research: implications for the derivation of pig pluripotent cells." Reprod Domest Anim **47 Suppl 4**: 98-106.

Pluripotent stem cells have the unique capacity to contribute to all the tissues of an adult animal after transfer into a host embryo. How pluripotency is acquired during early development and how it is maintained in stem cells have attracted the interest of many scientists for over three decades. Much progress in our understanding of how stem cells arise in culture and the signals required for homoeostasis has enabled the derivation of pluripotent cells in multiple species. Here, we discuss recent developments in stem cell biology that will impact the generation of pluripotent cells from different embryonic origins and will contribute to increase our capacity for generating transgenic animals.

Alipio, Z. A., et al. (2011). "Epithelial to mesenchymal transition (EMT) induced by bleomycin or TFG(b1)/EGF in murine induced pluripotent stem cell-derived alveolar Type II-like cells." Differentiation **82**(2): 89-98.

Induced pluripotent stem (iPS) cells are derived from reprogrammed somatic cells and are similar to embryonic stem (ES) cells in morphology, gene/protein expression, and pluripotency. In this study, we explored the potential of iPS cells to differentiate into alveolar Type II (ATII)-like epithelial cells. Analysis using quantitative real time polymerase chain reaction and immunofluorescence staining showed that pulmonary surfactant proteins commonly expressed by ATII cells such as surfactant protein A (SPA), surfactant protein B (SPB), and surfactant protein C (SPC) were upregulated in the differentiated cells. Microphilopodia characteristics and lamellar bodies were observed by transmission electron microscopy and lipid deposits were verified by Nile Red and Periodic Acid Schiff staining. C3 complement protein, a specific feature of ATII cells, was present at high levels in culture supernatants demonstrating functionality of these cells in culture. These data show that the differentiated cells generated from iPS cells using a culture method developed previously (Rippon et al., 2006) are ATII-like cells. To further characterize these ATII-like cells, we tested whether they could undergo epithelial to mesenchymal transition (EMT) by exposure to drugs that induce lung fibrosis in mice, such as bleomycin, and the combination of transforming growth factor beta1 (TGF(b1)) and epidermal growth factor (EGF). When the ATII-like cells were exposed to either bleomycin or a TGF(b1)-EGF cocktail, they underwent phenotypic changes including acquisition of a mesenchymal/fibroblastic morphology, upregulation of mesenchymal markers (Col1, Vim, a-Sma, and S100A4), and downregulation of surfactant proteins and E-cadherin. We have shown that ATII-like cells can be derived from skin fibroblasts and that they respond to fibrotic stimuli. These cells provide a valuable tool for screening of agents that can potentially ameliorate or prevent diseases involving lung fibrosis.

Allison, T. F., et al. (2018). "Identification and Single-Cell Functional Characterization of an Endodermally Biased Pluripotent Substate in Human Embryonic Stem Cells." Stem Cell Reports **10**(6): 1895-1907.

Human embryonic stem cells (hESCs) display substantial heterogeneity in gene expression, implying the existence of discrete substates within the stem cell compartment. To determine whether these substates impact fate decisions of hESCs we used a GFP reporter line to investigate the properties of fractions of putative undifferentiated cells defined by their differential expression of the endoderm transcription factor, GATA6, together with the hESC surface marker, SSEA3. By single-cell cloning, we confirmed that substates characterized by expression of GATA6 and SSEA3 include pluripotent stem cells capable of long-term self-renewal. When clonal stem cell colonies were formed from GATA6-positive and GATA6-negative

cells, more of those derived from GATA6-positive cells contained spontaneously differentiated endoderm cells than similar colonies derived from the GATA6-negative cells. We characterized these discrete cellular states using single-cell transcriptomic analysis, identifying a potential role for SOX17 in the establishment of the endoderm-biased stem cell state.

Altomare, C., et al. (2016). "Human-induced pluripotent stem cell-derived cardiomyocytes from cardiac progenitor cells: effects of selective ion channel blockade." Europace **18**(suppl 4): iv67-iv76.

AIM: Human-induced pluripotent stem cell (hiPSC)-derived cardiomyocytes are likely to revolutionize electrophysiological approaches to arrhythmias. Recent evidence suggests the somatic cell origin of hiPSCs may influence their differentiation potential. Owing to their cardiomyogenic potential, cardiac-stromal progenitor cells (CPCs) are an interesting cellular source for generation of hiPSC-derived cardiomyocytes. The effect of ionic current blockade in hiPSC-derived cardiomyocytes generated from CPCs has not been characterized yet. METHODS AND RESULTS: Human-induced pluripotent stem cell-derived cardiomyocytes were generated from adult CPCs and skin fibroblasts from the same individuals. The effect of selective ionic current blockade on spontaneously beating hiPSC-derived cardiomyocytes was assessed using multi-electrode arrays. Cardiac-stromal progenitor cells could be reprogrammed into hiPSCs, then differentiated into hiPSC-derived cardiomyocytes. Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin showed higher upregulation of cardiac-specific genes compared with those of fibroblastic origin. Human-induced pluripotent stem cell-derived cardiomyocytes of both somatic cell origins exhibited sensitivity to tetrodotoxin, a blocker of Na(+)current (INa), nifedipine, a blocker of L-type Ca(2+)current (ICaL), and E4031, a blocker of the rapid component of delayed rectifier K(+)current (IKr). Human-induced pluripotent stem cell-derived cardiomyocytes of cardiac origin exhibited sensitivity to JNJ303, a blocker of the slow component of delayed rectifier K(+)current (IKs). CONCLUSION: In hiPSC-derived cardiomyocytes of cardiac origin, INa, ICaL, IKr, and IKs were present as tetrodotoxin-, nifedipine-, E4031-, and JNJ303-sensitive currents, respectively. Although cardiac differentiation efficiency was improved in hiPSCs of cardiac vs. non-cardiac origin, no major functional differences were observed between hiPSC-derived cardiomyocytes of different somatic cell origins. Further studies are warranted to characterize electrophysiological properties of hiPSC-derived cardiomyocytes generated from CPCs.

Alvisi, G., et al. (2018). "Generation of a transgene-free human induced pluripotent stem cell line (UNIPDi001-A) from oral mucosa epithelial stem cells." Stem Cell Res **28**: 177-180.

Human oral mucosa epithelial stem cells (hOMESCs) were obtained from a fresh oral biopsy collected from a healthy subject at the Fondazione Banca degli Occhi del Veneto (FBOV). An integration-free reprogramming protocol was applied exploiting episomal plasmids transfected into cells using a Nucleofector device. Around day 20 post transfection, several human induced pluripotent stem cell (hiPSC) colonies were manually picked and expanded. One of these (UNIPDi001-A-hiPSCs) expressed undifferentiated state marker alkaline phosphatase along with a panel of pluripotency state markers and was able to differentiate into the derivatives of all the three germ layers.

Anand, T., et al. (2016). "Differentiation of Induced Pluripotent Stem Cells to Lentoid Bodies Expressing a Lens Cell-Specific Fluorescent Reporter." PLoS One **11**(6): e0157570.

Curative approaches for eye cataracts and other eye abnormalities, such as myopia and hyperopia currently suffer from a lack of appropriate models. Here, we present a new approach for in vitro growth of lentoid bodies from induced pluripotent stem (iPS) cells as a tool for ophthalmological research. We generated a transgenic mouse line with lens-specific expression of a fluorescent reporter driven by the alphaA crystallin promoter. Fetal fibroblasts were isolated from transgenic fetuses, reprogrammed to iPS cells, and differentiated to lentoid bodies exploiting the specific fluorescence of the lens cell-specific reporter. The employment of cell type-specific reporters for establishing and optimizing differentiation in vitro seems to be an efficient and generally applicable approach for developing differentiation protocols for desired cell populations.

Araki, R., et al. (2017). "The Number of Point Mutations in Induced Pluripotent Stem Cells and Nuclear Transfer Embryonic Stem Cells Depends on the Method and Somatic Cell Type Used for Their Generation." Stem Cells **35**(5): 1189-1196.

Induced pluripotent stem cells hold great promise for regenerative medicine but point mutations have been identified in these cells and have raised serious concerns about their safe use. We generated nuclear transfer embryonic stem cells (ntESCs) from both mouse embryonic fibroblasts (MEFs) and tail-tip fibroblasts (TTFs) and by whole genome sequencing found fewer mutations compared with iPSCs generated by retroviral gene transduction. Furthermore, TTF-derived ntESCs showed only a very small number of

point mutations, approximately 80% less than the number observed in iPSCs generated using retrovirus. Base substitution profile analysis confirmed this greatly reduced number of point mutations. The point mutations in iPSCs are therefore not a Yamanaka factor-specific phenomenon but are intrinsic to genome reprogramming. Moreover, the dramatic reduction in point mutations in ntESCs suggests that most are not essential for genome reprogramming. Our results suggest that it is feasible to reduce the point mutation frequency in iPSCs by optimizing various genome reprogramming conditions. We conducted whole genome sequencing of ntES cells derived from MEFs or TTFs. We thereby succeeded in establishing TTF-derived ntES cell lines with far fewer point mutations. Base substitution profile analysis of these clones also indicated a reduced point mutation frequency, moving from a transversion-predominance to a transition-predominance. Stem Cells 2017;35:1189-1196.

Armstrong, L., et al. (2010). "Human induced pluripotent stem cell lines show stress defense mechanisms and mitochondrial regulation similar to those of human embryonic stem cells." Stem Cells **28**(4): 661-673.

The generation of induced pluripotent stem cells (iPSC) has enormous potential for the development of patient-specific regenerative medicine. Human embryonic stem cells (hESC) are able to defend their genomic integrity by maintaining low levels of reactive oxygen species (ROS) through a combination of enhanced removal capacity and limited production of these molecules. Such limited ROS production stems partly from the small number of mitochondria present in hESC; thus, it was important to determine that human iPSC (hiPSC) generation is able to eliminate the extra mitochondria present in the parental fibroblasts (reminiscent of "bottleneck" situation after fertilization) and to show that hiPSC have antioxidant defenses similar to hESC. We were able to generate seven hiPSC lines from adult human dermal fibroblasts and have fully characterized two of those clones. Both hiPSC clones express pluripotency markers and are able to differentiate in vitro into cells belonging to all three germ layers. One of these clones is able to produce fully differentiated teratoma, whereas the other hiPSC clone is unable to silence the viral expression of OCT4 and c-MYC, produce fully differentiated teratoma, and unable to downregulate the expression of some of the pluripotency genes during the differentiation process. In spite of these differences, both clones show ROS stress defense mechanisms and mitochondrial biogenesis similar to hESC. Together our data suggest that, during the reprogramming process, certain cellular mechanisms are in place to ensure that hiPSC are provided with the same defense mechanisms against accumulation of ROS as the hESC.

Asgari, S., et al. (2013). "Differentiation and transplantation of human induced pluripotent stem cell-derived hepatocyte-like cells." Stem Cell Rev **9**(4): 493-504.

The generation of human induced pluripotent stem cells (hiPSCs) with a high differentiation potential provided a new source for hepatocyte generation not only for drug discovery and in vitro disease models, but also for cell replacement therapy. However, the reported hiPSC-derived hepatocyte-like cells (HLCs) were not well characterized and their transplantation, as the most promising clue of cell function was not reported. Here, we performed a growth factor-mediated differentiation of functional HLCs from hiPSCs and evaluated their potential for recovery of a carbon tetrachloride (CCl4)-injured mouse liver following transplantation. The hiPSC-derived hepatic lineage cells expressed hepatocyte-specific markers, showed glycogen and lipid storage activity, secretion of albumin (ALB), alpha-fetoprotein (AFP), urea, and CYP450 metabolic activity in addition to low-density lipoprotein (LDL) and indocyanin green (ICG) uptake. Similar results were observed with human embryonic stem cell (hESC)-derived HLCs. The transplantation of hiPSC-HLCs into a CCl4-injured liver showed incorporation of the hiPSC-HLCs into the mouse liver which resulted in a significant enhancement in total serum ALB after 1 week. A reduction of total serum LDH and bilirubin was seen when compared with the control and sham groups 1 and 5 weeks post-transplantation. Additionally, we detected human serum ALB and ALB-positive transplanted cells in both the host serum and livers, respectively, which showed functional integration of transplanted cells within the mouse livers. Therefore, our results have opened up a proof of concept that functional HLCs can be generated from hiPSCs, thus improving the general condition of a CCl4-injured mouse liver after their transplantation. These results may bring new insights in the clinical applications of hiPSCs once safety issues are overcome.

Astori, G., et al. (2001). "A novel protocol that allows short-term stem cell expansion of both committed and pluripotent hematopoietic progenitor cells suitable for clinical use." Blood Cells Mol Dis **27**(4): 715-724; discussion 725-717.

To obtain long-term engraftment and hematopoiesis in myeloablated patients, the cell population used for hematopoietic reconstitution should include a sufficient number of early pluripotent hematopoietic stem cells (HSCs), along with committed cells from the various lineages. For this

purpose, the small subset of CD34+ cells purified from different sources must be expanded ex vivo. Since cytokines may induce both proliferation and differentiation, expansion would provide a cell population comprising committed as well as uncommitted cells. Optimization of HSC expansion methods could be obtained by a combination of cytokines able to sustain renewal of pluripotent cells yet endowed with poor differentiation potential. We used variations of the combinations of cytokines described by Brugger et al. [W. Brugger, S. Heimfels, R. J. Berenson, R. Mertelsmann, and L. Kanz (1995) N. Engl. J. Med. 333, 283-287] and Piacibello et al. [W. Piacibello, F. Sanavio, L. Garetto, A. Severino, D. Bergandi, J. Ferrario, F. Fagioli, M. Berger, and M. Aglietta (1997) Blood 89, 2644-2653] to expand UCB CD34+ cells and monitored proliferation rate and phenotype after 14 days of culture. Several hematopoietic lineage-associated surface antigens were evaluated. Our data show that flt3L and thrombopoietin in combination with IL-3, while sustaining a high CD34+ proliferation rate, provide a relatively low enrichment in very early uncommitted CD34+/CD38- cells. Conversely, in the absence of IL-3, they are less effective in inducing proliferation yet significantly increase the number of CD34+/CD38- cells. A combination of the above protocols, applied simultaneously to aliquots of the same sample, would allow expansion of both committed and pluripotent HSC. This strategy may represent a significant improvement for clinical applications.

Ayoubi, S., et al. (2017). "Human induced pluripotent stem cell-derived vascular smooth muscle cells: differentiation and therapeutic potential." Cardiovasc Res **113**(11): 1282-1293.

Cardiovascular diseases remain the leading cause of death worldwide and current treatment strategies have limited effect of disease progression. It would be desirable to have better models to study developmental and pathological processes and model vascular diseases in laboratory settings. To this end, human induced pluripotent stem cells (hiPSCs) have generated great enthusiasm, and have been a driving force for development of novel strategies in drug discovery and regenerative cell-therapy for the last decade. Hence, investigating the mechanisms underlying the differentiation of hiPSCs into specialized cell types such as cardiomyocytes, endothelial cells, and vascular smooth muscle cells (VSMCs) may lead to a better understanding of developmental cardiovascular processes and potentiate progress of safe autologous regenerative therapies in pathological conditions. In this review, we summarize the latest trends on differentiation protocols of hiPSC-derived VSMCs and their potential application in vascular research and regenerative therapy.

Azhdari, M., et al. (2013). "Therapeutic potential of human-induced pluripotent stem cell-derived endothelial cells in a bleomycin-induced scleroderma mouse model." Stem Cell Res **10**(3): 288-300.

Vascular injury and destruction of endothelial cells (ECs) are the early events in scleroderma (SSc) patients. This study aims to investigate the therapeutic potential of human-induced pluripotent stem cell-derived ECs (hiPSC-ECs) to treat SSc. We have assessed the functional differentiation of hiPSC-ECs and compared them with human embryonic stem cell-derived ECs (hESC-ECs) by a variety of in vitro experimental approaches. Additionally, we evaluated the therapeutic potential of hiPSC-ECs in a bleomycin-induced SSc mouse model. Our results demonstrated that hiPSC-ECs and hESC-ECs showed similar maximum expressions of FLK1 (early EC marker) at day five during differentiation. After sorting and culturing, the FLK1-positive cells exhibited spindle and subsequent endothelial cobblestone morphology in EGM2 medium. The hESC-ECs and hiPSC-ECs also expressed late EC markers CD31 (68% and 75%), CD144 (50% and 61%), CD146 (46% and 61%), and DiI-labeled acetylated low-density lipoprotein (DiI-ac-LDL) uptake (55% and 63%), respectively. They additionally formed capillary-like structures on Matrigel. Analyses of the transplantation of sorted CD31-positive hiPSC-ECs into the bleomycin-induced SSc mouse model demonstrated that these cells participate in recovery of the damaged vessels. There was a reduction in collagen content; the number of total and degranulated mast cells returned to their normal state, and bleomycin-induced wounds as well as skin fibrosis improved four weeks after transplantation of hiPSC-ECs. Our findings have shown that the differentiation process from hESCs and hiPSCs to vascular cell components is similar. Additionally, this is the first study to determine the therapeutic potential of vascular cells from hiPSCs in the treatment of an SSc model. In the future, with further validation, these may be used as an appropriate source for the treatment of SSc patients.

Bae, K. M., et al. (2010). "Expression of pluripotent stem cell reprogramming factors by prostate tumor initiating cells." J Urol **183**(5): 2045-2053.

PURPOSE: We identified a discrete population of stem cell-like tumor cells expressing 5 essential transcription factors required to reprogram pluripotency in prostate tumor cell lines and primary prostate cancer tissue. MATERIALS AND METHODS: DU145 and PC3 human prostate cancer cell lines (ATCC), tumor tissue from patients with prostate

cancer and normal prostate tissue were evaluated for the reprogramming factors OCT3/4 (Cell Signaling Technology), SOX2, Klf4 (Santa Cruz Biotechnology, Santa Cruz, California), Nanog (BioLegend) and c-Myc (Cell Signaling) by semiquantitative reverse transcriptase-polymerase chain reaction, histological and immunohistochemical analysis. Stem cell-like tumor cells were enriched by flow cytometric cell sorting using E-cadherin (R&D Systems) as a surface marker, and soft agar, spheroid and tumorigenicity assays to confirm cancer stem cell-like characteristics. RESULTS: mRNA expression of transcription factors OCT3/4 and SOX2 highly correlated in primary prostate tumor tissue samples. The number of OCT3/4 or SOX2 expressing cells was significantly increased in prostate cancer tissue compared to that in normal prostate or benign prostate hyperplasia tissue ($p < 0.05$). When isolated from the DU145 and PC3 prostate cancer cell lines by flow cytometry, stem cell-like tumor cells expressing high OCT3/4 and SOX2 levels showed high tumorigenicity in immunodeficient mice. In vivo growth of the parental DU145 and PC3 prostate cancer cell lines was inhibited by short hairpin RNA knockdown of OCT3/4 or SOX2. CONCLUSIONS: Data suggest that prostate tumor cells expressing pluripotent stem cell transcription factors are highly tumorigenic. Identifying such cells and their importance in prostate cancer growth could provide opportunities for novel targeting strategies for prostate cancer therapy.

Baiu, D., et al. (2011). "Potential pathways to restore beta-cell mass: pluripotent stem cells, reprogramming, and endogenous regeneration." Curr Diab Rep **11**(5): 392-401.

Currently available beta-cell replacement therapies for patients with diabetes, including islet and pancreas transplantation, are largely successful in restoring normal glucose metabolism, but the scarcity of organ donors restricts their more widespread use. To solve this supply problem, several different strategies for achieving beta-cell mass restoration are being pursued. These include the generation of beta cells from stem cells and their subsequent transplantation, or regeneration-type approaches, such as stimulating endogenous regenerative mechanisms or inducing reprogramming of non-beta cells into beta cells. Because these strategies would ultimately generate allogeneic or syngeneic beta cells in humans, the control of alloimmunity and/or autoimmunity in addition to replacing lost beta cells will be of utmost importance. We briefly review the recent literature on these three promising strategies toward beta-cell replacement or restoration and point out the major issues impacting their translation to treating human diabetes.

Bajpai, V. K., et al. (2012). "Functional vascular smooth muscle cells derived from human induced pluripotent stem cells via mesenchymal stem cell intermediates." Cardiovasc Res **96**(3): 391-400.

AIMS: Smooth muscle cells (SMC) play an important role in vascular homeostasis and disease. Although adult mesenchymal stem cells (MSC) have been used as a source of contractile SMC, they suffer from limited proliferation potential and culture senescence, particularly when originating from older donors. By comparison, human induced pluripotent stem cells (hiPSC) can provide an unlimited source of functional SMC for autologous cell-based therapies and for creating models of vascular disease. Our goal was to develop an efficient strategy to derive functional, contractile SMC from hiPSC. METHODS AND RESULTS: We developed a robust, stage-wise, feeder-free strategy for hiPSC differentiation into functional SMC through an intermediate stage of multipotent MSC, which could be coaxed to differentiate into fat, bone, cartilage, and muscle. At this stage, the cells were highly proliferative and displayed higher clonogenic potential and reduced senescence when compared with parental hair follicle mesenchymal stem cells. In addition, when exposed to differentiation medium, the myogenic proteins such as alpha-smooth muscle actin, calponin, and myosin heavy chain were significantly upregulated and displayed robust fibrillar organization, suggesting the development of a contractile phenotype. Indeed, tissue constructs prepared from these cells exhibited high levels of contractility in response to receptor- and non-receptor-mediated agonists. CONCLUSION: We developed an efficient stage-wise strategy that enabled hiPSC differentiation into contractile SMC through an intermediate population of clonogenic and multipotent MSC. The high yield of MSC and SMC derivation suggests that our strategy may facilitate an acquisition of the large numbers of cells required for regenerative medicine or for studying vascular disease pathophysiology.

Balasubramanian, S., et al. (2009). "Non cell-autonomous reprogramming of adult ocular progenitors: generation of pluripotent stem cells without exogenous transcription factors." Stem Cells **27**(12): 3053-3062.

Direct reprogramming of differentiated cells to induced pluripotent stem (iPS) cells by ectopic expression of defined transcription factors (TFs) represents a significant breakthrough towards the use of stem cells in regenerative medicine (Takahashi and Yamanaka Cell 2006;126:663-676). However, the virus-mediated expression of exogenous transcription factors could be potentially harmful and, therefore, represents a barrier to the clinical use of iPS cells.

Several approaches, ranging from plasmid-mediated TF expression to introduction of recombinant TFs (Yamanaka Cell 2009;137:13-17; Zhou, Wu, Joo et al. Cell Stem Cell 2009;4:381-384), have been reported to address the risk associated with viral integration. We describe an alternative strategy of reprogramming somatic progenitors entirely through the recruitment of endogenous genes without the introduction of genetic materials or exogenous factors. To this end, we reprogrammed accessible and renewable progenitors from the limbal epithelium of adult rat eye by microenvironment-based induction of endogenous iPS cell genes. Non cell-autonomous reprogramming generates cells that are pluripotent and capable of differentiating into functional neurons, cardiomyocytes, and hepatocytes, which may facilitate autologous cell therapy to treat degenerative diseases.

Balboa, D., et al. (2018). "Human pluripotent stem cells for the modelling of pancreatic beta-cell pathology." Stem Cells.

Pancreatic beta-cells are the only source of insulin. Disturbances in beta-cell development or function may thus result in insulin deficiency or excess, presenting as hyper- or hypoglycemia. It is increasingly evident that common forms of diabetes (Type 1 and 2) are pathogenically heterogeneous. Development of efficient therapies is dependent on reliable disease models. Although animal models are remarkably useful research tools, they present limitations due to species differences. As an alternative, human pluripotent stem cell technologies offer multiple possibilities for the study of human diseases in vitro. In the last decade, advances in the derivation of induced pluripotent stem cells from diabetic patients, combined with beta-cell differentiation protocols, have resulted in the generation of useful disease models for diabetes. First disease models have been focusing on monogenic diabetes. The development of genome editing technologies, more advanced differentiation protocols and humanized mouse models based on transplanted cells have opened new horizons for the modelling of more complex forms of beta-cell dysfunction. We present here the incremental progress made in the modeling of diabetes using pluripotent stem cells. We discuss the current challenges and opportunities of these approaches to dissect beta-cell pathology and devise new pharmacological and cell replacement therapies. (c) AlphaMed Press 2018.

Bamba, Y., et al. (2014). "Differentiation, polarization, and migration of human induced pluripotent stem cell-derived neural progenitor cells co-cultured with a human glial cell line with radial glial-like characteristics." Biochem Biophys Res Commun **447**(4): 683-688.

Here we established a unique human glial cell line, GDC90, derived from a human glioma and demonstrated its utility as a glial scaffold for the polarization and differentiation of human induced pluripotent stem cell-derived neural progenitor cells (iPSC-NPCs). When co-cultured with GDC90 cells, iPSC-NPCs underwent rapid polarization and neurite extension along the radially spreading processes of the GDC90 cells, and showed migratory behavior. This method is potentially useful for detailed examination of neurites or for controlling neurites behavior for regenerative medicine.

Bao, S., et al. (2012). "The germ cell determinant Blimp1 is not required for derivation of pluripotent stem cells." Cell Stem Cell **11**(1): 110-117.

Blimp1 (Prdm1), the key determinant of primordial germ cells (PGCs), plays a combinatorial role with Prdm14 during PGC specification from postimplantation epiblast cells. They together initiate epigenetic reprogramming in early germ cells toward an underlying pluripotent state, which is equivalent to embryonic stem cells (ESCs). Whereas Prdm14 alone can promote reprogramming and is important for the propagation of the pluripotent state, it is not known whether Blimp1 is similarly involved. By using a genetic approach, we demonstrate that Blimp1 is dispensable for the derivation and maintenance of ESCs and postimplantation epiblast stem cells (epiSCs). Notably, Blimp1 is also dispensable for reprogramming epiSCs to ESCs. Thus, although Blimp1 is obligatory for PGC specification, it is not required for the reversion of epiSCs to ESCs and for their maintenance thereafter. This study suggests that reprogramming, including that of somatic cells to ESCs, may not entail an obligatory route through a Blimp1-positive PGC-like state.

Bao, X., et al. (2017). "Human pluripotent stem cell-derived epicardial progenitors can differentiate to endocardial-like endothelial cells." Bioeng Transl Med **2**(2): 191-201.

During heart development, epicardial progenitors contribute various cardiac lineages including smooth muscle cells, cardiac fibroblasts, and endothelial cells. However, their specific contribution to the human endothelium has not yet been resolved, at least in part due to the inability to expand and maintain human primary or pluripotent stem cell (hPSC)-derived epicardial cells. Here we first generated CDH5-2A-eGFP knock-in hPSC lines and differentiated them into self-renewing WT1+ epicardial cells, which gave rise to endothelial cells upon VEGF treatment in vitro. In addition, we found that the percentage of endothelial cells correlated with WT1 expression in a WT1-2A-eGFP reporter line. The resulting endothelial cells

displayed many endocardium-like endothelial cell properties, including high expression levels of endocardial-specific markers, nutrient transporters and well-organized tight junctions. These findings suggest that human epicardial progenitors may have the capacity to form endocardial endothelium during development and have implications for heart regeneration and cardiac tissue engineering.

Barbet, R., et al. (2012). "Expression of the 49 human ATP binding cassette (ABC) genes in pluripotent embryonic stem cells and in early- and late-stage multipotent mesenchymal stem cells: possible role of ABC plasma membrane transporters in maintaining human stem cell pluripotency." Cell Cycle **11**(8): 1611-1620.

The 49-member human ATP binding cassette (ABC) gene family encodes 44 membrane transporters for lipids, ions, peptides or xenobiotics, four translation factors without transport activity, as they lack transmembrane domains, and one pseudogene. To understand the roles of ABC genes in pluripotency and multipotency, we performed a sensitive qRT-PCR analysis of their expression in embryonic stem cells (hESCs), bone marrow-derived mesenchymal stem cells (hMSCs) and hESC-derived hMSCs (hES-MSCs). We confirm that hES-MSCs represent an intermediate developmental stage between hESCs and hMSCs. We observed that 44 ABCs were significantly expressed in hESCs, 37 in hES-MSCs and 35 in hMSCs. These variations are mainly due to plasma membrane transporters with low but significant gene expression: 18 are expressed in hESCs compared with 16 in hES-MSCs and 8 in hMSCs, suggesting important roles in pluripotency. Several of these ABCs shared similar substrates but differ regarding gene regulation. ABCA13 and ABCB4, similarly to ABCB1, could be new markers to select primitive hMSCs with specific plasma membrane transporter (low) phenotypes. ABC proteins performing basal intracellular functions, including translation factors and mitochondrial heme transporters, showed the highest constant gene expression among the three populations. Peptide transporters in the endoplasmic reticulum, Golgi and lysosome were well expressed in hESCs and slightly upregulated in hMSCs, which play important roles during the development of stem cell niches in bone marrow or meningeal tissue. These results will be useful to study specific cell cycle regulation of pluripotent stem cells or ABC dysregulation in complex pathologies, such as cancers or neurological disorders.

Barone, A., et al. (2014). "Sialyl-lactotetra, a novel cell surface marker of undifferentiated human pluripotent stem cells." J Biol Chem **289**(27): 18846-18859.

Cell surface glycoconjugates are used as markers for undifferentiated pluripotent stem cells. Here, antibody binding and mass spectrometry characterization of acid glycosphingolipids isolated from a large number (1 x 10(9) cells) of human embryonic stem cell (hESC) lines allowed identification of several novel acid glycosphingolipids, like the gangliosides sialyl-lactotetraosylceramide and sialyl-globotetraosylceramide, and the sulfated glycosphingolipids sulfatide, sulf-lactosylceramide, and sulf-globopentaosylceramide. A high cell surface expression of sialyl-lactotetra on hESC and human induced pluripotent stem cells (hiPSC) was demonstrated by flow cytometry, immunohistochemistry, and electron microscopy, whereas sulfated glycosphingolipids were only found in intracellular compartments. Immunohistochemistry showed distinct cell surface anti-sialyl-lactotetra staining on all seven hESC lines and three hiPSC lines analyzed, whereas no staining of hESC-derived hepatocyte-like or cardiomyocyte-like cells was obtained. Upon differentiation of hiPSC into hepatocyte-like cells, the sialyl-lactotetra epitope was rapidly down-regulated and not detectable after 14 days. These findings identify sialyl-lactotetra as a promising marker of undifferentiated human pluripotent stem cells.

Barrett, R., et al. (2014). "Reliable generation of induced pluripotent stem cells from human lymphoblastoid cell lines." Stem Cells Transl Med **3**(12): 1429-1434.

Patient-specific induced pluripotent stem cells (iPSCs) hold great promise for many applications, including disease modeling to elucidate mechanisms involved in disease pathogenesis, drug screening, and ultimately regenerative medicine therapies. A frequently used starting source of cells for reprogramming has been dermal fibroblasts isolated from skin biopsies. However, numerous repositories containing lymphoblastoid cell lines (LCLs) generated from a wide array of patients also exist in abundance. To date, this rich bioresource has been severely underused for iPSC generation. We first attempted to create iPSCs from LCLs using two existing methods but were unsuccessful. Here we report a new and more reliable method for LCL reprogramming using episomal plasmids expressing pluripotency factors and p53 shRNA in combination with small molecules. The LCL-derived iPSCs (LCL-iPSCs) exhibited identical characteristics to fibroblast-derived iPSCs (fib-iPSCs), wherein they retained their genotype, exhibited a normal pluripotency profile, and readily differentiated into all three germ-layer cell types. As expected, they also maintained rearrangement of the heavy chain immunoglobulin locus. Importantly, we also show

efficient iPSC generation from LCLs of patients with spinal muscular atrophy and inflammatory bowel disease. These LCL-iPSCs retained the disease mutation and could differentiate into neurons, spinal motor neurons, and intestinal organoids, all of which were virtually indistinguishable from differentiated cells derived from fib-iPSCs. This method for reliably deriving iPSCs from patient LCLs paves the way for using invaluable worldwide LCL repositories to generate new human iPSC lines, thus providing an enormous bioresource for disease modeling, drug discovery, and regenerative medicine applications.

Baxter, M. A., et al. (2010). "Generating hepatic cell lineages from pluripotent stem cells for drug toxicity screening." Stem Cell Res **5**(1): 4-22.

Hepatotoxicity is an enormous and increasing problem for the pharmaceutical industry. Early detection of problems during the drug discovery pathway is advantageous to minimize costs and improve patient safety. However, current cellular models are sub-optimal. This review addresses the potential use of pluripotent stem cells in the generation of hepatic cell lineages. It begins by highlighting the scale of the problem faced by the pharmaceutical industry, the precise nature of drug-induced liver injury and where in the drug discovery pathway the need for additional cell models arises. Current research is discussed, mainly for generating hepatocyte-like cells rather than other liver cell-types. In addition, an effort is made to identify where some of the major barriers remain in translating what is currently hypothesis-driven laboratory research into meaningful platform technologies for the pharmaceutical industry.

Belair, D. G., et al. (2015). "Human vascular tissue models formed from human induced pluripotent stem cell derived endothelial cells." Stem Cell Rev **11**(3): 511-525.

Here we describe a strategy to model blood vessel development using a well-defined induced pluripotent stem cell-derived endothelial cell type (iPSC-EC) cultured within engineered platforms that mimic the 3D microenvironment. The iPSC-ECs used here were first characterized by expression of endothelial markers and functional properties that included VEGF responsiveness, TNF-alpha-induced upregulation of cell adhesion molecules (MCAM/CD146; ICAM1/CD54), thrombin-dependent barrier function, shear stress-induced alignment, and 2D and 3D capillary-like network formation in Matrigel. The iPSC-ECs also formed 3D vascular networks in a variety of engineering contexts, yielded perfusable, interconnected lumen when co-cultured with primary human fibroblasts, and aligned with flow in microfluidics devices. iPSC-EC function during tubule network formation, barrier formation, and sprouting was consistent with that of primary ECs, and the results suggest a VEGF-independent mechanism for sprouting, which is relevant to therapeutic anti-angiogenesis strategies. Our combined results demonstrate the feasibility of using a well-defined, stable source of iPSC-ECs to model blood vessel formation within a variety of contexts using standard in vitro formats.

Ben-Ari, M., et al. (2014). "From beat rate variability in induced pluripotent stem cell-derived pacemaker cells to heart rate variability in human subjects." Heart Rhythm **11**(10): 1808-1818.

BACKGROUND: We previously reported that induced pluripotent stem cell-derived cardiomyocytes manifest beat rate variability (BRV) resembling heart rate variability (HRV) in the human sinoatrial node. We now hypothesized the BRV-HRV continuum originates in pacemaker cells. OBJECTIVE: To investigate whether cellular BRV is a source of HRV dynamics, we hypothesized 3 levels of interaction among different cardiomyocyte entities: (1) single pacemaker cells, (2) networks of electrically coupled pacemaker cells, and (3) the in situ sinoatrial node. METHODS: We measured BRV/HRV properties in single pacemaker cells, induced pluripotent stem cell-derived contracting embryoid bodies (EBs), and electrocardiograms from the same individual. RESULTS: Pronounced BRV/HRV was present at all 3 levels. The coefficient of variance of interbeat intervals and Poincare plot indices SD1 and SD2 for single cells were 20 times greater than those for EBs ($P < .05$) and the in situ heart (the latter two were similar; $P > .05$). We also compared BRV magnitude among single cells, small EBs (~5-10 cells), and larger EBs (>10 cells): BRV indices progressively increased with the decrease in the cell number ($P < .05$). Disrupting intracellular Ca(2+) handling markedly augmented BRV magnitude, revealing a unique bimodal firing pattern, suggesting that intracellular mechanisms contribute to BRV/HRV and the fractal behavior of heart rhythm. CONCLUSION: The decreased BRV magnitude in transitioning from the single cell to the EB suggests that the HRV of in situ hearts originates from the summation and integration of multiple cell-based oscillators. Hence, complex interactions among multiple pacemaker cells and intracellular Ca(2+) handling determine HRV in humans and cardiomyocyte networks.

Bendall, S. C., et al. (2007). "IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro." Nature **448**(7157): 1015-1021.

Distinctive properties of stem cells are not autonomously achieved, and recent evidence points to a level of external control from the microenvironment. Here, we demonstrate that self-renewal and pluripotent properties of human embryonic stem (ES) cells depend on a dynamic interplay between human ES cells and autologously derived human ES cell fibroblast-like cells (hdFs). Human ES cells and hdFs are uniquely defined by insulin-like growth factor (IGF)- and fibroblast growth factor (FGF)-dependence. IGF 1 receptor (IGF1R) expression was exclusive to the human ES cells, whereas FGF receptor 1 (FGFR1) expression was restricted to surrounding hdFs. Blocking the IGF-II/IGF1R pathway reduced survival and clonogenicity of human ES cells, whereas inhibition of the FGF pathway indirectly caused differentiation. IGF-II is expressed by hdFs in response to FGF, and alone was sufficient in maintaining human ES cell cultures. Our study demonstrates a direct role of the IGF-II/IGF1R axis on human ES cell physiology and establishes that hdFs produced by human ES cells themselves define the stem cell niche of pluripotent human stem cells.

Ben-David, U., et al. (2014). "Elimination of undifferentiated cancer cells by pluripotent stem cell inhibitors." J Mol Cell Biol **6**(3): 267-269.

Bershteyn, M., et al. (2014). "Cell-autonomous correction of ring chromosomes in human induced pluripotent stem cells." Nature **507**(7490): 99-103.

Ring chromosomes are structural aberrations commonly associated with birth defects, mental disabilities and growth retardation. Rings form after fusion of the long and short arms of a chromosome, and are sometimes associated with large terminal deletions. Owing to the severity of these large aberrations that can affect multiple contiguous genes, no possible therapeutic strategies for ring chromosome disorders have been proposed. During cell division, ring chromosomes can exhibit unstable behaviour leading to continuous production of aneuploid progeny with low viability and high cellular death rate. The overall consequences of this chromosomal instability have been largely unexplored in experimental model systems. Here we generated human induced pluripotent stem cells (iPSCs) from patient fibroblasts containing ring chromosomes with large deletions and found that reprogrammed cells lost the abnormal chromosome and duplicated the wild-type homologue through the compensatory uniparental disomy (UPD) mechanism. The karyotypically normal iPSCs with isodisomy for the corrected chromosome outgrew co-existing aneuploid populations, enabling rapid and efficient isolation of patient-derived iPSCs devoid of the original chromosomal aberration. Our results suggest a fundamentally different function for cellular reprogramming as a means of 'chromosome therapy' to reverse combined loss-of-function across many genes in cells with large-scale aberrations involving ring structures. In addition, our work provides an experimentally tractable human cellular system for studying mechanisms of chromosomal number control, which is of critical relevance to human development and disease.

Bharadwaj, P., et al. (2013). "Reprogramming of fetal cells by avian EE for generation of pluripotent stem cell like cells in caprine." Res Vet Sci **95**(2): 638-643.

The present work was carried out to study the ability of avian "Extract Egg" (EE) for reprogramming caprine fetal cells. The isolated caprine fetal cells were cultured in stem cell media supplemented with different percentages of either EE or FBS. The results indicated that the supplementation of 2-4% EE formed lesser but larger size stem cell like cell colonies as compared to 6% or 10% EE. The expression of pluripotent genes were comparatively higher in colonies developed in 2% or 4% as compared to 6% or 10% EE. Further, immunocytochemistry revealed that the colonies developed in all percentage of EE expressed pluripotent markers like Oct4, Nanog, TRA-1-60 and TRA-1-81. Our findings indicated that avian EE has the potentiality to reprogram caprine fetal cells into embryonic state which may help in generation of pluripotent stem cells without using viral vector.

Bieberich, E., et al. (2004). "Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants." J Cell Biol **167**(4): 723-734.

The formation of stem cell-derived tumors (teratomas) is observed when engrafting undifferentiated embryonic stem (ES) cells, embryoid body-derived cells (EBCs), or mammalian embryos and is a significant obstacle to stem cell therapy. We show that in tumors formed after engraftment of EBCs into mouse brain, expression of the pluripotency marker Oct-4 colocalized with that of prostate apoptosis response-4 (PAR-4), a protein mediating ceramide-induced apoptosis during neural differentiation of ES cells. We tested the ability of the novel ceramide analogue N-oleoyl serinol (S18) to eliminate mouse and human Oct-4(+)/PAR-4(+) cells and to increase the proportion of nestin(+) neuroprogenitors in EBC-derived cell cultures and grafts. S18-treated EBCs persisted in the hippocampal area and showed neuronal lineage differentiation as indicated by the expression of beta-tubulin III. However, untreated cells formed numerous teratomas that contained derivatives of endoderm, mesoderm, and

ectoderm. Our results show for the first time that ceramide-induced apoptosis eliminates residual, pluripotent EBCs, prevents teratoma formation, and enriches the EBCs for cells that undergo neural differentiation after transplantation.

Bilican, B., et al. (2013). "Unpicking neurodegeneration in a dish with human pluripotent stem cells: one cell type at a time." Cell Cycle **12**(15): 2339-2340.

Bobis-Wozowicz, S., et al. (2015). "Human Induced Pluripotent Stem Cell-Derived Microvesicles Transmit RNAs and Proteins to Recipient Mature Heart Cells Modulating Cell Fate and Behavior." Stem Cells **33**(9): 2748-2761.

Microvesicles (MVs) are membrane-enclosed cytoplasmic fragments released by normal and activated cells that have been described as important mediators of cell-to-cell communication. Although the ability of human induced pluripotent stem cells (hiPSCs) to participate in tissue repair is being increasingly recognized, the use of hiPSC-derived MVs (hiPSC-MVs) in this regard remains unknown. Accordingly, we investigated the ability of hiPSC-MVs to transfer bioactive molecules including mRNA, microRNA (miRNA), and proteins to mature target cells such as cardiac mesenchymal stromal cells (cMSCs), and we next analyzed effects of hiPSC-MVs on fate and behavior of such target cells. The results show that hiPSC-MVs derived from integration-free hiPSCs cultured under serum-free and feeder-free conditions are rich in mRNA, miRNA, and proteins originated from parent cells; however, the levels of expression vary between donor cells and MVs. Importantly, we found that transfer of hiPSC components by hiPSC-MVs impacted on transcriptome and proteomic profiles of target cells as well as exerted proliferative and protective effects on cMSCs, and enhanced their cardiac and endothelial differentiation potential. hiPSC-MVs also transferred exogenous transcripts from genetically modified hiPSCs that opens new perspectives for future strategies to enhance MV content. We conclude that hiPSC-MVs are effective vehicles for transferring iPSC attributes to adult somatic cells, and hiPSC-MV-mediated horizontal transfer of RNAs and proteins to injured tissues may be used for therapeutic tissue repair. In this study, for the first time, we propose a new concept of use of hiPSCs as a source of safe acellular bioactive derivatives for tissue regeneration.

Bodine, D. M., et al. (1994). "Efficient retrovirus transduction of mouse pluripotent hematopoietic stem cells mobilized into the peripheral blood by treatment with granulocyte colony-stimulating factor and stem cell factor." Blood **84**(5): 1482-1491.

Cytokine-mobilized peripheral blood cells have been shown to participate in hematopoietic recovery after bone marrow (BM) transplantation, and are proposed to be useful targets for retrovirus-mediated gene transfer protocols. We treated mice with granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) to mobilize hematopoietic progenitor cells into the peripheral blood. These cells were analyzed for the number and frequency of pluripotent hematopoietic stem cells (PHSC). We found that splenectomized animals treated for 5 days with G-CSF and SCF showed a threefold increase in the absolute number of PHSC over normal mice. The number of peripheral-blood PHSC increased 250-fold from 29 per untreated mouse to 7,200 in peripheral-blood PHSC in splenectomized animals treated for 5 days with G-CSF and SCF. Peripheral blood PHSC mobilized by treatment with G-CSF and SCF were analyzed for their ability to be transduced by retroviral vectors. Peripheral-blood PHSC from splenectomized animals G-CSF and SCF were transduced with a recombinant retrovirus containing the human MDR-1 gene. The frequency of gene transfer into peripheral blood PHSC from animals treated for 5 and 7 days was two-fold and threefold higher than gene transfer into PHSC from the BM of 5-fluorouracil-treated mice ($P < .01$). We conclude that peripheral blood stem cells mobilized by treatment with G-CSF and SCF are excellent targets for retrovirus-mediated gene transfer.

Bodine, D. M., et al. (1993). "In vivo administration of stem cell factor to mice increases the absolute number of pluripotent hematopoietic stem cells." Blood **82**(2): 445-455.

We have examined the effects of administration of stem cell-factor (SCF) on the number and distribution of pluripotent hematopoietic stem cells (PHSC) in normal mice. Using the competitive repopulation assay we found that in vivo administration of SCF increases the absolute number of PHSC per mouse threefold. The increased numbers of PHSC are found in the peripheral blood and spleen of the SCF-treated animals. The spleen and peripheral blood stem cells completely repopulated the erythroid, myeloid, and lymphoid lineages of irradiated or W/Wv hosts, similar to bone marrow PHSC. PHSC from the peripheral blood of SCF-treated mice have a lineage marker-negative, c-kit-positive phenotype that is indistinguishable from that of bone marrow PHSC. The increase in the absolute number of spleen PHSC is associated with efficient gene transfer to these cells without prior treatment with 5-fluorouracil. This is a US government work. There are no restrictions on its use.

Boonkaew, B., et al. (2018). "Establishment of an integration-free induced pluripotent stem cell line (MUSIi005-A) from exfoliated renal epithelial cells." Stem Cell Res **30**: 34-37.

Human induced pluripotent stem cells (iPSCs) were generated from exfoliated renal epithelial cells isolated from a urine sample of a 31-year-old healthy woman. Epithelial cells were characterized for the expression of E-cadherin and reprogrammed using non-integrating Sendai viral vectors. The urine-derived iPSC line (designated as MUSIi005-A) was karyotypically normal, expressed pluripotent markers, differentiated into cells of three embryonic germ layers, and showed no viral and transgene expressions at passage 29. Our protocol offers a non-invasive and efficient approach for iPSC generation from patients with genetic or acquired disorders.

Borestrom, C., et al. (2014). "Footprint-free human induced pluripotent stem cells from articular cartilage with redifferentiation capacity: a first step toward a clinical-grade cell source." Stem Cells Transl Med **3**(4): 433-447.

Human induced pluripotent stem cells (iPSCs) are potential cell sources for regenerative medicine; however, clinical applications of iPSCs are restricted because of undesired genomic modifications associated with most reprogramming protocols. We show, for the first time, that chondrocytes from autologous chondrocyte implantation (ACI) donors can be efficiently reprogrammed into iPSCs using a nonintegrating method based on mRNA delivery, resulting in footprint-free iPSCs (no genome-sequence modifications), devoid of viral factors or remaining reprogramming molecules. The search for universal allogeneic cell sources for the ACI regenerative treatment has been difficult because making chondrocytes with high matrix-forming capacity from pluripotent human embryonic stem cells has proven challenging and human mesenchymal stem cells have a predisposition to form hypertrophic cartilage and bone. We show that chondrocyte-derived iPSCs can be redifferentiated in vitro into cartilage matrix-producing cells better than fibroblast-derived iPSCs and on par with the donor chondrocytes, suggesting the existence of a differentiation bias toward the somatic cell origin and making chondrocyte-derived iPSCs a promising candidate universal cell source for ACI. Whole-genome single nucleotide polymorphism array and karyotyping were used to verify the genomic integrity and stability of the established iPSC lines. Our results suggest that RNA-based technology eliminates the risk of genomic integrations or aberrations, an important step toward a clinical-grade cell source for regenerative medicine such as treatment of cartilage defects and osteoarthritis.

Brafman, D. A. (2015). "Generation, Expansion, and Differentiation of Human Pluripotent Stem Cell (hPSC) Derived Neural Progenitor Cells (NPCs)." Methods Mol Biol **1212**: 87-102.

Human pluripotent stem cell (hPSC)-derived neural progenitor cells (NPCs), a multipotent cell population that is capable of near indefinite expansion and subsequent differentiation into the various cell types that comprise the central nervous system (CNS), could provide an unlimited source of cells for neural-related cell-based therapies and disease modeling. However, the use of NPCs for the study and treatment of a variety of debilitating neurological diseases requires the development of scalable and reproducible protocols for their generation, expansion, characterization, and neuronal differentiation. Here, we describe a serum-free method for the stepwise generation of NPCs from hPSCs through the sequential formation of embryoid bodies (EBs) and neuro-epithelial-like rosettes. NPCs isolated from neural rosette cultures can be homogenously expanded while maintaining high expression of pan-neural markers such as SOX1, SOX2, and Nestin. Finally, this protocol allows for the robust differentiation of NPCs into microtubule-associated protein 2 (MAP2) and beta-Tubulin-III (beta3T) positive neurons.

Brafman, D. A., et al. (2013). "Analysis of SOX2-expressing cell populations derived from human pluripotent stem cells." Stem Cell Reports **1**(5): 464-478.

SOX2 is involved in several cell and developmental processes, including maintenance of embryonic stem cells, differentiation of neural progenitor cells, and patterning of gut endoderm. To study its role in a human system, we generated a human embryonic stem cell (hESC) line harboring a reporter gene encoding GFP in the SOX2 locus. This SOX2 reporter line faithfully recapitulates expression of the SOX2 gene in undifferentiated human pluripotent stem cells (hPSCs), neural progenitor cells (NPCs), and anterior foregut endoderm (AFE). In undifferentiated hESCs, GFP expression corresponds to those cells with highest levels of expression of genes associated with the pluripotent state. In NPCs, expression of GFP can be employed to isolate cells expressing markers associated with NPC multipotency. In AFE, we used transcriptome-wide expression analysis to identify cell surface markers with elevated expression in this population, thereby facilitating isolation and purification of this hPSC-derived cell population.

Brauer, P. M., et al. (2016). "Modeling altered T-cell development with induced pluripotent stem cells from patients with RAG1-dependent immune deficiencies." Blood **128**(6): 783-793.

Primary immunodeficiency diseases comprise a group of heterogeneous genetic defects that affect immune system development and/or function. Here we use in vitro differentiation of human induced pluripotent stem cells (iPSCs) generated from patients with different recombination-activating gene 1 (RAG1) mutations to assess T-cell development and T-cell receptor (TCR) V(D)J recombination. RAG1-mutants from severe combined immunodeficient (SCID) patient cells showed a failure to sustain progression beyond the CD3(--)CD4(-)CD8(-)CD7(+)CD5(+)CD38(-)CD31(-/lo)CD45RA(+) stage of T-cell development to reach the CD3(-/+)CD4(+)CD8(+)CD7(+)CD5(+)CD38(+)CD31(+)CD45RA(-) stage. Despite residual mutant RAG1 recombination activity from an Omenn syndrome (OS) patient, similar impaired T-cell differentiation was observed, due to increased single-strand DNA breaks that likely occur due to heterodimers consisting of both an N-terminal truncated and a catalytically dead RAG1. Furthermore, deep-sequencing analysis of TCR-beta (TRB) and TCR-alpha (TRA) rearrangements of CD3(-)CD4(+)CD8(-) immature single-positive and CD3(+)CD4(+)CD8(+) double-positive cells showed severe restriction of repertoire diversity with preferential usage of few Variable, Diversity, and Joining genes, and skewed length distribution of the TRB and TRA complementary determining region 3 sequences from SCID and OS iPSC-derived cells, whereas control iPSCs yielded T-cell progenitors with a broadly diversified repertoire. Finally, no TRA/delta excision circles (TRECs), a marker of TRA/delta locus rearrangements, were detected in SCID and OS-derived T-lineage cells, consistent with a pre-TCR block in T-cell development. This study compares human T-cell development of SCID vs OS patients, and elucidates important differences that help to explain the wide range of immunologic phenotypes that result from different mutations within the same gene of various patients.

Brehany, J. (2005). "Nontraditional sources of pluripotent stem cells: a new chapter in the debate about embryonic stem cell research." Health Care Ethics USA **13**(2): E3.

Recent efforts to resolve the political impasse over human embryonic stem cells (ESC) have generated proposals for obtaining ESC while avoiding the destruction of human embryos. This new chapter in the scientific and ethical debate provides an important opportunity to introduce additional ethical considerations to enhance public discourse.

Bremer, S. and R. Vogel (1999). "Pluripotent stem cells of the mouse as a potential in vitro model for mammalian germ cells. Sister chromatid exchanges induced by MMC and ENU in undifferentiated cell lines compared to differentiated cell lines." Mutat Res **444**(1): 97-102.

We tried to develop an in-vitro test system which could serve as a model for mammalian germ cells in vivo. Two pluripotent cell types were used, because they express some germ cell specific immunological and biochemical markers: (1) Embryonal carcinoma cells (ECC) of the line P19 had been isolated from a teratocarcinoma of murine primordial germ cells (PGC). (2) Embryonal stem cells (ESC) are obtained from the inner cell mass of mouse blastocysts. Sister chromatid exchanges (SCE) induced by mitomycin C and ethylnitrosourea (ENU) were analysed in the two undifferentiated cell lines, ECC and ESC, to detect differences in their sensitivity compared with differentiated cell lines of the mouse. Neither of the model cell lines have shown a greater sensibility after exposure to MMC and ENU. In contrary, the carcinoma cell line was able to tolerate higher concentrations of these genotoxicants. Therefore, SCE analysis in the ECC and ESC lines used does not provide a suitable model for genotoxicity testing on mammalian germ cells.

Brenner, C. and W. M. Franz (2014). "Pluripotent-stem-cell-derived epicardial cells: a step toward artificial cardiac tissue." Cell Stem Cell **15**(5): 533-534.

Epicardial cells are crucial for heart development, function, and regeneration, but methods to study them have been lacking. Using WNT and BMP signaling pathways, Witty et al. (2014) have now developed an elaborate method to generate fully functional epicardial cells from human pluripotent stem cells, allowing their in vitro investigation.

Briggs, S. F., et al. (2015). "Single-Cell XIST Expression in Human Preimplantation Embryos and Newly Reprogrammed Female Induced Pluripotent Stem Cells." Stem Cells **33**(6): 1771-1781.

The process of X chromosome inactivation (XCI) during reprogramming to produce human induced pluripotent stem cells (iPSCs), as well as during the extensive programming that occurs in human preimplantation development, is not well-understood. Indeed, studies of XCI during reprogramming to iPSCs report cells with two active X chromosomes and/or cells with one inactive X chromosome. Here, we examine expression of the long noncoding RNA, XIST, in single cells of human embryos through the oocyte-to-embryo transition and in new mRNA reprogrammed iPSCs. We show that

XIST is first expressed beginning at the 4-cell stage, coincident with the onset of embryonic genome activation in an asynchronous manner. Additionally, we report that mRNA reprogramming produces iPSCs that initially express XIST transcript; however, expression is rapidly lost with culture. Loss of XIST and H3K27me3 enrichment at the inactive X chromosome at late passage results in X chromosome expression changes. Our data may contribute to applications in disease modeling and potential translational applications of female stem cells.

Bylund, J. B., et al. (2017). "Coordinated Proliferation and Differentiation of Human-Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells Depend on Bone Morphogenetic Protein Signaling Regulation by GREMLIN 2." Stem Cells Dev **26**(9): 678-693.

Heart development depends on coordinated proliferation and differentiation of cardiac progenitor cells (CPCs), but how the two processes are synchronized is not well understood. Here, we show that the secreted Bone Morphogenetic Protein (BMP) antagonist GREMLIN 2 (GREM2) is induced in CPCs shortly after cardiac mesoderm specification during differentiation of human pluripotent stem cells. GREM2 expression follows cardiac lineage differentiation independently of the differentiation method used, or the origin of the pluripotent stem cells, suggesting that GREM2 is linked to cardiogenesis. Addition of GREM2 protein strongly increases cardiomyocyte output compared to established procardiogenic differentiation methods. Our data show that inhibition of canonical BMP signaling by GREM2 is necessary to promote proliferation of CPCs. However, canonical BMP signaling inhibition alone is not sufficient to induce cardiac differentiation, which depends on subsequent JNK pathway activation specifically by GREM2. These findings may have broader implications in the design of approaches to orchestrate growth and differentiation of pluripotent stem cell-derived lineages that depend on precise regulation of BMP signaling.

Cai, S., et al. (2018). "Human Induced Pluripotent Stem Cell-Derived Sensory Neurons for Fate Commitment of Bone Marrow Stromal Cell-Derived Schwann Cells." Methods Mol Biol **1739**: 149-160.

Here we describe the in vitro derivation of sensory neurons for use in effecting fate commitment of Schwann cell-like cells derived from human bone marrow stromal cells (hBMSCs). We adopt a novel combination of small molecules in an 8-day program that induces the differentiation of human induced pluripotent stem cells into sensory neurons. In co-cultures, the derived sensory neurons present contact-dependent cues to direct hBMSC-derived Schwann cell-like cells toward the Schwann cell fate. These derived human Schwann cells survive passaging and cryopreservation, retain marker expression despite withdrawal of glia-inducing medium and neuronal cues, demonstrate capacity for myelination, and therefore promise application in autologous transplantation and re-myelination therapy.

Carcamo-Orive, I., et al. (2017). "Induced Pluripotent Stem Cell-Derived Endothelial Cells in Insulin Resistance and Metabolic Syndrome." Arterioscler Thromb Vasc Biol **37**(11): 2038-2042.

Insulin resistance leads to a number of metabolic and cellular abnormalities including endothelial dysfunction that increase the risk of vascular disease. Although it has been particularly challenging to study the genetic determinants that predispose to abnormal function of the endothelium in insulin-resistant states, the possibility of deriving endothelial cells from induced pluripotent stem cells generated from individuals with detailed clinical phenotyping, including accurate measurements of insulin resistance accompanied by multilevel omic data (eg, genetic and genomic characterization), has opened new avenues to study this relationship. Unfortunately, several technical barriers have hampered these efforts. In the present review, we summarize the current status of induced pluripotent stem cell-derived endothelial cells for modeling endothelial dysfunction associated with insulin resistance and discuss the challenges to overcoming these limitations.

Carlessi, L., et al. (2013). "Brain and induced pluripotent stem cell-derived neural stem cells as an in vitro model of neurodegeneration in ataxia-telangiectasia." Exp Biol Med (Maywood) **238**(3): 301-307.

The ataxia telangiectasia mutated (ATM) kinase is a key transducer of the cellular response to DNA double strand breaks and its deficiency causes ataxia-telangiectasia (A-T), a pleiotropic genetic disorder primarily characterized by cerebellar neuropathy, immunodeficiency and cancer predisposition. While enormous progress has been achieved in elucidating the biochemical and functional regulation of ATM in DNA damage response, and more recently in redox signalling and antioxidant defence, the factors that make neurons in A-T extremely vulnerable remain unclear. Given also that ATM knockout mice do not recapitulate the central nervous system phenotype, a number of human neural stem cell (hNSC) model systems have been developed to provide insights into the mechanisms of neurodegeneration associated with ATM dysfunction. Here we review the hNSC systems developed by us an others to model A-T.

Carpenter, L., et al. (2011). "Human induced pluripotent stem cells are capable of B-cell lymphopoiesis." Blood **117**(15): 4008-4011.

Induced pluripotent stem (iPS) cells offer a unique potential for understanding the molecular basis of disease and development. Here we have generated several human iPS cell lines, and we describe their pluripotent phenotype and ability to differentiate into erythroid cells, monocytes, and endothelial cells. More significantly, however, when these iPS cells were differentiated under conditions that promote lympho-hematopoiesis from human embryonic stem cells, we observed the formation of pre-B cells. These cells were CD45(+)CD19(+)CD10(+) and were positive for transcripts Pax5, IL7alphaR, lambda-like, and VpreB receptor. Although they were negative for surface IgM and CD5 expression, iPS-derived CD45(+)CD19(+) cells also exhibited multiple genomic D-J(H) rearrangements, which supports a pre-B-cell identity. We therefore have been able to demonstrate, for the first time, that human iPS cells are able to undergo hematopoiesis that contributes to the B-cell lymphoid lineage.

Casaroli-Marano, R. P., et al. (2015). "Potential Role of Induced Pluripotent Stem Cells (IPSCs) for Cell-Based Therapy of the Ocular Surface." J Clin Med **4**(2): 318-342.

The integrity and normal function of the corneal epithelium are crucial for maintaining the cornea's transparency and vision. The existence of a cell population with progenitor characteristics in the limbus maintains a dynamic of constant epithelial repair and renewal. Currently, cell-based therapies for bio replacement-cultured limbal epithelial transplantation (CLET) and cultured oral mucosal epithelial transplantation (COMET)-present very encouraging clinical results for treating limbal stem cell deficiency (LSCD) and restoring vision. Another emerging therapeutic approach consists of obtaining and implementing human progenitor cells of different origins in association with tissue engineering methods. The development of cell-based therapies using stem cells, such as human adult mesenchymal or induced pluripotent stem cells (IPSCs), represent a significant breakthrough in the treatment of certain eye diseases, offering a more rational, less invasive, and better physiological treatment option in regenerative medicine for the ocular surface. This review will focus on the main concepts of cell-based therapies for the ocular surface and the future use of IPSCs to treat LSCD.

Chan, Y. S., et al. (2013). "A PRC2-dependent repressive role of PRDM14 in human embryonic stem cells and induced pluripotent stem cell reprogramming." Stem Cells **31**(4): 682-692.

PRDM14 is an important determinant of the human embryonic stem cell (ESC) identity and works in concert with the core ESC regulators to activate pluripotency-associated genes. PRDM14 has been previously reported to exhibit repressive activity in mouse ESCs and primordial germ cells; and while PRDM14 has been implicated to suppress differentiation genes in human ESCs, the exact mechanism of this repressive activity remains unknown. In this study, we provide evidence that PRDM14 is a direct repressor of developmental genes in human ESCs. PRDM14 binds to silenced genes in human ESCs and its global binding profile is enriched for the repressive trimethylation of histone H3 lysine 27 (H3K27me3) modification. Further investigation reveals that PRDM14 interacts directly with the chromatin regulator polycomb repressive complex 2 (PRC2) and PRC2 binding is detected at PRDM14-bound loci in human ESCs. Depletion of PRDM14 reduces PRC2 binding at these loci and the concomitant reduction of H3K27me3 modification. Using reporter assays, we demonstrate that gene loci bound by PRDM14 exhibit repressive activity that is dependent on both PRDM14 and PRC2. In reprogramming human fibroblasts into induced pluripotent stem cells (iPSCs), ectopically expressed PRDM14 can repress these developmental genes in fibroblasts. In addition, we show that PRDM14 recruits PRC2 to repress a key mesenchymal gene ZEB1, which enhances mesenchymal-to-epithelial transition in the initiation event of iPSC reprogramming. In summary, our study reveals a repressive role of PRDM14 in the maintenance and induction of pluripotency and identifies PRDM14 as a new regulator of PRC2.

Chang, C. W., et al. (2013). "Engineering cell-material interfaces for long-term expansion of human pluripotent stem cells." Biomaterials **34**(4): 912-921.

Cost-effective and scalable synthetic matrices that support long-term expansion of human pluripotent stem cells (hPSCs) have many applications, ranging from drug screening platforms to regenerative medicine. Here, we report the development of a hydrogel-based matrix containing synthetic heparin-mimicking moieties that supports the long-term expansion of hPSCs (>/=20 passages) in a chemically defined medium. HPSCs expanded on this synthetic matrix maintained their characteristic morphology, colony forming ability, karyotypic stability, and differentiation potential. We also used the synthetic matrix as a platform to investigate the effects of various physicochemical properties of the extracellular environment on the adhesion, growth, and self-renewal

of hPSCs. The observed cellular responses can be explained in terms of matrix interface-mediated binding of extracellular matrix proteins, growth factors, and other cell-secreted factors, which create an instructive microenvironment to support self-renewal of hPSCs. These synthetic matrices, which comprise of "off-the-shelf" components and are easy to synthesize, provide an ideal tool to elucidate the molecular mechanisms that control stem cell fate.

Chang, C. W., et al. (2014). "Broad T-cell receptor repertoire in T-lymphocytes derived from human induced pluripotent stem cells." PLoS One **9**(5): e97335.

Human induced pluripotent stem cells (hiPSCs) have enormous potential for the treatment of inherited and acquired disorders. Recently, antigen-specific T lymphocytes derived from hiPSCs have been reported. However, T lymphocyte populations with broad T cell receptor (TCR) diversity have not been generated. We report that hiPSCs derived from skin biopsy are capable of producing T lymphocyte populations with a broad TCR repertoire. In vitro T cell differentiation follows a similar developmental program as observed in vivo, indicated by sequential expression of CD7, intracellular CD3 and surface CD3. The gammadelta TCR locus is rearranged first and is followed by rearrangement of the alphabeta locus. Both gammadelta and alphabeta T cells display a diverse TCR repertoire. Upon activation, the cells express CD25, CD69, cytokines (TNF-alpha, IFN-gamma, IL-2) and cytolytic proteins (Perforin and Granzyme-B). These results suggest that most, if not all, mechanisms required to generate functional T cells with a broad TCR repertoire are intact in our in vitro differentiation protocol. These data provide a foundation for production of patient-specific T cells for the treatment of acquired or inherited immune disorders and for cancer immunotherapy.

Chang, G., et al. (2010). "Linking incomplete reprogramming to the improved pluripotency of murine embryonal carcinoma cell-derived pluripotent stem cells." PLoS One **5**(4): e10320.

Somatic cell nuclear transfer (SCNT) has been proved capable of reprogramming various differentiated somatic cells into pluripotent stem cells. Recently, induced pluripotent stem cells (iPS) have been successfully derived from mouse and human somatic cells by the over-expression of a combination of transcription factors. However, the molecular mechanisms underlying the reprogramming mediated by either the SCNT or iPS approach are poorly understood. Increasing evidence indicates that many tumor pathways play roles in the derivation of iPS cells. Embryonal carcinoma (EC) cells have the characteristics of both stem cells and cancer cells and thus they might be the better candidates for elucidating the details of the reprogramming process. Although previous studies indicate that EC cells cannot be reprogrammed into real pluripotent stem cells, the reasons for this remain unclear. Here, nuclei from mouse EC cells (P19) were transplanted into enucleated oocytes and pluripotent stem cells (P19 NTES cells) were subsequently established. Interestingly, P19 NTES cells prolonged the development of tetraploid aggregated embryos compared to EC cells alone. More importantly, we found that the expression recovery of the imprinted H19 gene was dependent on the methylation state in the differential methylation region (DMR). The induction of Nanog expression, however, was independent of the promoter region DNA methylation state in P19 NTES cells. A whole-genome transcriptome analysis further demonstrated that P19 NTES cells were indeed the intermediates between P19 cells and ES cells and many interesting genes were uncovered that may be responsible for the failed reprogramming of P19 cells. To our knowledge, for the first time, we linked incomplete reprogramming to the improved pluripotency of EC cell-derived pluripotent stem cells. The candidate genes we discovered may be useful not only for understanding the mechanisms of reprogramming, but also for deciphering the transition between tumorigenesis and pluripotency.

Chao, H. M. and E. Chern (2018). "Patient-derived induced pluripotent stem cells for models of cancer and cancer stem cell research." J Formos Med Assoc.

Induced pluripotent stem cells (iPSCs) are embryonic stem cell-like cells reprogrammed from somatic cells by four transcription factors, OCT4, SOX2, KLF4 and c-MYC. iPSCs derived from cancer cells (cancer-iPSCs) could be a novel strategy for studying cancer. During cancer cell reprogramming, the epigenetic status of the cancer cell may be altered, such that it acquires stemness and pluripotency. The cellular behavior of the reprogrammed cells exhibits dynamic changes during the different stages of reprogramming. The cells may acquire the properties of cancer stem cells (CSCs) during the process of reprogramming, and lose their carcinogenic properties during reprogramming into a cancer-iPSCs. Differentiation of cancer-iPSCs by teratoma formation or organoid culturing could mimic the process of tumorigenesis. Some of the molecular mechanisms associated with cancer progression could be elucidated using the cancer-iPSC model. Furthermore, cancer-iPSCs could be expanded in culture system or bioreactors, and serve as cell sources for research, and as personal disease models for therapy and drug

screening. This article introduces cancer studies that used the cell reprogramming strategy.

Chatterjee, I., et al. (2016). "Induced Pluripotent Stem (iPS) Cell Culture Methods and Induction of Differentiation into Endothelial Cells." Methods Mol Biol **1357**: 311-327.

The study of stem cell behavior and differentiation in a developmental context is complex, time-consuming, and expensive, and for this reason, cell culture remains a method of choice for developmental and regenerative biology and mechanistic studies. Similar to ES cells, iPS cells have the ability to differentiate into endothelial cells (ECs), and the route for differentiation appears to mimic the developmental process that occurs during the formation of an embryo. Traditional EC induction methods from embryonic stem (ES) cells rely mostly on the formation of embryoid body (EB), which employs feeder or feeder-free conditions in the presence or absence of supporting cells. Similar to ES cells, iPS cells can be cultured in feeder layer or feeder-free conditions. Here, we describe the iPS cell culture methods and induction differentiation of these cells into ECs. We use anti-mouse Flk1 and anti-mouse VE-cadherin to isolate and characterize mouse ECs, because these antibodies are commercially available and their use has been described in the literature, including by our group. The ECs produced by this method have been used by our laboratory, and we have demonstrated their in vivo potential. We also discuss how iPS cells differ in their ability to differentiate into endothelial cells in culture.

Chen, C., et al. (2013). "Inhibition of neuronal nitric oxide synthase activity promotes migration of human-induced pluripotent stem cell-derived neural stem cells toward cancer cells." J Neurochem **126**(3): 318-330.

The breakthrough in derivation of human-induced pluripotent stem cells (hiPSCs) provides an approach that may help overcome ethical and allergenic challenges posed in numerous medical applications involving human cells, including neural stem/progenitor cells (NSCs). Considering the great potential of NSCs in targeted cancer gene therapy, we investigated in this study the tumor tropism of hiPSC-derived NSCs and attempted to enhance the tropism by manipulation of biological activities of proteins that are involved in regulating the migration of NSCs toward cancer cells. We first demonstrated that hiPSC-NSCs displayed tropism for both glioblastoma cells and breast cancer cells in vitro and in vivo. We then compared gene expression profiles between migratory and non-migratory hiPSC-NSCs toward these cancer cells and observed that the gene encoding neuronal nitric oxide synthase (nNOS) was down-regulated in migratory hiPSC-NSCs. Using nNOS inhibitors and nNOS siRNAs, we demonstrated that this protein is a relevant regulator in controlling migration of hiPSC-NSCs toward cancer cells, and that inhibition of its activity or down-regulation of its expression can sensitize poorly migratory NSCs and be used to improve their tumor tropism. These findings suggest a novel application of nNOS inhibitors in neural stem cell-mediated cancer therapy.

Chen, H. F., et al. (2011). "Surface marker epithelial cell adhesion molecule and E-cadherin facilitate the identification and selection of induced pluripotent stem cells." Stem Cell Rev **7**(3): 722-735.

The derivation of induced pluripotent stem cells (iPSCs) requires not only efficient reprogramming methods, but also reliable markers for identification and purification of iPSCs. Here, we demonstrate that surface markers, epithelial cells adhesion molecule (EpCAM) and epithelial cadherin (E-cadherin) can be used for efficient identification and/or isolation of reprogrammed mouse iPSCs. By viral transduction of Oct4, Sox2, Klf4 and n- or c-Myc into mouse embryonic fibroblasts, we observed that the conventional mouse embryonic stem cell (mESC) markers, alkaline phosphatase (AP) and stage-specific embryonic antigen 1 (SSEA1), were expressed in incompletely reprogrammed cells that did not express all the exogenous reprogramming factors or failed to acquire pluripotent status even though exogenous reprogramming factors were expressed. EpCAM and E-cadherin, however, remained inactivated in these cells. Expression of EpCAM and E-cadherin correlated with the activation of Nanog and endogenous Oct4, and was only seen in the successfully reprogrammed iPSCs. Furthermore, purification of EpCAM-expressing cells at late reprogramming stage by FACS enriched the Nanog-expressing cell population suggesting the feasibility of selecting successful reprogrammed mouse iPSCs by EpCAM expression. We have thus identified new surface markers that can efficiently identify successfully reprogrammed iPSCs and provide an effective means for iPSC isolation.

Chen, J., et al. (2018). "Fine Tuning of Canonical Wnt Stimulation Enhances Differentiation of Pluripotent Stem Cells Independent of beta-Catenin-Mediated T-Cell Factor Signaling." Stem Cells **36**(6): 822-833.

The canonical Wnt/beta-catenin pathway is crucial for early embryonic patterning, tissue homeostasis, and regeneration. While canonical Wnt/beta-catenin stimulation has been used extensively to modulate pluripotency and differentiation of pluripotent stem cells (PSCs), the mechanism of these two seemingly opposing roles has not been fully characterized and is currently largely attributed to activation of nuclear Wnt target genes. Here, we show

that low levels of Wnt stimulation via ectopic expression of Wnt1 or administration of glycogen synthase kinase-3 inhibitor CHIR99021 significantly increases PSC differentiation into neurons, cardiomyocytes and early endodermal intermediates. Our data indicate that enhanced differentiation outcomes are not mediated through activation of traditional Wnt target genes but by beta-catenin's secondary role as a binding partner of membrane bound cadherins ultimately leading to the activation of developmental genes. In summary, fine-tuning of Wnt signaling to subthreshold levels for detectable nuclear beta-catenin function appears to act as a switch to enhance differentiation of PSCs into multiple lineages. Our observations highlight a mechanism by which Wnt/beta-catenin signaling can achieve dosage dependent dual roles in regulating self-renewal and differentiation. Stem Cells 2018;36:822-833.

Chen, L., et al. (2014). "Mouse induced pluripotent stem cell microenvironment generates epithelial-mesenchymal transition in mouse Lewis lung cancer cells." Am J Cancer Res **4**(1): 80-88.

Induced pluripotent stem (iPS) cells may be a powerful tool in regenerative medicine, but their potential tumorigenicity is a significant challenge for the clinical use of iPS cells. Previously, we succeeded in converting miPS cells into cancer stem cells (CSCs) under the conditions of tumor microenvironment. Both stem cells and tumor cells are profoundly influenced by bi-directional communication with their respective microenvironment, which dictates cell fate determination and behavior. The microenvironment derived from iPS cells has not been well studied. In this paper, we have investigated the effects of secreted factors from Nanog-mouse iPS (miPS) cells on mouse Lewis lung cancer (LLC) cells that are found in the conditioned media. The results demonstrated that miPS cells secrete factors that can convert the epithelia phenotype of LLC cells to a mesenchymal phenotype, and that can promote tumorigenisity, migration and invasion. Furthermore, LLC cells that have been exposed to miPS conditioned medium became resistant to apoptosis. These various biological effects suggest that the miPS microenvironment contain factors that can promote an epithelial-mesenchymal transition (EMT) through an active Snail-MMP axis or by suppressing differentiation in LLC cells.

Chen, L. W., et al. (2011). "Potential application of induced pluripotent stem cells in cell replacement therapy for Parkinson's disease." CNS Neurol Disord Drug Targets **10**(4): 449-458.

Parkinson's disease (PD), a common degenerative disease in humans, is known to result from loss of dopamine neurons in the substantia nigra and is characterized by severe motor symptoms of tremor, rigidity, bradykinsia and postural instability. Although levodopa administration, surgical neural lesion, and deep brain stimulation have been shown to be effective in improving parkinsonian symptoms, cell replacement therapy such as transplantation of dopamine neurons or neural stem cells has shed new light on an alternative treatment strategy for PD. While the difficulty in securing donor dopamine neurons and the immuno-rejection of neural transplants largely hinder application of neural transplants in clinical treatment, induced pluripotent stem cells (iPS cells) derived from somatic cells may represent a powerful tool for studying the pathogenesis of PD and provide a source for replacement therapies in this neurodegenerative disease. Yamanaka et al. [2006, 2007] first succeeded in generating iPS cells by reprogramming fibroblasts with four transcription factors, Oct4, Sox2, Klf4, and c-Myc in both mouse and human. Animal studies have further shown that iPS cells from fibroblasts could be induced into dopamine neurons and transplantation of these cells within the central nervous system improved motor symptoms in the 6-OHDA model of PD. More interestingly, neural stem cells or fibroblasts from patients can be efficiently reprogrammed and subsequently differentiated into dopamine neurons. Derivation of patient-specific iPS cells and subsequent differentiation into dopamine neurons would provide a disease-specific in vitro model for disease pathology, drug screening and personalized stem cell therapy for PD. This review summarizes current methods and modifications in producing iPS cells from somatic cells as well as safety concerns of reprogramming procedures. Novel reprogramming strategies that deter abnormal permanent genetic and epigenetic alterations are essential for propagating clinically-qualified iPS cells. Future investigations into cell transforming and reprogramming processes are needed to generate the disease-specific iPS cells for personalized regeneration medicine of PD patients by disclosing detailed reprogramming mechanisms.

Chen, M., et al. (2018). "Central and Peripheral Nervous System Progenitors Derived from Human Pluripotent Stem Cells Reveal a Unique Temporal and Cell-Type Specific Expression of PMCAs." Front Cell Dev Biol **6**: 5.

The P-type ATPases family consists of ion and lipid transporters. Their unique diversity in function and expression is critical for normal development. In this study we investigated human pluripotent stem cells (hPSC) and different neural progenitor states to characterize the expression of the plasma membrane calcium ATPases (PMCAs) during human neural development and in mature

mesencephalic dopaminergic (mesDA) neurons. Our RNA sequencing data identified a dynamic change in ATPase expression correlating with the differentiation time of the neural progenitors, which was independent of the neuronal progenitor type. Expression of ATP2B1 and ATP2B4 were the most abundantly expressed, in accordance with their main role in Ca(2+) regulation and we observed all of the PMCAs to have a subcellular punctate localization. Interestingly in hPSCs ATP2B1 and ATP2B3 were highly expressed in a cell cycle specific manner and ATP2B2 and ATP2B4 were highly expressed in a hPSC sub-population. In neural rosettes a strong apical PMCA expression was identified in the luminal region. Lastly, we confirmed all PMCAs to be expressed in mesDA neurons, however at varying levels. Our results reveal that PMCA expression dynamically changes during stem cell differentiation and highlights the diverging needs of cell populations to regulate and properly integrate Ca(2+) changes, which can ultimately correspond to changes in specific stem cell transcription states.

Chen, P., et al. (2015). "Treatment with retinoic acid and lens epithelial cell-conditioned medium in vitro directed the differentiation of pluripotent stem cells towards corneal endothelial cell-like cells." Exp Ther Med **9**(2): 351-360.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have extensive self-renewal capacity and the potential to differentiate into all tissue-specific cell lineages, including corneal endothelial cells (CECs). They are a promising prospect for the future of regenerative medicine. The method of derivation of CECs from ESCs and iPSCs, however, remains to be elucidated. In this study, mouse ESCs and iPSCs were induced to differentiate into CECs using CEC embryonic development events as a guide. All-trans retinoic acid (RA) treatment during the embryoid body (EB) differentiation step was used to promote neural crest (NC) cell differentiation as first step and was followed by a second induction in CEC- or lens epithelial cell (LEC)-conditioned medium (CM) to ultimately generate CEC-like cells. During the corresponding differentiation stages, NC developmental markers and CEC differentiation markers were detected at the protein level using immunocytochemistry (ICC) and at the mRNA level by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). During the first stage, the data indicated that 4 days of treatment with 1 muM RA starting on day 4 of EB formation favored NC cell differentiation and that plating on gelatin-coated plates led to cell migration out of the EBs. The second-stage differentiation results showed that the CM, particularly the LEC-CM, enhanced the yield of polygonal cells with CEC-specific marker expression shown by ICC and RT-qPCR. This study demonstrates that mouse ESCs and iPSCs were induced and expressed CEC differentiation markers when subjected to a two-step inducement process, suggesting that they are a promising resource for corneal endothelium failure replacement therapy in the future.

Chen, W., et al. (2018). "Angiogenic and osteogenic regeneration in rats via calcium phosphate scaffold and endothelial cell co-culture with human bone marrow mesenchymal stem cells (MSCs), human umbilical cord MSCs, human induced pluripotent stem cell-derived MSCs and human embryonic stem cell-derived MSCs." J Tissue Eng Regen Med **12**(1): 191-203.

Angiogenesis is a limiting factor in regenerating large bone defects. The objective of this study was to investigate angiogenic and osteogenic effects of co-culture on calcium phosphate cement (CPC) scaffold using human umbilical vein endothelial cells (hUVECs) and mesenchymal stem cells (MSCs) from different origins for the first time. hUVECs were co-cultured with four types of cell: human umbilical cord MSCs (hUCMSCs), human bone marrow MSCs (hBMSCs) and MSCs from induced pluripotent stem cells (hiPSC-MSCs) and embryonic stem cells (hESC-MSCs). Constructs were implanted in 8 mm cranial defects of rats for 12 weeks. CPC without cells served as control 1. CPC with hBMSCs served as control 2. Microcapillary-like structures were successfully formed on CPC in vitro in all four co-cultured groups. Microcapillary lengths increased with time ($p < 0.05$). Osteogenic and angiogenic gene expressions were highly elevated and mineralization by co-cultured cells increased with time ($p < 0.05$). New bone amount and blood vessel density of co-cultured groups were much greater than controls ($p < 0.05$) in an animal study. hUVECs co-cultured with hUCMSCs, hiPSC-MSCs and hESC-MSCs achieved new bone and vessel density similar to hUVECs co-cultured with hBMSCs ($p > 0.1$). Therefore, hUCMSCs, hiPSC-MSCs and hESC-MSCs could serve as alternative cell sources to hBMSCs, which require an invasive procedure to harvest. In conclusion, this study showed for the first time that co-cultures of hUVECs with hUCMSCs, hiPSC-MSCs, hESC-MSCs and hBMSCs delivered via CPC scaffold achieved excellent osteogenic and angiogenic capabilities in vivo. The novel co-culture constructs are promising for bone reconstruction with improved angiogenesis for craniofacial/orthopaedic applications.

Chen, W., et al. (2013). "Nonviral cell labeling and differentiation agent for induced pluripotent stem cells based on mesoporous silica nanoparticles." ACS Nano **7**(10): 8423-8440.

The generation of induced pluripotent stem cells (iPSCs) is an innovative personalized-regenerative technology, which can transform own-self somatic cells into embryonic stem (ES)-like cells, which have the potential to differentiate into all cell types of three dermal lineages. However, how to quickly, efficiently, and safely produce specific-lineage differentiation from pluripotent-state cells and iPSCs is still an open question. The objective of the present study was to develop a platform of a nonviral gene delivery system of mesoporous silica nanoparticles (MSNs) to rapidly generate iPSC-derived definitive-lineage cells, including endodermal-differentiated cells. We also evaluated the feasibility and efficiency of FITC-conjugated MSNs (FMSNs) for labeling of iPSCs and utilized the multifunctional properties of FMSNs for a suitable carrier for biomolecule delivery. We showed that FMSNs of various surface charges could be efficiently internalized by iPSCs without causing cytotoxicity. The levels of reactive oxygen species and pluripotent status, including in vitro stemness signatures and in vivo teratoma formation, remained unaltered. Notably, positive-charged FMSN enhanced cellular uptake efficiency and retention time. Moreover, when using positive-charged FMSN to deliver hepatocyte nuclear factor 3beta (HNF3beta) plasmid DNA (pDNA), the treated iPSCs exhibited significantly improved definitive endoderm formation and further quickly differentiated into hepatocyte-like cells with mature functions (low-density lipoprotein uptake and glycogen storage) within 2 weeks in vitro. Double delivery of pHNF3beta further improved mRNA expression levels of liver-specific genes. These findings reveal the multiple advantages of FMSNs to serve as ideal vectors not only for stem cell labeling but also for safe gene delivery to promote the production of hepatocyte-like cells from iPSCs.

Chen, Y. C., et al. (2018). "ATOH1/RFX1/RFX3 transcription factors facilitate the differentiation and characterisation of inner ear hair cell-like cells from patient-specific induced pluripotent stem cells harbouring A8344G mutation of mitochondrial DNA." Cell Death Dis **9**(4): 437.

Degeneration or loss of inner ear hair cells (HCs) is irreversible and results in sensorineural hearing loss (SHL). Human-induced pluripotent stem cells (hiPSCs) have been employed in disease modelling and cell therapy. Here, we propose a transcription factor (TF)-driven approach using ATOH1 and regulatory factor of x-box (RFX) genes to generate HC-like cells from hiPSCs. Our results suggest that ATOH1/RFX1/RFX3 could significantly increase the differentiation capacity of iPSCs into MYO7A(mCherry)-positive cells, upregulate the mRNA expression levels of HC-related genes and promote the differentiation of HCs with more mature stereociliary bundles. To model the molecular and stereociliary structural changes involved in HC dysfunction in SHL, we further used ATOH1/RFX1/RFX3 to differentiate HC-like cells from the iPSCs from patients with myoclonus epilepsy associated with ragged-red fibres (MERRF) syndrome, which is caused by A8344G mutation of mitochondrial DNA (mtDNA), and characterised by myoclonus epilepsy, ataxia and SHL. Compared with isogenic iPSCs, MERRF-iPSCs possessed ~42-44% mtDNA with A8344G mutation and exhibited significantly elevated reactive oxygen species (ROS) production and CAT gene expression. Furthermore, MERRF-iPSC-differentiated HC-like cells exhibited significantly elevated ROS levels and MnSOD and CAT gene expression. These MERRF-HCs that had more single cilia with a shorter length could be observed only by using a non-TF method, but those with fewer stereociliary bundle-like protrusions than isogenic iPSCs-differentiated-HC-like cells could be further observed using ATOH1/RFX1/RFX3 TFs. We further analysed and compared the whole transcriptome of M1(ctrl)-HCs and M1-HCs after treatment with ATOH1 or ATOH1/RFX1/RFX3. We revealed that the HC-related gene transcripts in M1(ctrl)-iPSCs had a significantly higher tendency to be activated by ATOH1/RFX1/RFX3 than M1-iPSCs. The ATOH1/RFX1/RFX3 TF-driven approach for the differentiation of HC-like cells from iPSCs is an efficient and promising strategy for the disease modelling of SHL and can be employed in future therapeutic strategies to treat SHL patients.

Chen, Y. H. and S. M. Pruett-Miller (2018). "Improving single-cell cloning workflow for gene editing in human pluripotent stem cells." Stem Cell Res **31**: 186-192.

The availability of human pluripotent stem cells (hPSCs) and progress in genome engineering technology have altered the way we approach scientific research and drug development screens. Unfortunately, the procedures for genome editing of hPSCs often subject cells to harsh conditions that compromise viability: a major problem that is compounded by the innate challenge of single-cell culture. Here we describe a generally applicable workflow that supports single-cell cloning and expansion of hPSCs after genome editing and single-cell sorting. Stem-Flex and RevitaCell supplement, in combination with Geltrex or Vitronectin (VN), promote reliable single-cell growth in a feeder-free and defined environment. Characterization of final genome-edited clones reveals that pluripotency and normal karyotype are retained following this single-cell culture protocol. This time-efficient and simplified culture method paves the way

for high-throughput hPSC culture and will be valuable for both basic research and clinical applications.

Cheng, X., et al. (2013). "Endodermal stem cell populations derived from pluripotent stem cells." Curr Opin Cell Biol **25**(2): 265-271.

The generation of functional endodermal lineages, such as hepatocytes and pancreatic endocrine cells, from pluripotent stem cells (PSCs) remains a challenge. One strategy to enhance the purity, yield and maturity of endodermal derivatives is to expand endoderm committed stem or progenitor cell populations derived from PSCs before final differentiation. Recent studies have shown that this is in fact a viable option both for expanding pure populations of endodermal cells as well as for generating more mature derivative tissues, as highlighted in the case of pancreatic beta cells.

Chetty, S., et al. (2015). "A Src inhibitor regulates the cell cycle of human pluripotent stem cells and improves directed differentiation." J Cell Biol **210**(7): 1257-1268.

Driving human pluripotent stem cells (hPSCs) into specific lineages is an inefficient and challenging process. We show that a potent Src inhibitor, PP1, regulates expression of genes involved in the G1 to S phase transition of the cell cycle, activates proteins in the retinoblastoma family, and subsequently increases the differentiation propensities of hPSCs into all three germ layers. We further demonstrate that genetic suppression of Src regulates the activity of the retinoblastoma protein and enhances the differentiation potential of hPSCs across all germ layers. These positive effects extend beyond the initial germ layer specification and enable efficient differentiation at subsequent stages of differentiation.

Chhabra, A. (2017). "Inherent Immunogenicity or Lack Thereof of Pluripotent Stem Cells: Implications for Cell Replacement Therapy." Front Immunol **8**: 993.

Donor-specific induced pluripotent stem cells (iPSCs) offer opportunities for personalized cell replacement therapeutic approaches due to their unlimited self-renewal potential and ability to differentiate into different somatic cells. A significant progress has been made toward generating iPSC lines that are free of integrating viral vectors, development of xeno-free culture conditions, and differentiation of pluripotent stem cells (PSCs) into functional somatic cell lineages. Since donor-specific iPSC lines are genetically identical to the individual, they are expected to be immunologically matched and these iPSC lines and their cellular derivatives are not expected to be immunologically rejected. However, studies in mouse models, utilizing rejection of teratomas as a model, have claimed that syngenic iPSC lines, especially the iPSC lines derived with integrating viral vectors, could be inherently immunogenic. This manuscript reviews current understanding of inherent immunogenicity of PSC lines, especially that of the human iPSC lines and their cellular derivatives, and strategies to overcome it.

Chichagova, V., et al. (2016). "Generation of Human Induced Pluripotent Stem Cells Using RNA-Based Sendai Virus System and Pluripotency Validation of the Resulting Cell Population." Methods Mol Biol **1353**: 285-307.

Human induced pluripotent stem cells (hiPSCs) provide a platform for studying human disease in vitro, increase our understanding of human embryonic development, and provide clinically relevant cell types for transplantation, drug testing, and toxicology studies. Since their discovery, numerous advances have been made in order to eliminate issues such as vector integration into the host genome, low reprogramming efficiency, incomplete reprogramming and acquisition of genomic instabilities. One of the ways to achieve integration-free reprogramming is by using RNA-based Sendai virus. Here we describe a method to generate hiPSCs with Sendai virus in both feeder-free and feeder-dependent culture systems. Additionally, we illustrate methods by which to validate pluripotency of the resulting stem cell population.

Chikhovskaya, J. V., et al. (2012). "Human testis-derived embryonic stem cell-like cells are not pluripotent, but possess potential of mesenchymal progenitors." Hum Reprod **27**(1): 210-221.

BACKGROUND: Spontaneous in vitro transition of undifferentiated spermatogonia into the pluripotent cell state has been achieved using neonatal and adult mouse testis tissue. In an effort to establish an analogous source of human patient-specific pluripotent stem cells, several research groups have described the derivation of embryonic stem cell-like cells from primary cultures of human testis. These cells are characterized in all studies as growing in compact colonies, expressing pluripotency-associated markers and possessing multilineage differentiation capabilities in vitro, but only one study claimed their ability to induce teratomas. This controversy initiated a debate about the pluripotent state and origin of human testis-derived ES-like cells (htES-like cells). METHODS: htES-like cell colonies were obtained from primary testicular cultures of three individuals and selectively expanded using culture conditions known to support the propagation of blastocyst-derived human embryonic stem cells (ESCs), mouse epiblast stem cells and 'naive' human ESCs. The stem cell properties of

htES-like cells were subsequently assessed by testing the expression of ESC-specific markers, differentiation abilities in vitro and in vivo, and microarray profiling. RESULTS: The expression of pluripotency-associated markers in htES-like cells and their differentiation abilities differed significantly from those of ESCs. Gene expression microarray analysis revealed that htES-like cells possess a transcriptome distinct from human ESCs and fibroblasts, but closely resembling the transcriptome of mesenchymal stem cells (MSCs). The similarity to MSCs was confirmed by detection of SSEA4/CD146 expressing cells within htES-like colonies and efficient in vitro differentiation toward three mesodermal lineages (adipogenic, osteogenic, chondrogenic). CONCLUSIONS: Taken together, these results indicate that htES-like cells, in contrast to pluripotent stem cells derived from adult mouse testis, are not pluripotent and most likely not of germ cell but of mesenchymal origin.

Chiou, S. S., et al. (2013). "Control of Oxidative Stress and Generation of Induced Pluripotent Stem Cell-like Cells by Jun Dimerization Protein 2." Cancers (Basel) **5**(3): 959-984.

We report here that the Jun dimerization protein 2 (JDP2) plays a critical role as a cofactor for the transcription factors nuclear factor-erythroid 2-related factor 2 (Nrf2) and MafK in the regulation of the antioxidants and production of reactive oxygen species (ROS). JDP2 associates with Nrf2 and MafK (Nrf2-MafK) to increase the transcription of antioxidant response element-dependent genes. Oxidative-stress-inducing reagent led to an increase in the intracellular accumulation of ROS and cell proliferation in Jdp2 knock-out mouse embryonic fibroblasts. In Jdp2-Cre mice mated with reporter mice, the expression of JDP2 was restricted to granule cells in the brain cerebellum. The induced pluripotent stem cells (iPSC)-like cells were generated from DAOY medulloblastoma cell by introduction of JDP2, and the defined factor OCT4. iPSC-like cells expressed stem cell-like characteristics including alkaline phosphatase activity and some stem cell markers. However, such iPSC-like cells also proliferated rapidly, became neoplastic, and potentiated cell malignancy at a later stage in SCID mice. This study suggests that medulloblastoma cells can be reprogrammed successfully by JDP2 and OCT4 to become iPSC-like cells. These cells will be helpful for studying the generation of cancer stem cells and ROS homeostasis.

Chng, Z., et al. (2010). "SIP1 mediates cell-fate decisions between neuroectoderm and mesendoderm in human pluripotent stem cells." Cell Stem Cell **6**(1): 59-70.

Human embryonic stem cells (hESCs) rely on fibroblast growth factor and Activin-Nodal signaling to maintain their pluripotency. However, Activin-Nodal signaling is also known to induce mesendoderm differentiation. The mechanisms by which Activin-Nodal signaling can achieve these contradictory functions remain unknown. Here, we demonstrate that Smad-interacting protein 1 (SIP1) limits the mesendoderm-inducing effects of Activin-Nodal signaling without inhibiting the pluripotency-maintaining effects exerted by SMAD2/3. In turn, Activin-Nodal signaling cooperates with NANOG, OCT4, and SOX2 to control the expression of SIP1 in hESCs, thereby limiting the neuroectoderm-promoting effects of SIP1. Similar results were obtained with mouse epiblast stem cells, implying that these mechanisms are evolutionarily conserved and may operate in vivo during mammalian development. Overall, our results reveal the mechanisms by which Activin-Nodal signaling acts through SIP1 to regulate the cell-fate decision between neuroectoderm and mesendoderm in the progression from pluripotency to primary germ layer differentiation.

Cho, L. T., et al. (2012). "Conversion from mouse embryonic to extra-embryonic endoderm stem cells reveals distinct differentiation capacities of pluripotent stem cell states." Development **139**(16): 2866-2877.

The inner cell mass of the mouse pre-implantation blastocyst comprises epiblast progenitor and primitive endoderm cells of which cognate embryonic (mESCs) or extra-embryonic (XEN) stem cell lines can be derived. Importantly, each stem cell type retains the defining properties and lineage restriction of their in vivo tissue of origin. Recently, we demonstrated that XEN-like cells arise within mESC cultures. This raises the possibility that mESCs can generate self-renewing XEN cells without the requirement for gene manipulation. We have developed a novel approach to convert mESCs to XEN cells (cXEN) using growth factors. We confirm that the downregulation of the pluripotency transcription factor Nanog and the expression of primitive endoderm-associated genes Gata6, Gata4, Sox17 and Pdgfra are necessary for cXEN cell derivation. This approach highlights an important function for Fgf4 in cXEN cell derivation. Paracrine FGF signalling compensates for the loss of endogenous Fgf4, which is necessary to exit mESC self-renewal, but not for XEN cell maintenance. Our cXEN protocol also reveals that distinct pluripotent stem cells respond uniquely to differentiation promoting signals. cXEN cells can be derived from mESCs cultured with Erk and Gsk3 inhibitors (2i), and LIF, similar to conventional mESCs. However, we find that epiblast stem cells (EpiSCs) derived from the post-implantation embryo are

refractory to cXEN cell establishment, consistent with the hypothesis that EpiSCs represent a pluripotent state distinct from mESCs. In all, these findings suggest that the potential of mESCs includes the capacity to give rise to both extra-embryonic and embryonic lineages.

Choi, H. Y., et al. (2018). "Generation of a human induced pluripotent stem cell line, KSCBi003-A, from human adipose tissue-derived mesenchymal stem cells using a chromosomal integration-free system." Stem Cell Res **31**: 1-4.

We generated a human induced pluripotent stem cell (hiPSC) line, KSCBi003-A, from adipose tissue-derived mesenchymal stem cells (Ad-MSCs) using a Sendai virus-based gene delivery system. We confirmed that the KSCBi003-A has a normal karyotype and short tandem repeat (STR)-based identities that match the parent cells. We also confirmed that the cell line expresses pluripotent stem cell markers such as Nanog, OCT4, SSEA-4, TRA-1-60, and TRA-1-81. We also analyzed that the KSCBi003-A has an ability to differentiate three germ layers (ectoderm, mesoderm, endoderm). This cell line is registered and available at the National Stem Cell Bank, Korea National Institute of Health.

Choi, H. Y., et al. (2015). "G protein-coupled receptors in stem cell maintenance and somatic reprogramming to pluripotent or cancer stem cells." BMB Rep **48**(2): 68-80.

G protein-coupled receptors (GPCRs) are a large class of transmembrane receptors categorized into five distinct families: rhodopsin, secretin, adhesion, glutamate, and frizzled. They bind and regulate 80% of all hormones and account for 20-50% of the pharmaceuticals currently on the market. Hundreds of GPCRs integrate and coordinate the functions of individual cells, mediating signaling between various organs. GPCRs are crucial players in tumor progression, adipogenesis, and inflammation. Several studies have also confirmed their central roles in embryonic development and stem cell maintenance. Recently, GPCRs have emerged as key players in the regulation of cell survival, proliferation, migration, and self-renewal in pluripotent (PSCs) and cancer stem cells (CSCs). Our study and other reports have revealed that the expression of many GPCRs is modulated during the generation of induced PSCs (iPSCs) or CSCs as well as during CSC sphere formation. These GPCRs may have crucial roles in the regulation of selfrenewal and other biological properties of iPSCs and CSCs. This review addresses the current understanding of the role of GPCRs in stem cell maintenance and somatic reprogramming to PSCs or CSCs.

Choi, K. D., et al. (2009). "Generation of mature human myelomonocytic cells through expansion and differentiation of pluripotent stem cell-derived lin-CD34+CD43+CD45+ progenitors." J Clin Invest **119**(9): 2818-2829.

Basic research into human mature myelomonocytic cell function, myeloid lineage diversification and leukemic transformation, and assessment of myelotoxicity in preclinical drug development requires a constant supply of donor blood or bone marrow samples and laborious purification of mature myeloid cells or progenitors, which are present in very small quantities. To overcome these limitations, we have developed a protocol for efficient generation of neutrophils, eosinophils, macrophages, osteoclasts, DCs, and Langerhans cells from human embryonic stem cells (hESCs). As a first step, we generated lin-CD34+CD43+CD45+ hematopoietic cells highly enriched in myeloid progenitors through coculture of hESCs with OP9 feeder cells. After expansion in the presence of GM-CSF, these cells were directly differentiated with specific cytokine combinations toward mature cells of particular types. Morphologic, phenotypic, molecular, and functional analyses revealed that hESC-derived myelomonocytic cells were comparable to their corresponding somatic counterparts. In addition, we demonstrated that a similar protocol could be used to generate myelomonocytic cells from induced pluripotent stem cells (iPSCs). This technology offers an opportunity to generate large numbers of patient-specific myelomonocytic cells for in vitro studies of human disease mechanisms as well as for drug screening.

Choi, S. M., et al. (2011). "Reprogramming of EBV-immortalized B-lymphocyte cell lines into induced pluripotent stem cells." Blood **118**(7): 1801-1805.

EBV-immortalized B lymphocyte cell lines have been widely banked for studying a variety of diseases, including rare genetic disorders. These cell lines represent an important resource for disease modeling with the induced pluripotent stem cell (iPSC) technology. Here we report the generation of iPSCs from EBV-immortalized B-cell lines derived from multiple inherited disease patients via a nonviral method. The reprogramming method for the EBV cell lines involves a distinct protocol compared with that of patient fibroblasts. The B-cell line-derived iPSCs expressed pluripotency markers, retained the inherited mutation and the parental V(D)J rearrangement profile, and differentiated into all 3 germ layer cell types. There was no integration of the reprogramming-related transgenes or the EBV-associated genes in these iPSCs. The ability to reprogram the widely banked patient B-cell lines will offer an unprecedented opportunity to

generate human disease models and provide novel drug therapies.

Choi, Y. J., et al. (2017). "Deficiency of microRNA miR-34a expands cell fate potential in pluripotent stem cells." Science **355**(6325).

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) efficiently generate all embryonic cell lineages but rarely generate extraembryonic cell types. We found that microRNA miR-34a deficiency expands the developmental potential of mouse pluripotent stem cells, yielding both embryonic and extraembryonic lineages and strongly inducing MuERV-L (MERVL) endogenous retroviruses, similar to what is seen with features of totipotent two-cell blastomeres. miR-34a restricts the acquisition of expanded cell fate potential in pluripotent stem cells, and it represses MERVL expression through transcriptional regulation, at least in part by targeting the transcription factor Gata2. Our studies reveal a complex molecular network that defines and restricts pluripotent developmental potential in cultured ESCs and iPSCs.

Chow, L., et al. (2017). "Safety and immune regulatory properties of canine induced pluripotent stem cell-derived mesenchymal stem cells." Stem Cell Res **25**: 221-232.

Mesenchymal stem cells (MSCs) exhibit broad immune modulatory activity in vivo and can suppress T cell proliferation and dendritic cell activation in vitro. Currently, most MSC for clinical usage are derived from younger donors, due to ease of procurement and to the superior immune modulatory activity. However, the use of MSC from multiple unrelated donors makes it difficult to standardize study results and compare outcomes between different clinical trials. One solution is the use of MSC derived from induced pluripotent stem cells (iPSC); as iPSC-derived MSC have nearly unlimited proliferative potential and exhibit in vitro phenotypic stability. Given the value of dogs as a spontaneous disease model for pre-clinical evaluation of stem cell therapeutics, we investigated the functional properties of canine iPSC-derived MSC (iMSC), including immune modulatory properties and potential for teratoma formation. We found that canine iMSC downregulated expression of pluripotency genes and appeared morphologically similar to conventional MSC. Importantly, iMSC retained a stable phenotype after multiple passages, did not form teratomas in immune deficient mice, and did not induce tumor formation in dogs following systemic injection. We concluded therefore that iMSC were phenotypically stable, immunologically potent, safe with respect to tumor formation, and represented an important new source of cells for therapeutic modulation of inflammatory disorders.

Chuang, C. Y., et al. (2015). "Granulosa cell-derived induced pluripotent stem cells exhibit pro-trophoblastic differentiation potential." Stem Cell Res Ther **6**: 14.

INTRODUCTION: Human induced pluripotent stem cells (hiPSCs) have been derived from various somatic cell types. Granulosa cells, a group of cells which surround oocytes and are obtained from the (normally discarded) retrieved egg follicles of women undergoing infertility treatment, are a possible cell source for induced pluripotent stem cell (iPSC) generation. Here, we explored the possibility of using human granulosa cells as a donor cell type for iPSC reprogramming, and compared granulosa cell-derived iPSCs (iGRAs) with those derived from other cell sources, to determine the potential ability of iGRA differentiation. METHODS: Granulosa cells were collected from egg follicles retrieved from women undergoing infertility treatment. After short-term culture, the granulosa cells derived from different patients were mixed in culture, and infected with retroviruses encoding reprogramming factors. The resulting iPSC clones were selected and subjected to microsatellite DNA analysis to determine their parental origin. IGRAs were subjected to RT-PCR, immunofluorescence staining, and in vitro and in vivo differentiation assays to further establish their pluripotent characteristics. RESULTS: Microsatellite DNA analysis was used to demonstrate that hiPSCs with different parental origins can be simultaneously reprogrammed by retroviral transfection of a mixed human granulosa cell population obtained from multiple individuals. The iGRAs resemble human embryonic stem cells (hESCs) in many respects, including morphological traits, growth requirements, gene and marker expression profiles, and in vitro and in vivo developmental propensities. We also demonstrate that the iGRAs express low levels of NLRP2, and differentiating iGRAs possess a biased differentiation potential toward the trophoblastic lineage. Although NLRP2 knockdown in hESCs promotes trophoblastic differentiation of differentiating hESCs, it does not result in exit from pluripotency. These results imply that NLRP2 may play a role in regulating the trophoblastic differentiation of human pluripotent stem cells. CONCLUSIONS: These findings provide a means of generating iPSCs from multiple granulosa cell populations with different parental origins. The ability to generate iPSCs from granulosa cells not only enables modeling of infertility-associated disease, but also provides a means of identifying potential clinical interventions through iPSC-based drug screening.

Chung, H. C., et al. (2012). "Human induced pluripotent stem cells derived under feeder-free conditions display unique cell cycle and DNA replication gene profiles." Stem Cells Dev **21**(2): 206-216.

Use of animal feeder layers and serum containing media in the derivation and propagation of induced pluripotent stem cells (iPSCs) can hinder clinical translation, because of the presence of xeno-material/pathogens. A defined and standardized system would be ideal for generating a homogenous population of iPSCs, which closely resembles human embryonic stem cells (hESCs). This article presents a novel and extensive comparison between in-house produced iPSCs and hESCs under "feeder" and "feeder-free" conditions, using transcriptomic genome-wide microarray analysis. We generated a list of pluripotency-associated and bivalent domain-containing genes by meta-analysis to measure qualitatively the degree of reprogramming in feeder-free derived iPSCs, in which both profiles displayed similar levels of gene expression as in hESCs. Gene ontology analysis showed that feeder-free iPSCs have enriched terms belonging to DNA repair/replication and cell cycle, which are signature to pluripotent cells. Transcriptomic data combined with directed differentiation assays, indicated that variability among iPSC lines is minimized when using a feeder-free cultural system, which may serve as a platform for further developing regenerative medicine compliant human iPSCs.

Chung, Y. G., et al. (2015). "Histone Demethylase Expression Enhances Human Somatic Cell Nuclear Transfer Efficiency and Promotes Derivation of Pluripotent Stem Cells." Cell Stem Cell **17**(6): 758-766.

The extremely low efficiency of human embryonic stem cell (hESC) derivation using somatic cell nuclear transfer (SCNT) limits its potential application. Blastocyst formation from human SCNT embryos occurs at a low rate and with only some oocyte donors. We previously showed in mice that reduction of histone H3 lysine 9 trimethylation (H3K9me3) through ectopic expression of the H3K9me3 demethylase Kdm4d greatly improves SCNT embryo development. Here we show that overexpression of a related H3K9me3 demethylase KDM4A improves human SCNT, and that, as in mice, H3K9me3 in the human somatic cell genome is an SCNT reprogramming barrier. Overexpression of KDM4A significantly improves the blastocyst formation rate in human SCNT embryos by facilitating transcriptional reprogramming, allowing efficient derivation of SCNT-derived ESCs using adult Age-related Macular Degeneration (AMD) patient somatic nuclei donors. This conserved mechanistic insight has potential applications for improving SCNT in a variety of contexts, including regenerative medicine.

Cieslar-Pobuda, A., et al. (2016). "Human induced pluripotent stem cell differentiation and direct transdifferentiation into corneal epithelial-like cells." Oncotarget **7**(27): 42314-42329.

The corneal epithelium is maintained by a small pool of tissue stem cells located at the limbus. Through certain injuries or diseases this pool of stem cells may get depleted. This leads to visual impairment. Standard treatment options include autologous or allogeneic limbal stem cell (LSC) transplantation, however graft rejection and chronic inflammation lowers the success rate over long time. Induced pluripotent stem (iPS) cells have opened new possibilities for treating various diseases with patient specific cells, eliminating the risk of immune rejection. In recent years, several protocols have been developed, aimed at the differentiation of iPS cells into the corneal epithelial lineage by mimicking the environmental niche of limbal stem cells. However, the risk of teratoma formation associated with the use of iPS cells hinders most applications from lab into clinics. Here we show that the differentiation of iPS cells into corneal epithelial cells results in the expression of corneal epithelial markers showing a successful differentiation, but the process is long and the level of gene expression for the pluripotency markers does not vanish completely. Therefore we set out to determine a direct transdifferentiation approach to circumvent the intermediate state of pluripotency (iPS-stage). The resulting cells, obtained by direct transdifferentiation of fibroblasts into limbal cells, exhibited corneal epithelial cell morphology and expressed corneal epithelial markers. Hence we shows for the first time a direct transdifferentiation of human dermal fibroblasts into the corneal epithelial lineage that may serve as source for corneal epithelial cells for transplantation approaches.

Citro, L., et al. (2014). "Comparison of human induced pluripotent stem-cell derived cardiomyocytes with human mesenchymal stem cells following acute myocardial infarction." PLoS One **9**(12): e116281.

INTRODUCTION: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have recently been shown to express key cardiac proteins and improve in vivo cardiac function when administered following myocardial infarction. However, the efficacy of hiPSC-derived cell therapies, in direct comparison to current, well-established stem cell-based therapies, is yet to be elucidated. The goal of the current study was to compare the therapeutic efficacy of human mesenchymal stem cells (hMSCs) with hiPSC-CMs in mitigating myocardial infarction

(MI). METHODS: Male athymic nude hyrats were subjected to permanent ligation of the left-anterior-descending (LAD) coronary artery to induce acute MI. Four experimental groups were studied: 1) control (non-MI), 2) MI, 3) hMSCs (MI+MSC), and 4) hiPSC-CMs (MI+hiPSC-derived cardiomyocytes). The hiPSC-CMs and hMSCs were labeled with superparamagnetic iron oxide (SPIO) in vitro to track the transplanted cells in the ischemic heart by high-field cardiac MRI. These cells were injected into the ischemic heart 30-min after LAD ligation. Four-weeks after MI, cardiac MRI was performed to track the transplanted cells in the infarct heart. Additionally, echocardiography (M-mode) was performed to evaluate the cardiac function. Immunohistological and western blot studies were performed to assess the cell tracking, engraftment and cardiac fibrosis in the infarct heart tissues. RESULTS: Echocardiography data showed a significantly improved cardiac function in the hiPSC-CMs and hMSCs groups, when compared to MI. Immunohistological studies showed expression of connexin-43, alpha-actinin and myosin heavy chain in engrafted hiPSC-CMs. Cardiac fibrosis was significantly decreased in hiPSC-CMs group when compared to hMSCs or MI groups. Overall, this study demonstrated improved cardiac function with decreased fibrosis with both hiPSC-CMs and hMSCs groups when compared with MI group.

Clayton, Z. E., et al. (2015). "Generating induced pluripotent stem cell derived endothelial cells and induced endothelial cells for cardiovascular disease modelling and therapeutic angiogenesis." Int J Cardiol **197**: 116-122.

Standard therapy for atherosclerotic coronary and peripheral arterial disease is insufficient in a significant number of patients because extensive disease often precludes effective revascularization. Stem cell therapy holds promise as a supplementary treatment for these patients, as pre-clinical and clinical research has shown transplanted cells can promote angiogenesis via direct and paracrine mechanisms. Induced pluripotent stem cells (iPSCs) are a novel cell type obtained by reprogramming somatic cells using exogenous transcription factor cocktails, which have been introduced to somatic cells via viral or plasmid constructs, modified mRNA or small molecules. IPSCs are now being used in disease modelling and drug testing and are undergoing their first clinical trial, but despite recent advances, the inefficiency of the reprogramming process remains a major limitation, as does the lack of consensus regarding the optimum transcription factor combination and delivery method and the uncertainty surrounding the genetic and epigenetic stability of iPSCs. IPSCs have been successfully differentiated into vascular endothelial cells (iPSC-ECs) and, more recently, induced endothelial cells (iECs) have also been generated by direct differentiation, which bypasses the pluripotent intermediate. IPSC-ECs and iECs demonstrate endothelial functionality in vitro and have been shown to promote neovessel growth and enhance blood flow recovery in animal models of myocardial infarction and peripheral arterial disease. Challenges remain in optimising the efficiency, safety and fidelity of the reprogramming and endothelial differentiation processes and establishing protocols for large-scale production of clinical-grade, patient-derived cells.

Clayton, Z. E., et al. (2018). "Induced pluripotent stem cell-derived endothelial cells promote angiogenesis and accelerate wound closure in a murine excisional wound healing model." Biosci Rep **38**(4).

Chronic wounds are a major complication in patients with cardiovascular diseases. Cell therapies have shown potential to stimulate wound healing, but clinical trials using adult stem cells have been tempered by limited numbers of cells and invasive procurement procedures. Induced pluripotent stem cells (iPSCs) have several advantages of other cell types, for example they can be generated in abundance from patients' somatic cells (autologous) or those from a matched donor. iPSCs can be efficiently differentiated to functional endothelial cells (iPSC-ECs). Here, we used a murine excisional wound model to test the pro-angiogenic properties of iPSC-ECs in wound healing. Two full-thickness wounds were made on the dorsum of NOD-SCID mice and splinted. iPSC-ECs (5 x 10(5)) were topically applied to one wound, with the other serving as a control. Treatment with iPSC-ECs significantly increased wound perfusion and accelerated wound closure. Expression of endothelial cell (EC) surface marker, platelet endothelial cell adhesion molecule (PECAM-1) (CD31), and pro-angiogenic EC receptor, Tie1, mRNA was up-regulated in iPSC-EC treated wounds at 7 days post-wounding. Histological analysis of wound sections showed increased capillary density in iPSC-EC wounds at days 7 and 14 post-wounding, and increased collagen content at day 14. Anti-GFP fluorescence confirmed presence of iPSC-ECs in the wounds. Bioluminescent imaging (BLI) showed progressive decline of iPSC-ECs over time, suggesting that iPSC-ECs are acting primarily through short-term paracrine effects. These results highlight the pro-regenerative effects of iPSC-ECs and demonstrate that they are a promising potential therapy for intractable wounds.

Clayton, Z. E., et al. (2017). "A comparison of the pro-angiogenic potential of human induced pluripotent stem cell derived endothelial cells and induced

endothelial cells in a murine model of peripheral arterial disease." Int J Cardiol **234**: 81-89.

BACKGROUND: Endothelial cells derived from human induced pluripotent stem cells (iPSC-ECs) promote angiogenesis, and more recently induced endothelial cells (iECs) have been generated via fibroblast trans-differentiation. These cell types have potential as treatments for peripheral arterial disease (PAD). However, it is unknown whether different reprogramming methods produce cells that are equivalent in terms of their pro-angiogenic capabilities. OBJECTIVES: We aimed to directly compare iPSC-ECs and iECs in an animal model of PAD, in order to identify which cell type, if any, displays superior therapeutic potential. METHODS: IPSC-ECs and iECs were generated from human fibroblasts, and transduced with a reporter construct encoding GFP and firefly luciferase for bioluminescence imaging (BLI). Endothelial phenotype was confirmed using in vitro assays. NOD-SCID mice underwent hindlimb ischaemia surgery and received an intramuscular injection of either 1x10(6) iPSC-ECs, 1x10(6) iECs or control vehicle only. Perfusion recovery was measured by laser Doppler. Hindlimb muscle samples were taken for histological analyses. RESULTS: Perfusion recovery was enhanced in iPSC-EC treated mice on day 14 (Control vs. iPSC-EC; 0.35+/-0.04 vs. 0.54+/-0.08, $p<0.05$) and in iEC treated mice on days 7 (Control vs. iEC; 0.23+/-0.02 vs. 0.44+/-0.06, $p<0.05$), 10 (0.31+/-0.04 vs. 0.64+/-0.07, $p<0.001$) and 14 (0.35+/-0.04 vs. 0.68+/-0.07, $p<0.001$) post-treatment. IEC-treated mice also had greater capillary density in the ischaemic gastrocnemius muscle (Control vs. iEC; 125+/-10 vs. 179+/-11 capillaries/image; $p<0.05$). BLI detected iPSC-EC and iEC presence in vivo for two weeks post-treatment. CONCLUSIONS: IPSC-ECs and iECs exhibit similar, but not identical, endothelial functionality and both cell types enhance perfusion recovery after hindlimb ischaemia.

Clemens, G., et al. (2013). "The action of all-trans-retinoic acid (ATRA) and synthetic retinoid analogues (EC19 and EC23) on human pluripotent stem cells differentiation investigated using single cell infrared microspectroscopy." Mol Biosyst **9**(4): 677-692.

All trans-retinoic acid (ATRA) is widely used to direct the differentiation of cultured stem cells. When exposed to the pluripotent human embryonal carcinoma (EC) stem cell line, TERA2.cl.SP12, ATRA induces ectoderm differentiation and the formation of neuronal cell types. We have previously generated synthetic analogues of retinoic acid (EC23 and EC19) which also induce the differentiation of EC cells. Even though EC23 and EC19 have similar chemical structures, they have differing biochemical effects in terms of EC cell differentiation. EC23 induces neuronal differentiation in a manner similar to ATRA, whereas EC19 directs the cells to form epithelial-like derivatives. Previous MALDI-TOF MS analysis examined the response of TERA2.cl.SP12 cells after exposure to ATRA, EC23 and EC19 and further demonstrated the similarly in the effect of ATRA and EC23 activity whilst responses to EC19 were very different. In this study, we show that Fourier Transform Infrared Micro-Spectroscopy (FT-IRMS) coupled with appropriate scatter correction and multivariate analysis can be used as an effective tool to further investigate the differentiation of human pluripotent stem cells and monitor the alternative affects different retinoid compounds have on the induction of differentiation. FT-IRMS detected differences between cell populations as early as 3 days of compound treatment. Populations of cells treated with different retinoid compounds could easily be distinguished from one another during the early stages of cell differentiation. These data demonstrate that FT-IRMS technology can be used as a sensitive screening technique to monitor the status of the stem cell phenotype and progression of differentiation along alternative pathways in response to different compounds.

Collado, M. S., et al. (2017). "Exposure of Induced Pluripotent Stem Cell-Derived Vascular Endothelial and Smooth Muscle Cells in Coculture to Hemodynamics Induces Primary Vascular Cell-Like Phenotypes." Stem Cells Transl Med **6**(8): 1673-1683.

Human induced pluripotent stem cells (iPSCs) can be differentiated into vascular endothelial (iEC) and smooth muscle (iSMC) cells. However, because iECs and iSMCs are not derived from an intact blood vessel, they represent an immature phenotype. Hemodynamics and heterotypic cell:cell communication play important roles in vascular cell phenotypic modulation. Here we tested the hypothesis that hemodynamic exposure of iECs in coculture with iSMCs induces an in vivo-like phenotype. iECs and iSMCs were cocultured under vascular region-specific blood flow hemodynamics, and compared to hemodynamic cocultures of blood vessel-derived endothelial (pEC) and smooth muscle (pSMC) cells. Hemodynamic flow-induced gene expression positively correlated between pECs and iECs as well as pSMCs and iSMCs. While endothelial nitric oxide synthase 3 protein was lower in iECs than pECs, iECs were functionally mature as seen by acetylated-low-density lipoprotein (LDL) uptake. SMC contractile protein markers were also positively correlated between pSMCs and iSMCs. Exposure of iECs and pECs to atheroprone hemodynamics with oxidized-LDL induced an inflammatory response in both. Dysfunction of the transforming growth factor beta (TGFbeta) pathway is seen in several vascular diseases, and iECs

and iSMCs exhibited a transcriptomic prolife similar to pECs and pSMCs, respectively, in their responses to LY2109761-mediated transforming growth factor beta receptor I/II (TGFbetaRI/II) inhibition. Although there are differences between ECs and SMCs derived from iPSCs versus blood vessels, hemodynamic coculture restores a high degree of similarity in their responses to pathological stimuli associated with vascular diseases. Thus, iPSC-derived vascular cells exposed to hemodynamics may provide a viable system for modeling rare vascular diseases and testing new therapeutic approaches. Stem Cells Translational Medicine 2017;6:1673-1683.

Collier, A. J. and P. J. Rugg-Gunn (2018). "Identifying Human Naive Pluripotent Stem Cells - Evaluating State-Specific Reporter Lines and Cell-Surface Markers." Bioessays **40**(5): e1700239.

Recent reports that human pluripotent stem cells can be captured in a spectrum of states with variable properties has prompted a re-evaluation of how pluripotency is acquired and stabilised. The latest additions to the stem cell hierarchy open up opportunities for understanding human development, reprogramming, and cell state transitions more generally. Many of the new cell lines have been collectively termed 'naive' human pluripotent stem cells to distinguish them from the conventional 'primed' cells. Here, several transcriptional and epigenetic hallmarks of human pluripotent states in the recently described cell lines are reviewed and evaluated. Methods to derive and identify human naive pluripotent stem cells are also discussed, with a focus on the uses and future developments of state-specific reporter cell lines and cell-surface proteins. Finally, opportunities and uncertainties in naive stem cell biology are highlighted, and the current limitations of human naive pluripotent stem cells considered, particularly in the context of differentiation.

Collin, J. and M. Lako (2011). "Concise review: putting a finger on stem cell biology: zinc finger nuclease-driven targeted genetic editing in human pluripotent stem cells." Stem Cells **29**(7): 1021-1033.

Human pluripotent stem cells (hPSCs) encompassing human embryonic stem cells and human induced pluripotent stem cells (hiPSCs) have a wide appeal for numerous basic biology studies and for therapeutic applications because of their potential to give rise to almost any cell type in the human body and immense ability to self-renew. Much attention in the stem cell field is focused toward the study of gene-based anomalies relating to the causative affects of human disease and their correction with the potential for patient-specific therapies using gene corrected hiPSCs. Therefore, the genetic manipulation of stem cells is clearly important for the development of future medicine. Although successful targeted genetic engineering in hPSCs has been reported, these cases are surprisingly few because of inherent technical limitations with the methods used. The development of more robust and efficient means by which to achieve specific genomic modifications in hPSCs has far reaching implications for stem cell research and its applications. Recent proof-of-principle reports have shown that genetic alterations with minimal toxicity are now possible through the use of zinc finger nucleases (ZFNs) and the inherent DNA repair mechanisms within the cell. In light of recent comprehensive reviews that highlight the applications, methodologies, and prospects of ZFNs, this article focuses on the application of ZFNs to stem cell biology, discussing the published work to date, potential problems, and future uses for this technology both experimentally and therapeutically.

Collin, J., et al. (2016). "Using Zinc Finger Nuclease Technology to Generate CRX-Reporter Human Embryonic Stem Cells as a Tool to Identify and Study the Emergence of Photoreceptors Precursors During Pluripotent Stem Cell Differentiation." Stem Cells **34**(2): 311-321.

The purpose of this study was to generate human embryonic stem cell (hESC) lines harboring the green fluorescent protein (GFP) reporter at the endogenous loci of the Cone-Rod Homeobox (CRX) gene, a key transcription factor in retinal development. Zinc finger nucleases (ZFNs) designed to cleave in the 3' UTR of CRX were transfected into hESCs along with a donor construct containing homology to the target region, eGFP reporter, and a puromycin selection cassette. Following selection, polymerase chain reaction (PCR) and sequencing analysis of antibiotic resistant clones indicated targeted integration of the reporter cassette at the 3' of the CRX gene, generating a CRX-GFP fusion. Further analysis of a clone exhibiting homozygote integration of the GFP reporter was conducted suggesting genomic stability was preserved and no other copies of the targeting cassette were inserted elsewhere within the genome. This clone was selected for differentiation towards the retinal lineage. Immunocytochemistry of sections obtained from embryoid bodies and quantitative reverse transcriptase PCR of GFP positive and negative subpopulations purified by fluorescence activated cell sorting during the differentiation indicated a significant correlation between GFP and endogenous CRX expression. Furthermore, GFP expression was found in photoreceptor precursors emerging during hESC differentiation, but not in the retinal pigmented epithelium, retinal ganglion cells, or neurons of the developing inner nuclear layer. Together our data

demonstrate the successful application of ZFN technology to generate CRX-GFP labeled hESC lines, which can be used to study and isolate photoreceptor precursors during hESC differentiation.

Conway, A. E., et al. (2009). "A self-renewal program controls the expansion of genetically unstable cancer stem cells in pluripotent stem cell-derived tumors." Stem Cells **27**(1): 18-28.

Human germ cell tumors are often metastatic, presumably due to distal site tumor growth by cancer stem cells. To determine whether cancer stem cells can be identified in a transplantation model of testicular germ cell tumor, we transplanted murine embryonic germ cells (EGCs) into the testis of adult severe combined immunodeficient mice. Transplantation resulted in a locally invasive solid tumor, with a cellular component that generated secondary tumors upon serial transplantation. The secondary tumors were invariably metastatic, a feature not observed in the primary tumors derived from EGCs. To characterize the differences between EGCs and the tumor-derived stem cells, we performed karyotype and microarray analysis. Our results show that generation of cancer stem cells is associated with the acquisition of nonclonal genomic rearrangements not found in the originating population. Furthermore, pretreatment of EGCs with a potent inhibitor of self-renewal, retinoic acid, prevented tumor formation and the emergence of these genetically unstable cancer stem cells. Microarray analysis revealed that EGCs and first- and second-generation cancer stem cells were highly similar; however, approximately 1,000 differentially expressed transcripts could be identified corresponding to alterations in oncogenes and genes associated with motility and development. Combined, the data suggest that the activation of oncogenic pathways in a cellular background of genetic instability, coupled with an inherent ability to self-renew, is involved in the acquisition of metastatic behavior in the cancer stem cell population of tumors derived from pluripotent cells.

Conway, A. E., et al. (2016). "Enhanced CLIP Uncovers IMP Protein-RNA Targets in Human Pluripotent Stem Cells Important for Cell Adhesion and Survival." Cell Rep **15**(3): 666-679.

Human pluripotent stem cells (hPSCs) require precise control of post-transcriptional RNA networks to maintain proliferation and survival. Using enhanced UV crosslinking and immunoprecipitation (eCLIP), we identify RNA targets of the IMP/IGF2BP family of RNA-binding proteins in hPSCs. At the broad region and binding site levels, IMP1 and IMP2 show reproducible binding to a large and overlapping set of 3' UTR-enriched targets. RNA Bind-N-seq applied to recombinant full-length IMP1 and IMP2 reveals CA-rich motifs that are enriched in eCLIP-defined binding sites. We observe that IMP1 loss in hPSCs recapitulates IMP1 phenotypes, including a reduction in cell adhesion and increase in cell death. For cell adhesion, we find IMP1 maintains levels of integrin mRNA specifically regulating RNA stability of ITGB5 in hPSCs. Additionally, we show that IMP1 can be linked to hPSC survival via direct target BCL2. Thus, transcriptome-wide binding profiles identify hPSC targets modulating well-characterized IMP1 roles.

Cooke, J. A. and J. S. Meyer (2015). "Human Pluripotent Stem Cell-Derived Retinal Ganglion Cells: Applications for the Study and Treatment of Optic Neuropathies." Curr Ophthalmol Rep **3**(3): 200-206.

Correia, C., et al. (2016). "Effective Hypothermic Storage of Human Pluripotent Stem Cell-Derived Cardiomyocytes Compatible With Global Distribution of Cells for Clinical Applications and Toxicology Testing." Stem Cells Transl Med **5**(5): 658-669.

UNLABELLED: To fully explore the potential of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), efficient methods for storage and shipment of these cells are required. Here, we evaluated the feasibility to cold store monolayers and aggregates of functional CMs obtained from different PSC lines using a fully defined clinical-compatible preservation formulation and investigated the time frame that hPSC-CMs could be subjected to hypothermic storage. We showed that two-dimensional (2D) monolayers of hPSC-CMs can be efficiently stored at 4 degrees C for 3 days without compromising cell viability. However, cell viability decreased when the cold storage interval was extended to 7 days. We demonstrated that hPSC-CMs are more resistant to prolonged hypothermic storage-induced cell injury in three-dimensional aggregates than in 2D monolayers, showing high cell recoveries (>70%) after 7 days of storage. Importantly, hPSC-CMs maintained their typical (ultra)structure, gene and protein expression profile, electrophysiological profiles, and drug responsiveness. SIGNIFICANCE: The applicability of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) in the clinic/industry is highly dependent on the development of efficient methods for worldwide shipment of these cells. This study established effective clinically compatible strategies for cold (4 degrees C) storage of hPSC-CMs cultured as two-dimensional (2D) monolayers and three-dimensional (3D) aggregates. Cell recovery of 2D monolayers of hPSC-CMs was found to be dependent on the time of storage, and 3D cell aggregates were more resistant to prolonged cold storage than 2D monolayers. Of note, it was demonstrated that 7 days of cold storage did not affect hPSC-CM ultrastructure, phenotype, or function. This

study provides important insights into the cold preservation of PSC-CMs that could be valuable in improving global commercial distribution of hPSC-CMs.

Critchley, C. R., et al. (2013). "The impact of commercialisation on public perceptions of stem cell research: exploring differences across the use of induced pluripotent cells, human and animal embryos." Stem Cell Rev **9**(5): 541-554.

The development of pluripotent cells that enable stem cell research (SCR) without destroying human embryos is now a leading priority for science. Public and political controversies associated with human embryonic SCR experienced in the recent past should be alleviated if scientists no longer need to harvest cells from human embryos. This research suggests however additional issues needing attention in order to gain the public's trust and support: the use of mouse embryos and the commercialisation of research. Using a representative sample of 2,800 Australians, and an experimental telephone survey design, this research compared levels and predictors of public support for stem cell research across three cell source conditions: human embryo (HE), mouse embryo (ME) and induced pluripotent cells (iPSCs). The results revealed that the public were significantly more likely to support research using iPSCs than HE and ME cells and public compared to private research (regardless of the cell source). There was no significant difference in support for HE compared to ME research, but the former was viewed as more likely to lead to accessible health care benefits and to be associated with more trustworthy scientists. The results of a multimediation structural equation model showed that the primary reason support for SCR significantly dropped in a private compared to public context (i.e., the commercialisation effect) was because public scientists were trusted more than private scientists. This effect was consistent across all three SCR materials, suggesting that the use of mouse embryos or even iPSCs will not reduce the publics' concern with commercialised science. The implications these results have for public acceptance of stem cell and animal research are discussed in relation to possible solutions such as increasing public awareness of the regulation of animal research and benefit sharing.

Csobonyeiova, M., et al. (2016). "Induced pluripotent stem cells for modeling and cell therapy of Parkinson's disease." Neural Regen Res **11**(5): 727-728.

Csobonyeiova, M., et al. (2017). "Induced pluripotent stem cells in modeling and cell-based therapy of amyotrophic lateral sclerosis." J Physiol Pharmacol **68**(5): 649-657.

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease characterized by neuromuscular degeneration and the progressive loss of upper and lower motor neurons (MNs), causing weakness and paralysis. However, the underlying mechanisms of this disease are still unknown and there is no cure, or even treatment to stop or reverse its pathology. Consequently, most ALS patients die within 3 - 5 years after disease onset. While considerable progress has been made in studying animal models of ALS, they lack clinical suitability due to genetic differences. However, the recent development of induced pluripotent stem cells (iPSCs) has made it possible to study human disease-specific neuronal and glial cells to identify disease mechanisms and develop phenotypic screens for drug discovery. iPSCs provide researchers with a model of naturally occurring pathology under the human genetic background and MNs differentiated from human iPSCs bearing ALS-associated mutations offer a powerful model to study disease pathology. This paper reviews recent methods of differentiating iPSCs into neuronal cells and suggests further applications of these iPSCs-derived cells for ALS disease modeling, drug screening, and possible cell-based therapy.

Czaplewski, S. K., et al. (2014). "Tenogenic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells dictated by properties of braided submicron fibrous scaffolds." Biomaterials **35**(25): 6907-6917.

Tendon and ligament (T/L) engineering is a growing area of research with potential to address the inadequacies of current T/L defect treatments. Our group previously developed braided submicron fibrous scaffolds (BSMFSs) and demonstrated the viability of BSMFSs for T/L tissue engineering. The objective of this study was to investigate the effect of fiber chemistry and braiding angle on BSMFS mechanical properties and in turn, tenogenic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs) seeded on BSMFSs subjected to cyclic tensile stimulation in the absence of tenogenic medium. By varying fiber chemistry and/or braiding angle, BSMFSs with a range of mechanical properties were produced. We found that fiber chemistry dictated cell adhesion while braiding angle dictated the tissue-specific lineage commitment of hiPSC-MSCs. Scaffolds braided with large angles better supported hiPSC-MSC tenogenic differentiation as evidenced by the production of T/L-associated markers, downregulation of osteogenic markers, and expression of fibroblast-like, spindle cell morphology compared to scaffolds braided with small angles. Our results demonstrate the importance of substrate properties and mechanical stimulation on tenogenic

differentiation. These results also demonstrate the versatility of BSMFSs and the potential of hiPSC-MSCs for T/L tissue engineering.

D'Aiuto, L., et al. (2012). "Human induced pluripotent stem cell-derived models to investigate human cytomegalovirus infection in neural cells." PLoS One **7**(11): e49700.

Human cytomegalovirus (HCMV) infection is one of the leading prenatal causes of congenital mental retardation and deformities world-wide. Access to cultured human neuronal lineages, necessary to understand the species specific pathogenic effects of HCMV, has been limited by difficulties in sustaining primary human neuronal cultures. Human induced pluripotent stem (iPS) cells now provide an opportunity for such research. We derived iPS cells from human adult fibroblasts and induced neural lineages to investigate their susceptibility to infection with HCMV strain Ad169. Analysis of iPS cells, iPS-derived neural stem cells (NSCs), neural progenitor cells (NPCs) and neurons suggests that (i) iPS cells are not permissive to HCMV infection, i.e., they do not permit a full viral replication cycle; (ii) Neural stem cells have impaired differentiation when infected by HCMV; (iii) NPCs are fully permissive for HCMV infection; altered expression of genes related to neural metabolism or neuronal differentiation is also observed; (iv) most iPS-derived neurons are not permissive to HCMV infection; and (v) infected neurons have impaired calcium influx in response to glutamate.

Daley, G. Q. (2007). "Towards the generation of patient-specific pluripotent stem cells for combined gene and cell therapy of hematologic disorders." Hematology Am Soc Hematol Educ Program: 17-22.

Hematopoietic stem cell transplantation (HSCT) has proven successful for the treatment of a host of genetic and malignant diseases of the blood, but immune barriers to allogeneic tissue transplantation have hindered wider application. Likewise, gene therapy now appears effective in the treatment of various forms of immune deficiency, and yet insertional mutagenesis from viral gene transfer has raised safety concerns. One strategy for addressing the limitations of both gene therapy and allogeneic transplantation entails the creation of pluripotent stem cells from a patient's own somatic cells, thereby enabling precise in situ gene repair via homologous recombination in cultured cells, followed by autologous tissue transplantation. In murine model systems, the methods of somatic cell nuclear transfer, parthenogenesis, and direct somatic cell reprogramming with defined genetic factors have been used to generate pluripotent stem cells, and initial efforts at therapeutic gene repair and tissue transplantation suggest that the technology is feasible. Generating patient-specific autologous pluripotent stem cells provides an opportunity to combine gene therapy with autologous cell therapy to treat a host of human conditions. However, a number of technical hurdles must be overcome before therapies based on pluripotent human stem cells will appear in the clinic.

Dannenmann, B., et al. (2015). "High glutathione and glutathione peroxidase-2 levels mediate cell-type-specific DNA damage protection in human induced pluripotent stem cells." Stem Cell Reports **4**(5): 886-898.

Pluripotent stem cells must strictly maintain genomic integrity to prevent transmission of mutations. In human induced pluripotent stem cells (iPSCs), we found that genome surveillance is achieved via two ways, namely, a hypersensitivity to apoptosis and a very low accumulation of DNA lesions. The low apoptosis threshold was mediated by constitutive p53 expression and a marked upregulation of proapoptotic p53 target genes of the BCL-2 family, ensuring the efficient iPSC removal upon genotoxic insults. Intriguingly, despite the elevated apoptosis sensitivity, both mitochondrial and nuclear DNA lesions induced by genotoxins were less frequent in iPSCs compared to fibroblasts. Gene profiling identified that mRNA expression of several antioxidant proteins was considerably upregulated in iPSCs. Knockdown of glutathione peroxidase-2 and depletion of glutathione impaired protection against DNA lesions. Thus, iPSCs ensure genomic integrity through enhanced apoptosis induction and increased antioxidant defense, contributing to protection against DNA damage.

Das, A. K. and R. Pal (2010). "Induced pluripotent stem cells (iPSCs): the emergence of a new champion in stem cell technology-driven biomedical applications." J Tissue Eng Regen Med **4**(6): 413-421.

Pluripotent stem cells possess the unique property of differentiating into all other cell types of the human body. Further, the discovery of induced pluripotent stem cells (iPSCs) in 2006 has opened up new avenues in clinical medicine. In simple language, iPSCs are nothing but somatic cells reprogrammed genetically to exhibit pluripotent characteristics. This process utilizes retroviruses/lentiviruses/adenovirus/plasmids to incorporate candidate genes into somatic cells isolated from any part of the human body. It is also possible to develop disease-specific iPSCs which are most likely to revolutionize research in respect to the pathophysiology of most debilitating diseases, as these can be mimicked ex vivo in the laboratory. These models can also be used to study the safety and efficacy of known drugs or potential drug candidates

for a particular diseased condition, limiting the need for animal studies and considerably reducing the time and money required to develop new drugs. Recently, functional neurons, cardiomyocytes, pancreatic islet cells, hepatocytes and retinal cells have been derived from human iPSCs, thus re-confirming the pluripotency and differentiation capacity of these cells. These findings further open up the possibility of using iPSCs in cell replacement therapy for various degenerative disorders. In this review we highlight the development of iPSCs by different methods, their biological characteristics and their prospective applications in regenerative medicine and drug screening. We further discuss some practical limitations pertaining to this technology and how they can be averted for the betterment of human life.

Dash, B. C., et al. (2015). "Induced pluripotent stem cell-derived vascular smooth muscle cells: methods and application." Biochem J **465**(2): 185-194.

Vascular smooth muscle cells (VSMCs) play a major role in the pathophysiology of cardiovascular diseases. The advent of induced pluripotent stem cell (iPSC) technology and the capability of differentiating into virtually every cell type in the human body make this field a ray of hope for vascular regenerative therapy and understanding of the disease mechanism. In the present review, we first discuss the recent iPSC technology and vascular smooth muscle development from an embryo and then examine different methodologies to derive VSMCs from iPSCs, and their applications in regenerative therapy and disease modelling.

David, R., et al. (2009). "Forward programming of pluripotent stem cells towards distinct cardiovascular cell types." Cardiovasc Res **84**(2): 263-272.

AIMS: The proliferative potential of pluripotent stem cell-derived cardiomyocytes is limited, and reasonable yields for novel therapeutic options have yet to be achieved. In addition, various clinical applications will require the generation of specific cardiac cell types. Whereas early cardiovascular precursors appear to be important for novel approaches such as reseeding decellularized hearts, direct cell transplantation may require ventricular cells. Our recent work demonstrated that MesP1 represents a master regulator sufficient to induce cardiovasculogenesis in pluripotent cells. This led to our hypothesis that 'forward programming' towards specific subtypes may be feasible via overexpression of distinct early cardiovascular transcription factors. METHODS AND RESULTS: Here we demonstrate that forced expression of Nkx2.5 similar to MesP1 is sufficient to enhance cardiogenesis in murine embryonic stem cells (mES). In comparison to control transfected mES cells, a five-fold increased appearance of beating foci was observed as well as upregulated mRNA and protein expression levels. In contrast to MesP1, no increase of the endothelial lineage within the cardiovasculogenic mesoderm was observed. Likewise, Flk-1, the earliest known cardiovascular surface marker, was not induced via Nkx2.5 as opposed to MesP1. Detailed patch clamping analyses showed electrophysiological characteristics corresponding to all subtypes of cardiac ES cell differentiation in Nkx2.5 as well as MesP1 programmed embryoid bodies, but fractions of cardiomyocytes had distinct characteristics: MesP1 forced the appearance of early/intermediate type cardiomyocytes in comparison to control transfected ES cells whereas Nkx2.5 led to preferentially differentiated ventricular cells. CONCLUSION: Our findings show proof of principle for cardiovascular subtype-specific programming of pluripotent stem cells and confirm the molecular hierarchy for cardiovascular specification initiated via MesP1 with differentiation factors such as Nkx2.5 further downstream.

de Lazaro, I., et al. (2014). "Induced pluripotent stem (iPS) cells: a new source for cell-based therapeutics?" J Control Release **185**: 37-44.

The generation of induced pluripotent stem (iPS) cells from somatic cells by the ectopic expression of defined transcription factors has provided the regenerative medicine field with a new tool for cell replacement strategies. The advantages that these pluripotent cells can offer in comparison to other sources of stem cells include the generation of patient-derived cells and the lack of embryonic tissue while maintaining a versatile differentiation potential. The promise of iPS cell derivatives for therapeutic applications is encouraging albeit very early in development, with the first clinical study currently ongoing in Japan. Many challenges are yet to be circumvented before this technology can be clinically translated widely though. The delivery and expression of the reprogramming factors, the genomic instability, epigenetic memory and impact of cell propagation in culture are only some of the concerns. This article aims to critically discuss the potential of iPS cells as a new source of cell therapeutics.

de Peppo, G. M. and D. Marolt (2012). "State of the art in stem cell research: human embryonic stem cells, induced pluripotent stem cells, and transdifferentiation." J Blood Transfus **2012**: 317632.

Stem cells divide by asymmetric division and display different degrees of potency, or ability to differentiate into various specialized cell types. Owing to their unique regenerative capacity, stem cells have generated great enthusiasm worldwide and represent an

invaluable tool with unprecedented potential for biomedical research and therapeutic applications. Stem cells play a central role in the understanding of molecular mechanisms regulating tissue development and regeneration in normal and pathological conditions and open large possibilities for the discovery of innovative pharmaceuticals to treat the most devastating diseases of our time. Not least, their intrinsic characteristics allow the engineering of functional tissues for replacement therapies that promise to revolutionize the medical practice in the near future. In this paper, the authors present the characteristics of pluripotent stem cells and new developments of transdifferentiation technologies and explore some of the biomedical applications that this emerging technology is expected to empower.

de Rham, C. and J. Villard (2014). "Potential and limitation of HLA-based banking of human pluripotent stem cells for cell therapy." J Immunol Res **2014**: 518135.

Great hopes have been placed on human pluripotent stem (hPS) cells for therapy. Tissues or organs derived from hPS cells could be the best solution to cure many different human diseases, especially those who do not respond to standard medication or drugs, such as neurodegenerative diseases, heart failure, or diabetes. The origin of hPS is critical and the idea of creating a bank of well-characterized hPS cells has emerged, like the one that already exists for cord blood. However, the main obstacle in transplantation is the rejection of tissues or organ by the receiver, due to the three main immunological barriers: the human leukocyte antigen (HLA), the ABO blood group, and minor antigens. The problem could be circumvented by using autologous stem cells, like induced pluripotent stem (iPS) cells, derived directly from the patient. But iPS cells have limitations, especially regarding the disease of the recipient and possible difficulties to handle or prepare autologous iPS cells. Finally, reaching standards of good clinical or manufacturing practices could be challenging. That is why well-characterized and universal hPS cells could be a better solution. In this review, we will discuss the interest and the feasibility to establish hPS cells bank, as well as some economics and ethical issues.

Delaney, C. and I. D. Bernstein (2004). "Establishment of a pluripotent preleukaemic stem cell line by expression of the AML1-ETO fusion protein in Notch1-immortalized HSCN1cl10 cells." Br J Haematol **125**(3): 353-357.

The AML1-ETO fusion has been associated with up to 40% of acute myeloid leukaemia French-American-British classified M2 cases. This chimaeric protein interferes with normal AML1 function and disrupts critical transcriptional regulation of haematopoiesis. Current evidence suggests that AML1-ETO alone is insufficient to induce leukaemia, but rather is a co-operating event in leukaemogenesis. We developed a pluripotent murine haematopoietic stem cell line expressing the AML1-ETO fusion protein that displays in vitro and in vivo properties consistent with a preleukaemic state, including inhibition of terminal granulocytic differentiation in vitro and the development of non-lymphoid leukaemias in vivo. This cell line represents a potential platform for the introduction and in vitro rapid screening of candidate genes thought to co-operate with AML1-ETO in developing frank leukaemia.

Deng, J., et al. (2018). "Cell Transplantation for Spinal Cord Injury: Tumorigenicity of Induced Pluripotent Stem Cell-Derived Neural Stem/Progenitor Cells." Stem Cells Int **2018**: 5653787.

Spinal cord injury (SCI) is an intractable and worldwide difficult medical challenge with limited treatments. Neural stem/progenitor cell (NS/PC) transplantation derived from fetal tissues or embryonic stem cells (ESCs) has demonstrated therapeutic effects via replacement of lost neurons and severed axons and creation of permissive microenvironment to promote repair of spinal cord and axon regeneration but causes ethnical concerns and immunological rejections as well. Thus, the implementation of induced pluripotent stem cells (iPSCs), which can be generated from adult somatic cells and differentiated into NS/PCs, provides an effective alternation in the treatment of SCI. However, as researches further deepen, there is accumulating evidence that the use of iPSC-derived NS/PCs shows mounting concerns of safety, especially the tumorigenicity. This review discusses the tumorigenicity of iPSC-derived NS/PCs focusing on the two different routes of tumorigenicity (teratomas and true tumors) and underlying mechanisms behind them, as well as possible solutions to circumvent them.

Deshmukh, R. S., et al. (2012). "Drug discovery models and toxicity testing using embryonic and induced pluripotent stem-cell-derived cardiac and neuronal cells." Stem Cells Int **2012**: 379569.

Development of induced pluripotent stem cells (iPSCs) using forced expression of specific sets of transcription factors has changed the field of stem cell research extensively. Two important limitations for research application of embryonic stem cells (ESCs), namely, ethical and immunological issues, can be circumvented using iPSCs. Since the development of first iPSCs, tremendous effort has been directed to the development of methods to increase the efficiency of the process and to reduce the extent of genomic

modifications associated with the reprogramming procedure. The established lineage-specific differentiation protocols developed for ESCs are being applied to iPSCs, as they have great potential in regenerative medicine for cell therapy, disease modeling either for drug development or for fundamental science, and, last but not least, toxicity testing. This paper reviews efforts aimed at practical development of iPSC differentiation to neural/cardiac lineages and further the use of these iPSCs-derived cells for drug development and toxicity testing.

Devolder, K. (2010). "Complicity in stem cell research: the case of induced pluripotent stem cells." Hum Reprod **25**(9): 2175-2180.

Many who object to human embryonic stem cell (hESC) research because they believe it involves complicity in embryo destruction have welcomed induced pluripotent stem cell (iPSC) research as an ethical alternative. This opinion article aims to show that complicity arguments against hESC research are prima facie inconsistent with accepting iPSC research as it is currently done. Those who oppose hESC research on grounds of complicity should either (i) oppose iPSC research as well, (ii) advocate a radical change in the way iPSC research is done, (iii) demonstrate that complicity arguments against iPSC research are weaker than those against hESC research or (iv) reject complicity arguments against both hESC and iPSC research, either by adopting a more limited conception of complicity that allows acceptance of some hESC research, or by accepting that destroying embryos for important scientific research is not wrong.

Dianat, N., et al. (2013). "Human pluripotent stem cells for modelling human liver diseases and cell therapy." Curr Gene Ther **13**(2): 120-132.

The liver is affected by many types of diseases, including metabolic disorders and acute liver failure. Orthotopic liver transplantation (OLT) is currently the only effective treatment for life-threatening liver diseases but transplantation of allogeneic hepatocytes has now become an alternative as it is less invasive than OLT and can be performed repeatedly. However, this approach is hampered by the shortage of organ donors, and the problems related to the isolation of high quality adult hepatocytes, their cryopreservation and their absence of proliferation in culture. Liver is also a key organ to assess the pharmacokinetics and toxicology of xenobiotics and for drug discovery, but appropriate cell culture systems are lacking. All these problems have highlighted the need to explore other sources of cells such as stem cells that could be isolated, expanded to yield sufficiently large populations and then induced to differentiate into functional hepatocytes. The presence of a niche of "facultative" progenitor and stem cells in the normal liver has recently been confirmed but they display no telomerase activity. The recent discovery that human induced pluripotent stem cells can be generated from somatic cells has renewed hopes for regenerative medicine and in vitro disease modelling, as these cells are easily accessible. We review here the present progresses, limits and challenges for the generation of functional hepatocytes from human pluripotent stem cells in view of their potential use in regenerative medicine and drug discovery.

Diederichs, S. and R. S. Tuan (2014). "Functional comparison of human-induced pluripotent stem cell-derived mesenchymal cells and bone marrow-derived mesenchymal stromal cells from the same donor." Stem Cells Dev **23**(14): 1594-1610.

Mesenchymal stem cells (MSCs) have a high potential for therapeutic efficacy in treating diverse musculoskeletal injuries and cardiovascular diseases, and for ameliorating the severity of graft-versus-host and autoimmune diseases. While most of these clinical applications require substantial cell quantities, the number of MSCs that can be obtained initially from a single donor is limited. Reports on the derivation of MSC-like cells from pluripotent stem cells (PSCs) are, thus, of interest, as the infinite proliferative capacity of PSCs opens the possibility to generate large amounts of uniform batches of MSCs. However, characterization of such MSC-like cells is currently inadequate, especially with regard to the question of whether these cells are equivalent or identical to MSCs. In this study, we have derived MSC-like cells [induced PSC-derived MSC-like progenitor cells (iMPCs)] using four different methodologies from a newly established induced PSC line reprogrammed from human bone marrow stromal cells (BMSCs), and compared the iMPCs directly with the originating parental BMSCs. The iMPCs exhibited typical MSC/fibroblastic morphology and MSC-typical surface marker profile, and they were capable of differentiation in vitro along the osteogenic, chondrogenic, and adipogenic lineages. However, compared with the parental BMSCs, iMPCs displayed a unique expression pattern of mesenchymal and pluripotency genes and were less responsive to traditional BMSC differentiation protocols. We, therefore, conclude that iMPCs generated from PSCs via spontaneous differentiation represent a distinct population of cells which exhibit MSC-like characteristics.

Diekman, B. O., et al. (2015). "Knockdown of the cell cycle inhibitor p21 enhances cartilage formation by induced pluripotent stem cells." Tissue Eng Part A **21**(7-8): 1261-1274.

The limited regenerative capacity of articular cartilage contributes to progressive joint dysfunction associated with cartilage injury or osteoarthritis. Cartilage tissue engineering seeks to provide a biological substitute for repairing damaged or diseased cartilage, but requires a cell source with the capacity for extensive expansion without loss of chondrogenic potential. In this study, we hypothesized that decreased expression of the cell cycle inhibitor p21 would enhance the proliferative and chondrogenic potential of differentiated induced pluripotent stem cells (iPSCs). Murine iPSCs were directed to differentiate toward the chondrogenic lineage with an established protocol and then engineered to express a short hairpin RNA (shRNA) to reduce the expression of p21. Cells expressing the p21 shRNA demonstrated higher proliferative potential during monolayer expansion and increased synthesis of glycosaminoglycans (GAGs) in pellet cultures. Furthermore, these cells could be expanded approximately 150-fold over three additional passages without a reduction in the subsequent production of GAGs, while control cells showed reduced potential for GAG synthesis with three additional passages. In pellets from extensively passaged cells, knockdown of p21 attenuated the sharp decrease in cell number that occurred in control cells, and immunohistochemical analysis showed that p21 knockdown limited the production of type I and type X collagen while maintaining synthesis of cartilage-specific type II collagen. These findings suggest that manipulating the cell cycle can augment the monolayer expansion and preserve the chondrogenic capacity of differentiated iPSCs, providing a strategy for enhancing iPSC-based cartilage tissue engineering.

Diekmann, U., et al. (2017). "Purification of Definitive Endoderm Generated from Pluripotent Stem Cells by Magnetic Cell Sorting." Curr Protoc Stem Cell Biol **40**: 1D 9 1-1D 9 17.

Pluripotent stem cells have the capability to differentiate into any somatic cell type of the human body. The generation of surrogate cells for the treatment of liver, lung, and pancreatic diseases is of great medical interest. First, the in vitro formation into cells of the definitive endoderm is required. Upon commitment into this lineage, the cells express transcription factors such as FOXA2, SOX17, HNF1B; GATA family members; and the surface protein CXCR4. Unfortunately, some pluripotent stem cells resist the differentiation and contaminate the culture. Thus, we describe here an endoderm differentiation protocol, which yields endoderm-committed cells in high numbers in a 4-day treatment protocol. Second, a method for the purification of CXCR4-positive endoderm cells by magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) is described. The purification by MACS is quick and reliable and can be used to obtain pure endoderm cells either meant for downstream analysis such as omics or further differentiation experiments into endoderm-derived somatic cells. (c) 2017 by John Wiley & Sons, Inc.

Diekmann, U., et al. (2015). "A reliable and efficient protocol for human pluripotent stem cell differentiation into the definitive endoderm based on dispersed single cells." Stem Cells Dev **24**(2): 190-204.

Differentiation of pluripotent cells into endoderm-related cell types initially requires in vitro gastrulation into the definitive endoderm (DE). Most differentiation protocols are initiated from colonies of pluripotent cells complicating their adaption due to insufficiently defined starting conditions. The protocol described here was initiated from a defined cell number of dispersed single cells and tested on three different human embryonic stem cell lines and one human induced pluripotent stem cell line. Combined activation of ActivinA/Nodal signaling and GSK3 inhibition for the first 24 h, followed by ActivinA/Nodal signaling efficiently induced the DE state. Activation of ActivinA/Nodal signaling alone was not effective. Efficient GSK3 inhibition allowed the reduction of the ActivinA concentration during the entire protocol. A feeder-independent cultivation of pluripotent cells was preferred to achieve the high efficiency and robustness since feeder cells hindered the differentiation process. Additionally, inhibition of the phosphatidylinositol 3-kinase (PI3K) signaling pathway was not required, nonetheless yielding high cell numbers efficiently committed toward the DE. Finally, the endoderm generated could be differentiated further into PDX1-positive pan-pancreatic cells and NGN3-positive endocrine progenitors. Thus, this efficient and robust DE differentiation protocol is a step forward toward better reproducibility due to the well-defined conditions based on dispersed single cells from feeder-free-cultivated human pluripotent cells.

Ding, Q., et al. (2018). "Protective effects of human induced pluripotent stem cell-derived exosomes on high glucose-induced injury in human endothelial cells." Exp Ther Med **15**(6): 4791-4797.

Exosomes are a family of extracellular vesicles that are secreted from almost all types of cells and are associated with cell-to-cell communication. The present study was performed to investigate the effects of human induced pluripotent stem cell-derived exosomes (hiPSC-exo) on cell viability, capillary-like structure formation and senescence in endothelial cells exposed to high glucose. Exosomes were isolated from the conditional medium of hiPSCs and confirmed by transmission electron microscopy, nanoparticle

tracking analysis and western blot analysis using Alix and cluster of differentiation-63 as markers. hiPSC-exo were labeled with PKH26 for tracking, and it was determined that spherical exosomes, with a typical cup-shape, were absorbed by human umbilical vascular endothelial cells (HUVECs). Cultured HUVECs were treated with high glucose (33 mM) with or without hiPSC-exo (20 microg/ml) for 48 h, and cell viability, capillary tube formation and senescence were assessed. When exposed to high glucose, viability and tube formation in HUVECs was significantly reduced ($P<0.0001$), whereas the proportion of senescent cells was higher compared with that in control HUVECs ($P<0.0001$). Furthermore, hiPSC-exo restored cell viability and capillary-like structure formation, and reduced senescence in HUVECs exposed to high glucose ($P<0.0001$). However, hiPSC-exo had minimal effects on normal HUVECs. These findings suggest that stem cell-derived exosomes are able to promote cell proliferation, enhance capillary-like structure formation and reduce senescence in endothelial cells exposed to high glucose.

Dirja, B. T., et al. (2016). "Potential of laryngeal muscle regeneration using induced pluripotent stem cell-derived skeletal muscle cells." Acta Otolaryngol **136**(4): 391-396.

Conclusion Induced pluripotent stem (iPS) cells may be a new potential cell source for laryngeal muscle regeneration in the treatment of vocal fold atrophy after recurrent laryngeal nerve paralysis. Objectives Unilateral vocal fold paralysis can lead to degeneration, atrophy, and loss of force of the thyroarytenoid muscle. At present, there are some treatments such as thyroplasty, arytenoid adduction, and vocal fold injection. However, such treatments cannot restore reduced mass of the thyroarytenoid muscle. iPS cells have been recognized as supplying a potential resource for cell transplantation. The aim of this study was to assess the effectiveness of the use of iPS cells for the regeneration of laryngeal muscle through the evaluation of both in vitro and in vivo experiments. Methods Skeletal muscle cells were generated from tdTomato-labeled iPS cells using embryoid body formation. Differentiation into skeletal muscle cells was analyzed by gene expression and immunocytochemistry. The tdTomato-labeled iPS cell-derived skeletal muscle cells were transplanted into the left atrophied thyroarytenoid muscle. To evaluate the engraftment of these cells after transplantation, immunohistochemistry was performed. Results The tdTomato-labeled iPS cells were successfully differentiated into skeletal muscle cells through an in vitro experiment. These cells survived in the atrophied thyroarytenoid muscle after transplantation.

Dolatshad, N. F., et al. (2015). "G-protein Coupled Receptor Signaling in Pluripotent Stem Cell-derived Cardiovascular Cells: Implications for Disease Modeling." Front Cell Dev Biol **3**: 76.

Human pluripotent stem cell derivatives show promise as an in vitro platform to study a range of human cardiovascular diseases. A better understanding of the biology of stem cells and their cardiovascular derivatives will help to understand the strengths and limitations of this new model system. G-protein coupled receptors (GPCRs) are key regulators of stem cell maintenance and differentiation and have an important role in cardiovascular cell signaling. In this review, we will therefore describe the state of knowledge concerning the regulatory role of GPCRs in both the generation and function of pluripotent stem cell derived-cardiomyocytes, -endothelial, and -vascular smooth muscle cells. We will consider how far the in vitro disease models recapitulate authentic GPCR signaling and provide a useful basis for discovery of disease mechanisms or design of therapeutic strategies.

Dominguez, A. A., et al. (2014). "Human germ cell formation in xenotransplants of induced pluripotent stem cells carrying X chromosome aneuploidies." Sci Rep **4**: 6432.

Turner syndrome is caused by complete or partial loss of the second sex chromosome and is characterized by spontaneous fetal loss in >90% of conceptions. Survivors possess an array of somatic and germline clinical characteristics. Induced pluripotent stem cells (iPSCs) offer an opportunity for insight into genetic requirements of the X chromosome linked to Turner syndrome. We derived iPSCs from Turner syndrome and control individuals and examined germ cell development as a function of X chromosome composition. We demonstrate that two X chromosomes are not necessary for reprogramming or maintenance of pluripotency and that there are minimal differences in gene expression, at the single cell level, linked to X chromosome aneuploidies. Formation of germ cells, as assessed in vivo through a murine xenotransplantation model, indicated that undifferentiated iPSCs, independent of X chromosome composition, are capable of forming germ-cell-like cells (GCLCs) in vivo. In combination with clinical data regarding infertility in women with X chromosome aneuploidies, results suggest that two intact X chromosomes are not required for human germ cell formation, qualitatively or quantitatively, but rather are likely to be required for maintenance of human germ cells to adulthood.

Dono, R. (2015). "Glypican 4 down-regulation in pluripotent stem cells as a potential strategy to improve

differentiation and to impair tumorigenicity of cell transplants." Neural Regen Res **10**(10): 1576-1577.

Dorn, I., et al. (2015). "Erythroid differentiation of human induced pluripotent stem cells is independent of donor cell type of origin." Haematologica **100**(1): 32-41.

Epigenetic memory in induced pluripotent stem cells, which is related to the somatic cell type of origin of the stem cells, might lead to variations in the differentiation capacities of the pluripotent stem cells. In this context, induced pluripotent stem cells from human CD34(+) hematopoietic stem cells might be more suitable for hematopoietic differentiation than the commonly used fibroblast-derived induced pluripotent stem cells. To investigate the influence of an epigenetic memory on the ex vivo expansion of induced pluripotent stem cells into erythroid cells, we compared induced pluripotent stem cells from human neural stem cells and human cord blood-derived CD34(+) hematopoietic stem cells and evaluated their potential for differentiation into hematopoietic progenitor and mature red blood cells. Although genome-wide DNA methylation profiling at all promoter regions demonstrates that the epigenetic memory of induced pluripotent stem cells is influenced by the somatic cell type of origin of the stem cells, we found a similar hematopoietic induction potential and erythroid differentiation pattern of induced pluripotent stem cells of different somatic cell origin. All human induced pluripotent stem cell lines showed terminal maturation into normoblasts and enucleated reticulocytes, producing predominantly fetal hemoglobin. Differences were only observed in the growth rate of erythroid cells, which was slightly higher in the induced pluripotent stem cells derived from CD34(+) hematopoietic stem cells. More detailed methylation analysis of the hematopoietic and erythroid promoters identified similar CpG methylation levels in the induced pluripotent stem cell lines derived from CD34(+) cells and those derived from neural stem cells, which confirms their comparable erythroid differentiation potential.

Drowley, L., et al. (2016). "Human Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells in Phenotypic Screening: A Transforming Growth Factor-beta Type 1 Receptor Kinase Inhibitor Induces Efficient Cardiac Differentiation." Stem Cells Transl Med **5**(2): 164-174.

Several progenitor cell populations have been reported to exist in hearts that play a role in cardiac turnover and/or repair. Despite the presence of cardiac stem and progenitor cells within the myocardium, functional repair of the heart after injury is inadequate. Identification of the signaling pathways involved in the expansion and differentiation of cardiac progenitor cells (CPCs) will broaden insight into the fundamental mechanisms playing a role in cardiac homeostasis and disease and might provide strategies for in vivo regenerative therapies. To understand and exploit cardiac ontogeny for drug discovery efforts, we developed an in vitro human induced pluripotent stem cell-derived CPC model system using a highly enriched population of KDR(pos)/CKIT(neg)/NKX2.5(pos) CPCs. Using this model system, these CPCs were capable of generating highly enriched cultures of cardiomyocytes under directed differentiation conditions. In order to facilitate the identification of pathways and targets involved in proliferation and differentiation of resident CPCs, we developed phenotypic screening assays. Screening paradigms for therapeutic applications require a robust, scalable, and consistent methodology. In the present study, we have demonstrated the suitability of these cells for medium to high-throughput screens to assess both proliferation and multilineage differentiation. Using this CPC model system and a small directed compound set, we identified activin-like kinase 5 (transforming growth factor-beta type 1 receptor kinase) inhibitors as novel and potent inducers of human CPC differentiation to cardiomyocytes. Significance: Cardiac disease is a leading cause of morbidity and mortality, with no treatment available that can result in functional repair. This study demonstrates how differentiation of induced pluripotent stem cells can be used to identify and isolate cell populations of interest that can translate to the adult human heart. Two separate examples of phenotypic screens are discussed, demonstrating the value of this biologically relevant and reproducible technology. In addition, this assay system was able to identify novel and potent inducers of differentiation and proliferation of induced pluripotent stem cell-derived cardiac progenitor cells.

Du, C., et al. (2016). "Functional Kidney Bioengineering with Pluripotent Stem-Cell-Derived Renal Progenitor Cells and Decellularized Kidney Scaffolds." Adv Healthc Mater **5**(16): 2080-2091.

Recent advances in developmental biology and stem cell technology have led to the engineering of functional organs in a dish. However, the limited size of these organoids and absence of a large circulatory system poses limits to its clinical translation. To overcome these issues, decellularized whole kidney scaffolds with native microstructure and extracellular matrix (ECM) are employed for kidney bioengineering, using human-induced pluripotent-stem-cell-derived renal progenitor cells and endothelial cells. To demonstrate ECM-guided cellular assembly, the present work is focused on generating the functional unit of the kidney, the glomerulus. In the repopulated

organ, the presence of endothelial cells broadly upregulates the expression level of genes related to renal development. When the cellularized native scaffolds are implanted in SCID mice, glomeruli assembly can be achieved by co-culture of the renal progenitors and endothelial cells. These individual glomerular units are shown to be functional in the context of the whole organ using a simulated bio-reactor set-up with urea and creatinine excretion and albumin reabsorption. Our results indicate that the repopulation of decellularized native kidney using clinically relevant, expandable patient-specific renal progenitors and endothelial cells may be a viable approach for the generation of a functional whole kidney.

Du, C., et al. (2014). "Induced pluripotent stem cell-derived hepatocytes and endothelial cells in multi-component hydrogel fibers for liver tissue engineering." Biomaterials **35**(23): 6006-6014.

Liver tissue engineering requires a suitable cell source, methodologies to assemble the cells within their niche microenvironments in a spatially defined manner, and vascularization of the construct in vivo for maintenance of hepatocyte viability and function. Recently, we have developed methods of encapsulating cells within separate domains in multi-component hydrogel fibers and methods of assembling fibers to form 3D-patterned tissue constructs. In the present work, we have combined these approaches to encapsulate hepatocytes and endothelial cells within their specific niches, and to assemble them into endothelialized liver tissue constructs. The hepatocytes and endothelial cells were obtained in parallel by differentiating human recombinant protein-induced human pluripotent stem cells, resulting in a construct which contained genetically identical endothelial and parenchymal elements. We were able to demonstrate that the presence of endothelial cells in the scaffold significantly improved hepatocyte function in vitro and facilitated vascularization of the scaffold when implanted in a mouse partial hepatectomy model. The in vivo studies further asserted that integration of the scaffold with host vasculature had occurred, as demonstrated by the presence of human albumin in the mouse serum.

Du, Y., et al. (2017). "Exosomes from Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stromal Cells (hiPSC-MSCs) Protect Liver against Hepatic Ischemia/ Reperfusion Injury via Activating Sphingosine Kinase and Sphingosine-1-Phosphate Signaling Pathway." Cell Physiol Biochem **43**(2): 611-625.

BACKGROUND/AIMS: This study aimed to evaluate the effects of exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) on hepatic ischemia-reperfusion (I/R) injury, as well as the underlying mechanisms. METHODS: Exosomes derived from hiPSC-MSCs were isolated and characterized both biochemically and biophysically. hiPSC-MSCs-Exo were injected systemically into a murine ischemia/reperfusion injury model via the inferior vena cava, and then the therapeutic effects were evaluated. The serum levels of transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as histological changes were examined. Primary hepatocytes and human hepatocyte cell line HL7702 were used to test whether exosomes could induce hepatocytes proliferation in vitro. In addition, the expression levels of proliferation markers (proliferation cell nuclear antigen, PCNA; Phosphohistone-H3, PHH3) were measured by immunohistochemistry and Western blot. Moreover, SK inhibitor (SKI-II) and S1P1 receptor antagonist (VPC23019) were used to investigate the role of sphingosine kinase and sphingosine-1-phosphate-dependent pathway in the effects of hiPSC-MSCs-Exo on hepatocytes. RESULTS: hiPSCs were efficiently induced into hiPSC-MSCs that had typical MSC characteristics. hiPSC-MSCs-Exo had diameters ranging from 100 to 200 nm and expressed exosome markers (Alix, CD63 and CD81). After hiPSC-MSCs-Exo administration, hepatocyte necrosis and sinusoidal congestion were markedly suppressed in the ischemia/reperfusion injury model, with lower histopathological scores. The levels of hepatocyte injury markers AST and ALT were significantly lower in the treatment group compared to control, and the expression levels of proliferation markers (PCNA and PHH3) were greatly induced after hiPSC-MSCs-Exo administration. Moreover, hiPSC-MSCs-Exo also induced primary hepatocytes and HL7702 cells proliferation in vitro in a dose-dependent manner. We found that hiPSC-MSCs-Exo could directly fuse with target hepatocytes or HL7702 cells and increase the activity of sphingosine kinase and synthesis of sphingosine-1-phosphate (S1P). Furthermore, the inhibition of SK1 or S1P1 receptor completely abolished the protective and proliferative effects of hiPSC-MSCs-Exo on hepatocytes, both in vitro and in vivo. CONCLUSIONS: Our results demonstrated that hiPSC-MSCs-Exo could alleviate hepatic I/R injury via activating sphingosine kinase and sphingosine-1-phosphate pathway in hepatocytes and promote cell proliferation. These findings represent a novel mechanism that potentially contributes to liver regeneration and have important implications for new therapeutic approaches to acute liver disease.

Dubois, N. C., et al. (2011). "SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells." Nat Biotechnol **29**(11): 1011-1018.

To identify cell-surface markers specific to human cardiomyocytes, we screened cardiovascular cell populations derived from human embryonic stem cells (hESCs) against a panel of 370 known CD antibodies. This screen identified the signal-regulatory protein alpha (SIRPA) as a marker expressed specifically on cardiomyocytes derived from hESCs and human induced pluripotent stem cells (hiPSCs), and PECAM, THY1, PDGFRB and ITGA1 as markers of the nonmyocyte population. Cell sorting with an antibody against SIRPA allowed for the enrichment of cardiac precursors and cardiomyocytes from hESC/hiPSC differentiation cultures, yielding populations of up to 98% cardiac troponin T-positive cells. When plated in culture, SIRPA-positive cells were contracting and could be maintained over extended periods of time. These findings provide a simple method for isolating populations of cardiomyocytes from human pluripotent stem cell cultures, and thereby establish a readily adaptable technology for generating large numbers of enriched cardiomyocytes for therapeutic applications.

Dukhovny, A., et al. (2012). "Varicella-zoster virus infects human embryonic stem cell-derived neurons and neurospheres but not pluripotent embryonic stem cells or early progenitors." J Virol **86**(6): 3211-3218.

Pluripotent human stem cells are a powerful tool for the generation of differentiated cells that can be used for the study of human disease. We recently demonstrated that neurons derived from pluripotent human embryonic stem cells (hESC) can be infected by the highly host-restricted human alphaherpesvirus varicella-zoster virus (VZV), permitting the interaction of VZV with neurons to be readily evaluated in culture. In the present study, we examine whether pluripotent hESC and neural progenitors at intermediate stages of differentiation are permissive for VZV infection. We demonstrate here that VZV infection is blocked in naive hESC. A block to VZV replication is also seen when a bacterial artificial chromosome (BAC) containing the VZV genome is transfected into hESC. In contrast, related alphaherpesviruses herpes simplex virus 1 (HSV-1) and pseudorabies virus (PrV) productively infect naive hESC in a cell-free manner, and PrV replicates from a BAC transfected into hESC. Neurons differentiate from hESC via neural progenitor intermediates, as is the case in the embryo. The first in vitro stage at which permissiveness of hESC-derived neural precursors to VZV replication is observed is upon formation of "neurospheres," immediately after detachment from the inductive stromal feeder layer. These findings suggest that hESC may be useful in deciphering the yet enigmatic mechanisms of specificity of VZV infection and replication.

Duran, A. G., et al. (2018). "Regenerative Medicine/Cardiac Cell Therapy: Pluripotent Stem Cells." Thorac Cardiovasc Surg **66**(1): 53-62.

For more than 20 years, tremendous efforts have been made to develop cell-based therapies for treatment of heart failure. However, the results of clinical trials using somatic, nonpluripotent stem or progenitor cells have been largely disappointing in both cardiology and cardiac surgery scenarios. Surgical groups were among the pioneers of experimental and clinical myocyte transplantation ("cellular cardiomyoplasty"), but little translational progress was made prior to the development of cellular reprogramming for creation of induced pluripotent stem cells (iPSC). Ever since, protocols have been developed which allow for the derivation of large numbers of autologous cardiomyocytes (CMs) from patient-specific iPSC, moving translational research closer toward clinical pilot trials. However, compared with somatic cell therapy, the technology required for safe and efficacious pluripotent stem cell (PSC)-based therapies is extremely complex and requires tremendous resources and close interactions between basic scientists and clinicians. This review summarizes PSC sources, strategies to derive CMs, current cardiac tissue engineering approaches, concerns regarding immunogenicity and cellular maturity, and highlights the contributions made by surgical groups.

Easley, C. A., et al. (2014). "Gamete derivation from embryonic stem cells, induced pluripotent stem cells or somatic cell nuclear transfer-derived embryonic stem cells: state of the art." Reprod Fertil Dev **27**(1): 89-92.

Generating gametes from pluripotent stem cells (PSCs) has many scientific justifications and several biomedical rationales. Here, we consider several strategies for deriving gametes from PSCs from mice and primates (human and non-human) and their anticipated strengths, challenges and limitations. Although the 'Weismann barrier', which separates the mortal somatic cell lineages from the potentially immortal germline, has long existed, breakthroughs first in mice and now in humans are artificially creating germ cells from somatic cells. Spermatozoa with full reproductive viability establishing multiple generations of seemingly normal offspring have been reported in mice and, in humans, haploid spermatids with correct parent-of-origin imprints have been obtained. Similar progress with making oocytes has been published using mouse PSCs differentiated in vitro into primordial germ cells, which are then cultured after xenografting reconstructed artificial ovaries. Progress in making

human oocytes artificially is proving challenging. The usefulness of these artificial gametes, from assessing environmental exposure toxicity to optimising medical treatments to prevent negative off-target effects on fertility, may prove invaluable, as may basic discoveries on the fundamental mechanisms of gametogenesis.

Eckert, A., et al. (2015). "Bystander Effect Fuels Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells to Quickly Attenuate Early Stage Neurological Deficits After Stroke." Stem Cells Transl Med **4**(7): 841-851.

UNLABELLED: : Present therapies for stroke rest with tissue plasminogen activator (tPA), the sole licensed antithrombotic on the market; however, tPA's effectiveness is limited in that the drug not only must be administered less than 3-5 hours after stroke but often exacerbates blood-brain barrier (BBB) leakage and increases hemorrhagic incidence. A potentially promising therapy for stroke is transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs). To date, the effects of iPSCs on injuries that take place during early stage ischemic stroke have not been well studied. Consequently, we engrafted iPSC-NSCs into the ipsilesional hippocampus, a natural niche of NSCs, at 24 hours after stroke (prior to secondary BBB opening and when inflammatory signature is abundant). At 48 hours after stroke (24 hours after transplant), hiPSC-NSCs had migrated to the stroke lesion and quickly improved neurological function. Transplanted mice showed reduced expression of proinflammatory factors (tumor necrosis factor-alpha, interleukin 6 [IL-6], IL-1beta, monocyte chemotactic protein 1, macrophage inflammatory protein 1alpha), microglial activation, and adhesion molecules (intercellular adhesion molecule 1, vascular cell adhesion molecule 1) and attenuated BBB damage. We are the first to report that engrafted hiPSC-NSCs rapidly improved neurological function (less than 24 hours after transplant). Rapid hiPSC-NSC therapeutic activity is mainly due to a bystander effect that elicits reduced inflammation and BBB damage. SIGNIFICANCE: Clinically, cerebral vessel occlusion is rarely permanent because of spontaneous or thrombolytic therapy-mediated reperfusion. These results have clinical implications indicating a much extended therapeutic window for transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs; 24 hours after stroke as opposed to the 5-hour window with tissue plasminogen activator [tPA]). In addition, there is potential for a synergistic effect by combining hiPSC-NSC transplantation with tPA to attenuate stroke's adverse effects.

Efthymiou, A., et al. (2014). "Functional screening assays with neurons generated from pluripotent stem cell-derived neural stem cells." J Biomol Screen **19**(1): 32-43.

Rapid and effective drug discovery for neurodegenerative disease is currently impeded by an inability to source primary neural cells for high-throughput and phenotypic screens. This limitation can be addressed through the use of pluripotent stem cells (PSCs), which can be derived from patient-specific samples and differentiated to neural cells for use in identifying novel compounds for the treatment of neurodegenerative diseases. We have developed an efficient protocol to culture pure populations of neurons, as confirmed by gene expression analysis, in the 96-well format necessary for screens. These differentiated neurons were subjected to viability assays to illustrate their potential in future high-throughput screens. We have also shown that organelles such as nuclei and mitochondria could be live-labeled and visualized through fluorescence, suggesting that we should be able to monitor subcellular phenotypic changes. Neurons derived from a green fluorescent protein-expressing reporter line of PSCs were live-imaged to assess markers of neuronal maturation such as neurite length and co-cultured with astrocytes to demonstrate further maturation. These studies confirm that PSC-derived neurons can be used effectively in viability and functional assays and pave the way for high-throughput screens on neurons derived from patients with neurodegenerative disorders.

Efthymiou, A. G., et al. (2014). "Self-renewal and cell lineage differentiation strategies in human embryonic stem cells and induced pluripotent stem cells." Expert Opin Biol Ther **14**(9): 1333-1344.

INTRODUCTION: Since the initial discoveries of human embryonic and induced pluripotent stem cells, many strategies have been developed to utilize the potential of these cells for translational research and disease modeling. The success of these aims and the development of future applications in this area will depend on the ability to generate high-quality and large numbers of differentiated cell types that genetically, epigenetically, and functionally mimic the cells found in the body. AREAS COVERED: In this review, we highlight the current strategies used to maintain stem cell pluripotency (a measure of stem cell quality), as well as provide an overview of the various differentiation strategies being used to generate cells from all three germ lineages. We also discuss the particular considerations that must be addressed when utilizing these cells for translational therapy, and provide an example of a cell type currently used in clinical trials. EXPERT OPINION: The major challenge in

regenerative medicine and disease modeling will be in generating functional cells of sufficient quality that are physiologically and epigenetically similar to the diverse cells that they are modeled after. By meeting these criteria, these differentiated products can be successfully used in disease modeling, drug/toxicology screens, and cellular replacement therapy.

Emborg, M. E., et al. (2013). "Induced pluripotent stem cell-derived neural cells survive and mature in the nonhuman primate brain." Cell Rep **3**(3): 646-650.

The generation of induced pluripotent stem cells (iPSCs) opens up the possibility for personalized cell therapy. Here, we show that transplanted autologous rhesus monkey iPSC-derived neural progenitors survive for up to 6 months and differentiate into neurons, astrocytes, and myelinating oligodendrocytes in the brains of MPTP-induced hemiparkinsonian rhesus monkeys with a minimal presence of inflammatory cells and reactive glia. This finding represents a significant step toward personalized regenerative therapies.

Fagoonee, S., et al. (2015). "Long Term Liver Engraftment of Functional Hepatocytes Obtained from Germline Cell-Derived Pluripotent Stem Cells." PLoS One **10**(8): e0136762.

One of the major hurdles in liver gene and cell therapy is availability of ex vivo-expanded hepatocytes. Pluripotent stem cells are an attractive alternative. Here, we show that hepatocyte precursors can be isolated from male germline cell-derived pluripotent stem cells (GPSCs) using the hepatoblast marker, Liv2, and induced to differentiate into hepatocytes in vitro. These cells expressed hepatic-specific genes and were functional as demonstrated by their ability to secrete albumin and produce urea. When transplanted in the liver parenchyma of partially hepatectomised mice, Liv2-sorted cells showed regional and heterogeneous engraftment in the injected lobe. Moreover, approximately 50% of Y chromosome-positive, GPSC-derived cells were found in the female livers, in the region of engraftment, even one month after cell injection. This is the first study showing that Liv2-sorted GPSCs-derived hepatocytes can undergo long lasting engraftment in the mouse liver. Thus, GPSCs might offer promise for regenerative medicine.

Fagoonee, S., et al. (2010). "Generation of functional hepatocytes from mouse germ line cell-derived pluripotent stem cells in vitro." Stem Cells Dev **19**(8): 1183-1194.

Germ line cell-derived pluripotent stem cells (GPSCs) are similar to embryonic stem (ES) cells in that they can proliferate intensively and differentiate into a variety of cell types. Previous studies have revealed some inherent differences in gene expression between undifferentiated mouse ES cells and GPSCs. Our aims were to generate functional hepatocytes from mouse GPSCs in vitro and to investigate whether the differences in gene expression may impact on the hepatocyte differentiation capacity of the GPSCs compared with ES cells. Mouse GPSCs and ES cells were induced to differentiate into hepatocytes through embryoid body formation, with very high efficiency. These hepatocytes were characterized at cellular, molecular, and functional levels. The GPSC-derived hepatocytes expressed hepatic markers and were metabolically active as shown by albumin and haptoglobin secretion, urea synthesis, glycogen storage, and indocyanine green uptake. We also performed an unprecedented DNA microarray analysis comparing different stages of hepatocyte differentiation. Gene expression profiling demonstrated a strong similarity between GPSC and ES cells at different stages of induced hepatic differentiation. Moreover, Pearson correlation analysis of the microarray datasets suggested that, at late hepatic differentiation stages, the in vitro-derived cells were closer to fetal mouse primary hepatocytes than to those obtained from neonates. We have shown for the first time that adult GPSCs can be induced to differentiate into functional hepatocytes in vitro. These GPSC-derived hepatocytes offer great potential for cell replacement therapy for a wide variety of liver diseases.

Fagoonee, S., et al. (2011). "Potential applications of germline cell-derived pluripotent stem cells in organ regeneration." Organogenesis **7**(2): 116-122.

Impressive progress has been made since the turn of the century in the field of stem cells. Different types of stem cells have now been isolated from different types of tissues. Pluripotent stem cells are the most promising cell source for organ regeneration. One such cell type is the germline cell-derived pluripotent cell, which is derived from adult spermatogonial stem cells. The germline cell-derived pluripotent stem cells have been obtained from both human and mouse and, importantly, are adult stem cells with embryonic stem cell-like properties that do not require specific manipulations for pluripotency acquisition, hence bypassing problems related to induced pluripotent stem cells and embryonic stem cells. The germline cell-derived pluripotent stem cells have been induced to differentiate into cells deriving from the three germ layers and shown to be functional in vitro. This review will discuss the plasticity of the germline cell-derived pluripotent stem cells and their potential applications in human organ regeneration, with special emphasis on liver regeneration. Potential problems related to their use are also highlighted.

Fan, F., et al. (2018). "Induction of Pluripotent Stem Cell-Derived Cardiomyocyte Toxicity by Supernatant of Long Term-Stored Red Blood Cells in Vitro." Cell Physiol Biochem **46**(3): 1230-1240.

BACKGROUND/AIMS: Preserved red blood cells (RBCs) in vitro undergo a series of morphological, functional and metabolic changes during storage. RBC metabolites accumulate over time during storage, the toxicity of the supernatants of RBCs (SSRBCs) on tissue cells is largely unknown. Here, we aimed to study cardiomyocyte toxicity by supernatant of long term-stored RBCs in vitro and to discover elements involved in the mechanism. METHODS: Using human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) and real-time cell analyzing (RTCA), we analyzed the cardiotoxicity of d0, d14 and d35 SSRBCs. To analyze the cardiotoxicity of potassium (K) and lactic acid (LA) in SSRBCs, solutions containing the same concentrations of K and LA were respectively prepared and co-cultured with hiPS-CMs. Immunofluorescence and Gene Expression Array of hiPS-CMs were performed to evaluate the effects of d35 K and d35 SSRBCs. RESULTS: The beating of hiPS-CM was stopped by d14, d35 SSRBCs, or d35 K solution. Beating resumed within 48 hours in the presence of d14 SSRBC or d35 K but not d35 SSRBC; d0, d14 and d35 LA solution had no effect on beating patterns. At 48h after treatment, the immunofluorescence results showed that the integrity of the filament and sarcomere were intact. Gene Expression Array results found 14 differentially expressed genes which were likely to play an important role in the cytotoxic effect. CONCLUSION: Our results demonstrated cardiomyocyte toxicity by long term-stored SSRBCs in vitro. Besides high K-induced cardiotoxicity, there must be other unknown components in long term-stored SSRBCs that are cytotoxic to hiPS-CMs.

Fan, X. L., et al. (2018). "Induced pluripotent stem cell-derived mesenchymal stem cells activate quiescent T cells and elevate regulatory T cell response via NF-kappaB in allergic rhinitis patients." Stem Cell Res Ther **9**(1): 170.

BACKGROUND: It has been demonstrated previously that induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs) have immunosuppressive effects on activated T cells. However, the effects of iPSC-MSCs on quiescent T cells are still unknown. The aim of this study was to identify the immunomodulatory role of iPSC-MSCs on resting peripheral blood mononuclear cells (PBMCs) from allergic rhinitis (AR) patients. METHODS: PBMCs were cocultured with iPSC-MSCs without any stimulation, following which lymphocyte proliferation, activation of T cells, TH1/TH2 and regulatory T (Treg) cell differentiation, and Treg cell function were analyzed. The roles of soluble factors and cell-cell contact were examined to investigate the mechanisms involved. RESULTS: iPSC-MSCs promoted the proliferation of resting lymphocytes, activated CD4(+) and CD8(+) T cells, and upregulated and activated Treg cells without any additional stimulation. In addition, iPSC-MSCs balanced biased TH1/TH2 cytokine levels. Cell-cell contact was confirmed to be a possible mechanism involved. NF-kappaB was identified to play an important role in the immunomodulatory effects of iPSC-MSCs on quiescent T cells. CONCLUSIONS: iPSC-MSCs activate quiescent T cells and elevate regulatory T-cell response in AR patients, suggesting different immunomodulatory functions of iPSC-MSCs according to the phases of diseases. Therefore, iPSC-MSCs are a potential therapeutic candidate for treating allergic airway inflammation.

Fan, Y., et al. (2012). "Generation of human beta-thalassemia induced pluripotent stem cells from amniotic fluid cells using a single excisable lentiviral stem cell cassette." J Reprod Dev **58**(4): 404-409.

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients represent a powerful tool for biomedical research and may have a wide range of applications in cell and gene therapy. However, the safety issues and the low efficiency associated with generating human iPSCs have limited their usage in clinical settings. The cell type used to create iPSCs can significantly influence the reprogramming efficiency and kinetics. Here, we show that amniotic fluid cells from the prenatal diagnosis of a beta-thalassemia patient can be efficiently reprogrammed using a doxycycline (DOX)-inducible humanized version of the single lentiviral "stem cell cassette" vector flanked by loxP sites, which can be excised with Cre recombinase. We also demonstrated that the patient-derived iPSCs can be characterized based on the expression of pluripotency markers, and they can be differentiated into various somatic cell types in vitro and in vivo. Moreover, microarray analysis demonstrates a high correlation coefficient between human beta-thalassemia iPS cells and human embryonic stem (hES) cells but a low correlation coefficient between human beta-thalassemia amniotic fluid cells and human beta-thalassemia iPS cells. Our data suggest that amniotic fluid cells may be an ideal human somatic cell resource for rapid and efficient generation of patient-specific iPS cells.

Fauser, A. A., et al. (1982). "Suppressor T-cell clones derived from pluripotent stem cells (CFU-GEMM) of a patient with Hodgkin's lymphoma." Blut **45**(2): 97-102.

Pluripotent stem cells (CFU-GEMM) give rise to multilineage hemopoietic colonies in culture. The cellular composition revealed that mixed colonies contain cells of different myeloid lineages and mononuclear cells with T-cell surface antigens. T-lymphocytes of primary colonies, replated secondary and tertiary colonies from a patient with Hodgkin's Lymphoma were identified by their reaction with the monoclonal antibody OKT 8. Evidence for a common progenitor of myeloid and lymphoid cells is provided by analysis of individual secondary and tertiary colonies using OKT 3, OKT 4, OKT 8, VIM-D 5, and Ig M + D antibodies for each individual colony. Primary mixed, replated secondary and tertiary colonies revealed OKT 8 positive cells. No reaction with OKT 3, OKT 4, VIM-D 5, or Ig M + D was observed.

Fauser, A. A., et al. (1985). "B cell lineage: part of the differentiation program of human pluripotent stem cells." Haematol Blood Transfus **29**: 433-435.

Fauser, A. A., et al. (1982). "Cytotoxic T-cell clones derived from pluripotent stem cells (CFU-GEMM) of patients with Hodgkin's lymphoma." Blood **60**(6): 1317-1320.

Pluripotent stem cells (CFU-GEMM) give rise to multilineage hemopoietic colonies in culture. The cellular composition revealed that mixed colonies contain cells of different myeloid lineages and mononuclear cells with T-cell surface antigens. T lymphocytes of primary colonies and replated secondary clones from 5 patients with Hodgkin's lymphoma (stage I--II) were identified by their reaction with the monoclonal antibody OKT-8. Replated secondary clones do act functionally as cytotoxic cells using K562 as target cells. Evidence for a common progenitor of myeloid and lymphoid cells is provided by analysis of individual secondary colonies with the use of OKT-3, OKT-4, OKT-8, VIM-D5, and IgM + D antibodies for each individual clone. Primary mixed and replated secondary colonies revealed OKT-8-positive cells. No reaction with OKT-3, OKT-4, VIM-D 5, or IgM + D was observed. In mixed colonies grown from putative bone marrow transplant donors, only OKT-3-positive cells could be observed. Secondary replated colonies did not stain for OKT-8 and failed to lyse 51Cr-labeled K562 cells.

Fernandes, T. G. (2015). "Engineering at the microscale: A step towards single-cell analysis of human pluripotent stem cells." Biotechnol J **10**(10): 1511-1512.

Ferreira, A. F., et al. (2018). "Hematopoietic stem cells from induced pluripotent stem cells - considering the role of microRNA as a cell differentiation regulator." J Cell Sci **131**(4).

Although hematopoietic stem cell (HSC) therapy for hematological diseases can lead to a good outcome from the clinical point of view, the limited number of ideal donors, the comorbidity of patients and the increasing number of elderly patients may limit the application of this therapy. HSCs can be generated from induced pluripotent stem cells (iPSCs), which requires the understanding of the bone marrow and liver niches components and function in vivo iPSCs have been extensively applied in several studies involving disease models, drug screening and cellular replacement therapies. However, the somatic reprogramming by transcription factors is a low-efficiency process. Moreover, the reprogramming process is also regulated by microRNAs (miRNAs), which modulate the expression of the transcription factors OCT-4 (also known as POU5F1), SOX-2, KLF-4 and MYC, leading somatic cells to a pluripotent state. In this Review, we present an overview of the challenges of cell reprogramming protocols with regard to HSC generation from iPSCs, and highlight the potential role of miRNAs in cell reprogramming and in the differentiation of induced pluripotent stem cells.

Firth, A. L. and J. X. Yuan (2012). ""Ether-a-go-go" proliferation of iPSC-derived mesenchymal stem cells. Focus on "Regulation of cell proliferation of human induced pluripotent stem cell-derived mesenchymal stem cells via ether-a-go-go 1 (hEAG1) potassium channel"." Am J Physiol Cell Physiol **303**(2): C113-114.

Fligor, C. M., et al. (2018). "Three-Dimensional Retinal Organoids Facilitate the Investigation of Retinal Ganglion Cell Development, Organization and Neurite Outgrowth from Human Pluripotent Stem Cells." Sci Rep **8**(1): 14520.

Retinal organoids are three-dimensional structures derived from human pluripotent stem cells (hPSCs) which recapitulate the spatial and temporal differentiation of the retina, serving as effective in vitro models of retinal development. However, a lack of emphasis has been placed upon the development and organization of retinal ganglion cells (RGCs) within retinal organoids. Thus, initial efforts were made to characterize RGC differentiation throughout early stages of organoid development, with a clearly defined RGC layer developing in a temporally-appropriate manner expressing a complement of RGC-associated markers. Beyond studies of RGC development, retinal organoids may also prove useful for cellular replacement in which extensive axonal outgrowth is necessary to reach post-synaptic targets. Organoid-derived RGCs could help to elucidate factors

promoting axonal outgrowth, thereby identifying approaches to circumvent a formidable obstacle to RGC replacement. As such, additional efforts demonstrated significant enhancement of neurite outgrowth through modulation of both substrate composition and growth factor signaling. Additionally, organoid-derived RGCs exhibited diverse phenotypes, extending elaborate growth cones and expressing numerous guidance receptors. Collectively, these results establish retinal organoids as a valuable tool for studies of RGC development, and demonstrate the utility of organoid-derived RGCs as an effective platform to study factors influencing neurite outgrowth from organoid-derived RGCs.

Focosi, D. and G. Amabile (2017). "Induced Pluripotent Stem Cell-Derived Red Blood Cells and Platelet Concentrates: From Bench to Bedside." Cells **7**(1).

Red blood cells and platelets are anucleate blood components indispensable for oxygen delivery and hemostasis, respectively. Derivation of these blood elements from induced pluripotent stem (iPS) cells has the potential to develop blood donor-independent and genetic manipulation-prone products to complement or replace current transfusion banking, also minimizing the risk of alloimmunization. While the production of erythrocytes from iPS cells has challenges to overcome, such as differentiation into adult-type phenotype that functions properly after transfusion, platelet products are qualitatively and quantitatively approaching a clinically-applicable level owing to advances in expandable megakaryocyte (MK) lines, platelet-producing bioreactors, and novel reagents. Guidelines that assure the quality of iPS cells-derived blood products for clinical application represent a novel challenge for regulatory agencies. Considering the minimal risk of tumorigenicity and the expected significant demand of such products, ex vivo production of iPS-derived blood components can pave the way for iPS translation into the clinic.

Forster, R., et al. (2014). "Human intestinal tissue with adult stem cell properties derived from pluripotent stem cells." Stem Cell Reports **2**(6): 838-852.

Genetically engineered human pluripotent stem cells (hPSCs) have been proposed as a source for transplantation therapies and are rapidly becoming valuable tools for human disease modeling. However, many applications are limited due to the lack of robust differentiation paradigms that allow for the isolation of defined functional tissues. Here, using an endogenous LGR5-GFP reporter, we derived adult stem cells from hPSCs that gave rise to functional human intestinal tissue comprising all major cell types of the intestine. Histological and functional analyses revealed that such human organoid cultures could be derived with high purity and with a composition and morphology similar to those of cultures obtained from human biopsies. Importantly, hPSC-derived organoids responded to the canonical signaling pathways that control self-renewal and differentiation in the adult human intestinal stem cell compartment. This adult stem cell system provides a platform for studying human intestinal disease in vitro using genetically engineered hPSCs.

Foster, A. A., et al. (2018). "Protein-engineered hydrogels enhance the survival of induced pluripotent stem cell-derived endothelial cells for treatment of peripheral arterial disease." Biomater Sci **6**(3): 614-622.

A key feature of peripheral arterial disease (PAD) is damage to endothelial cells (ECs), resulting in lower limb pain and restricted blood flow. Recent preclinical studies demonstrate that the transplantation of ECs via direct injection into the affected limb can result in significantly improved blood circulation. Unfortunately, the clinical application of this therapy has been limited by low cell viability and poor cell function. To address these limitations we have developed an injectable, recombinant hydrogel, termed SHIELD (Shear-thinning Hydrogel for Injectable Encapsulation and Long-term Delivery) for cell transplantation. SHIELD provides mechanical protection from cell membrane damage during syringe flow. Additionally, secondary in situ crosslinking provides a reinforcing network to improve cell retention, thereby augmenting the therapeutic benefit of cell therapy. In this study, we demonstrate the improved acute viability of human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) following syringe injection delivery in SHIELD, compared to saline. Using a murine hind limb ischemia model of PAD, we demonstrate enhanced iPSC-EC retention in vivo and improved neovascularization of the ischemic limb based on arteriogenesis following transplantation of iPSC-ECs delivered in SHIELD.

Fox, I. J., et al. (2014). "Stem cell therapy. Use of differentiated pluripotent stem cells as replacement therapy for treating disease." Science **345**(6199): 1247391.

Pluripotent stem cells (PSCs) directed to various cell fates holds promise as source material for treating numerous disorders. The availability of precisely differentiated PSC-derived cells will dramatically affect blood component and hematopoietic stem cell therapies and should facilitate treatment of diabetes, some forms of liver disease and neurologic disorders, retinal diseases, and possibly heart disease. Although an unlimited supply of specific cell types is needed, other barriers must be overcome. This review of the state of cell therapies highlights

important challenges. Successful cell transplantation will require optimizing the best cell type and site for engraftment, overcoming limitations to cell migration and tissue integration, and occasionally needing to control immunologic reactivity, as well as a number of other challenges. Collaboration among scientists, clinicians, and industry is critical for generating new stem cell-based therapies.

Frederich, B. J., et al. (2017). "Electrotaxis of cardiac progenitor cells, cardiac fibroblasts, and induced pluripotent stem cell-derived cardiac progenitor cells requires serum and is directed via PI3'K pathways." Heart Rhythm **14**(11): 1685-1692.

BACKGROUND: The limited regenerative capacity of cardiac tissue has long been an obstacle to treating damaged myocardium. Cell-based therapy offers an enormous potential to the current treatment paradigms. However, the efficacy of regenerative therapies remains limited by inefficient delivery and engraftment. Electrotaxis (electrically guided cell movement) has been clinically used to improve recovery in a number of tissues but has not been investigated for treating myocardial damage. OBJECTIVE: The purpose of this study was to test the electrotactic behaviors of several types of cardiac cells. METHODS: Cardiac progenitor cells (CPCs), cardiac fibroblasts (CFs), and human induced pluripotent stem cell-derived cardiac progenitor cells (hiPSC-CPCs) were used. RESULTS: CPCs and CFs electrotax toward the anode of a direct current electric field, whereas hiPSC-CPCs electrotax toward the cathode. The voltage-dependent electrotaxis of CPCs and CFs requires the presence of serum in the media. Addition of soluble vascular cell adhesion molecule to serum-free media restores directed migration. We provide evidence that CPC and CF electrotaxis is mediated through phosphatidylinositide 3-kinase signaling. In addition, very late antigen-4, an integrin and growth factor receptor, is required for electrotaxis and localizes to the anodal edge of CPCs in response to direct current electric field. The hiPSC-derived CPCs do not express very late antigen-4, migrate toward the cathode in a voltage-dependent manner, and, similar to CPCs and CFs, require media serum and phosphatidylinositide 3-kinase activity for electrotaxis. CONCLUSION: The electrotactic behaviors of these therapeutic cardiac cells may be used to improve cell-based therapy for recovering function in damaged myocardium.

Freund, C., et al. (2010). "The first reported generation of human induced pluripotent stem cells (iPS cells) and iPS cell-derived cardiomyocytes in the Netherlands." Neth Heart J **18**(1): 51-54.

One of the recent breakthroughs in stem cell research has been the reprogramming of human somatic cells to an embryonic stem cell (ESC)-like state (induced pluripotent stem cells, iPS cells). Similar to ESCs, iPS cells can differentiate into derivatives of the three germ layers, for example cardiomyocytes, pancreatic cells or neurons. This technique offers a new approach to investigating disease pathogenesis and to the development of novel therapies. It may now be possible to generate iPS cells from somatic cells of patients who suffer from vascular genetic diseases, such as hereditary haemorrhagic telangiectasia (HHT). The iPS cells will have a similar genotype to that of the patient and can be differentiated in vitro into the cell type(s) that are affected in the patient. Thus they will serve as excellent models for a better understanding of mechanisms underlying the disease. This, together with the ability to test new drugs, could potentially lead to novel therapeutic concepts in the near future. Here we report the first derivation of three human iPS cell lines from two healthy individuals and one HHT patient in the Netherlands. The iPS cells resembled ESCs in morphology and expressed typical ESC markers. In vitro, iPS cells could be differentiated into cells of the three germ layers, including beating cardiomyocytes and vascular cells. With this technique it will be possible to establish human cardiovascular disease models from patient biopsies provided by the principal hospitals in the Netherlands. (Neth Heart J 2010;18:51-4.).

Fu, Q. L., et al. (2012). "Mesenchymal stem cells derived from human induced pluripotent stem cells modulate T-cell phenotypes in allergic rhinitis." Allergy **67**(10): 1215-1222.

BACKGROUND: Human induced pluripotent stem cells (iPSCs) possess remarkable self-renewal capacity and the potential to differentiate into novel cell types, such as mesenchymal stem cells (MSCs). iPSC-MSCs have been shown to enhance tissue regeneration and attenuate tissue ischaemia; however, their contribution to the immune regulation of Th2-skewed allergic rhinitis (AR) and asthma remains unclear. OBJECTIVE: This study compared the immunomodulatory effects of iPSC-MSCs and bone marrow-derived MSCs (BM-MSCs) on lymphocyte proliferation, T-cell phenotypes and cytokine production in peripheral blood mononuclear cells (PBMCs) in patients with AR, and investigated the possible molecular mechanisms underlying the immunomodulatory properties of iPSC-MSCs. METHODS: In co-cultures of PBMCs with iPSC-MSCs or BM-MSCs, lymphocyte proliferation was evaluated using 3H-thymidine (3H-TdR) uptake, carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) assays; the regulatory T-cell (Treg) phenotype was determined by flow cytometry, and cytokine levels were measured using an enzyme-linked

immunosorbent assay. The immunomodulatory properties of both MSCs were further evaluated using NS398 and transwell experiments. RESULTS: Similar to BM-MSCs, we determined that iPSC-MSCs significantly inhibit lymphocyte proliferation and promote Treg response in PBMCs ($P < 0.05$). Accordingly, the cytokine milieu (IFN-gamma, IL-4, IL-5, IL-10 and IL-13) in the supernatants of PBMCs changed significantly ($P < 0.05$). The immunomodulatory properties of iPSC-MSCs and BM-MSCs were associated with prostaglandin E2 (PGE2) production and cell-cell contact. CONCLUSIONS: These data demonstrate that iPSC-MSCs are capable of modulating T-cell phenotypes towards Th2 suppression through inducing Treg expansion, suggesting that iPSC-MSCs can be used as an alternative candidate to adult MSCs to treat allergic airway diseases.

Fuerstenau-Sharp, M., et al. (2015). "Generation of highly purified human cardiomyocytes from peripheral blood mononuclear cell-derived induced pluripotent stem cells." PLoS One **10**(5): e0126596.

Induced pluripotent stem (iPS) cells have an enormous potential for physiological studies. A novel protocol was developed combining the derivation of iPS from peripheral blood with an optimized directed differentiation to cardiomyocytes and a subsequent metabolic selection. The human iPS cells were retrovirally dedifferentiated from activated T cells. The subsequent optimized directed differentiation protocol yielded 30-45% cardiomyocytes at day 16 of differentiation. The derived cardiomyocytes expressed appropriate structural markers like cardiac troponin T, alpha-actinin and myosin light chain 2 (MLC2V). In a subsequent metabolic selection with lactate, the cardiomyocytes content could be increased to more than 90%. Loss of cardiomyocytes during metabolic selection were less than 50%, whereas alternative surface antibody-based selection procedures resulted in loss of up to 80% of cardiomyocytes. Electrophysiological characterization confirmed the typical cardiac features and the presence of ventricular, atrial and nodal-like action potentials within the derived cardiomyocyte population. Our combined and optimized protocol is highly robust and applicable for scalable cardiac differentiation. It provides a simple and cost-efficient method without expensive equipment for generating large numbers of highly purified, functional cardiomyocytes. It will further enhance the applicability of iPS cell-derived cardiomyocytes for disease modeling, drug discovery, and regenerative medicine.

Fuhrmann, T., et al. (2018). "Combined delivery of chondroitinase ABC and human induced pluripotent stem cell-derived neuroepithelial cells promote tissue repair in an animal model of spinal cord injury." Biomed Mater **13**(2): 024103.

The lack of tissue regeneration after traumatic spinal cord injury in animal models is largely attributed to the local inhibitory microenvironment. To overcome this inhibitory environment while promoting tissue regeneration, we investigated the combined delivery of chondroitinase ABC (chABC) with human induced pluripotent stem cell-derived neuroepithelial stem cells (NESCs). ChABC was delivered to the injured spinal cord at the site of injury by affinity release from a crosslinked methylcellulose (MC) hydrogel by injection into the intrathecal space. NESCs were distributed in a hydrogel comprised of hyaluronan and MC and injected into the spinal cord tissue both rostral and caudal to the site of injury. Cell transplantation led to reduced cavity formation, but did not improve motor function. While few surviving cells were found 2 weeks post injury, the majority of live cells were neurons, with only few astrocytes, oligodendrocytes, and progenitor cells. At 9 weeks post injury, there were more progenitor cells and a more even distribution of cell types compared to those at 2 weeks post injury, suggesting preferential survival and differentiation. Interestingly, animals that received cells and chABC had more neurons than animals that received cells alone, suggesting that chABC influenced the injury environment such that neuronal differentiation or survival was favoured.

Fujimaki, S., et al. (2013). "Intrinsic ability of adult stem cell in skeletal muscle: an effective and replenishable resource to the establishment of pluripotent stem cells." Stem Cells Int **2013**: 420164.

Adult stem cells play an essential role in mammalian organ maintenance and repair throughout adulthood since they ensure that organs retain their ability to regenerate. The choice of cell fate by adult stem cells for cellular proliferation, self-renewal, and differentiation into multiple lineages is critically important for the homeostasis and biological function of individual organs. Responses of stem cells to stress, injury, or environmental change are precisely regulated by intercellular and intracellular signaling networks, and these molecular events cooperatively define the ability of stem cell throughout life. Skeletal muscle tissue represents an abundant, accessible, and replenishable source of adult stem cells. Skeletal muscle contains myogenic satellite cells and muscle-derived stem cells that retain multipotent differentiation abilities. These stem cell populations have the capacity for long-term proliferation and high self-renewal. The molecular mechanisms associated with deficits in skeletal muscle and stem cell function have been extensively studied. Muscle-derived stem cells are an obvious, readily available cell resource that offers

promise for cell-based therapy and various applications in the field of tissue engineering. This review describes the strategies commonly used to identify and functionally characterize adult stem cells, focusing especially on satellite cells, and discusses their potential applications.

Fujimoto, Y., et al. (2012). "Treatment of a mouse model of spinal cord injury by transplantation of human induced pluripotent stem cell-derived long-term self-renewing neuroepithelial-like stem cells." Stem Cells **30**(6): 1163-1173.

Because of their ability to self-renew, to differentiate into multiple lineages, and to migrate toward a damaged site, neural stem cells (NSCs), which can be derived from various sources such as fetal tissues and embryonic stem cells, are currently considered to be promising components of cell replacement strategies aimed at treating injuries of the central nervous system, including the spinal cord. Despite their efficiency in promoting functional recovery, these NSCs are not homogeneous and possess variable characteristics depending on their derivation protocols. The advent of induced pluripotent stem (iPS) cells has provided new prospects for regenerative medicine. We used a recently developed robust and stable protocol for the generation of long-term, self-renewing, neuroepithelial-like stem cells from human iPS cells (hiPS-lt-NES cells), which can provide a homogeneous and well-defined population of NSCs for standardized analysis. Here, we show that transplanted hiPS-lt-NES cells differentiate into neural lineages in the mouse model of spinal cord injury (SCI) and promote functional recovery of hind limb motor function. Furthermore, using two different neuronal tracers and ablation of the transplanted cells, we revealed that transplanted hiPS-lt-NES cell-derived neurons, together with the surviving endogenous neurons, contributed to restored motor function. Both types of neurons reconstructed the corticospinal tract by forming synaptic connections and integrating neuronal circuits. Our findings indicate that hiPS-lt-NES transplantation represents a promising avenue for effective cell-based treatment of SCI.

Fujita, A., et al. (2016). "beta-Globin-Expressing Definitive Erythroid Progenitor Cells Generated from Embryonic and Induced Pluripotent Stem Cell-Derived Sacs." Stem Cells **34**(6): 1541-1552.

Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells represent a potential alternative source for red blood cell transfusion. However, when using traditional methods with embryoid bodies, ES cell-derived erythroid cells predominantly express embryonic type varepsilon-globin, with lesser fetal type gamma-globin and very little adult type beta-globin. Furthermore, no beta-globin expression is detected in iPS cell-derived erythroid cells. ES cell-derived sacs (ES sacs) have been recently used to generate functional platelets. Due to its unique structure, we hypothesized that ES sacs serve as hemangioblast-like progenitors capable to generate definitive erythroid cells that express beta-globin. With our ES sac-derived erythroid differentiation protocol, we obtained approximately 120 erythroid cells per single ES cell. Both primitive (varepsilon-globin expressing) and definitive (gamma- and beta-globin expressing) erythroid cells were generated from not only ES cells but also iPS cells. Primitive erythropoiesis is gradually switched to definitive erythropoiesis during prolonged ES sac maturation, concurrent with the emergence of hematopoietic progenitor cells. Primitive and definitive erythroid progenitor cells were selected on the basis of glycophorin A or CD34 expression from cells within the ES sacs before erythroid differentiation. This selection and differentiation strategy represents an important step toward the development of in vitro erythroid cell production systems from pluripotent stem cells. Further optimization to improve expansion should be required for clinical application. Stem Cells 2016;34:1541-1552.

Fujiwara, M., et al. (2011). "Induction and enhancement of cardiac cell differentiation from mouse and human induced pluripotent stem cells with cyclosporin-A." PLoS One **6**(2): e16734.

Induced pluripotent stem cells (iPSCs) are novel stem cells derived from adult mouse and human tissues by reprogramming. Elucidation of mechanisms and exploration of efficient methods for their differentiation to functional cardiomyocytes are essential for developing cardiac cell models and future regenerative therapies. We previously established a novel mouse embryonic stem cell (ESC) and iPSC differentiation system in which cardiovascular cells can be systematically induced from Flk1(+) common progenitor cells, and identified highly cardiogenic progenitors as Flk1(+)/CXCR4(+)/VE-cadherin(-) (FCV) cells. We have also reported that cyclosporin-A (CSA) drastically increases FCV progenitor and cardiomyocyte induction from mouse ESCs. Here, we combined these technologies and extended them to mouse and human iPSCs. Co-culture of purified mouse iPSC-derived Flk1(+) cells with OP9 stroma cells induced cardiomyocyte differentiation whilst addition of CSA to Flk1(+) cells dramatically increased both cardiomyocyte and FCV progenitor cell differentiation. Spontaneously beating colonies were obtained from human iPSCs by co-culture with END-2 visceral endoderm-like cells. Appearance of beating colonies from human iPSCs was increased approximately 4.3

times by addition of CSA at mesoderm stage. CSA-expanded human iPSC-derived cardiomyocytes showed various cardiac marker expressions, synchronized calcium transients, cardiomyocyte-like action potentials, pharmacological reactions, and ultra-structural features as cardiomyocytes. These results provide a technological basis to obtain functional cardiomyocytes from iPSCs.

Fukuda, T., et al. (2017). "Isolation and expansion of human pluripotent stem cell-derived hepatic progenitor cells by growth factor defined serum-free culture conditions." Exp Cell Res **352**(2): 333-345.

Limited growth potential, narrow ranges of sources, and difference in variability and functions from batch to batch of primary hepatocytes cause a problem for predicting drug-induced hepatotoxicity during drug development. Human pluripotent stem cell (hPSC)-derived hepatocyte-like cells in vitro are expected as a tool for predicting drug-induced hepatotoxicity. Several studies have already reported efficient methods for differentiating hPSCs into hepatocyte-like cells, however its differentiation process is time-consuming, labor-intensive, cost-intensive, and unstable. In order to solve this problem, expansion culture for hPSC-derived hepatic progenitor cells, including hepatic stem cells and hepatoblasts which can self-renewal and differentiate into hepatocytes should be valuable as a source of hepatocytes. However, the mechanisms of the expansion of hPSC-derived hepatic progenitor cells are not yet fully understood. In this study, to isolate hPSC-derived hepatic progenitor cells, we tried to develop serum-free growth factor defined culture conditions using defined components. Our culture conditions were able to isolate and grow hPSC-derived hepatic progenitor cells which could differentiate into hepatocyte-like cells through hepatoblast-like cells. We have confirmed that the hepatocyte-like cells prepared by our methods were able to increase gene expression of cytochrome P450 enzymes upon encountering rifampicin, phenobarbital, or omeprazole. The isolation and expansion of hPSC-derived hepatic progenitor cells in defined culture conditions should have advantages in terms of detecting accurate effects of exogenous factors on hepatic lineage differentiation, understanding mechanisms underlying self-renewal ability of hepatic progenitor cells, and stably supplying functional hepatic cells.

Galat, V., et al. (2016). "Transgene Reactivation in Induced Pluripotent Stem Cell Derivatives and Reversion to Pluripotency of Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells." Stem Cells Dev **25**(14): 1060-1072.

Induced pluripotent stem cells (iPSCs) have enormous potential in regenerative medicine and disease modeling. It is now felt that clinical trials should be performed with iPSCs derived with nonintegrative constructs. Numerous studies, however, including those describing disease models, are still being published using cells derived from iPSCs generated with integrative constructs. Our experimental work presents the first evidence of spontaneous transgene reactivation in vitro in several cellular types. Our results show that the transgenes were predominantly silent in parent iPSCs, but in mesenchymal and endothelial iPSC derivatives, the transgenes experienced random upregulation of Nanog and c-Myc. Additionally, we provide evidence of spontaneous secondary reprogramming and reversion to pluripotency in mesenchymal stem cells derived from iPSCs. These findings strongly suggest that the studies, which use cellular products derived from iPSCs generated with retro- or lentiviruses, should be evaluated with consideration of the possibility of transgene reactivation. The in vitro model described here provides insight into the earliest events of culture transformation and suggests the hypothesis that reversion to pluripotency may be responsible for the development of tumors in cell replacement experiments. The main goal of this work, however, is to communicate the possibility of transgene reactivation in retro- or lenti-iPSC derivatives and the associated loss of cellular fidelity in vitro, which may impact the outcomes of disease modeling and related experimentation.

Gamez Escalona, J. A. and N. Lopez Moratalla (2014). "[Pluripotent stem cells on cell therapy]." An Sist Sanit Navar **37**(1): 129-136.

Induced pluripotent stem (iPS) cells are a novel stem cell population derived from human somatic cells through reprogramming using a set of transcription factors. These iPS cells were shown to share the characteristics of embryonic stem cells, including the ability to give rise to differentiated cells of every tissue type of the body. In the shorter term, iPS cells will be useful for creating patient-identical disease model cells in which the pathological process can be studied and drugs can be tested. Despite critical attitudes, accumulating preclinical evidence supports the effectiveness of iPSC-based cell therapy on the selection of appropriate iPSC clones. The production of iPS cells has also spurred the development of other techniques, for example, transdifferentiation by researchers can now convert heart fibroblasts directly in vivo into myocytes by similar methods. This pluripotent cells is indeed of great value in medical research and it is opening new possibilities in cell therapy.

Gao, L., et al. (2018). "Large Cardiac Muscle Patches Engineered From Human Induced-Pluripotent Stem Cell-Derived Cardiac Cells Improve Recovery From Myocardial Infarction in Swine." Circulation **137**(16): 1712-1730.

BACKGROUND: Here, we generated human cardiac muscle patches (hCMPs) of clinically relevant dimensions (4 cm x 2 cm x 1.25 mm) by suspending cardiomyocytes, smooth muscle cells, and endothelial cells that had been differentiated from human induced-pluripotent stem cells in a fibrin scaffold and then culturing the construct on a dynamic (rocking) platform. METHODS: In vitro assessments of hCMPs suggest maturation in response to dynamic culture stimulation. In vivo assessments were conducted in a porcine model of myocardial infarction (MI). Animal groups included: MI hearts treated with 2 hCMPs (MI+hCMP, n=13), MI hearts treated with 2 cell-free open fibrin patches (n=14), or MI hearts with neither experimental patch (n=15); a fourth group of animals underwent sham surgery (Sham, n=8). Cardiac function and infarct size were evaluated by MRI, arrhythmia incidence by implanted loop recorders, and the engraftment rate by calculation of quantitative polymerase chain reaction measurements of expression of the human Y chromosome. Additional studies examined the myocardial protein expression profile changes and potential mechanisms of action that related to exosomes from the cell patch. RESULTS: The hCMPs began to beat synchronously within 1 day of fabrication, and after 7 days of dynamic culture stimulation, in vitro assessments indicated the mechanisms related to the improvements in electronic mechanical coupling, calcium-handling, and force generation, suggesting a maturation process during the dynamic culture. The engraftment rate was 10.9+/-1.8% at 4 weeks after the transplantation. The hCMP transplantation was associated with significant improvements in left ventricular function, infarct size, myocardial wall stress, myocardial hypertrophy, and reduced apoptosis in the periscar boarder zone myocardium. hCMP transplantation also reversed some MI-associated changes in sarcomeric regulatory protein phosphorylation. The exosomes released from the hCMP appeared to have cytoprotective properties that improved cardiomyocyte survival. CONCLUSIONS: We have fabricated a clinically relevant size of hCMP with trilineage cardiac cells derived from human induced-pluripotent stem cells. The hCMP matures in vitro during 7 days of dynamic culture. Transplantation of this type of hCMP results in significantly reduced infarct size and improvements in cardiac function that are associated with reduction in left ventricular wall stress. The hCMP treatment is not associated with significant changes in arrhythmogenicity.

Garcia-Reitboeck, P., et al. (2018). "Human Induced Pluripotent Stem Cell-Derived Microglia-Like Cells Harboring TREM2 Missense Mutations Show Specific Deficits in Phagocytosis." Cell Rep **24**(9): 2300-2311.

Dysfunction of microglia, the brain's immune cells, is linked to neurodegeneration. Homozygous missense mutations in TREM2 cause Nasu-Hakola disease (NHD), an early-onset dementia. To study the consequences of these TREM2 variants, we generated induced pluripotent stem cell-derived microglia-like cells (iPSC-MGLCs) from patients with NHD caused by homozygous T66M or W50C missense mutations. iPSC-MGLCs expressed microglial markers and secreted higher levels of TREM2 than primary macrophages. TREM2 expression and secretion were reduced in variant lines. LPS-mediated cytokine secretion was comparable between control and TREM2 variant iPSC-MGLCs, whereas survival was markedly reduced in cells harboring missense mutations when compared with controls. Furthermore, TREM2 missense mutations caused a marked impairment in the phagocytosis of apoptotic bodies, but not in Escherichia coli or zymosan substrates. Coupled with changes in apoptotic cell-induced cytokine release and migration, these data identify specific deficits in the ability of iPSC-MGLCs harboring TREM2 missense mutations to respond to specific pathogenic signals.

Ge, X., et al. (2012). "Human amniotic mesenchymal stem cell-derived induced pluripotent stem cells may generate a universal source of cardiac cells." Stem Cells Dev **21**(15): 2798-2808.

Human amniotic mesenchymal stem cells (hAMSCs) demonstrated partially pluripotent characteristics with a strong expression of Oct4 and Nanog genes and immunomodulatory properties characterized by the absence of HLA-DR and the presence of HLA-G and CD59. The hAMSCs were reprogrammed into induced pluripotent stem cells (iPSCs) that generate a promising source of universal cardiac cells. The hAMSC-derived iPSCs (MiPSCs) successfully underwent robust cardiac differentiation to generate cardiomyocytes. This study investigated 3 key properties of the hAMSCs and MiPSCs: (1) the reprogramming efficiency of the partially pluripotent hAMSCs to generate MiPSCs; (2) immunomodulatory properties of the hAMSCs and MiPSCs; and (3) the cardiac differentiation potential of the MiPSCs. The characteristic iPSC colony formation was observed within 10 days after the transduction of the hAMSCs with a single integration polycistronic vector containing 4 Yamanaka factors. Immunohistology and reverse transcription-polymerase chain reaction assays revealed that the MiPSCs expressed stem cell surface markers and pluripotency-specific genes. Furthermore,

the hAMSCs and MiPSCs demonstrated immunomodulatory properties enabling successful engraftment in the SVJ mice. Finally, the cardiac differentiation of MiPSCs exhibited robust spontaneous contractility, characteristic calcium transience across the membrane, a high expression of cardiac genes and mature cardiac phenotypes, and a contractile force comparable to cardiomyocytes. Our results demonstrated that the hAMSCs are reprogrammed with a high efficiency into MiPSCs, which possess pluripotent, immunomodulatory, and precardiac properties. The MiPSC-derived cardiac cells express a c-kit cell surface marker, which may be employed to purify the cardiac cell population and enable allogeneic cardiac stem cell therapy.

Ghasemi-Dehkordi, P., et al. (2015). "Comparison between the cultures of human induced pluripotent stem cells (hiPSCs) on feeder-and serum-free system (Matrigel matrix), MEF and HDF feeder cell lines." J Cell Commun Signal **9**(3): 233-246.

Human induced pluripotent stem cells (hiPSCs) are a type of pluripotent stem cells artificially derived from an adult somatic cell (typically human fibroblast) by forced expression of specific genes. In recent years, different feeders like inactivated mouse embryonic fibroblasts (MEFs), human dermal fibroblasts (HDFs), and feeder free system have commonly been used for supporting the culture of stem cells in undifferentiated state. In the present work, the culture of hiPSCs and their characterizations on BD Matrigel (feeder-and serum-free system), MEF and HDF feeders using cell culture methods and molecular techniques were evaluated and compared. The isolated HDFs from foreskin samples were reprogrammed to hiPSCs using gene delivery system. Then, the pluripotency ability of hiPSCs cultured on each layer was determined by teratoma formation and immunohistochemical staining. After EBs generation the expression level of three germ layers genes were evaluated by Q-real-time PCR. Also, the cytogenetic stability of hiPSCs cultured on each condition was analyzed by karyotyping and comet assay. Then, the presence of pluripotency antigens were confirmed by Immunocytochemistry (ICC) test and alkaline phosphatase staining. This study were showed culturing of hiPSCs on BD Matrigel, MEF and HDF feeders had normal morphology and could maintain in undifferentiated state for prolonged expansion. The hiPSCs cultured in each system had normal karyotype without any chromosomal abnormalities and the DNA lesions were not observed by comet assay. Moreover, up-regulation in three germ layers genes in cultured hiPSCs on each layer (same to ESCs) compare to normal HDFs were observed ($p < 0.05$). The findings of the present work were showed in stem cells culturing especially hiPSCs both MEF and HDF feeders as well as feeder free system like Matrigel are proper despite benefits and disadvantages. Although, MEFs is suitable for supporting of stem cell culturing but it can animal pathogens transferring and inducing immune response. Furthermore, HDFs have homologous source with hiPSCs and can be used as feeder instead of MEF but in therapeutic approaches the cells contamination is a problem. So, this study were suggested feeder free culturing of hiPSCs on Matrigel in supplemented media (without using MEF conditioned medium) resolves these problems and could prepare easy applications of hiPSCs in therapeutic approaches of regenerative medicine such as stem-cell therapy and somatic cell nuclear in further researches.

Ghosh, Z., et al. (2010). "Persistent donor cell gene expression among human induced pluripotent stem cells contributes to differences with human embryonic stem cells." PLoS One **5**(2): e8975.

Human induced pluripotent stem cells (hiPSCs) generated by de-differentiation of adult somatic cells offer potential solutions for the ethical issues surrounding human embryonic stem cells (hESCs), as well as their immunologic rejection after cellular transplantation. However, although hiPSCs have been described as "embryonic stem cell-like", these cells have a distinct gene expression pattern compared to hESCs, making incomplete reprogramming a potential pitfall. It is unclear to what degree the difference in tissue of origin may contribute to these gene expression differences. To answer these important questions, a careful transcriptional profiling analysis is necessary to investigate the exact reprogramming state of hiPSCs, as well as analysis of the impression, if any, of the tissue of origin on the resulting hiPSCs. In this study, we compare the gene profiles of hiPSCs derived from fetal fibroblasts, neonatal fibroblasts, adipose stem cells, and keratinocytes to their corresponding donor cells and hESCs. Our analysis elucidates the overall degree of reprogramming within each hiPSC line, as well as the "distance" between each hiPSC line and its donor cell. We further identify genes that have a similar mode of regulation in hiPSCs and their corresponding donor cells compared to hESCs, allowing us to specify core sets of donor genes that continue to be expressed in each hiPSC line. We report that residual gene expression of the donor cell type contributes significantly to the differences among hiPSCs and hESCs, and adds to the incompleteness in reprogramming. Specifically, our analysis reveals that fetal fibroblast-derived hiPSCs are closer to hESCs, followed by adipose, neonatal fibroblast, and keratinocyte-derived hiPSCs.

Ghule, P. N., et al. (2011). "Reprogramming the pluripotent cell cycle: restoration of an abbreviated G1 phase in human induced pluripotent stem (iPS) cells." J Cell Physiol **226**(5): 1149-1156.

Induced pluripotent stem (iPS) cells derived from terminally differentiated human fibroblasts are reprogrammed to possess stem cell like properties. However, the extent to which iPS cells exhibit unique properties of the human embryonic stem (hES) cell cycle remains to be established. hES cells are characterized by an abbreviated G1 phase (approximately 2.5 h) and accelerated organization of subnuclear domains that mediate the assembly of regulatory machinery for histone gene expression [i.e., histone locus bodies (HLBs)]. We therefore examined cell cycle parameters of iPS cells in comparison to hES cells. Analysis of DNA synthesis [5-bromo-2'-deoxy-uridine (BrdU) incorporation], cell cycle distribution (FACS analysis and Ki67 staining) and subnuclear organization of HLBs [immunofluorescence microscopy and fluorescence in situ hybridization (FISH)] revealed that human iPS cells have a short G1 phase (approximately 2.5 h) and an abbreviated cell cycle (16-18 h). Furthermore, HLBs are formed and reorganized rapidly after mitosis (within 1.5-2 h). Thus, reprogrammed iPS cells have cell cycle kinetics and dynamic subnuclear organization of regulatory machinery that are principal properties of pluripotent hES cells. Our findings support the concept that the abbreviated cell cycle of hES and iPS cells is functionally linked to pluripotency.

Gill, K. P., et al. (2014). "Methods of Retinal Ganglion Cell Differentiation From Pluripotent Stem Cells." Transl Vis Sci Technol **3**(4): 7.

Glaucoma, the worldwide leading cause of irreversible blindness, is characterized by progressive degeneration of the optic nerve and loss of retinal ganglion cells. Research into glaucoma pathogenesis has been hampered by difficulties in isolating and culturing retinal ganglion cells in vitro. However, recent improvements in laboratory techniques have enabled the generation of a variety of mature cell types from pluripotent stem cells, including retinal ganglion cells. Indeed, stem cell-based approaches have the potential to revolutionize the field by providing an unlimited source of cells for replacement therapies and by enabling development of in vitro disease models for drug screening and research. Consequently, research aimed at directing pluripotent stem cells to differentiate into retinal ganglion cells has expanded dramatically during the past decade, resulting in significant advances in technique and efficiency. In this paper, we review the methodology for retinal ganglion cell differentiation from pluripotent stem cells of both mouse and human origin and summarize how these techniques have opened up new avenues for modelling glaucoma. Generation of stem cell-derived retinal ganglion cells will have significant translational values, providing an in vitro platform to study the mechanisms responsible for pathogenesis and for drug screening to improve treatment options, as well as for the development of cell therapies for optic neuropathies such as glaucoma.

Giordano, S., et al. (2017). "Induced Pluripotent Stem Cell-Derived Endothelial Cells Overexpressing Interleukin-8 Receptors A/B and/or C-C Chemokine Receptors 2/5 Inhibit Vascular Injury Response." Stem Cells Transl Med **6**(4): 1168-1177.

Recruitment of neutrophils and monocytes/macrophages to the site of vascular injury is mediated by binding of chemoattractants to interleukin (IL) 8 receptors RA and RB (IL8RA/B) C-C chemokine receptors (CCR) 2 and 5 expressed on neutrophil and monocyte/macrophage membranes. Endothelial cells (ECs) derived from rat-induced pluripotent stem cells (RiPS) were transduced with adenovirus containing cDNA of IL8RA/B and/or CCR2/5. We hypothesized that RiPS-ECs overexpressing IL8RA/B (RiPS-IL8RA/B-ECs), CCR2/5 (RiPS-CCR2/5-ECs), or both receptors (RiPS-IL8RA/B+CCR2/5-ECs) will inhibit inflammatory responses and neointima formation in balloon-injured rat carotid artery. Twelve-week-old male Sprague-Dawley rats underwent balloon injury of the right carotid artery and intravenous infusion of (a) saline vehicle, (b) control RiPS-Null-ECs (ECs transduced with empty virus), (c) RiPS-IL8RA/B-ECs, (d) RiPS-CCR2/5-ECs, or (e) RiPS-IL8RA/B+CCR2/5-ECs. Inflammatory mediator expression and leukocyte infiltration were measured in injured and uninjured arteries at 24 hours postinjury by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry, respectively. Neointima formation was assessed at 14 days postinjury. RiPS-ECs expressing the IL8RA/B or CCR2/5 homing device targeted the injured arteries and decreased injury-induced inflammatory cytokine expression, neutrophil/macrophage infiltration, and neointima formation. Transfused RiPS-ECs overexpressing IL8RA/B and/or CCR2/5 prevented inflammatory responses and neointima formation after vascular injury. Targeted delivery of iPS-ECs with a homing device to inflammatory mediators in injured arteries provides a novel strategy for the treatment of cardiovascular diseases. Stem Cells Translational Medicine 2017;6:1168-1177.

Giuliani, M., et al. (2011). "Human mesenchymal stem cells derived from induced pluripotent stem cells

down-regulate NK-cell cytolytic machinery." Blood **118**(12): 3254-3262.

A major issue in immunosuppressive biotherapy is the use of mesenchymal stem cells (MSCs) that harbor regulatory capacity. However, currently used bone marrow-derived MSCs (BM-MSCs) are short-lived and cannot assure long lasting immunoregulatory function both in vitro and in vivo. Consequently, we have generated MSCs from human induced pluripotent stem (IPS-MSCs) cells that share similar properties with embryonic stem cells (ES-MSCs). Herein, we compared the immunoregulatory properties of ES/IPS-MSCs with those of BM-MSCs and showed, for the first time, that IPS-derived MSCs display remarkable inhibition of NK-cell proliferation and cytolytic function in a similar way to ES-MSCs. Both MSCs disrupt NK-cell cytolytic machinery in the same fashion that BM-MSCs, by down-regulating the expression of different activation markers and ERK1/2 signaling, leading to an impairment to form immunologic synapses with target cells and, therefore, secretion of cytotoxic granules. In addition, they are more resistant than adult BM-MSCs to preactivated NK cells. IPS-MSCs could represent an attractive alternative source of immunoregulatory cells, and their capacity to impair NK-cell cytotoxicity constitutes a complex mechanism to prevent allograft rejection.

Glicksman, M. A. (2018). "Induced Pluripotent Stem Cells: The Most Versatile Source for Stem Cell Therapy." Clin Ther **40**(7): 1060-1065.

Cell therapy has existed since the first bone marrow transplant in the 1950s involving identical twins. The blood-forming stem cells were used to restore healthy blood cells for the twin with leukemia. It was not until 1968 that genetic matching (known as human leukocyte antigen matching) was known to be important, and not until 1973 that bone marrow transplants were performed from non-twin-related and nonrelated donors. The most important application of human stem cells is for the generation of cells and tissues for cell-based therapies. Currently, donated organs and tissues are often the only option to replace diseased, injured, or destroyed tissue. The availability for these transplantable tissues and organs is very limited, however. To satisfy the demand for a source for these cells and tissues, induced pluripotent stem cells that have been differentiated into specific cell types can serve as a renewable source of replacement cells and tissues. A bank of suitable human leukocyte antigen-matched cells will be an important source providing immediate availability of cells that are readily scalable, economical, and well characterized. Areas of active pursuit with stem cell therapy is being investigated for treating diseases such as macular degeneration, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, rheumatoid arthritis, and neurodegenerative diseases. This article describes the advantages and hurdles for the use of induced pluripotent cells as the starting material for a source of replacement cells for regenerative medicine.

Gnedeva, K., et al. (2015). "Derivation of hair-inducing cell from human pluripotent stem cells." PLoS One **10**(1): e0116892.

Dermal Papillae (DP) is a unique population of mesenchymal cells that was shown to regulate hair follicle formation and growth cycle. During development most DP cells are derived from mesoderm, however, functionally equivalent DP cells of cephalic hairs originate from Neural Crest (NC). Here we directed human embryonic stem cells (hESCs) to generate first NC cells and then hair-inducing DP-like cells in culture. We showed that hESC-derived DP-like cells (hESC-DPs) express markers typically found in adult human DP cells (e.g., p-75, nestin, versican, SMA, alkaline phosphatase) and are able to induce hair follicle formation when transplanted under the skin of immunodeficient NUDE mice. Engineered to express GFP, hESC-derived DP-like cells incorporate into DP of newly formed hair follicles and express appropriate markers. We demonstrated that BMP signaling is critical for hESC-DP derivation since BMP inhibitor dorsomorphin completely eliminated hair-inducing activity from hESC-DP cultures. DP cells were proposed as the cell-based treatment for hair loss diseases. Unfortunately human DP cells are not suitable for this purpose because they cannot be obtained in necessary amounts and rapidly loose their ability to induce hair follicle formation when cultured. In this context derivation of functional hESC-DP cells capable of inducing a robust hair growth for the first time shown here can become an important finding for the biomedical science.

Godfrey, K. J., et al. (2012). "Stem cell-based treatments for Type 1 diabetes mellitus: bone marrow, embryonic, hepatic, pancreatic and induced pluripotent stem cells." Diabet Med **29**(1): 14-23.

Type 1 diabetes mellitus--characterized by the permanent destruction of insulin-secreting beta-cells--is responsive to cell-based treatments that replace lost beta-cell populations. The current gold standard of pancreas transplantation provides only temporary independence from exogenous insulin and is fraught with complications, including increased mortality. Stem cells offer a number of theoretical advantages over current therapies. Our review will focus on the development of treatments involving tissue stem cells from bone marrow, liver and pancreatic cells, as well as the potential use of embryonic and induced pluripotent stem cells for Type 1 diabetes therapy.

While the body of research involving stem cells is at once promising and inconsistent, bone marrow-derived mesenchymal stem cell transplantation seems to offer the most compelling evidence of efficacy. These cells have been demonstrated to increase endogenous insulin production, while partially mitigating the autoimmune destruction of newly formed beta-cells. However, recently successful experiments involving induced pluripotent stem cells could quickly move them into the foreground of therapeutic research. We address the limitations encountered by present research and look toward the future of stem cell treatments for Type 1 diabetes.

Goldman, O. and V. Gouon-Evans (2016). "Human Pluripotent Stem Cells: Myths and Future Realities for Liver Cell Therapy." Cell Stem Cell **18**(6): 703-706.

The severe shortage of organ donors for treating patients with liver disease has prompted in vitro efforts to produce the main functional cells of the liver: hepatocyte-like cells (Hep cells). We consider the key challenges posed by various stem cell technologies and liver pathologies for developing clinically useful Hep cells.

Goldschneider, I., et al. (1980). "Analysis of rat hemopoietic cells on the fluorescence-activated cell sorter. I. Isolation of pluripotent hemopoietic stem cells and granulocyte-macrophage progenitor cells." J Exp Med **152**(2): 419-437.

A scheme is presented whereby pluripotent hemopoietic stem cells (PHSC) from rat bone marrow can be enriched 320-fold with the aid of the fluorescence- activated cell sorter. This scheme is based on the observations that PHSC are strongly positive for Thy-1 antigen (upper 10th percentile); have light- scattering properties (size distribution) between those of bone marrow lymphocytes and myeloid progenitor cells; and are relatively resistant to cortisone. It is estimated that PHSC may constitute 80 percent of the cells isolated according to these parameters. Candidate PHSC are described at the light and electron microscopic levels. At least two populations of accessory cells appear to influence the number and/or the nature of the hemopoietic colonies that form in the in vivo spleen colony-forming unit assay. Putative amplifier cells are strongly Thy-1(+) and cortisone sensitive; putative suppressor cells are weakly Thy-1(+) and cortisone resistant. Three subsets of granulocyte (G) -macrophage (M) progenitor cells (in vitro colony-forming cells [CFC]) are identified on the basis of relative fluorescence intensity for Thy-1 antigen: G-CFC are strongly Thy-l(+); M-CFC are weakly Thy-l(+); and cells that produce mixed G and M CFC have intermediate levels of Thy-1. GM-cluster-forming cells and mature G and M are Thy-1(-). The results suggest that G-CFC are bipotential cells that give rise to G and M-CFC; and that the latter produce mature M through a cluster- forming cell intermediate. Thy-1 antigen is also demonstrated on members of the eosinophil, megakaryocyte, erythrocyte, and lymphocyte cell series in rat bone marrow. In each instance, the relative concentration of Thy-1 antigen is inversely related to the state of cellular differentiation.

Goldstein, L. S. (2012). "New frontiers in human cell biology and medicine: can pluripotent stem cells deliver?" J Cell Biol **199**(4): 577-581.

Human pluripotent stem cells provide enormous opportunities to treat disease using cell therapy. But human stem cells can also drive biomedical and cell biological discoveries in a human model system, which can be directly linked to understanding disease or developing new therapies. Finally, rigorous scientific studies of these cells can and should inform the many science and medical policy issues that confront the translation of these technologies to medicine. In this paper, I discuss these issues using amyotrophic lateral sclerosis as an example.

Gomez-Cabello, D., et al. (2017). "CtIP-Specific Roles during Cell Reprogramming Have Long-Term Consequences in the Survival and Fitness of Induced Pluripotent Stem Cells." Stem Cell Reports **8**(2): 432-445.

Acquired genomic instability is one of the major concerns for the clinical use of induced pluripotent stem cells (iPSCs). All reprogramming methods are accompanied by the induction of DNA damage, of which double-strand breaks are the most cytotoxic and mutagenic. Consequently, DNA repair genes seem to be relevant for accurate reprogramming to minimize the impact of such DNA damage. Here, we reveal that reprogramming is associated with high levels of DNA end resection, a critical step in homologous recombination. Moreover, the resection factor CtIP is essential for cell reprogramming and establishment of iPSCs, probably to repair reprogramming-induced DNA damage. Our data reveal a new role for DNA end resection in maintaining genomic stability during cell reprogramming, allowing DNA repair fidelity to be retained in both human and mouse iPSCs. Moreover, we demonstrate that reprogramming in a resection-defective environment has long-term consequences on stem cell self-renewal and differentiation.

Gomez-Lechon, M. J. and L. Tolosa (2016). "Human hepatocytes derived from pluripotent stem cells: a promising cell model for drug hepatotoxicity screening." Arch Toxicol **90**(9): 2049-2061.

Drug-induced liver injury (DILI) is a frequent cause of failure in both clinical and post-approval stages of drug development, and poses a key challenge to the pharmaceutical industry. Current animal models offer poor prediction of human DILI. Although several human cell-based models have been proposed for the detection of human DILI, human primary hepatocytes remain the gold standard for preclinical toxicological screening. However, their use is hindered by their limited availability, variability and phenotypic instability. In contrast, pluripotent stem cells, which include embryonic and induced pluripotent stem cells (iPSCs), proliferate extensively in vitro and can be differentiated into hepatocytes by the addition of soluble factors. This provides a stable source of hepatocytes for multiple applications, including early preclinical hepatotoxicity screening. In addition, iPSCs also have the potential to establish genotype-specific cells from different individuals, which would increase the predictivity of toxicity assays allowing more successful clinical trials. Therefore, the generation of human hepatocyte-like cells derived from pluripotent stem cells seems to be promising for overcoming limitations of hepatocyte preparations, and it is expected to have a substantial repercussion in preclinical hepatotoxicity risk assessment in early drug development stages.

Gong, J., et al. (2015). "Differentiation of Human Protein-Induced Pluripotent Stem Cells toward a Retinal Pigment Epithelial Cell Fate." PLoS One **10**(11): e0143272.

Compared with many induced pluripotent stem cell (iPSC) lines generated using retrovirus and other non-integrating methods, the utilization of human protein-induced iPSC (piPSC) lines may provide a safer alternative for the generation of retinal pigment epithelial (RPE) cells for transplantation in retinal degenerative diseases. Here we assess the ability of piPSCs to differentiate into RPE cells, and to perform native RPE cell behavior. piPSCs were seeded in 6-well low-attachment plates to allow embryoid body formation, and then analyzed for pluripotent stem cell markers NANOG, SSEA4 and TRA-1-60 by immunofluorescence. Following colony formation, piPSCs were assessed for confirmation of RPE cell differentiation by staining for zonula occludens (ZO-1), bestrophin, microphthalmia-associated transcription factor (MITF) and retinal pigment epithelium specific protein-65 (RPE65). To evaluate piPSC-RPE cell phagocytic ability, adult bovine photoreceptor rod outer segments (ROS) were fed to piPSC-RPE cells, which were analyzed by fluorescent microscopy and flow cytometry. Undifferentiated piPSCs expressed all pluripotent markers assessed and formed embryoid body aggregates after 7 days. Differentiated piPSC-RPE cells expressed ZO-1, bestrophin, MITF and RPE65, typical RPE cell markers. Flow cytometry revealed robust ingestion of fluorescently-labeled ROS by piPSC-RPE cells, which was over four-times greater than that of undifferentiated piPSCs and comparable to that of an immortalized RPE cell line. Phagocytosis activity by piPSC-RPE cells was significantly reduced after the addition of anti-integrin alphaVbeta5. In conclusion, piPSCs can be differentiated toward an RPE cell fate, expressing RPE cell markers and resembling native RPE cells in behavior. These results demonstrate that piPSCs can be differentiated into RPE-like cells using a method that has an increased safety profile, a critical consideration for the development of better treatments for retinal degenerative diseases such as age-related macular degeneration (AMD).

Gong, Y., et al. (2015). "[Establishment of induced pluripotent stem cell lines from human amniotic fluid cells with 1q21.1 microdeletion]." Zhonghua Yi Xue Yi Chuan Xue Za Zhi **32**(2): 175-179.

OBJECTIVE: To reprogram the 1q21.1 microdeletion pluripotent stem cells in order to establish an ideal model for further studying its pathogenesis. METHODS: Human amniotic fluid-derived cells induced pluripotent stem cells (hAF-iPSCs) were induced from amniotic fluid cells harboring the 1q21.1 microdeletion by retroviral vectors encoding Oct4, Sox2, c-Myc and Klf4. Characteristics of the 1q21.1 microdeletion hAF-iPSCs were determined, which included in vitro pluripotency, karyotype, microarray analysis, the capacity of differentiation in vivo and in vitro, etc. RESULTS: hAF-iPSCs derived from amniotic fluid cells harboring the 1q21.1 microdeletion have maintained self renewal, with expression of pluripotency marker genes detectable at mRNA level. Stem cell surface antigens were tested by immunocytochemistry. The 1q21.1 microdeletion hAF-iPSCs showed a normal karyotype after long-term culturing in vitro, and harbored the same microdeletion as confirmed by microarray analysis. The cells have maintained their differentiation capacity both in vivo and in vitro. CONCLUSION: The hAF-iPSCs harboring the 1q21.1 microdeletion have all the characteristics of normal pluripotent stem cells, and can be used for directed differentiation into specific cells, which may provide an ideal model for studying the pathogenesis of 1q21.1 microdeletion in vitro.

Gonzales, K. A. and H. Liang (2015). "Transcriptomic profiling of human embryonic stem cells upon cell cycle manipulation during pluripotent state dissolution." Genom Data **6**: 118-119.

While distinct cell cycle structures have been known to correlate with pluripotent or differentiated cell states [1], there is no evidence on how the cell cycle machinery directly contributes to human embryonic stem cell (hESC) pluripotency. We established a determinant role of cell cycle machineries on the pluripotent state by demonstrating that the specific perturbation of the S and G2 phases can prevent pluripotent state dissolution (PSD) [2]. Active mechanisms in these phases, such as the DNA damage checkpoint and Cyclin B1, promote the pluripotent state [2]. To understand the mechanisms behind the effect on PSD by these pathways in hESCs, we performed comprehensive gene expression analysis by time-course microarray experiments. From these datasets, we observed expression changes in genes involved in the TGFbeta signaling pathway, which has a well-established role in hESC maintenance [3], [4], [5]. The microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO) and can be accessed through GEO Series accession numbers GSE62062 and GSE63215.

Gonzalez-Cordero, A., et al. (2018). "Assessment of AAV Vector Tropisms for Mouse and Human Pluripotent Stem Cell-Derived RPE and Photoreceptor Cells." Hum Gene Ther **29**(10): 1124-1139.

Adeno-associated viral vectors are showing great promise as gene therapy vectors for a wide range of retinal disorders. To date, evaluation of therapeutic approaches has depended almost exclusively on the use of animal models. With recent advances in human stem cell technology, stem cell-derived retina now offers the possibility to assess efficacy in human organoids in vitro. Here we test six adeno-associated virus (AAV) serotypes [AAV2/2, AAV2/9, AAV2/8, AAV2/8T(Y733F), AAV2/5, and ShH10] to determine their efficiency in transducing mouse and human pluripotent stem cell-derived retinal pigment epithelium (RPE) and photoreceptor cells in vitro. All the serotypes tested were capable of transducing RPE and photoreceptor cells in vitro. AAV ShH10 and AAV2/5 are the most efficient vectors at transducing both mouse and human RPE, while AAV2/8 and ShH10 achieved similarly robust transduction of human embryonic stem cell-derived cone photoreceptors. Furthermore, we show that human embryonic stem cell-derived photoreceptors can be used to establish promoter specificity in human cells in vitro. The results of this study will aid capsid selection and vector design for preclinical evaluation of gene therapy approaches, such as gene editing, that require the use of human cells and tissues.

Gordeeva, O., et al. (2005). "Differentiation of embryonic stem cells after transplantation into peritoneal cavity of irradiated mice and expression of specific germ cell genes in pluripotent cells." Transplant Proc **37**(1): 295-298.

Permanent embryonic stem cell lines (ES cells) are considered as one of the most promising cellular sources for regenerative medicine. ES cells have a high proliferative potency and ability to differentiate into all kinds of somatic and germ cells. However, transplantation of undifferentiated ES cells into adult recipient tissue results in the formation of teratomas. To understand the mechanisms underlying self-renewal and determination of pluripotent cells, we investigated differentiation potencies of undifferentiated ES cells and differentiating embryoid bodies (EB). ES cells and EBs growing on acetate-cellulose membranes were transplanted into the peritoneal cavity of irradiated mice. Behavior and differentiation of transplanted cells were studied within 1, 2, 3, and 6 weeks after transplantation. No differences in the cell composition were found in the teratomas formed by ES cells and differentiating EBs. The pattern of expression of the genes specific for pluripotent and germ cells was studied in all types of experimental teratomas. The expression of oct4, stella, fragilis was detected in the teratomas, but nanog was not expressed. We conclude that pluripotent cells are retained in the experimental teratomas formed after transplantation of ES cells and EBs but the pattern of expression of the studied genes underwent changes.

Gordeeva, O. F., et al. (2006). "Analysis of expression of genes specific for pluripotent and primordial germ cells in human and mouse embryonic stem cell lines." Dokl Biol Sci **406**: 115-118.

Gordeeva, O. F. and M. Mitalipov Sh (2008). "[Pluripotent stem cells: maintenance of genetic and epigenetic stability and prospects of cell technologies]." Ontogenez **39**(6): 405-419.

Permanent lines of pluripotent stem cells can be obtained from humans and monkeys using different techniques and from different sources--inner cell mass of the blastocyst, primary germ cells, parthenogenetic oocytes, and mature spermatogonia--as well as by transgenic modification of various adult somatic cells. Despite different origin, all pluripotent lines demonstrate considerable similarity of the major biological properties: active self-renewal and differentiation into various somatic and germ cells in vitro and in vivo, similar gene expression profiles, and similar cell cycle structure. Ten years of intense studies on the stability of different human and monkey embryonic stem cells demonstrated that, irrespective of their origin, long-term in vitro cultures lead to the accumulation of chromosomal and gene mutations as well as epigenetic changes that can cause oncogenic

transformation of cells. This review summarizes the research data on the genetic and epigenetic stability of different lines of pluripotent stem cells after long-term in vitro culture. These data were used to analyze possible factors of the genome and epigenome instability in pluripotent lines. The prospects of using pluripotent stem cells of different origin in cell therapy and pharmacological studies were considered.

Gorris, R., et al. (2015). "Pluripotent stem cell-derived radial glia-like cells as stable intermediate for efficient generation of human oligodendrocytes." Glia **63**(12): 2152-2167.

Neural precursor cells (NPCs) derived from human pluripotent stem cells (hPSCs) represent an attractive tool for the in vitro generation of various neural cell types. However, the developmentally early NPCs emerging during hPSC differentiation typically show a strong propensity for neuronal differentiation, with more limited potential for generating astrocytes and, in particular, for generating oligodendrocytes. This phenomenon corresponds well to the consecutive and protracted generation of neurons and GLIA during normal human development. To obtain a more gliogenic NPC type, we combined growth factor-mediated expansion with pre-exposure to the differentiation-inducing agent retinoic acid and subsequent immunoisolation of CD133-positive cells. This protocol yields an adherent and self-renewing population of hindbrain/spinal cord radial glia (RG)-like neural precursor cells (RGL-NPCs) expressing typical neural stem cell markers such as nestin, ASCL1, SOX2, and PAX6 as well as RG markers BLBP, GLAST, vimentin, and GFAP. While RGL-NPCs maintain the ability for tripotential differentiation into neurons, astrocytes, and oligodendrocytes, they exhibit greatly enhanced propensity for oligodendrocyte generation. Under defined differentiation conditions promoting the expression of the major oligodendrocyte fate-determinants OLIG1/2, NKX6.2, NKX2.2, and SOX10, RGL-NPCs efficiently convert into NG2-positive oligodendroglial progenitor cells (OPCs) and are subsequently capable of in vivo myelination. Representing a stable intermediate between PSCs and OPCs, RGL-NPCs expedite the generation of PSC-derived oligodendrocytes with O4-, 4860-, and myelin basic protein (MBP)-positive cells that already appear within 7 weeks following growth factor withdrawal-induced differentiation. Thus, RGL-NPCs may serve as robust tool for time-efficient generation of human oligodendrocytes from embryonic and induced pluripotent stem cells.

Gouadon, E., et al. (2016). "Concise Review: Pluripotent Stem Cell-Derived Cardiac Cells, A Promising Cell Source for Therapy of Heart Failure: Where Do We Stand?" Stem Cells **34**(1): 34-43.

Heart failure is still a major cause of hospitalization and mortality in developed countries. Many clinical trials have tested the use of multipotent stem cells as a cardiac regenerative medicine. The benefit for the patients of this therapeutic intervention has remained limited. Herein, we review the pluripotent stem cells as a cell source for cardiac regeneration. We more specifically address the various challenges of this cell therapy approach. We question the cell delivery systems, the immune tolerance of allogenic cells, the potential proarrhythmic effects, various drug mediated interventions to facilitate cell grafting and, finally, we describe the pathological conditions that may benefit from such an innovative approach. As members of a transatlantic consortium of excellence of basic science researchers and clinicians, we propose some guidelines to be applied to cell types and modes of delivery in order to translate pluripotent stem cell cardiac derivatives into safe and effective clinical trials.

Goyal, A., et al. (2013). "Generation of human induced pluripotent stem cells using epigenetic regulators reveals a germ cell-like identity in partially reprogrammed colonies." PLoS One **8**(12): e82838.

Previous studies have shown that induced pluripotent stem cells (iPSCs) can be derived from fibroblasts by ectopic expression of four transcription factors, OCT4, SOX2, KLF4 and c-MYC using various methods. More recent studies have focused on identifying alternative approaches and factors that can be used to increase reprogramming efficiency of fibroblasts to pluripotency. Here, we use nucleofection, morpholino technologies and novel epigenetic factors, which were chosen based on their expression profile in human embryos, fibroblasts and undifferentiated/differentiated human embryonic stem cells (hESCs) and conventionally generated iPSCs, to reprogram human fibroblasts into iPSCs. By over expressing DNMT3B, AURKB, PRMT5 and/or silencing SETD7 in human fibroblasts with and without NANOG, hTERT and/or SV40 overexpression, we observed the formation of colonies resembling iPSCs that were positive for certain pluripotency markers, but exhibited minimal proliferation. More importantly, we also demonstrate that these partially-reprogrammed colonies express high levels of early to mid germ cell-specific genes regardless of the transfection approach, which suggests conversion to a germ cell-like identity is associated with early reprogramming. These findings may provide an additional means to evaluate human germ cell differentiation in vitro, particularly in the context of pluripotent stem cell-derived germ cell development,

and contribute to our understanding of the epigenetic requirements of the reprogramming process.

Graffmann, N., et al. (2016). "Modeling Nonalcoholic Fatty Liver Disease with Human Pluripotent Stem Cell-Derived Immature Hepatocyte-Like Cells Reveals Activation of PLIN2 and Confirms Regulatory Functions of Peroxisome Proliferator-Activated Receptor Alpha." Stem Cells Dev **25**(15): 1119-1133.

Nonalcoholic fatty liver disease (NAFLD/steatosis) is a metabolic disease characterized by the incorporation of fat into hepatocytes. In this study, we developed an in vitro model for NAFLD based on hepatocyte-like cells (HLCs) differentiated from human pluripotent stem cells. We induced fat storage in these HLCs and detected major expression changes of metabolism-associated genes, as well as an overall reduction of liver-related microRNAs. We observed an upregulation of the lipid droplet coating protein Perilipin 2 (PLIN2), as well as of numerous genes of the peroxisome proliferator-activated receptor (PPAR) pathway, which constitutes a regulatory hub for metabolic processes. Interference with PLIN2 and PPARalpha resulted in major alterations in gene expression, especially affecting lipid, glucose, and purine metabolism. Our model recapitulates many metabolic changes that are characteristic for NAFLD. It permits the dissection of disease-promoting molecular pathways and allows us to investigate the influences of distinct genetic backgrounds on disease progression.

Greber, B. and H. Scholer (2008). "[A breakthrough in stem cell research? Reprogramming somatic cells into pluripotent stem cells]." Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz **51**(9): 1005-1013.

Embryonic stem (ES) cells are capable of generating all cell types and tissues of the body. As such they represent an attractive source for therapeutic approaches. However, transplanted cells may be rejected by the immune system. One way to address this problem is to generate patient-specific ES cells. This, however, requires the transformation of the genetic program of somatic cells back to that of an early embryonic state. The field of stem cell research and reprogramming is rapidly evolving. This article aims at providing background information to understand some of the most exciting recent developments. Subsequently, the different existing strategies of converting somatic cells into ES-like cells are reviewed and evaluated.

Greenhough, S., et al. (2010). "Pluripotent stem cell derived hepatocyte like cells and their potential in toxicity screening." Toxicology **278**(3): 250-255.

Despite considerable progress in modelling human liver toxicity, the requirement still exists for efficient, predictive and cost effective in vitro models to reduce attrition during drug development. Thousands of compounds fail in this process, with hepatotoxicity being one of the significant causes of failure. The cost of clinical studies is substantial, therefore it is essential that toxicological screening is performed early on in the drug development process. Human hepatocytes represent the gold standard model for evaluating drug toxicity, but are a limited resource. Current alternative models are based on immortalised cell lines and animal tissue, but these are limited by poor function, exhibit species variability and show instability in culture. Pluripotent stem cells are an attractive alternative as they are capable of self-renewal and differentiation to all three germ layers, and thereby represent a potentially inexhaustible source of somatic cells. The differentiation of human embryonic stem cells and induced pluripotent stem cells to functional hepatocyte like cells has recently been reported. Further development of this technology could lead to the scalable production of hepatocyte like cells for liver toxicity screening and clinical therapies. Additionally, induced pluripotent stem cell derived hepatocyte like cells may permit in vitro modelling of gene polymorphisms and genetic diseases.

Gregory, S., et al. (2016). "Autophagic response to cell culture stress in pluripotent stem cells." Biochem Biophys Res Commun **473**(3): 758-763.

Autophagy is an important conserved cellular process, both constitutively as a recycling pathway for long lived proteins and as an upregulated stress response. Recent findings suggest a fundamental role for autophagic processes in the maintenance of pluripotent stem cell function. In human embryonic stem cells (hESCS), autophagy was investigated by transfection of LC3-GFP to visualize autophagosomes and with an antibody to LC3B protein. The presence of the primary cilium (PC) in hESCs as the site of recruitment of autophagy-related proteins was also assessed. HESCs (mShef11) in vitro displayed basal autophagy which was upregulated in response to deprivation of culture medium replacement. Significantly higher levels of autophagy were exhibited on spontaneous differentiation of hESCs in vitro. The PC was confirmed to be present in hESCs and therefore may serve to coordinate autophagy function.

Griscelli, F., et al. (2012). "Malignant germ cell-like tumors, expressing Ki-1 antigen (CD30), are revealed during in vivo differentiation of partially reprogrammed human-induced pluripotent stem cells." Am J Pathol **180**(5): 2084-2096.

Because many of the genes used to produce induced pluripotent stem cells (iPSCs) from somatic cells are either outright established oncogenes, such as c-myc and Klf4, or potentially related to tumorigenesis in various cancers, both the safety and the risks of tumorigenesis linked to iPSC generation require evaluation. In this work, we generated, by lentivirus-mediated gene transfer of Oct4, Sox2, Nanog, and Lin28, two types of iPSCs from human mesenchymal stem cells and human amniotic fluid-derived cells: fully reprogrammed iPSCs with silencing of the four transgenes and partially reprogrammed iPSCs that still express one or several transgenes. We assessed the behavior of these cells during both their differentiation and proliferation using in vivo teratoma assays in nonobese diabetic mice with severe combined immunodeficiency. In contrast to fully reprogrammed iPSCs, 43% of partially reprogrammed iPSC cases (6 of 14 teratomas) generated major dysplasia and malignant tumors, with yolk sac tumors and embryonal carcinomas positive for alpha-fetoprotein, cytokeratin AE1/AE3, and CD30. This correlated with the expression of one or several transgenes used for the reprogramming, down-regulation of CDK 1A mRNA (p21/CDKN1A), and up-regulation of antiapoptotic Bcl-2 mRNA. Therefore, the oncogenicity of therapeutically valuable patient-specific iPSC-derived cells should be scrupulously evaluated before they are used for any clinical applications.

Gu, M., et al. (2015). "Pravastatin reverses obesity-induced dysfunction of induced pluripotent stem cell-derived endothelial cells via a nitric oxide-dependent mechanism." Eur Heart J **36**(13): 806-816.

AIMS: High-fat diet-induced obesity (DIO) is a major contributor to type II diabetes and micro- and macro-vascular complications leading to peripheral vascular disease (PVD). Metabolic abnormalities of induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) from obese individuals could potentially limit their therapeutic efficacy for PVD. The aim of this study was to compare the function of iPSC-ECs from normal and DIO mice using comprehensive in vitro and in vivo assays. METHODS AND RESULTS: Six-week-old C57Bl/6 mice were fed with a normal or high-fat diet. At 24 weeks, iPSCs were generated from tail tip fibroblasts and differentiated into iPSC-ECs using a directed monolayer approach. In vitro functional analysis revealed that iPSC-ECs from DIO mice had significantly decreased capacity to form capillary-like networks, diminished migration, and lower proliferation. Microarray and ELISA confirmed elevated apoptotic, inflammatory, and oxidative stress pathways in DIO iPSC-ECs. Following hindlimb ischaemia, mice receiving intramuscular injections of DIO iPSC-ECs had significantly decreased reperfusion compared with mice injected with control healthy iPSC-ECs. Hindlimb sections revealed increased muscle atrophy and presence of inflammatory cells in mice receiving DIO iPSC-ECs. When pravastatin was co-administered to mice receiving DIO iPSC-ECs, a significant increase in reperfusion was observed; however, this beneficial effect was blunted by co-administration of the nitric oxide synthase inhibitor, N(omega)-nitro-l-arginine methyl ester. CONCLUSION: This is the first study to provide evidence that iPSC-ECs from DIO mice exhibit signs of endothelial dysfunction and have suboptimal efficacy following transplantation in a hindlimb ischaemia model. These findings may have important implications for future treatment of PVD using iPSC-ECs in the obese population.

Gu, M., et al. (2012). "Microfluidic single-cell analysis shows that porcine induced pluripotent stem cell-derived endothelial cells improve myocardial function by paracrine activation." Circ Res **111**(7): 882-893.

RATIONALE: Induced pluripotent stem cells (iPSCs) hold great promise for the development of patient-specific therapies for cardiovascular disease. However, clinical translation will require preclinical optimization and validation of large-animal iPSC models. OBJECTIVE: To successfully derive endothelial cells from porcine iPSCs and demonstrate their potential utility for the treatment of myocardial ischemia. METHODS AND RESULTS: Porcine adipose stromal cells were reprogrammed to generate porcine iPSCs (piPSCs). Immunohistochemistry, quantitative PCR, microarray hybridization, and angiogenic assays confirmed that piPSC-derived endothelial cells (piPSC-ECs) shared similar morphological and functional properties as endothelial cells isolated from the autologous pig aorta. To demonstrate their therapeutic potential, piPSC-ECs were transplanted into mice with myocardial infarction. Compared with control, animals transplanted with piPSC-ECs showed significant functional improvement measured by echocardiography (fractional shortening at week 4: 27.2+/-1.3% versus 22.3+/-1.1%; P<0.001) and MRI (ejection fraction at week 4: 45.8+/-1.3% versus 42.3+/-0.9%; P<0.05). Quantitative protein assays and microfluidic single-cell PCR profiling showed that piPSC-ECs released proangiogenic and antiapoptotic factors in the ischemic microenvironment, which promoted neovascularization and cardiomyocyte survival, respectively. Release of paracrine factors varied significantly among subpopulations of transplanted cells, suggesting that transplantation of specific cell populations may result in greater functional recovery. CONCLUSIONS: In summary, this is the first study to successfully differentiate piPSCs-ECs from piPSCs and demonstrate that

transplantation of piPSC-ECs improved cardiac function after myocardial infarction via paracrine activation. Further development of these large animal iPSC models will yield significant insights into their therapeutic potential and accelerate the clinical translation of autologous iPSC-based therapy.

Guadix, J. A., et al. (2017). "Human Pluripotent Stem Cell Differentiation into Functional Epicardial Progenitor Cells." Stem Cell Reports **9**(6): 1754-1764.

Human pluripotent stem cells (hPSCs) are widely used to study cardiovascular cell differentiation and function. Here, we induced differentiation of hPSCs (both embryonic and induced) to proepicardial/epicardial progenitor cells that cover the heart during development. Addition of retinoic acid (RA) and bone morphogenetic protein 4 (BMP4) promoted expression of the mesodermal marker PDGFRalpha, upregulated characteristic (pro)epicardial progenitor cell genes, and downregulated transcription of myocardial genes. We confirmed the (pro)epicardial-like properties of these cells using in vitro co-culture assays and in ovo grafting of hPSC-epicardial cells into chick embryos. Our data show that RA + BMP4-treated hPSCs differentiate into (pro)epicardial-like cells displaying functional properties (adhesion and spreading over the myocardium) of their in vivo counterpart. The results extend evidence that hPSCs are an excellent model to study (pro)epicardial differentiation into cardiovascular cells in human development and evaluate their potential for cardiac regeneration.

Guan, L., et al. (2014). "[Study on the induced differentiation of induced pluripotent stem cells into cochlear hair cell-like cells and spiral ganglion neuron-like cells in vitro]." Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi **49**(8): 680-686.

OBJECTIVE: In this study, we investigated the potential of mouse induced pluripotent stem cells (iPSC) for use as a source of transplants for the restoration of auditory hair cells and spiral ganglion neurons. METHODS: We co-cultured the mouse iPSC with the cells of the cochlear organ of Corti or the modiolus in vitro. The cochlear organ of Corti (which contains cochlear hair cells) and the modiolus (which contains auditory spiral ganglion neurons) were obtained from postnatal day 3 (P3) CD-1 ICR mice. After 18 days of coculture with the cells of newborn mouse cochleae. The expressions of hair cell markers (Myosin VIIa, Math1, Calretinin, Espin) and Spiral ganglion neuron markers [Nestin, Neurofilament-M, beta-III Tubulin, Vesicular glutamate transporter 1(VGluT1)] were detected by immunocytochemical analysis. RESULTS: Immunocytochemical analysis results indicated that the differentiated iPSC expressed auditory hair cell markers (MyosinVIIa,Math1, Calretinin, Espin) and spiral ganglion markers (Nestin, Neurofilament-M,beta-III Tubulin,VGluT1). CONCLUSION: Mouse iPSC in virto cultured could successfully be induced to differentiate into hair cell-like cells and spiral ganglion-like cells with hair cell and spiral ganglion molecular markers.

Guhr, A., et al. (2018). "Recent Trends in Research with Human Pluripotent Stem Cells: Impact of Research and Use of Cell Lines in Experimental Research and Clinical Trials." Stem Cell Reports **11**(2): 485-496.

The human pluripotent stem cell (hPSC) research landscape is rapidly evolving. To assess possible novel trends in hPSC usage, we analyzed experimental hPSC research published from 2014 to 2016 and compared our data with those of earlier periods. The number of papers describing experimental work involving hPSCs increased further with clear differences in the scientific impact of publications from different countries. Our results confirm the leading position of US-based hPSC research, although to a lesser degree than observed previously. Our data reveal that research into human induced pluripotent stem cells alone surpassed human embryonic stem cell (hESC) research by 2015 and rapidly grew after that. We also report on continuing and even slightly growing research activities in the hESC field as well as on a generally declining rate of the generation of new hESC lines. An increasing portion of new hESC lines represents disease-specific and clinical-grade cell lines. The previously noted usage of only a few early established hESC lines in the vast majority of scientific work is sustained. We also provide a comprehensive overview on clinical trials on the basis of hPSCs. We find that the vast majority of those trials are based on hESC-derived cell products that were generated from an only limited number of relatively old cell lines.

Gunaratne, P. H. (2009). "Embryonic stem cell microRNAs: defining factors in induced pluripotent (iPS) and cancer (CSC) stem cells?" Curr Stem Cell Res Ther **4**(3): 168-177.

The discovery of microRNAs (miRNAs - small non-coding RNAs of approximately 22 nt) heralded a new and exciting era in biology. During this period miRNAs have gone from ignominy due to their origin mainly in 'junk DNA' to notoriety where they can be at once characterized as being all powerful (a single miRNA can target and potentially silence several hundred genes) and yet marginal (a given gene can be targeted by several miRNAs such that a given miRNA typically exerts a modest repression) [1-4]. The emerging paradox is exemplified by miRNAs that are prominently expressed in embryonic stem (ES) cells.

The collective importance of miRNAs is firmly established by the fact that Dicer-/- mouse embryos die on day 7.5 due to defects in differentiation [5]. However, oppositely correlated expression that is expected of conventional repressors is increasingly being defied in multiple systems in relation to miRNA-mRNA target pairs. This is most evident in ES cells where miR-290-295 and 302 clusters the most abundant ES cell miRNAs are found to be driven by pluripotency genes Oct4, Nanog and Sox2 and also target these genes in 'incoherent feed-forward loops' [7]. Here the miRNAs are co-expressed and positively correlated with these targets that they repress suggesting that one of their primary roles is to fine tune gene expression rather than act as ON/OFF switches. On the other hand, let-7 family members that are notably low in ES cells and rapidly induced upon differentiation exhibit more conventional anti-correlated expression patterns with their targets [7-8]. In an intricately designed auto-regulatory loop, LIN28, a key 'keeper' of the pluripotent state binds and represses the processing of let-7 (a key 'keeper' of the differentiated state) [9-11]. One of the let-7 family members, let-7g targets and represses LIN28 through four 3'-UTR binding sites [12]. We propose that LIN28/let-7 pair has the potential to act as a 'toggle switch' that balances the decision to maintain pluripotency vs. differentiation. We also propose that the c-Myc/E2F driven miR17-92 cluster that together controls the G1 to S transition is fundamental for ES self-renewal and cell proliferation [13-18]. In that context it is no surprise that LIN28 and c-Myc (and therefore let-7 and miR-17-92 by association) and more recently Oct4/Sox2 regulated miR-302 has been shown to be among a handful of factors shown to be necessary and sufficient to convert differentiated cells to induced pluripotent stem (iPS) cells [19-29]. It is also no surprise that activation of miR-17-92 (OncomiRs) and down-regulation of let-7 (tumor suppressors) is a recurring theme in relation to cancers from multiple systems [30-48]. We speculate that the LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 networks are fundamental to properties of pluripotency and self-renewal associated with embryonic stem cells. We also speculate that ES cell miRNA-mRNA associations may also regulate tissue homeostasis and regeneration in the fully developed adult. Consequently, the appropriate regulation of LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 gene networks will be critical for the success of regenerative strategies that involve iPS cells. Any perturbation in key ES cell miRNA-mRNA networks during any of the above processes maybe a hallmark of (CSCs).

Gundry, R. L., et al. (2012). "A cell surfaceome map for immunophenotyping and sorting pluripotent stem cells." Mol Cell Proteomics **11**(8): 303-316.

Induction of a pluripotent state in somatic cells through nuclear reprogramming has ushered in a new era of regenerative medicine. Heterogeneity and varied differentiation potentials among induced pluripotent stem cell (iPSC) lines are, however, complicating factors that limit their usefulness for disease modeling, drug discovery, and patient therapies. Thus, there is an urgent need to develop nonmutagenic rapid throughput methods capable of distinguishing among putative iPSC lines of variable quality. To address this issue, we have applied a highly specific chemoproteomic targeting strategy for de novo discovery of cell surface N-glycoproteins to increase the knowledge-base of surface exposed proteins and accessible epitopes of pluripotent stem cells. We report the identification of 500 cell surface proteins on four embryonic stem cell and iPSCs lines and demonstrate the biological significance of this resource on mouse fibroblasts containing an oct4-GFP expression cassette that is active in reprogrammed cells. These results together with immunophenotyping, cell sorting, and functional analyses demonstrate that these newly identified surface marker panels are useful for isolating iPSCs from heterogeneous reprogrammed cultures and for isolating functionally distinct stem cell subpopulations.

Gunewardene, N., et al. (2016). "Innervation of Cochlear Hair Cells by Human Induced Pluripotent Stem Cell-Derived Neurons In Vitro." Stem Cells Int **2016**: 1781202.

Induced pluripotent stem cells (iPSCs) may serve as an autologous source of replacement neurons in the injured cochlea, if they can be successfully differentiated and reconnected with residual elements in the damaged auditory system. Here, we explored the potential of hiPSC-derived neurons to innervate early postnatal hair cells, using established in vitro assays. We compared two hiPSC lines against a well-characterized hESC line. After ten days' coculture in vitro, hiPSC-derived neural processes contacted inner and outer hair cells in whole cochlear explant cultures. Neural processes from hiPSC-derived neurons also made contact with hair cells in denervated sensory epithelia explants and expressed synapsin at these points of contact. Interestingly, hiPSC-derived neurons cocultured with hair cells at an early stage of differentiation formed synapses with a higher number of hair cells, compared to hiPSC-derived neurons cocultured at a later stage of differentiation. Notable differences in the innervation potentials of the hiPSC-derived neurons were also observed and variations existed between the hiPSC lines in their innervation

efficiencies. Collectively, these data illustrate the promise of hiPSCs for auditory neuron replacement and highlight the need to develop methods to mitigate variabilities observed amongst hiPSC lines, in order to achieve reliable clinical improvements for patients.

Guo, G., et al. (2016). "Naive Pluripotent Stem Cells Derived Directly from Isolated Cells of the Human Inner Cell Mass." Stem Cell Reports **6**(4): 437-446.

Conventional generation of stem cells from human blastocysts produces a developmentally advanced, or primed, stage of pluripotency. In vitro resetting to a more naive phenotype has been reported. However, whether the reset culture conditions of selective kinase inhibition can enable capture of naive epiblast cells directly from the embryo has not been determined. Here, we show that in these specific conditions individual inner cell mass cells grow into colonies that may then be expanded over multiple passages while retaining a diploid karyotype and naive properties. The cells express hallmark naive pluripotency factors and additionally display features of mitochondrial respiration, global gene expression, and genome-wide hypomethylation distinct from primed cells. They transition through primed pluripotency into somatic lineage differentiation. Collectively these attributes suggest classification as human naive embryonic stem cells. Human counterparts of canonical mouse embryonic stem cells would argue for conservation in the phased progression of pluripotency in mammals.

Hackett, C. H., et al. (2012). "Comparison of gene-specific DNA methylation patterns in equine induced pluripotent stem cell lines with cells derived from equine adult and fetal tissues." Stem Cells Dev **21**(10): 1803-1811.

Cellular pluripotency is associated with expression of the homeobox transcription factor genes NANOG, SOX2, and POU5F1 (OCT3/4 protein). Some reports suggest that mesenchymal progenitor cells (MPCs) may express increased quantities of these genes, creating the possibility that MPCs are more "pluripotent" than other adult cell types. The objective of this study was to determine whether equine bone marrow-derived MPCs had gene expression or DNA methylation patterns that differed from either early fetal-derived or terminally differentiated adult cells. Specifically, this study compared DNA methylation of the NANOG and SOX2 promoter regions and concurrent gene expression of NANOG, SOX2, and POU5F1 in equine induced pluripotent stem (iPS) cells, fetal fibroblasts, fetal brain cells, adult chondrocytes, and MPCs. Results indicate that NANOG and POU5F1 were not detectable in appreciable quantities in tissues other than the equine iPS cell lines. Equine iPS cells expressed large quantities of all three genes examined. Significantly increased quantities of SOX2 were noted in iPS cells and both fetal-derived cell types compared with adult cells. MPCs and adult chondrocytes expressed equivalent, low quantities of SOX2. Further, NANOG and SOX2 expression inversely correlated with the DNA methylation pattern in the promoter region, such that as gene expression increased, DNA methylation decreased. The equine iPS cell lines examined demonstrated DNA methylation and gene expression patterns that were consistent with pluripotency features described in other species. Results do not support previous reports that NANOG, SOX2, and POU5F1 are poised for increased activity in MPCs compared with other adult cells.

Hagman, S., et al. (2018). "Effects of inflammatory cytokines IFN-gamma, TNF-alpha and IL-6 on the viability and functionality of human pluripotent stem cell-derived neural cells." J Neuroimmunol.

Multiple Sclerosis (MS) is an inflammatory neurodegenerative disease, where neural progenitor cell (NPC) transplantation has been suggested as a potential neuroprotective therapeutic strategy. Since the effect of inflammation on NPCs is poorly known, their effect on the survival and functionality of human NPCs were studied. Treatment with interleukin (IL)-6, tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma did not induced cytotoxicity, but IFN-gamma treatment showed decreased proliferation and neuronal migration. By contrast, increased proliferation and inhibition of electrical activity were detected after TNF-alpha treatment. Treatments induced secretion of inflammatory factors. Inflammatory cytokines appear to modulate proliferation as well as the cellular and functional properties of human NPCs.

Hamano, S., et al. (2018). "Extracellular Matrix from Periodontal Ligament Cells Could Induce the Differentiation of Induced Pluripotent Stem Cells to Periodontal Ligament Stem Cell-Like Cells." Stem Cells Dev **27**(2): 100-111.

The periodontal ligament (PDL) plays an important role in anchoring teeth in the bone socket. Damage to the PDL, such as after severe inflammation, can be treated with a therapeutic strategy that uses stem cells derived from PDL tissue (PDLSCs), a strategy that has received intense scrutiny over the past decade. However, there is an insufficient number of PDLSCs within the PDL for treating such damage. Therefore, we sought to induce the differentiation of induced pluripotent stem (iPS) cells into PDLSCs as an initial step toward PDL therapy. To this end, we first induced iPS cells into neural crest (NC)-like cells. We then captured the p75 neurotrophic receptor-positive cells (iPS-NC cells) and cultured them on an extracellular

matrix (ECM) produced by human PDL cells (iPS-NC-PDL cells). These iPS-NC-PDL cells showed reduced expression of embryonic stem cell and NC cell markers as compared with iPS and iPS-NC cells, and enrichment of mesenchymal stem cell markers. The cells also had a higher proliferative capacity, multipotency, and elevated expression of PDL-related markers than iPS-NC cells cultured on fibronectin and laminin (iPS-NC-FL cells) or ECM produced by human skin fibroblast cells (iPS-NC-SF cells). Overall, we present a culture method to produce high number of PDLSC-like cells from iPS cells as a first step toward a strategy for PDL regeneration.

Harb, N., et al. (2008). "The Rho-Rock-Myosin signaling axis determines cell-cell integrity of self-renewing pluripotent stem cells." PLoS One **3**(8): e3001.

BACKGROUND: Embryonic stem (ES) cells self-renew as coherent colonies in which cells maintain tight cell-cell contact. Although intercellular communications are essential to establish the basis of cell-specific identity, molecular mechanisms underlying intrinsic cell-cell interactions in ES cells at the signaling level remain underexplored. METHODOLOGY/PRINCIPAL FINDINGS: Here we show that endogenous Rho signaling is required for the maintenance of cell-cell contacts in ES cells. siRNA-mediated loss of function experiments demonstrated that Rock, a major effector kinase downstream of Rho, played a key role in the formation of cell-cell junctional assemblies through regulation of myosin II by controlling a myosin light chain phosphatase. Chemical engineering of this signaling axis by a Rock-specific inhibitor revealed that cell-cell adhesion was reversibly controllable and dispensable for self-renewal of mouse ES cells as confirmed by chimera assay. Furthermore, a novel culture system combining a single synthetic matrix, defined medium, and the Rock inhibitor fully warranted human ES cell self-renewal independent of animal-derived matrices, tight cell contacts, or fibroblastic niche-forming cells as determined by teratoma formation assay. CONCLUSIONS/SIGNIFICANCE: These findings demonstrate an essential role of the Rho-Rock-Myosin signaling axis for the regulation of basic cell-cell communications in both mouse and human ES cells, and would contribute to advance in medically compatible xeno-free environments for human pluripotent stem cells.

Hariharan, K., et al. (2018). "Parallel generation of easily selectable multiple nephronal cell types from human pluripotent stem cells." Cell Mol Life Sci.

Human pluripotent stem cells (hPSCs) provide a source for the generation of defined kidney cells and renal organoids applicable in regenerative medicine, disease modeling, and drug screening. These applications require the provision of hPSC-derived renal cells by reproducible, scalable, and efficient methods. We established a chemically defined protocol by application of Activin A, BMP4, and Retinoic acid followed by GDNF, which steered hPSCs to the renal lineage and resulted in populations of SIX2(+)/CITED1(+) metanephric mesenchyme- (MM) and of HOXB7(+)/GRHL2(+) ureteric bud (UB)-like cells already by 6 days. Transcriptome analysis corroborated that the PSC-derived cell types at day 8 resemble their renal vesicle and ureteric epithelial counterpart in vivo, forming tubular and glomerular renal cells 6 days later. We demonstrate that starting from hPSCs, our in vitro protocol generates a pool of nephrogenic progenitors at the renal vesicle stage, which can be further directed into specialized nephronal cell types including mesangial-, proximal tubular-, distal tubular, collecting duct epithelial cells, and podocyte precursors after 14 days. This simple and rapid method to produce renal cells from a common precursor pool in 2D culture provides the basis for scaled-up production of tailored renal cell types, which are applicable for drug testing or cell-based regenerative therapies.

Hartman, M. E., et al. (2016). "Human pluripotent stem cells: Prospects and challenges as a source of cardiomyocytes for in vitro modeling and cell-based cardiac repair." Adv Drug Deliv Rev **96**: 3-17.

Human pluripotent stem cells (PSCs) represent an attractive source of cardiomyocytes with potential applications including disease modeling, drug discovery and safety screening, and novel cell-based cardiac therapies. Insights from embryology have contributed to the development of efficient, reliable methods capable of generating large quantities of human PSC-cardiomyocytes with cardiac purities ranging up to 90%. However, for human PSCs to meet their full potential, the field must identify methods to generate cardiomyocyte populations that are uniform in subtype (e.g. homogeneous ventricular cardiomyocytes) and have more mature structural and functional properties. For in vivo applications, cardiomyocyte production must be highly scalable and clinical grade, and we will need to overcome challenges including graft cell death, immune rejection, arrhythmogenesis, and tumorigenic potential. Here we discuss the types of human PSCs, commonly used methods to guide their differentiation into cardiomyocytes, the phenotype of the resultant cardiomyocytes, and the remaining obstacles to their successful translation.

Hayashi, K., et al. (2011). "Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells." Cell **146**(4): 519-532.

The generation of properly functioning gametes in vitro requires reconstitution of the multistepped pathway of germ cell development. We demonstrate here the generation of primordial germ cell-like cells (PGCLCs) in mice with robust capacity for spermatogenesis. PGCLCs were generated from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) through epiblast-like cells (EpiLCs), a cellular state highly similar to pregastrulating epiblasts but distinct from epiblast stem cells (EpiSCs). Reflecting epiblast development, EpiLC induction from ESCs/iPSCs is a progressive process, and EpiLCs highly competent for the PGC fate are a transient entity. The global transcription profiles, epigenetic reprogramming, and cellular dynamics during PGCLC induction from EpiLCs meticulously capture those associated with PGC specification from the epiblasts. Furthermore, we identify Integrin-beta3 and SSEA1 as markers that allow the isolation of PGCLCs with spermatogenic capacity from tumorigenic undifferentiated cells. Our findings provide a paradigm for the first step of in vitro gametogenesis.

Haynes, J. M., et al. (2018). "Induced Pluripotent Stem Cell-Derived Podocyte-Like Cells as Models for Assessing Mechanisms Underlying Heritable Disease Phenotype: Initial Studies Using Two Alport Syndrome Patient Lines Indicate Impaired Potassium Channel Activity." J Pharmacol Exp Ther **367**(2): 335-347.

Renal podocyte survival depends upon the dynamic regulation of a complex cell architecture that links the glomerular basement membrane to integrins, ion channels, and receptors. Alport syndrome is a heritable chronic kidney disease where mutations in alpha3, alpha4, or alpha5 collagen genes promote podocyte death. In rodent models of renal failure, activation of the calcium-sensing receptor (CaSR) can protect podocytes from stress-related death. In this study, we assessed CaSR function in podocyte-like cells derived from induced-pluripotent stem cells from two patients with Alport Syndrome (AS1 & AS2) and a renal disease free individual [normal human mesangial cell (NHMC)], as well as a human immortalized podocyte-like (HIP) cell line. Extracellular calcium elicited concentration-dependent elevations of intracellular calcium in all podocyte-like cells. NHMC and HIP, but not AS1 or AS2 podocyte-like cells, also showed acute reductions in intracellular calcium prior to elevation. In NHMC podocyte-like cells this acute reduction was blocked by the large-conductance potassium channel (KCNMA1) inhibitors iberiotoxin (10 nM) and tetraethylammonium (5 mM), as well as the focal adhesion kinase inhibitor PF562271 (N-methyl-N-(3-((2-(2-oxo-2,3-dihydro-1H-indol-5-ylamino)-5-trifluoromethyl-pyrim idin-4-ylamino)-methyl)-pyridin-2-yl)-methanesulfonamide, 10 nM). Quantitative polymerase chain reaction (qPCR) and immunolabeling showed the presence of KCNMA1 transcript and protein in all podocyte-like cells tested. Cultivation of AS1 podocytes on decellularized plates of NHMC podocyte-like cells partially restored acute reductions in intracellular calcium in response to extracellular calcium. We conclude that the AS patient-derived podocyte-like cells used in this study showed dysfunctional integrin signaling and potassium channel function, which may contribute to podocyte death seen in Alport syndrome.

Hermanson, D. L., et al. (2016). "Induced Pluripotent Stem Cell-Derived Natural Killer Cells for Treatment of Ovarian Cancer." Stem Cells **34**(1): 93-101.

Natural killer (NK) cells can provide effective immunotherapy for ovarian cancer. Here, we evaluated the ability of NK cells isolated from peripheral blood (PB) and NK cells derived from induced pluripotent stem cell (iPSC) to mediate killing of ovarian cancer cells in a mouse xenograft model. A mouse xenograft model was used to evaluate the intraperitoneal delivery of three different NK cell populations: iPSC-derived NK cells, PB-NK cells that had been activated and expanded in long-term culture, and overnight activated PB-NK cells that were isolated through CD3/CD19 depletion of PB B and T cells. Bioluminescent imaging was used to monitor tumor burden of luciferase expressing tumor lines. Tumors were allowed to establish prior to administering NK cells via intraperitoneal injection. These studies demonstrate a single dose of any of the three NK cell populations significantly reduced tumor burden. When mice were given three doses of either iPSC-NK cells or expanded PB-NK cells, the median survival improved from 73 days in mice untreated to 98 and 97 days for treated mice, respectively. From these studies, we conclude iPSC-derived NK cells mediate antiovarian cancer killing at least as well as PB-NK cells, making these cells a viable resource for immunotherapy for ovarian cancer. Due to their ability to be easily differentiated into NK cells and their long-term expansion potential, iPSCs can be used to produce large numbers of well-defined NK cells that can be banked and used to treat a large number of patients including treatment with multiple doses if necessary.

Heslop, J. A., et al. (2017). "Donor-Dependent and Other Nondefined Factors have Greater Influence on the Hepatic Phenotype than the Starting Cell Type in Induced Pluripotent Stem Cell Derived Hepatocyte-Like Cells." Stem Cells Transl Med **6**(8): 1751.

Heslop, J. A., et al. (2017). "Donor-Dependent and Other Nondefined Factors Have Greater Influence on the Hepatic Phenotype Than the Starting Cell Type in Induced Pluripotent Stem Cell Derived Hepatocyte-Like Cells." Stem Cells Transl Med **6**(5): 1321-1331.

Drug-induced liver injury is the greatest cause of post-marketing drug withdrawal; therefore, substantial resources are directed toward triaging potentially dangerous new compounds at all stages of drug development. One of the major factors preventing effective screening of new compounds is the lack of a predictive in vitro model of hepatotoxicity. Primary human hepatocytes offer a metabolically relevant model for which the molecular initiating events of hepatotoxicity can be examined; however, these cells vary greatly between donors and dedifferentiate rapidly in culture. Induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells (HLCs) offer a reproducible, physiologically relevant and genotypically normal model cell; however, current differentiation protocols produce HLCs with a relatively immature phenotype. During the reprogramming of somatic cells, the epigenome undergoes dramatic changes; however, this "resetting" is a gradual process, resulting in an altered differentiation propensity, skewed toward the lineage of origin, particularly in early passage cultures. We, therefore, performed a comparison of human hepatocyte- and dermal fibroblast-derived iPSCs, assessing the impact of epigenetic memory at all stages of HLC differentiation. These results provide the first isogenic assessment of the starting cell type in human iPSC-derived HLCs. Despite a trend toward improvement in hepatic phenotype in albumin secretion and gene expression, few significant differences in hepatic differentiation capacity were found between hepatocyte and fibroblast-derived iPSCs. We conclude that the donor and inter-clonal differences have a greater influence on the hepatocyte phenotypic maturity than the starting cell type. Therefore, it is not necessary to use human hepatocytes for generating iPSC-derived HLCs. Stem Cells Translational Medicine 2017;6:1321-1331.

Hibaoui, Y. and A. Feki (2015). "Concise Review: Methods and Cell Types Used to Generate Down Syndrome Induced Pluripotent Stem Cells." J Clin Med **4**(4): 696-714.

Down syndrome (DS, trisomy 21), is the most common viable chromosomal disorder, with an incidence of 1 in 800 live births. Its phenotypic characteristics include intellectual impairment and several other developmental abnormalities, for the majority of which the pathogenetic mechanisms remain unknown. Several models have been used to investigate the mechanisms by which the extra copy of chromosome 21 leads to the DS phenotype. In the last five years, several laboratories have been successful in reprogramming patient cells carrying the trisomy 21 anomaly into induced pluripotent stem cells, i.e., T21-iPSCs. In this review, we summarize the different T21-iPSCs that have been generated with a particular interest in the technical procedures and the somatic cell types used for the reprogramming.

Hidema, S., et al. (2016). "Transgenic expression of Telomerase reverse transcriptase (Tert) improves cell proliferation of primary cells and enhances reprogramming efficiency into the induced pluripotent stem cell." Biosci Biotechnol Biochem **80**(10): 1925-1933.

The enzymatic activity of telomerase is important for the extension of the telomere repeat sequence and overcoming cellular senescence. We generated a conditional transgenic mouse line, carrying the telomerase reverse transcriptase (Tert) expression cassette, controlled by the Cre-loxP-mediated recombination. In our study, Cre recombinase expression efficiently activated Tert expression, resulting in its increased enzymatic activity, which extended the period of cellular proliferation until the keratinocytes entered senescence. This suggests that transgenic Tert expression is effective in enhancing primary cell proliferation. Notably, Tert expression increased colony formation of induced pluripotent stem (iPS) cells after the introduction of four reprogramming factors, Oct-4, klf4, SOX-2, and c-Myc into the transgenic fibroblasts. To the best of our knowledge, this is the first study to show that the transgenic Tert expression enhances reprogramming efficiency of iPS cells, which indicates a critical role for Tert in the reprogramming process.

Hildebrand, L., et al. (2015). "Selective cell targeting and lineage tracing of human induced pluripotent stem cells using recombinant avian retroviruses." Cell Mol Life Sci **72**(23): 4671-4680.

Human induced pluripotent stem cells (hiPSC) differentiate into multiple cell types. Selective cell targeting is often needed for analyzing gene function by overexpressing proteins in a distinct population of hiPSC-derived cell types and for monitoring cell fate in response to stimuli. However, to date, this has not been possible, as commonly used viruses enter the hiPSC via ubiquitously expressed receptors. Here, we report for the first time the application of a heterologous avian receptor, the tumor virus receptor A (TVA), to selectively transduce TVA(+) cells in a mixed cell population. Expression of the TVA surface receptor via genetic engineering renders cells susceptible for infection by avian leucosis virus (ALV). We generated hiPSC lines with this stably integrated, ectopic TVA receptor gene that expressed the receptor while

retaining pluripotency. The undifferentiated hiPSC(TVA+) as well as their differentiating progeny could be infected by recombinant ALV (so-called RCAS virus) with high efficiency. Due to incomplete receptor blocking, even sequential infection of differentiating or undifferentiated TVA(+) cells was possible. In conclusion, the TVA/RCAS system provides an efficient and gentle gene transfer system for hiPSC and extends our possibilities for selective cell targeting and lineage tracing studies.

Himeno, T., et al. (2013). "Mesenchymal stem cell-like cells derived from mouse induced pluripotent stem cells ameliorate diabetic polyneuropathy in mice." Biomed Res Int **2013**: 259187.

BACKGROUND: Although pathological involvements of diabetic polyneuropathy (DPN) have been reported, no dependable treatment of DPN has been achieved. Recent studies have shown that mesenchymal stem cells (MSCs) ameliorate DPN. Here we demonstrate a differentiation of induced pluripotent stem cells (iPSCs) into MSC-like cells and investigate the therapeutic potential of the MSC-like cell transplantation on DPN. RESEARCH DESIGN AND METHODS: For induction into MSC-like cells, GFP-expressing iPSCs were cultured with retinoic acid, followed by adherent culture for 4 months. The MSC-like cells, characterized with flow cytometry and RT-PCR analyses, were transplanted into muscles of streptozotocin-diabetic mice. Three weeks after the transplantation, neurophysiological functions were evaluated. RESULTS: The MSC-like cells expressed MSC markers and angiogenic/neurotrophic factors. The transplanted cells resided in hindlimb muscles and peripheral nerves, and some transplanted cells expressed S100 beta in the nerves. Impairments of current perception thresholds, nerve conduction velocities, and plantar skin blood flow in the diabetic mice were ameliorated in limbs with the transplanted cells. The capillary number-to-muscle fiber ratios were increased in transplanted hindlimbs of diabetic mice. CONCLUSIONS: These results suggest that MSC-like cell transplantation might have therapeutic effects on DPN through secreting angiogenic/neurotrophic factors and differentiation to Schwann cell-like cells.

Hirata, M. and T. Yamaoka (2018). "Effect of stem cell niche elasticity/ECM protein on the self-beating cardiomyocyte differentiation of induced pluripotent stem (iPS) cells at different stages." Acta Biomater **65**: 44-52.

Stem cell-based myocardial regeneration therapies have emerged as alternative strategies to heart transplantation for serious heart diseases, but autologous beating mature cardiomyocytes are not available. Here we investigated the effect of culture substrates on the cardiomyocyte differentiation of induced pluripotent stem cells (iPSs) in vitro by separately evaluating the following continuous three steps: (1) cardiac marker gene expression, (2) contractile gene expression and self-beating, and (3) beating duration. To this end, we used iPS cells to study the cardiac differentiation, and neonatal rat cardiomyocytes (NCMs) to study beating behavior. These cells were cultured on substrates with different natures, i.e., an elastic substrate (Es) with the modulus of 9, 20, or 180kPa, and hard tissue culture polystyrene dishes (TCPS) coated with collagen type I (Col), gelatin (Gel), or fibronectin (FN). The results revealed that the effective niches in each step were very different. The cardiac marker gene (GATA4, Tbx5, MEF2C) expression of iPSs at the 1st step was very high on the TCPS coated with FN or Gel, whereas on the FN-coated Es (especially with the 9kPa modulus), the undifferentiated marker gene (Nanog) expression of iPSs was maintained. The expression of the contractile genes alpha-MHC, TnC1, and TnT2 and the self-beating (the 2nd step) of the NCMs were high on FN-coated TCPS and Col-coated Es. The 3rd step (beating duration) of the NCMs was effective on the Es, and at 21 days both the iPSs and NCMs stopped beating on the TCPS but were still beating on the Es. Overall, cardiac differentiation 'preferred' ECM-rigid culture substrates, and beating-behavior 'preferred' Col-soft culture substrates. These results are important for understanding and designing cardiac differentiation niches for regenerative medicine, and they suggest that a single culture substrate is not suitable for preparing self-beating cardiomyocytes. STATEMENT OF SIGNIFICANCE: The transplantation of beating cardiomyocytes (BCMs) is expected to be made more effective for serious heart diseases. The identification of the appropriate engineering processes and suitable culture substrates for inducing stem cell differentiation into BCMs is thus indispensable. The differentiation can be divided into three major processes, the cardiac differentiation step, the beating-induction step and the beating-duration step. A protocol with the higher efficiency in all of the steps must be useful. In this study, we separately evaluated the effect of culture substrates at each three step. We clarified that the biological and the physical properties of the culture substrates required at these steps were different. We found useful criteria for effective cardiac cell niche systems design.

Hitomi, H., et al. (2017). "Human pluripotent stem cell-derived erythropoietin-producing cells ameliorate renal anemia in mice." Sci Transl Med **9**(409).

The production of erythropoietin (EPO) by the kidneys, a principal hormone for the hematopoietic system, is reduced in patients with chronic kidney

disease (CKD), eventually resulting in severe anemia. Although recombinant human EPO treatment improves anemia in patients with CKD, returning to full red blood cell production without fluctuations does not always occur. We established a method to generate EPO-producing cells from human induced pluripotent stem cells (hiPSCs) by modifying previously reported hepatic differentiation protocols. These cells showed increased EPO expression and secretion in response to low oxygen conditions, prolyl hydroxylase domain-containing enzyme inhibitors, and insulin-like growth factor 1. The EPO protein secreted from hiPSC-derived EPO-producing (hiPSC-EPO) cells induced the erythropoietic differentiation of human umbilical cord blood progenitor cells in vitro. Furthermore, transplantation of hiPSC-EPO cells into mice with CKD induced by adenine treatment improved renal anemia. Thus, hiPSC-EPO cells may be a useful tool for clarifying the mechanisms of EPO production and may be useful as a therapeutic strategy for treating renal anemia.

Hitomi, T., et al. (2013). "Downregulation of Securin by the variant RNF213 R4810K (rs112735431, G>A) reduces angiogenic activity of induced pluripotent stem cell-derived vascular endothelial cells from moyamoya patients." Biochem Biophys Res Commun **438**(1): 13-19.

Moyamoya disease (MMD) is a cerebrovascular disease characterized by occlusive lesions in the circle of Willis. The RNF213 R4810K polymorphism increases susceptibility to MMD. Induced pluripotent stem cells (iPSCs) were established from unaffected fibroblast donors with wild-type RNF213 alleles, and from carriers/patients with one or two RNF213 R4810K alleles. Angiogenic activities of iPSC-derived vascular endothelial cells (iPSECs) from patients and carriers were lower (49.0 +/- 19.4%) than from wild-type subjects ($p<0.01$). Gene expression profiles in iPSECs showed that Securin was down-regulated ($p<0.01$) in carriers and patients. Overexpression of RNF213 R4810K downregulated Securin, inhibited angiogenic activity (36.0 +/- 16.9%) and proliferation of humanumbilical vein endothelial cells (HUVECs) while overexpression of RNF213 wild type did not. Securin expression was downregulated using RNA interference techniques, which reduced the level of tube formation in iPSECs and HUVECs without inhibition of proliferation. RNF213 R4810K reduced angiogenic activities of iPSECs from patients with MMD, suggesting that it is a promising in vitro model for MMD.

Ho, R., et al. (2013). "Stage-specific regulation of reprogramming to induced pluripotent stem cells by Wnt signaling and T cell factor proteins." Cell Rep **3**(6): 2113-2126.

Wnt signaling is intrinsic to mouse embryonic stem cell self-renewal. Therefore, it is surprising that reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) is not strongly enhanced by Wnt signaling. Here, we demonstrate that active Wnt signaling inhibits the early stage of reprogramming to iPSCs, whereas it is required and even stimulating during the late stage. Mechanistically, this biphasic effect of Wnt signaling is accompanied by a change in the requirement of all four of its transcriptional effectors: T cell factor 1 (Tcf1), Lef1, Tcf3, and Tcf4. For example, Tcf3 and Tcf4 are stimulatory early but inhibitory late in the reprogramming process. Accordingly, ectopic expression of Tcf3 early in reprogramming combined with its loss of function late enables efficient reprogramming in the absence of ectopic Sox2. Together, our data indicate that the stepwise process of reprogramming to iPSCs is critically dependent on the stage-specific control and action of all four Tcfs and Wnt signaling.

Holm, S. (2008). "Time to reconsider stem cell ethics--the importance of induced pluripotent cells." J Med Ethics **34**(2): 63-64.

Honda, A., et al. (2017). "Discrimination of Stem Cell Status after Subjecting Cynomolgus Monkey Pluripotent Stem Cells to Naive Conversion." Sci Rep **7**: 45285.

Experimental animal models have played an indispensable role in the development of human induced pluripotent stem cell (iPSC) research. The derivation of high-quality (so-called "true naive state") iPSCs of non-human primates enhances their application and safety for human regenerative medicine. Although several attempts have been made to convert human and non-human primate PSCs into a truly naive state, it is unclear which evaluation methods can discriminate them as being truly naive. Here we attempted to derive naive cynomolgus monkey (Cm) (Macaca fascicularis) embryonic stem cells (ESCs) and iPSCs. Several characteristics of naive Cm ESCs including colony morphology, appearance of naive-related mRNAs and proteins, leukaemia inhibitory factor dependency, and mitochondrial respiration were confirmed. Next, we generated Cm iPSCs and converted them to a naive state. Transcriptomic comparison of PSCs with early Cm embryos elucidated the partial achievement (termed naive-like) of their conversion. When these were subjected to in vitro neural differentiation, enhanced differentiating capacities were observed after naive-like conversion, but some lines exhibited heterogeneity. The difficulty of achieving contribution to chimeric mouse embryos

was also demonstrated. These results suggest that Cm PSCs could ameliorate their in vitro neural differentiation potential even though they could not display true naive characteristics.

Hong, S. H., et al. (2011). "Cell fate potential of human pluripotent stem cells is encoded by histone modifications." Cell Stem Cell **9**(1): 24-36.

Human embryonic stem cells (hESCs) expressing pluripotency markers are assumed to possess equipotent developmental potential. However, disparate responses to differentiation stimuli functionally illustrate that hESCs generate a spectrum of differentiated cell types, suggestive of lineage bias. Here, we reveal specific cell surface markers that allow subfractionation of hESCs expressing hallmark markers of pluripotency. By direct de novo isolation of these subsets, we demonstrate that propensities for lineage differentiation are balanced with reduced clonogenic self-renewal. Histone modification marks of gene loci associated with pluripotency versus lineage specificity predicted cell fate potential of these subfractions, thereby supporting the absence of uniform bivalency as a molecular paradigm to describe cell fate determination of pluripotent cells. Our study reveals that cell fate potential is encoded within cells comprising hESC cultures, highlighting them as a means to understand the mechanisms of lineage specification of pluripotent cells.

Hongisto, H., et al. (2017). "Xeno- and feeder-free differentiation of human pluripotent stem cells to two distinct ocular epithelial cell types using simple modifications of one method." Stem Cell Res Ther **8**(1): 291.

BACKGROUND: Human pluripotent stem cells (hPSCs) provide a promising cell source for ocular cell replacement therapy, but often lack standardized and xenogeneic-free culture and differentiation protocols. We aimed to develop a xeno- and feeder cell-free culture system for undifferentiated hPSCs along with efficient methods to derive ocular therapy target cells: retinal pigment epithelial (RPE) cells and corneal limbal epithelial stem cells (LESCs). METHODS: Multiple genetically distinct hPSC lines were adapted to a defined, xeno-, and feeder-free culture system of Essential 8 medium and laminin-521 matrix. Thereafter, two-stage differentiation methods toward ocular epithelial cells were established utilizing xeno-free media and a combination of extracellular matrix proteins. Both differentiation methods shared the same basal elements, using only minor inductive modifications during early differentiation towards desired cell lineages. The resulting RPE cells and LESCs were characterized after several independent differentiation experiments and recovery after xeno-free cryopreservation. RESULTS: The defined, xeno-, and feeder-free culture system provided a robust means to generate high-quality hPSCs with chromosomal stability limited to early passages. Inductive cues introduced during the first week of differentiation had a substantial effect on lineage specification, cell survival, and even mature RPE properties. Derivative RPE formed functional epithelial monolayers with mature tight junctions and expression of RPE genes and proteins, as well as phagocytosis and key growth factor secretion capacity after 9 weeks of maturation on inserts. Efficient LESC differentiation led to cell populations expressing LESC markers such as p40/p63alpha by day 24. Finally, we established xeno-free cryobanking protocols for pluripotent hPSCs, hPSC-RPE cells, and hPSC-LESCs, and demonstrated successful recovery after thawing. CONCLUSIONS: We propose methods for efficient and scalable, directed differentiation of high-quality RPE cells and LESCs. The two clinically relevant cell types are generated with simple inductive modification of the same basal method, followed by adherent culture, passaging, and cryobanking.

Hossini, A. M., et al. (2015). "Erratum: Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks." BMC Genomics **16**: 433.

Hossini, A. M., et al. (2015). "Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks." BMC Genomics **16**: 84.

BACKGROUND: Alzheimer's disease (AD) is a complex, irreversible neurodegenerative disorder. At present there are neither reliable markers to diagnose AD at an early stage nor therapy. To investigate underlying disease mechanisms, induced pluripotent stem cells (iPSCs) allow the generation of patient-derived neuronal cells in a dish. RESULTS: In this study, employing iPS technology, we derived and characterized iPSCs from dermal fibroblasts of an 82-year-old female patient affected by sporadic AD. The AD-iPSCs were differentiated into neuronal cells, in order to generate disease-specific protein association networks modeling the molecular pathology on the transcriptome level of AD, to analyse the reflection of the disease phenotype in gene expression in AD-iPS neuronal cells, in particular in the ubiquitin-proteasome system (UPS), and to address expression of typical AD proteins. We detected the expression of p-tau and GSK3B, a physiological kinase of tau, in neuronal cells derived from AD-iPSCs. Treatment of neuronal cells differentiated from AD-iPSCs with an inhibitor of

gamma-secretase resulted in the down-regulation of p-tau. Transcriptome analysis of AD-iPS derived neuronal cells revealed significant changes in the expression of genes associated with AD and with the constitutive as well as the inducible subunits of the proteasome complex. The neuronal cells expressed numerous genes associated with sub-regions within the brain thus suggesting the usefulness of our in-vitro model. Moreover, an AD-related protein interaction network composed of APP and GSK3B among others could be generated using neuronal cells differentiated from two AD-iPS cell lines. CONCLUSIONS: Our study demonstrates how an iPSC-based model system could represent (i) a tool to study the underlying molecular basis of sporadic AD, (ii) a platform for drug screening and toxicology studies which might unveil novel therapeutic avenues for this debilitating neuronal disorder.

Hou, L., et al. (2016). "Combinatorial extracellular matrix microenvironments promote survival and phenotype of human induced pluripotent stem cell-derived endothelial cells in hypoxia." Acta Biomater **44**: 188-199.

UNLABELLED: Recent developments in cell therapy using human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) hold great promise for treating ischemic cardiovascular tissues. However, poor post-transplantation viability largely limits the potential of stem cell therapy. Although the extracellular matrix (ECM) has become increasingly recognized as an important cell survival factor, conventional approaches primarily rely on single ECMs for in vivo co-delivery with cells, even though the endothelial basement membrane is comprised of a milieu of different ECMs. To address this limitation, we developed a combinatorial ECM microarray platform to simultaneously interrogate hundreds of micro-scale multi-component chemical compositions of ECMs on iPSC-EC response. After seeding iPSC-ECs onto ECM microarrays, we performed high-throughput analysis of the effects of combinatorial ECMs on iPSC-EC survival, endothelial phenotype, and nitric oxide production under conditions of hypoxia (1% O2) and reduced nutrients (1% fetal bovine serum), as is present in ischemic injury sites. Using automated image acquisition and analysis, we identified combinatorial ECMs such as collagen IV+gelatin+heparan sulfate+laminin and collagen IV+fibronectin+gelatin+heparan sulfate+laminin that significantly improved cell survival, nitric oxide production, and CD31 phenotypic expression, in comparison to single-component ECMs. These results were further validated in conventional cell culture platforms and within three-dimensional scaffolds. Furthermore, this approach revealed complex ECM interactions and non-intuitive cell behavior that otherwise could not be easily determined using conventional cell culture platforms. Together these data suggested that iPSC-EC delivery within optimal combinatorial ECMs may improve their survival and function under the condition of hypoxia with reduced nutrients. STATEMENT OF SIGNIFICANCE: Human endothelial cells (ECs) derived from induced pluripotent stem cells (iPSC-ECs) are promising for treating diseases associated with reduced nutrient and oxygen supply like heart failure. However, diminished iPSC-EC survival after implantation into diseased environments limits their therapeutic potential. Since native ECs interact with numerous extracellular matrix (ECM) proteins for functional maintenance, we hypothesized that combinatorial ECMs may improve cell survival and function under conditions of reduced oxygen and nutrients. We developed a high-throughput system for simultaneous screening of iPSC-ECs cultured on multi-component ECM combinations under the condition of hypoxia and reduced serum. Using automated image acquisition and analytical algorithms, we identified combinatorial ECMs that significantly improved cell survival and function, in comparison to single ECMs. Furthermore, this approach revealed complex ECM interactions and non-intuitive cell behavior that otherwise could not be easily determined.

Hough, S. R., et al. (2014). "Single-cell gene expression profiles define self-renewing, pluripotent, and lineage primed states of human pluripotent stem cells." Stem Cell Reports **2**(6): 881-895.

Pluripotent stem cells display significant heterogeneity in gene expression, but whether this diversity is an inherent feature of the pluripotent state remains unknown. Single-cell gene expression analysis in cell subsets defined by surface antigen expression revealed that human embryonic stem cell cultures exist as a continuum of cell states, even under defined conditions that drive self-renewal. The majority of the population expressed canonical pluripotency transcription factors and could differentiate into derivatives of all three germ layers. A minority subpopulation of cells displayed high self-renewal capacity, consistently high transcripts for all pluripotency-related genes studied, and no lineage priming. This subpopulation was characterized by its expression of a particular set of intercellular signaling molecules whose genes shared common regulatory features. Our data support a model of an inherently metastable self-renewing population that gives rise to a continuum of intermediate pluripotent states, which ultimately become primed for lineage specification.

Hoveizi, E., et al. (2015). "In vitro comparative survey of cell adhesion and proliferation of human induced

pluripotent stem cells on surfaces of polymeric electrospun nanofibrous and solution-cast film scaffolds." J Biomed Mater Res A **103**(9): 2952-2958.

Extracellular matrix (ECM) components play a critical role in regulating cell behaviors. Interactions between ECM components and cells are important in various biological processes, including cell attachment, survival, morphogenesis, spreading, proliferation, and gene expression. In this study the in vitro responses of human induced pluripotent stem cells (hiPSCs) on polycaprolactone (PCL) electrospun nanofibrous scaffold were reported in comparison with those of the cells on corresponding solution-cast film scaffold. Our results demonstrated that the nanofibrous scaffold showed better support for the attachment and proliferation of hiPSCs than their corresponding film scaffold. Consequently, we emphasize that hiPSCs can sense the physical properties and chemical composition of the materials and regulate their behaviors accordingly.

Hu, G. W., et al. (2015). "Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells attenuate limb ischemia by promoting angiogenesis in mice." Stem Cell Res Ther **6**: 10.

INTRODUCTION: 'Patient-specific' induced pluripotent stem cells (iPSCs) are attractive because they can generate abundant cells without the risk of immune rejection for cell therapy. Studies have shown that iPSC-derived mesenchymal stem cells (iMSCs) possess powerful proliferation, differentiation, and therapeutic effects. Recently, most studies indicate that stem cells exert their therapeutic effect mainly through a paracrine mechanism other than transdifferentiation, and exosomes have emerged as an important paracrine factor for stem cells to reprogram injured cells. The objective of this study was to evaluate whether exosomes derived from iMSCs (iMSCs-Exo) possess the ability to attenuate limb ischemia and promote angiogenesis after transplantation into limbs of mice with femoral artery excision. METHODS: Human iPSCs (iPS-S-01, C1P33, and PCKDSF001C1) were used to differentiate into iMSCs in a modified one-step method. iMSCs were characterized by flow cytometry and multipotent differentiation potential analysis. Ultrafiltration combined with a purification method was used to isolate iMSCs-Exo, and transmission electron microscopy and Western blotting were used to identify iMSCs-Exo. After establishment of mouse hind-limb ischemia with excision of femoral artery and iMSCs-Exo injection, blood perfusion was monitored at days 0, 7, 14, and 21; microvessel density in ischemic muscle was also analyzed. In vitro migration, proliferation, and tube formation experiments were used to analyze the ability of pro-angiogenesis in iMSCs-Exo, and quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay were used to identify expression levels of angiogenesis-related molecules in human umbilical vein endothelial cells (HUVECs) after being cultured with iMSCs-Exo. RESULTS: iPSCs were efficiently induced into iMSC- with MSC-positive and -negative surface antigens and osteogenesis, adipogenesis, and chondrogenesis differentiation potential. iMSCs-Exo with a diameter of 57 +/- 11 nm and expressed CD63, CD81, and CD9. Intramuscular injection of iMSCs-Exo markedly enhanced microvessel density and blood perfusion in mouse ischemic limbs, consistent with an attenuation of ischemic injury. In addition, iMSCs-Exo could activate angiogenesis-related molecule expression and promote HUVEC migration, proliferation, and tube formation. CONCLUSION: Implanted iMSCs-Exo was able to protect limbs from ischemic injury via the promotion of angiogenesis, which indicated that iMSCs-Exo may be a novel therapeutic approach in the treatment of ischemic diseases.

Hu, J., et al. (2018). "Embryonic germ cell extracts erase imprinted genes and improve the efficiency of induced pluripotent stem cells." Sci Rep **8**(1): 10955.

Patient-specific induced pluripotent stem cells (iPSCs) have the potential to be useful in the treatment of human diseases. While prior studies have reported multiple methods to generate iPSCs, DNA methylation continues to limit the totipotency and reprogramming efficiency of iPSCs. Here, we first show the competency of embryonic germ cells (EGCs) as a reprogramming catalyst capable of effectively promoting reprogramming induced by four defined factors, including Oct4, Sox2, Klf4 and c-Myc. Combining EGC extracts with these four factors resulted in formation of more embryonic stem cell-like colonies than did factors alone. Notably, expression of imprinted genes was higher with combined induction than with factors alone. Moreover, iPSCs derived from the combined inductors tended to have more global hypomethylation. Our research not only provides evidence that EGC extracts could activate DNA demethylation and reprogram imprinted genes, but also establishes a new way to enhance reprogramming of iPSCs, which remains a critical safety concern for potential use of iPSCs in regenerative medicine.

Hu, S., et al. (2016). "Effects of cellular origin on differentiation of human induced pluripotent stem cell-derived endothelial cells." JCI Insight **1**(8).

Human induced pluripotent stem cells (iPSCs) can be derived from various types of somatic cells by transient overexpression of 4 Yamanaka factors (OCT4, SOX2, C-MYC, and KLF4). Patient-specific iPSC

derivatives (e.g., neuronal, cardiac, hepatic, muscular, and endothelial cells [ECs]) hold great promise in drug discovery and regenerative medicine. In this study, we aimed to evaluate whether the cellular origin can affect the differentiation, in vivo behavior, and single-cell gene expression signatures of human iPSC-derived ECs. We derived human iPSCs from 3 types of somatic cells of the same individuals: fibroblasts (FB-iPSCs), ECs (EC-iPSCs), and cardiac progenitor cells (CPC-iPSCs). We then differentiated them into ECs by sequential administration of Activin, BMP4, bFGF, and VEGF. EC-iPSCs at early passage (10 < P < 20) showed higher EC differentiation propensity and gene expression of EC-specific markers (PECAM1 and NOS3) than FB-iPSCs and CPC-iPSCs. In vivo transplanted EC-iPSC-ECs were recovered with a higher percentage of CD31(+) population and expressed higher EC-specific gene expression markers (PECAM1, KDR, and ICAM) as revealed by microfluidic single-cell quantitative PCR (qPCR). In vitro EC-iPSC-ECs maintained a higher CD31(+) population than FB-iPSC-ECs and CPC-iPSC-ECs with long-term culturing and passaging. These results indicate that cellular origin may influence lineage differentiation propensity of human iPSCs; hence, the somatic memory carried by early passage iPSCs should be carefully considered before clinical translation.

Hu, W. T., et al. (2014). "Transient folate deprivation in combination with small-molecule compounds facilitates the generation of somatic cell-derived pluripotent stem cells in mice." J Huazhong Univ Sci Technolog Med Sci **34**(2): 151-156.

Induced pluripotent stem cells (iPSCs) can be propagated indefinitely, while maintaining the capacity to differentiate into all cell types in the body except for the extra-embryonic tissues. This iPSC technology not only represents a new way to use individual-specific stem cells for regenerative medicine but also constitutes a novel method to obtain large numbers of disease-specific cells for biomedical research. However, the low efficiency of reprogramming and genomic integration of oncogenes and viral vectors limit the potential application of iPSCs. Chemical-induced reprogramming offers a novel approach to generating iPSCs. In this study, a new combination of small-molecule compounds (SMs) (sodium butyrate, A-83-01, CHIR99021, Y-27632) under conditions of transient folate deprivation was used to generate iPSC. It was found that transient folate deprivation combined with SMs was sufficient to permit reprogramming from mouse embryonic fibroblasts (MEFs) in the presence of transcription factors, Oct4 and Klf4, within 25 days, replacing Sox2 and c-Myc, and accelerated the generation of mouse iPSCs. The resulting cell lines resembled mouse embryonic stem (ES) cells with respect to proliferation rate, morphology, pluripotency-associated markers and gene expressions. Deprivation of folic acid, combined with treating MEFs with SMs, can improve the inducing efficiency of iPSCs and reduce their carcinogenicity and the use of exogenous reprogramming factors.

Huang, B., et al. (2013). "Generation of human epidermis-derived mesenchymal stem cell-like pluripotent cells (hEMSCPCs)." Sci Rep **3**: 1933.

We isolated human epidermis-derived mesenchymal stem cell-like pluripotent cells (hEMSCPCs) and demonstrate efficient harvesting, maintenance in vitro for at least 30 passages, reprogramming into multiple phenotypes in vivo, and integration into adult host tissues after injection into the mouse blastocyst to create chimeras. Cell phenotype was examined by karyotyping, immunostaining, immunofluorescence, and flow cytometry. A nested PCR protocol using primers specific for human SRY genes was designed to detect hEMSCPC-derived cells in female chimeric mice. FISH was used to validate the results of nested PCR. Results indicated that hEMSCPCs were derived from epidermis but were distinct from epidermal cells; they resembled mesenchymal stem cells (MSCs) morphologically and expressed the main markers of MSCs. About half of all female offspring of mice implanted with embryos injected with hEMSCPCs at the blastocyst stage harbored the human Y chromosome and tissue-specific human protein, thereby demonstrating the transdifferentiation of hEMSCPCs.

Huang, H. P., et al. (2010). "Factors from human embryonic stem cell-derived fibroblast-like cells promote topology-dependent hepatic differentiation in primate embryonic and induced pluripotent stem cells." J Biol Chem **285**(43): 33510-33519.

The future clinical use of embryonic stem cell (ESC)-based hepatocyte replacement therapy depends on the development of an efficient procedure for differentiation of hepatocytes from ESCs. Here we report that a high density of human ESC-derived fibroblast-like cells (hESdFs) supported the efficient generation of hepatocyte-like cells with functional and mature hepatic phenotypes from primate ESCs and human induced pluripotent stem cells. Molecular and immunocytochemistry analyses revealed that hESdFs caused a rapid loss of pluripotency and induced a sequential endoderm-to-hepatocyte differentiation in the central area of ESC colonies. Knockdown experiments demonstrated that pluripotent stem cells were directed toward endodermal and hepatic lineages by FGF2 and activin A secreted from hESdFs. Furthermore, we found that the central region of ESC colonies was essential for the hepatic endoderm-

specific differentiation, because its removal caused a complete disruption of endodermal differentiation. In conclusion, we describe a novel in vitro differentiation model and show that hESdF-secreted factors act in concert with regional features of ESC colonies to induce robust hepatic endoderm differentiation in primate pluripotent stem cells.

Huang, K., et al. (2014). "The naive state of human pluripotent stem cells: a synthesis of stem cell and preimplantation embryo transcriptome analyses." Cell Stem Cell **15**(4): 410-415.

Here we use a systems biology approach to comprehensively assess the conservation of gene networks in naive pluripotent stem cells (PSCs) with preimplantation embryos. While gene networks in murine naive and primed pluripotent states are reproducible across data sets, different sources of human stem cells display high degrees of variation, partly reflecting disparities in culture conditions. Finally, naive gene networks between human and mouse PSCs are not well conserved and better resemble their respective blastocysts.

Huang, M. L., et al. (2018). "Small Molecule Antagonist of Cell Surface Glycosaminoglycans Restricts Mouse Embryonic Stem Cells in a Pluripotent State." Stem Cells **36**(1): 45-54.

Recently, the field of stem cell-based regeneration has turned its attention toward chemical approaches for controlling the pluripotency and differentiation of embryonic stem cells (ESCs) using drug-like small molecule modulators. Growth factor receptors or their associated downstream kinases that regulate intracellular signaling pathways during differentiation are typically the targets for these molecules. The glycocalyx, which plays an essential role in actuating responses to growth factors at the cellular boundary, offers an underexplored opportunity for intervention using small molecules to influence differentiation. Here, we show that surfen, an antagonist of cell-surface glycosaminoglycans required for growth factor association with cognate receptors, acts as a potent and general inhibitor of differentiation and promoter of pluripotency in mouse ESCs. This finding shows that drugging the stem cell Glycome with small molecules to silence differentiation cues can provide a powerful new alternative to existing techniques for controlling stem cell fate. Stem Cells 2018;36:45-54.

Huang, N. F., et al. (2013). "Chemotaxis of human induced pluripotent stem cell-derived endothelial cells." Am J Transl Res **5**(5): 510-520.

This study examined the homing capacity of human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) and their response to chemotactic gradients of stromal derived factor-1alpha (SDF). We have previously shown that EC derived from murine pluripotent stem cells can home to the ischemic hindlimb of the mouse. In the current study, we were interested to understand if ECs derived from human induced pluripotent stem cells are capable of homing. The homing capacity of iPSC-ECs was assessed after systemic delivery into immunodeficient mice with unilateral hindlimb ischemia. Furthermore, the iPSC-ECs were evaluated for their expression of CXCR4 and their ability to respond to SDF chemotactic gradients in vitro. Upon systemic delivery, the iPSC-ECs transiently localized to the lungs but did not home to the ischemic limb over the course of 14 days. To understand the mechanism of the lack of homing, the expression levels of the homing receptor, CXCR4, was examined at the transcriptional and protein levels. Furthermore, their ability to migrate in response to chemokines was assessed using microfluidic and scratch assays. Unlike ECs derived from syngeneic mouse pluripotent stem cells, human iPSC-ECs do not home to the ischemic mouse hindlimb. This lack of functional homing may represent an impairment of interspecies cellular communication or a difference in the differentiation state of the human iPSC-ECs. These results may have important implications in therapeutic delivery of iPSC-ECs.

Huang, X. and S. M. Wu (2010). "Isolation and functional characterization of pluripotent stem cell-derived cardiac progenitor cells." Curr Protoc Stem Cell Biol **Chapter 1**: Unit 1F 10.

The use of transgenic markers in pluripotent stem cells allows the facile isolation of transient cell populations that appear at certain phases of embryonic development. Here, we describe a procedure for deriving cardiac progenitors from mouse pluripotent stem cells carrying a GFP reporter under the control of an Nkx2.5 enhancer sequence. The cells are propagated under standard conditions and are differentiated using the hanging-droplet method with medium optimized for commitment to the cardiac lineage. Cardiac progenitors are isolated from the differentiation culture using fluorescence-activated cell sorting (FACS) and can be cultured further for functional characterization and experimentation. The protocols described here can be applied to both embryonic and induced pluripotent stem cells and can easily be adapted to transgenic lines carrying other cardiac cell lineage reporters.

Hynes, K., et al. (2014). "Generation of functional mesenchymal stem cells from different induced pluripotent stem cell lines." Stem Cells Dev **23**(10): 1084-1096.

The therapeutic potential of mesenchymal stem cells (MSC) has highlighted the need for identifying easily accessible and reliable sources of these cells. An alternative source for obtaining large populations of MSC is through the controlled differentiation of induced pluripotent stem cells (iPSC). In the present study, colonies of iPSC were cultured in MSC culture media for 2 weeks. Serial passaging then selected for fast growing MSC-like cells with a typical fibroblastic morphology and the capacity to proliferate on standard culture flasks without feeder cells. MSC-like cells were developed from iPSC lines arising from three different somatic tissues: gingiva, periodontal ligament (PDL), and lung. The iPSC-MSC like cells expressed key MSC-associated markers (CD73, CD90, CD105, CD146, and CD166) and lacked expression of pluripotent markers (TRA160, TRA181, and alkaline phosphatase) and hematopoietic markers (CD14, CD34, and CD45). In vitro iPSC-MSC-like cells displayed the capacity to differentiate into osteoblasts, adipocytes, and chondrocytes. In vivo subcutaneous implantation of the iPSC-MSC-like cells into NOD/SCID mice demonstrated that only the PDL-derived iPSC-MSC-like cells exhibited the capacity to form mature mineralized structures which were histologically similar to mature bone. These findings demonstrate that controlled induction of iPSC into fibroblastic-like cells that phenotypically and functionally resemble adult MSC is an attractive approach to obtain a readily available source of progenitor cells for orthopedic and dental-related tissue-engineering applications. However, a detailed characterization of the iPSC-MSC-like cells will be important, as MSC-like cells derived from different iPSC lines exhibit variability in their differentiation capacity.

Hyysalo, A., et al. (2017). "Aligned Poly(epsilon-caprolactone) Nanofibers Guide the Orientation and Migration of Human Pluripotent Stem Cell-Derived Neurons, Astrocytes, and Oligodendrocyte Precursor Cells In Vitro." Macromol Biosci **17**(7).

Stem cell transplantations for spinal cord injury (SCI) have been studied extensively for the past decade in order to replace the damaged tissue with human pluripotent stem cell (hPSC)-derived neural cells. Transplanted cells may, however, benefit from supporting and guiding structures or scaffolds in order to remain viable and integrate into the host tissue. Biomaterials can be used as supporting scaffolds, as they mimic the characteristics of the natural cellular environment. In this study, hPSC-derived neurons, astrocytes, and oligodendrocyte precursor cells (OPCs) are cultured on aligned poly(epsilon-caprolactone) nanofiber platforms, which guide cell orientation to resemble that of spinal cord in vivo. All cell types are shown to efficiently spread over the nanofiber platform and orient according to the fiber alignment. Human neurons and astrocytes require extracellular matrix molecule coating for the nanofibers, but OPCs grow on nanofibers without additional treatment. Furthermore, the nanofiber platform is combined with a 3D hydrogel scaffold with controlled thickness, and nanofiber-mediated orientation of hPSC-derived neurons is also demonstrated in a 3D environment. In this work, clinically relevant materials and substrates for nanofibers, fiber coatings, and hydrogel scaffolds are used and combined with cells suitable for developing functional cell grafts for SCI repair.

Ikeda, K., et al. (2017). "Cell fiber-based three-dimensional culture system for highly efficient expansion of human induced pluripotent stem cells." Sci Rep **7**(1): 2850.

Human pluripotent stem cells are a potentially powerful cellular resource for application in regenerative medicine. Because such applications require large numbers of human pluripotent stem cell-derived cells, a scalable culture system of human pluripotent stem cell needs to be developed. Several suspension culture systems for human pluripotent stem cell expansion exist; however, it is difficult to control the thickness of cell aggregations in these systems, leading to increased cell death likely caused by limited diffusion of gases and nutrients into the aggregations. Here, we describe a scalable culture system using the cell fiber technology for the expansion of human induced pluripotent stem (iPS) cells. The cells were encapsulated and cultured within the core region of core-shell hydrogel microfibers, resulting in the formation of rod-shaped or fiber-shaped cell aggregations with sustained thickness and high viability. By encapsulating the cells with type I collagen, we demonstrated a long-term culture of the cells by serial passaging at a high expansion rate (14-fold in four days) while retaining its pluripotency. Therefore, our culture system could be used for large-scale expansion of human pluripotent stem cells for use in regenerative medicine.

Ikeda, M., et al. (2017). "Implantation of Induced Pluripotent Stem Cell-Derived Tracheal Epithelial Cells." Ann Otol Rhinol Laryngol **126**(7): 517-524.

OBJECTIVES: Compared with using autologous tissue, the use of artificial materials in the regeneration of tracheal defects is minimally invasive. However, this technique requires early epithelialization on the inner side of the artificial trachea. After differentiation from induced pluripotent stem cells (iPSCs), tracheal epithelial tissues may be used to produce artificial tracheas. Herein, we aimed to demonstrate that after differentiation from fluorescent protein-labeled iPSCs, tracheal epithelial tissues

survived in nude rats with tracheal defects. METHODS: Red fluorescent tdTomato protein was electroporated into mouse iPSCs to produce tdTomato-labeled iPSCs. Embryoid bodies derived from these iPSCs were then cultured in differentiation medium supplemented with growth factors, followed by culture on air-liquid interfaces for further differentiation into tracheal epithelium. The cells were implanted with artificial tracheas into nude rats with tracheal defects on day 26 of cultivation. On day 7 after implantation, the tracheas were exposed and examined histologically. RESULTS: Tracheal epithelial tissue derived from tdTomato-labeled iPSCs survived in the tracheal defects. Moreover, immunochemical analyses showed that differentiated tissues had epithelial structures similar to those of proximal tracheal tissues. CONCLUSIONS: After differentiation from iPSCs, tracheal epithelial tissues survived in rat bodies, warranting the use of iPSCs for epithelial regeneration in tracheal defects.

Ikemoto, Y., et al. (2017). "Somatic mosaicism containing double mutations in PTCH1 revealed by generation of induced pluripotent stem cells from nevoid basal cell carcinoma syndrome." J Med Genet **54**(8): 579-584.

BACKGROUND: Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterised by developmental defects and tumorigenesis, such as medulloblastomas and basal cell carcinomas, caused by mutations of the patched-1 (PTCH1) gene. In this article, we seek to demonstrate a mosaicism containing double mutations in PTCH1 in an individual with NBCCS. METHODS AND RESULTS: A de novo germline mutation of PTCH1 (c.272delG) was detected in a 31-year-old woman with NBCCS. Gene analysis of two out of four induced pluripotent stem cell (iPSC) clones established from the patient unexpectedly revealed an additional mutation, c.274delT. Deep sequencing confirmed a low-prevalence somatic mutation (5.5%-15.6% depending on the tissue) identical to the one found in iPSC clones. CONCLUSIONS: This is the first case of mosaicism unequivocally demonstrated in NBCCS. Furthermore, the mosaicism is unique in that the patient carries one normal and two mutant alleles. Because these mutations are located in close proximity, reversion error is likely to be involved in this event rather than a spontaneous mutation. In addition, this study indicates that gene analysis of iPSC clones can contribute to the detection of mosaicism containing a minor population carrying a second mutation.

Imagawa, K., et al. (2017). "Generation of a bile salt export pump deficiency model using patient-specific induced pluripotent stem cell-derived hepatocyte-like cells." Sci Rep **7**: 41806.

Bile salt export pump (BSEP) plays an important role in hepatic secretion of bile acids and its deficiency results in severe cholestasis and liver failure. Mutation of the ABCB11 gene encoding BSEP induces BSEP deficiency and progressive familial intrahepatic cholestasis type 2 (PFIC2). Because liver transplantation remains standard treatment for PFIC2, the development of a novel therapeutic option is desired. However, a well reproducible model, which is essential for the new drug development for PFIC2, has not been established. Therefore, we attempted to establish a PFIC2 model by using iPSC technology. Human iPSCs were generated from patients with BSEP-deficiency (BD-iPSC), and were differentiated into hepatocyte-like cells (HLCs). In the BD-iPSC derived HLCs (BD-HLCs), BSEP was not expressed on the cell surface and the biliary excretion capacity was significantly impaired. We also identified a novel mutation in the 5'-untranslated region of the ABCB11 gene that led to aberrant RNA splicing in BD-HLCs. Furthermore, to evaluate the drug efficacy, BD-HLCs were treated with 4-phenylbutyrate (4PBA). The membrane BSEP expression level and the biliary excretion capacity in BD-HLCs were rescued by 4PBA treatment. In summary, we succeeded in establishing a PFIC2 model, which may be useful for its pathophysiological analysis and drug development.

Irie, N. and M. A. Surani (2017). "Efficient Induction and Isolation of Human Primordial Germ Cell-Like Cells from Competent Human Pluripotent Stem Cells." Methods Mol Biol **1463**: 217-226.

We recently reported a robust and defined culture system for the specification of human primordial germ cell-like cells (hPGCLCs) from human pluripotent stem cells (hPSCs), both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) in vitro (Irie et al. Cell 160: 253-268, 2015). Similar attempts previously produced hPGCLCs from hPSCs at a very low efficiency, and the resulting cells were not fully characterized. A key step, which facilitated efficient hPGCLC specification from hPSCs, was the induction of a "competent" state for PGC fate via the medium containing a cocktail of four inhibitors. The competency of hPSCs can be maintained indefinitely and interchangeably with the conventional/low-competent hPSCs. Specification of hPGCLC occurs following sequential expression of key germ cell fate regulators, notably SOX17 and BLIMP1, as well as initiation of epigenetic resetting over 5 days. The hPGCLCs can be isolated using specific cell surface markers without the need for generating germ cell-specific reporter hPSC lines. This powerful method for the induction and isolation of hPGCLCs can be applied to both hESCs and iPSCs, which can be used for advances in human germ line biology.

Iseki, M., et al. (2017). "Muse Cells, Nontumorigenic Pluripotent-Like Stem Cells, Have Liver Regeneration Capacity Through Specific Homing and Cell Replacement in a Mouse Model of Liver Fibrosis." Cell Transplant **26**(5): 821-840.

Muse cells, a novel type of nontumorigenic pluripotent-like stem cells, reside in the bone marrow, skin, and adipose tissue and are collectable as cells positive for pluripotent surface marker SSEA-3. They are able to differentiate into cells representative of all three germ layers. The capacity of intravenously injected human bone marrow-derived Muse cells to repair an immunodeficient mouse model of liver fibrosis was evaluated in this study. The cells exhibited the ability to spontaneously differentiate into hepatoblast/hepatocyte lineage cells in vitro. They demonstrated a high migration capacity toward the serum and liver section of carbon tetrachloride-treated mice in vitro. In vivo, they specifically accumulated in the liver, but not in other organs except, to a lesser extent, in the lungs at 2 weeks after intravenous injection in the liver fibrosis model. After homing, Muse cells spontaneously differentiated in vivo into HepPar-1 (71.1 +/- 15.2%), human albumin (54.3 +/- 8.2%), and anti-trypsin (47.9 +/- 4.6%)-positive cells without fusing with host hepatocytes, and expressed mature functional markers such as human CYP1A2 and human Glc-6-Pase at 8 weeks after injection. Recovery in serum, total bilirubin, and albumin and significant attenuation of fibrosis were recognized with statistical differences between the Muse cell-transplanted group and the control groups, which received the vehicle or the same number of a non-Muse cell population of MSCs (MSCs in which Muse cells were eliminated). Thus, unlike ESCs and iPSCs, Muse cells are unique in their efficient migration and integration into the damaged liver after intravenous injection, nontumorigenicity, and spontaneous differentiation into hepatocytes, rendering induction into hepatocytes prior to transplantation unnecessary. They may repair liver fibrosis by two simple steps: expansion after collection from the bone marrow and intravenous injection. A therapeutic strategy such as this is feasible and may provide significant advancements toward liver regeneration in patients with liver disease.

Ishikura, Y., et al. (2016). "In Vitro Derivation and Propagation of Spermatogonial Stem Cell Activity from Mouse Pluripotent Stem Cells." Cell Rep **17**(10): 2789-2804.

The in vitro derivation and propagation of spermatogonial stem cells (SSCs) from pluripotent stem cells (PSCs) is a key goal in reproductive science. We show here that when aggregated with embryonic testicular somatic cells (reconstituted testes), primordial germ cell-like cells (PGCLCs) induced from mouse embryonic stem cells differentiate into spermatogonia-like cells in vitro and are expandable as cells that resemble germline stem cells (GSCs), a primary cell line with SSC activity. Remarkably, GSC-like cells (GSCLCs), but not PGCLCs, colonize adult testes and, albeit less effectively than GSCs, contribute to spermatogenesis and fertile offspring. Whole-genome analyses reveal that GSCLCs exhibit aberrant methylation at vulnerable regulatory elements, including those critical for spermatogenesis, which may restrain their spermatogenic potential. Our study establishes a strategy for the in vitro derivation of SSC activity from PSCs, which, we propose, relies on faithful epigenomic regulation.

Ishizuka, T. and Y. Watanabe (2014). "[Involvement of cell membrane receptors on proliferation and differentiation of pluripotent stem cells]." Nihon Yakurigaku Zasshi **144**(1): 13-16.

Itakura, G., et al. (2017). "Low immunogenicity of mouse induced pluripotent stem cell-derived neural stem/progenitor cells." Sci Rep **7**(1): 12996.

Resolving the immunogenicity of cells derived from induced pluripotent stem cells (iPSCs) remains an important challenge for cell transplant strategies that use banked allogeneic cells. Thus, we evaluated the immunogenicity of mouse fetal neural stem/progenitor cells (fetus-NSPCs) and iPSC-derived neural stem/progenitor cells (iPSC-NSPCs) both in vitro and in vivo. Flow cytometry revealed the low expression of immunological surface antigens, and these cells survived in all mice when transplanted syngeneically into subcutaneous tissue and the spinal cord. In contrast, an allogeneic transplantation into subcutaneous tissue was rejected in all mice, and allogeneic cells transplanted into intact and injured spinal cords survived for 3 months in approximately 20% of mice. In addition, cell survival was increased after co-treatment with an immunosuppressive agent. Thus, the immunogenicity and post-transplantation immunological dynamics of iPSC-NSPCs resemble those of fetus-NSPCs.

Itoh, M., et al. (2016). "Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC1." Stem Cell Res **17**(1): 22-24.

Expanded human T cells from a Japanese healthy male were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, WT-iPSC1, was confirmed by the expressions of stem

cell markers and the differentiation capability into three germ layers. WT-iPSC1 may be a useful cell resource as a normal control for the comparative study using disease-specific iPSCs.

Itoh, M., et al. (2016). "Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC2." Stem Cell Res **17**(1): 16-18.

Expanded human T cells from a Japanese healthy male were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, WT-iPSC2, was confirmed by the expressions of stem cell markers and the differentiation capability into three germ layer. WT-iPSC2 may be a useful cell resource as a normal control for the comparative study using disease-specific iPSCs.

Itoh, M., et al. (2016). "Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a healthy individual: WT-iPSC4." Stem Cell Res **17**(1): 19-21.

Expanded human T cells from a Japanese healthy male were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, WT-iPSC4, was confirmed by the expressions of stem cell markers and the differentiation capability into three germ layer. WT-iPSC4 may be a useful cell resource as a normal control for the comparative study using disease-specific iPSCs.

Itoh, M., et al. (2016). "Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a patient with lymphedema-distichiasis syndrome (LDS) carrying an insertion-deletion complex mutation in the FOXC2 gene." Stem Cell Res **16**(3): 611-613.

Expanded human T cells from a Japanese male with lymphedema-distichiasis syndrome (LDS) were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, LDS-iPSC8, was confirmed by the expression of stem cell markers and the differentiation capability into three germ layers. LDS-iPSC8 may be a useful cell resource for the establishment of in vitro LDS modeling and the study for vascular and lymph vessel development.

Itoh, M., et al. (2016). "Integration-free T cell-derived human induced pluripotent stem cells (iPSCs) from a patient with recessive dystrophic epidermolysis bullosa (RDEB) carrying two compound heterozygous mutations in the COL7A1 gene." Stem Cell Res **17**(1): 32-35.

Expanded human T cells from a Japanese female with recessive dystrophic epidermolysis bullosa (RDBE) were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, RDEB-iPSC26, was confirmed by the expressions of stem cell markers and the differentiation capability into three germ layer. RDEB-iPSC26 may be a useful cell resource for the establishment of in vitro RDEB modeling and the study for developing gene and cell therapy.

Iwamoto, H., et al. (2013). "[Cancer vaccine therapy using genetically modified induced pluripotent stem cell-derived dendritic cells expressing the TAA gene]." Gan To Kagaku Ryoho **40**(12): 1575-1577.

It is generally accepted that the difficulty in obtaining a sufficient number of functional dendritic cells (DCs) poses a serious problem in DC-based immunotherapy. Therefore, we used induced pluripotent stem (iPS) cell-derived DCs (iPSDCs) instead. If the therapeutic efficacy of iPSDCs was equivalent to that of bone marrow-derived DCs(BMDCs), then the above-mentioned problems may be solved. In this study, we generated iPSDCs from iPS cells and compared their capacity to mature and migrate to the regional lymph nodes with that of BMDCs. We adenovirally transduced the hgp100 gene, which codes for a natural tumor antigen, into the DCs and immunized the mice with these genetically modified DCs. The cytotoxic activity of CD8(+) cytotoxic T lymphocytes(CTLs) was assayed using a 51Cr-release assay. The therapeutic efficacy of the vaccination was examined in a subcutaneous tumor model. Our results demonstrated that iPSDCs equaled BMDCs in terms of their maturation and migration capacity. Furthermore, hgp100-specific CTLs were generated in mice that were immunized with the genetically modified iPSDCs. These CTLs exhibited a high level of cytotoxicity against B16 cells, which is similar to that exhibited by CTLs generated in BMDCs immunized mice. Moreover, vaccination with genetically modified iPSDCs elicited a high level of therapeutic efficacy equaling that of vaccination with BMDCs. This study clarified experimentally that genetically modified iPSDCs are equivalent to BMDCs in terms of tumor-associated antigen-specific therapeutic antitumor immunity. This vaccination

strategy may therefore be useful for future clinical application as a cancer vaccine.

Iwao, T. (2018). "[Development of an In Vitro System for Evaluating Intestinal Drug Disposition Using Human Induced Pluripotent Stem Cell-Derived Intestinal Epithelial Cells]." Yakugaku Zasshi **138**(10): 1241-1247.

Tissues of the small intestine are crucial to understanding drug disposition because these tissues regulate the bioavailability of drugs. However, no evaluation system is currently available for precise and comprehensive analysis of intestinal pharmacokinetics. To address this, functional intestinal epithelial cells were generated from human induced pluripotent stem (iPS) cells for use in pharmacokinetic studies. An improved intestinal differentiation method was established by screening a variety of small molecule compounds against cells during differentiation. The mRNA expression levels of intestinal markers, drug transporters, and CYP3A4 were found to increase following treatment with compounds that act as inhibitors of mitogen-activated protein kinase, DNA methyltransferase, and transforming growth factor-beta. Therefore, we inferred that these compounds enhanced differentiation into intestinal epithelial cells. The differentiated intestinal epithelial cells in the presence of these compounds possessed drug-metabolizing enzyme activities, such as those of CYPs, UDP-glucuronosyltransferase, and sulfotransferase. In addition, these cells had the ability to induce CYP3A4 in the presence of 1alpha,25-dihydroxyvitamin D3. The differentiated intestinal epithelial cells seeded on cell culture inserts formed loose-tight junctions, similar to those in the human small intestine, rather than Caco-2 cells. The cells exhibited polarity, such as apical and basal sides. We also demonstrated that the uptake and efflux transport activities in the cells occurred via peptide transporter and breast cancer resistance protein, respectively. Taken together, it was suggested that human iPS cell-derived intestinal epithelial cells are pharmacokinetically functional, and represent a promising model system for pharmacokinetic studies of drug candidates.

Iwata, Y., et al. (2017). "High-Content Assay Multiplexing for Vascular Toxicity Screening in Induced Pluripotent Stem Cell-Derived Endothelial Cells and Human Umbilical Vein Endothelial Cells." Assay Drug Dev Technol **15**(6): 267-279.

Endothelial cells (ECs) play a major role in blood vessel formation and function. While there is longstanding evidence for the potential of chemical exposures to adversely affect EC function and vascular development, the hazard potential of chemicals with respect to vascular effects is not routinely evaluated in safety assessments. Induced pluripotent stem cell (iPSC)-derived ECs promise to provide a physiologically relevant, organotypic culture model that is amenable for high-throughput (HT) EC toxicant screening and may represent a viable alternative to traditional in vitro models, including human umbilical vein endothelial cells (HUVECs). To evaluate the utility of iPSC-ECs for multidimensional HT toxicity profiling of chemicals, both iPSC-ECs and HUVECs were exposed to selected positive (angiogenesis inhibitors, cytotoxic agents) and negative compounds in concentration response for either 16 or 24 h in a 384-well plate format. Furthermore, chemical effects on vascularization were quantified using EC angiogenesis on biological (Geltrex) and synthetic (SP-105 angiogenesis hydrogel) extracellular matrices. Cellular toxicity was assessed using high-content live cell imaging and the CellTiter-Glo((R)) assay. Assay performance indicated good to excellent assay sensitivity and reproducibility for both cell types investigated. Both iPSC-derived ECs and HUVECs formed tube-like structures on Geltrex and hydrogel, an effect that was inhibited by angiogenesis inhibitors and cytotoxic agents in a concentration-dependent manner. The quality of HT assays in HUVECs was generally higher than that in iPSC-ECs. Altogether, this study demonstrates the capability of ECs for comprehensive assessment of the biological effects of chemicals on vasculature in a HT compatible format.

Iyer, D., et al. (2016). "Robust derivation of epicardium and its differentiated smooth muscle cell progeny from human pluripotent stem cells." Development **143**(5): 904.

Iyer, D., et al. (2015). "Robust derivation of epicardium and its differentiated smooth muscle cell progeny from human pluripotent stem cells." Development **142**(8): 1528-1541.

The epicardium has emerged as a multipotent cardiovascular progenitor source with therapeutic potential for coronary smooth muscle cell, cardiac fibroblast (CF) and cardiomyocyte regeneration, owing to its fundamental role in heart development and its potential ability to initiate myocardial repair in injured adult tissues. Here, we describe a chemically defined method for generating epicardium and epicardium-derived smooth muscle cells (EPI-SMCs) and CFs from human pluripotent stem cells (HPSCs) through an intermediate lateral plate mesoderm (LM) stage. HPSCs were initially differentiated to LM in the presence of FGF2 and high levels of BMP4. The LM was robustly differentiated to an epicardial lineage by activation of WNT, BMP and retinoic acid signalling pathways. HPSC-derived epicardium displayed enhanced expression of epithelial- and epicardium-

specific markers, exhibited morphological features comparable with human foetal epicardial explants and engrafted in the subepicardial space in vivo. The in vitro-derived epicardial cells underwent an epithelial-to-mesenchymal transition when treated with PDGF-BB and TGFbeta1, resulting in vascular SMCs that displayed contractile ability in response to vasoconstrictors. Furthermore, the EPI-SMCs displayed low density lipoprotein uptake and effective lowering of lipoprotein levels upon treatment with statins, similar to primary human coronary artery SMCs. Cumulatively, these findings suggest that HPSC-derived epicardium and EPI-SMCs could serve as important tools for studying human cardiogenesis, and as a platform for vascular disease modelling and drug screening.

Jacobson, E. F. and E. S. Tzanakakis (2017). "Human pluripotent stem cell differentiation to functional pancreatic cells for diabetes therapies: Innovations, challenges and future directions." J Biol Eng **11**: 21.

Recent advances in the expansion and directed pancreatogenic differentiation of human pluripotent stem cells (hPSCs) have intensified efforts to generate functional pancreatic islet cells, especially insulin-secreting beta-cells, for cell therapies against diabetes. However, the consistent generation of glucose-responsive insulin-releasing cells remains challenging. In this article, we first present basic concepts of pancreatic organogenesis, which frequently serves as a basis for engineering differentiation regimens. Next, past and current efforts are critically discussed for the conversion of hPSCs along pancreatic cell lineages, including endocrine beta-cells and alpha-cells, as well as exocrine cells with emphasis placed on the later stages of commitment. Finally, major challenges and future directions are examined, such as the identification of factors for in vivo maturation, large-scale culture and post processing systems, cell loss during differentiation, culture economics, efficiency, and efficacy and exosomes and miRNAs in pancreatic differentiation.

Jaroonwitchawan, T., et al. (2016). "Inhibition of FGF signaling accelerates neural crest cell differentiation of human pluripotent stem cells." Biochem Biophys Res Commun **481**(1-2): 176-181.

Neural crest (NC) is a transient population, arising during embryonic development and capable of differentiating into various somatic cells. The defects of neural crest development leads to neurocristopathy. Several signaling pathways were revealed their significance in NC cell specification. Fibroblast growth factor (FGF) is recognized as an important signaling during NC development, for instance Xenopus and avian; however, its contributions in human species are remained elusive. Here we used human pluripotent stem cells (hPSCs) to investigate the consequences of FGF inhibition during NC cell differentiation. The specific-FGF receptor inhibitor, SU5402, was used in this investigation. The inhibition of FGF did not found to affect the proliferation or death of hPSC-derived NC cells, but promoted hPSCs to commit NC cell fate. NC-specific genes, including PAX3, SLUG, and TWIST1, were highly upregulated, while hPSC genes, such as OCT4, and E-CAD, rapidly reduced upon FGF signaling blockage. Noteworthy, TFAP-2alpha, a marker of migratory NC cells, abundantly presented in SU5402-induced cells. This accelerated NC cell differentiation could be due to the activation of Notch signaling upon the blockage of ERK1/2 phosphorylation, since NICD was increased by SU5402. Altogether, this study proposed the contributions of FGF signaling in controlling human NC cell differentiation from hPSCs, the crosstalk between FGF and Notch, and might imply to the influences of FGF signaling in neurocristophatic diseases.

Javed, M. S., et al. (2014). "Generation of hepatocyte-like cells from human induced pluripotent stem (iPS) cells by co-culturing embryoid body cells with liver non-parenchymal cell line TWNT-1." J Coll Physicians Surg Pak **24**(2): 91-96.

OBJECTIVE: To generate a homogeneous population of patient-specific hepatocyte-like cells (HLCs) from human iPS cells those show the morphologic and phenotypic properties of primary human hepatocytes. STUDY DESIGN: An experimental study. PLACE AND DURATION OF STUDY: Department of Surgery, Okayama University, Graduate School of Medicine, Japan, from April to December 2011. METHODOLOGY: Human iPS cells were generated and maintained on ES qualified matrigel coated plates supplemented with mTeSR medium or alternatively on mitotically inactivated MEF feeder layer in DMEM/F12 medium containing 20% KOSR, 4ng/ml bFGF-2, 1 x 10-4 M 2-mercaptoethanol, 1 mmol/L NEAA, 2mM L-glutamine and 1% penicillin-streptomycin. iPS cells were differentiated to HLCs by sequential culture using a four step differentiation protocol: (I) Generation of embryoid bodies (EBs) in suspension culture; (II) Induction of definitive endoderm (DE) from 2 days old EBs by growth in human activin-A (100 ng/ml) and basic fibroblasts growth factor (bFGF2) (100 ng/ml) on matrigel coated plates; (III) Induction of hepatic progenitors by co-culture with non-parenchymal human hepatic stellate cell line (TWNT-1); and (IV) Maturation by culture in dexamethasone. Characterization was performed by RT-PCR and functional assays. RESULTS: The generated HLCs

showed microscopically morphological phenotype of human hepatocytes, expressed liver-specific genes (ASGPR, Albumin, AFP, Sox17, Fox A2), secreted human liver-specific proteins such as albumin, synthesized urea and metabolized ammonia. CONCLUSION: Functional HLCs were generated from human iPS cells, which could be used for autologus hepatocyte transplantation for liver failure and as in vitro model for determining the metabolic and toxicological properties of drug compounds.

Jeong, H. C., et al. (2017). "Technical approaches to induce selective cell death of pluripotent stem cells." Cell Mol Life Sci **74**(14): 2601-2611.

Despite the recent promising results of clinical trials using human pluripotent stem cell (hPSC)-based cell therapies for age-related macular degeneration (AMD), the risk of teratoma formation resulting from residual undifferentiated hPSCs remains a serious and critical hurdle for broader clinical implementation. To mitigate the tumorigenic risk of hPSC-based cell therapy, a variety of approaches have been examined to ablate the undifferentiated hPSCs based on the unique molecular properties of hPSCs. In the present review, we offer a brief overview of recent attempts at selective elimination of undifferentiated hPSCs to decrease the risk of teratoma formation in hPSC-based cell therapy.

Jerebtsova, M., et al. (2012). "HIV-1 Resistant CDK2-Knockdown Macrophage-Like Cells Generated from 293T Cell-Derived Human Induced Pluripotent Stem Cells." Biology (Basel) **1**(2): 175-195.

A major challenge in studies of human diseases involving macrophages is low yield and heterogeneity of the primary cells and limited ability of these cells for transfections and genetic manipulations. To address this issue, we developed a simple and efficient three steps method for somatic 293T cells reprogramming into monocytes and macrophage-like cells. First, 293T cells were reprogrammed into induced pluripotent stem cells (iPSCs) through a transfection-mediated expression of two factors, Oct-4 and Sox2, resulting in a high yield of iPSC. Second, the obtained iPSC were differentiated into monocytes using IL-3 and M-CSF treatment. And third, monocytes were differentiated into macrophage-like cells in the presence of M-CSF. As an example, we developed HIV-1-resistant macrophage-like cells from 293T cells with knockdown of CDK2, a factor critical for HIV-1 transcription. Our study provides a proof-of-principle approach that can be used to study the role of host cell factors in HIV-1 infection of human macrophages.

Ji, H., et al. (2017). "Application of induced pluripotent stem cells to model smooth muscle cell function in vascular diseases." Curr Opin Biomed Eng **1**: 38-44.

Vascular smooth muscle cells (SMC) play an essential role in remodeling the vasculature during disease progression. Induced pluripotent stem cells (iPSC) provide an attractive approach to obtain autologous SMC source for patient-specific disease modeling. Here we discuss the current methods to 1) derive functional SMC from iPSC, 2) model vascular diseases using SMC generated from patient-derived iPSC, and 3) modulate microenvironmental cues to enhance cellular differentiation and functionality and better mimic the physiological environment. We emphasize that continuous exploration of biomaterial technologies to engineer a more SMC-specific microenvironment will provide further insight on complex vascular diseases.

Ji, Z. and B. Tian (2009). "Reprogramming of 3' untranslated regions of mRNAs by alternative polyadenylation in generation of pluripotent stem cells from different cell types." PLoS One **4**(12): e8419.

BACKGROUND: The 3' untranslated regions (3'UTRs) of mRNAs contain cis elements involved in post-transcriptional regulation of gene expression. Over half of all mammalian genes contain multiple polyadenylation sites that lead to different 3'UTRs for a gene. Studies have shown that the alternative polyadenylation (APA) pattern varies across tissues, and is dynamically regulated in proliferating or differentiating cells. Generation of induced pluripotent stem (iPS) cells, in which differentiated cells are reprogrammed to an embryonic stem (ES) cell-like state, has been intensively studied in recent years. However, it is not known how 3'UTRs are regulated during cell reprogramming. METHODS/MAIN FINDINGS: Using a computational method that robustly examines APA across DNA microarray data sets, we analyzed 3'UTR dynamics in generation of iPS cells from different cell types. We found that 3'UTRs shorten during reprogramming of somatic cells, the extent of which depends on the type of source cell. By contrast, reprogramming of spermatogonial cells involves 3'UTR lengthening. The alternative polyadenylation sites that are highly responsive to change of cell state in generation of iPS cells are also highly regulated during embryonic development in opposite directions. Compared with other sites, they are more conserved, can lead to longer alternative 3'UTRs, and are associated with more cis elements for polyadenylation. Consistently, reprogramming of somatic cells and germ cells involves significant upregulation and downregulation, respectively, of mRNAs encoding polyadenylation factors, and RNA processing is one of the most significantly regulated

biological processes during cell reprogramming. Furthermore, genes containing target sites of ES cell-specific microRNAs (miRNAs) in different portions of 3'UTR are distinctively regulated during cell reprogramming, suggesting impact of APA on miRNA targeting. CONCLUSIONS/SIGNIFICANCE: Taken together, these findings indicate that reprogramming of 3'UTRs by APA, which result from regulation of both general polyadenylation activity and cell type-specific factors and can reset post-transcriptional gene regulatory programs in the cell, is an integral part of iPS cell generation, and the APA pattern can be a good biomarker for cell type and state, useful for sample classification. The results also suggest that perturbation of the mRNA polyadenylation machinery or RNA processing activity may facilitate generation of iPS cells.

Jiang, B., et al. (2015). "SIRT1 Overexpression Maintains Cell Phenotype and Function of Endothelial Cells Derived from Induced Pluripotent Stem Cells." Stem Cells Dev **24**(23): 2740-2745.

Endothelial cells (ECs) that are differentiated from induced pluripotent stem cells (iPSCs) can be used in establishing disease models for personalized drug discovery or developing patient-specific vascularized tissues or organoids. However, a number of technical challenges are often associated with iPSC-ECs in culture, including instability of the endothelial phenotype and limited cell proliferative capacity over time. Early senescence is believed to be the primary mechanism underlying these limitations. Sirtuin1 (SIRT1) is an NAD(+)-dependent deacetylase involved in the regulation of cell senescence, redox state, and inflammatory status. We hypothesize that overexpression of the SIRT1 gene in iPSC-ECs will maintain EC phenotype, function, and proliferative capacity by overcoming early cell senescence. SIRT1 gene was packaged into a lentiviral vector (LV-SIRT1) and transduced into iPSC-ECs at passage 4. Beginning with passage 5, iPSC-ECs exhibited a fibroblast-like morphology, whereas iPSC-ECs overexpressing SIRT1 maintained EC cobblestone morphology. SIRT1 overexpressing iPSC-ECs also exhibited a higher percentage of canonical markers of endothelia (LV-SIRT1 61.8% CD31(+) vs. LV-empty 31.7% CD31(+), $P < 0.001$; LV-SIRT1 46.3% CD144(+) vs. LV-empty 20.5% CD144(+), $P < 0.02$), with a higher nitric oxide synthesis, lower beta-galactosidase production indicating decreased senescence (3.4% for LV-SIRT1 vs. 38.6% for LV-empty, $P < 0.001$), enhanced angiogenesis, increased deacetylation activity, and higher proliferation rate. SIRT1 overexpressing iPSC-ECs continued to proliferate through passage 9 with high purity of EC-like characteristics, while iPSC-ECs without SIRT1 overexpression became senescent after passage 5. Taken together, SIRT1 overexpression in iPSC-ECs maintains EC phenotype, improves EC function, and extends cell lifespan, overcoming critical hurdles associated with the use of iPSC-ECs in translational research.

Jiang, G., et al. (2014). "Human transgene-free amniotic-fluid-derived induced pluripotent stem cells for autologous cell therapy." Stem Cells Dev **23**(21): 2613-2625.

The establishment of a reliable prenatal source of autologous, transgene-free progenitor cells has enormous potential in the development of regenerative-medicine-based therapies for infants born with devastating birth defects. Here, we show that a largely CD117-negative population of human amniotic fluid mesenchymal stromal cells (AF-MSCs) obtained from fetuses with or without prenatally diagnosed anomalies are readily abundant and have limited baseline differentiation potential when compared with bone-marrow-derived MSCs and other somatic cell types. Nonetheless, the AF-MSCs could be easily reprogrammed into induced pluripotent stem cells (iPSCs) using nonintegrating Sendai viral vectors encoding for OCT4, SOX2, KLF4, and cMYC. The iPSCs were virtually indistinguishable from human embryonic stem cells in multiple assays and could be used to generate a relatively homogeneous population of neural progenitors, expressing PAX6, SOX2, SOX3, Musashi-1, and PSA-NCAM, for potential use in neurologic diseases. Further, these neural progenitors showed engraftment potential in vivo and were capable of differentiating into mature neurons and astrocytes in vitro. This study demonstrates the usefulness of AF-MSCs as an excellent source for the generation of human transgene-free iPSCs ideally suited for autologous perinatal regenerative medicine applications.

Jin, S., et al. (2016). "A Novel Role for miR-1305 in Regulation of Pluripotency-Differentiation Balance, Cell Cycle, and Apoptosis in Human Pluripotent Stem Cells." Stem Cells **34**(9): 2306-2317.

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are defined as pluripotent in view of their self-renewal ability and potential to differentiate to cells of all three germ layers. Recent studies have indicated that microRNAs (miRNAs) play an important role in the maintenance of pluripotency and cell cycle regulation. We used a microarray based approach to identify miRNAs that were enriched in hESCs when compared to differentiated cells and at the same time showed significant expression changes between different phases of cell cycle. We identified 34 candidate miRNAs and performed functional studies on one of these, miR-1305, which showed the highest expression

change during cell cycle transition. Overexpression of miR-1305 induced differentiation of pluripotent stem cells, increased cell apoptosis and sped up G1/S transition, while its downregulation facilitated the maintenance of pluripotency and increased cell survival. Using target prediction software and luciferase based reporter assays we identified POLR3G as a downstream target by which miR-1305 regulates the fine balance between maintenance of pluripotency and onset of differentiation. Overexpression of POLR3G rescued pluripotent stem cell differentiation induced by miR-1305 overexpression. In contrast, knock-down of POLR3G expression abolished the miR-1305-knockdown mediated enhancement of pluripotency, thus validating its role as miR-1305 target in human pluripotent stem cells. Together our data point to an important role for miR-1305 as a novel regulator of pluripotency, cell survival and cell cycle and uncovers new mechanisms and networks by which these processes are intertwined in human pluripotent stem cells. Stem Cells 2016;34:2306-2317.

Joubin, K., et al. (2012). "The endothelial cell line bEnd.3 maintains human pluripotent stem cells." Stem Cells Dev **21**(12): 2312-2321.

Endothelial cells line blood vessels and coordinate many aspects of vascular biology. More recent work has shown that endothelial cells provide a key niche in vivo for neural stem cells. In vitro, endothelial cells secrete a factor that expands neural stem cells while inhibiting their differentiation. Here, we show that a transformed mouse endothelial cell line (bEnd.3) maintains human pluripotent stem cells in an undifferentiated state. bEnd.3 cells have a practical advantage over mouse embryonic fibroblasts for pluripotent stem cell maintenance since they can be expanded in vitro and engineered to express genes of interest. We demonstrate this capability by producing fluorescent and drug-resistant feeder cells. Further, we show that bEnd.3 secretes an activity that maintains human embryonic stem cells without direct contact.

Joyce, D., et al. (2018). "Induced pluripotent stem cells-derived myeloid-derived suppressor cells regulate the CD8(+) T cell response." Stem Cell Res **29**: 32-41.

Myeloid-derived suppressor cells (MDSCs) are markedly increased in cancer patients and tumor-bearing mice and promote tumor growth and survival by inhibiting host innate and adaptive immunity. In this study, we generated and characterized MDSCs from murine-induced pluripotent stem cells (iPSCs). The iPSCs were co-cultured with OP9 cells, stimulated with GM-CSF, and became morphologically heterologous under co-culturing with hepatic stellate cells. Allogeneic and OVA-specific antigen stimulation demonstrated that iPS-MDSCs have a T-cell regulatory function. Furthermore, a popliteal lymph node assay and autoimmune hepatitis model showed that iPS-MDSCs also regulate immune responsiveness in vivo and have a therapeutic effect against hepatitis. Taken together, our results demonstrated a method of generating functional MDSCs from iPSCs and highlighted the potential of iPS-MDSCs as a key cell therapy resource for transplantation and autoimmune diseases.

Jozefczuk, J., et al. (2011). "Comparative analysis of human embryonic stem cell and induced pluripotent stem cell-derived hepatocyte-like cells reveals current drawbacks and possible strategies for improved differentiation." Stem Cells Dev **20**(7): 1259-1275.

Hepatocytes derived from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) could provide a defined and renewable source of human cells relevant for cell replacement therapies and toxicology studies. However, before patient-specific iPSCs can be routinely used for these purposes, there is a dire need to critically compare these cells to the golden standard--hESCs. In this study, we aimed at investigating the differences and similarities at the transcriptional level between hepatocyte-like cells (HLCs) derived from both hESCs and iPSCs. Two independent protocols for deriving HLCs from hESCs and iPSCs were adopted and further characterization included immunocytochemistry, real-time (RT)-polymerase chain reaction, and in vitro functional assays. Comparative microarray-based gene expression profiling was conducted on these cells and compared to the transcriptomes of human fetal liver and adult liver progenitors. HLCs derived from hESCs and human iPSCs showed significant functional similarities, similar expression of genes important for liver physiology and common pathways. However, specific differences between the 2 cell types could be observed. For example, among the cytochrome P450 gene family, CYP19A1, CYP1A1, and CYP11A1 were enriched in hESC-derived HLCs, and CYP46A1 and CYP26A1 in iPSC-derived HLCs. HLCs derived from hESCs and human iPSCs exhibited broad similarities but as well meaningful differences. We identified common upregulated transcription factors, which might serve as a source for generating a cocktail of factors able to directly transdifferentiate somatic cells into HLCs. The findings may be vital to the refinement of protocols for the efficient derivation of functional patient-specific HLCs for regenerative and toxicology studies.

Julian, L. M., et al. (2017). "Human Pluripotent Stem Cell-Derived TSC2-Haploinsufficient Smooth Muscle Cells Recapitulate Features of Lymphangioleiomyomatosis." Cancer Res **77**(20): 5491-5502.

Lymphangioleiomyomatosis (LAM) is a progressive destructive neoplasm of the lung associated with inactivating mutations in the TSC1 or TSC2 tumor suppressor genes. Cell or animal models that accurately reflect the pathology of LAM have been challenging to develop. Here, we generated a robust human cell model of LAM by reprogramming TSC2 mutation-bearing fibroblasts from a patient with both tuberous sclerosis complex (TSC) and LAM (TSC-LAM) into induced pluripotent stem cells (iPSC), followed by selection of cells that resemble those found in LAM tumors by unbiased in vivo differentiation. We established expandable cell lines under smooth muscle cell (SMC) growth conditions that retained a patient-specific genomic TSC2(+/-) mutation and recapitulated the molecular and functional characteristics of pulmonary LAM cells. These include multiple indicators of hyperactive mTORC1 signaling, presence of specific neural crest and SMC markers, expression of VEGF-D and female sex hormone receptors, reduced autophagy, and metabolic reprogramming. Intriguingly, the LAM-like features of these cells suggest that haploinsufficiency at the TSC2 locus contributes to LAM pathology, and demonstrated that iPSC reprogramming and SMC lineage differentiation of somatic patient cells with germline mutations was a viable approach to generate LAM-like cells. The patient-derived SMC lines we have developed thus represent a novel cellular model of LAM that can advance our understanding of disease pathogenesis and develop therapeutic strategies against LAM. Cancer Res; 77(20); 5491-502. (c)2017 AACR.

Jung, D. W., et al. (2014). "Reprogram or reboot: small molecule approaches for the production of induced pluripotent stem cells and direct cell reprogramming." ACS Chem Biol **9**(1): 80-95.

Stem cell transplantation is a potential therapy for regenerative medicine, which aims to restore tissues damaged by trauma, aging, and diseases. Since its conception in the late 1990s, chemical biology has provided powerful and diverse small molecule tools for modulating stem cell function. Embryonic stem cells could be an ideal source for transplantation, but ethical concerns restrict their development for cell therapy. The seminal advance of induced pluripotent stem cell (iPSC) technology provided an attractive alternative to human embryonic stem cells. However, iPSCs are not yet considered an ideal stem cell source, due to limitations associated with the reprogramming process and their potential tumorigenic behavior. This is an area of research where chemical biology has made a significant contribution to facilitate the efficient production of high quality iPSCs and elucidate the biological mechanisms governing their phenotype. In this review, we summarize these advances and discuss the latest progress in developing small molecule modulators. Moreover, we also review a new trend in stem cell research, which is the direct reprogramming of readily accessible cell types into clinically useful cells, such as neurons and cardiac cells. This is a research area where chemical biology is making a pivotal contribution and illustrates the many advantages of using small molecules in stem cell research.

Jung, Y., et al. (2012). "Concise review: Induced pluripotent stem cell-derived mesenchymal stem cells: progress toward safe clinical products." Stem Cells **30**(1): 42-47.

Adult stem cell therapies have provided success for more than 50 years, through reconstitution of the hematopoietic system using bone marrow, umbilical cord blood, and mobilized peripheral blood transplantation. Mesenchymal stem cell (MSC)-mediated therapy is a fast-growing field that has proven safe and effective in the treatment of various degenerative diseases and tissue injuries. Since the first derivation of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), there has been impressive progress toward developing safe clinical applications from PSCs. Recent successes in transgene-free iPSC reprogramming have brought attention to the potential of clinical applications of these pluripotent cells, but key hurdles must be overcome, which are discussed in this review. Looking to the future, it could be advantageous to derive MSC from iPSC or human ESC in cases where genetic engineering is needed, since in the PSCs, clones with "safe harbor" vector integration could be selected, expanded, and differentiated. Here, we describe the status of the progress of the use of MSC and PSCs in clinical trials and analyze the challenges that should be overcome before iPSC-derived MSC therapy can be used widely in the clinic.

Juuti-Uusitalo, K., et al. (2013). "Aquaporin expression and function in human pluripotent stem cell-derived retinal pigmented epithelial cells." Invest Ophthalmol Vis Sci **54**(5): 3510-3519.

PURPOSE: Aquaporins (AQPs), a family of transmembrane water channel proteins, are essential for allowing passive water transport through retinal pigmented epithelial (RPE) cells. Even though human native RPE cells and immortalized human RPEs have been shown to express AQPs, the expression of AQPs during the differentiation in stem cell-derived RPE remains to be elucidated. METHODS: In human embryonic (hESCs) and induced pluripotent stem cells (hiPSCs)-derived RPE cells, the expression of several AQPs was determined by quantitative real-time PCR and the localization of AQP1 was assessed with

confocal microscopy. The functionality of AQP water channels was determined by cell volume assay in hESC-derived RPE cells. RESULTS: AQP1, AQP3, AQP4, AQP5, AQP6, AQP7, AQP10, AQP11, and AQP12 were expressed in hESC- and hiPSC-derived RPE cells. Furthermore, the expression of AQP1 and AQP11 genes were significantly upregulated during the maturation of both hESC and iPSC into RPE. Confocal microscopy shows the expression of AQP1 at the apical plasma membrane of polarized cobblestone hESC- and hiPSC-derived RPE cells. Lastly, aquaporin inhibitors significantly reduced AQP functionality in hESC-RPE cells. CONCLUSIONS: hESC-RPE and hiPSC-RPE cells express several AQP genes, which are functional in mature hESC-derived RPE cells. The localization of AQP1 on the apical plasma membrane in mature RPE cells derived from both hESC and hiPSC suggests its functionality. These data propose that hESC- and hiPSC-derived RPE cells, grown and differentiated under serum-free conditions, resemble their native counterpart in the human eye.

Kaichi, S., et al. (2010). "Cell line-dependent differentiation of induced pluripotent stem cells into cardiomyocytes in mice." Cardiovasc Res **88**(2): 314-323.

AIMS: Mouse and human fibroblasts can be directly reprogrammed to pluripotency by the ectopic expression of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) to yield induced pluripotent stem (iPS) cells. iPS cells can be generated even without the expression of c-Myc. The present study examined patterns of differentiation of mouse iPS cells into cardiomyocytes in three different cell lines reprogrammed by three or four factors. METHODS AND RESULTS: During the induction of differentiation on feeder-free gelatinized dishes, genes involved in cardiogenesis were expressed as in embryonic stem cells and myogenic contraction occurred in two iPS cell lines. However, in one iPS cell line (20D17) generated by four factors, the expression of cardiac-specific genes and the beating activity were extremely low. Treating iPS cells with trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, increased Nkx2.5 expression in all iPS cell lines. While the basal Nkx2.5 expression was very low in 20D17, the TSA-induced increase was the greatest. TSA also induced the expression of contractile proteins in 20D17. Furthermore, we demonstrated the increased mRNA level of Oct3/4 and nuclear protein level of HDAC4 in 20D17 compared with the other two iPS cell lines. DNA microarray analysis identified genes whose expression is up- or down-regulated in 20D17. CONCLUSIONS: Mouse iPS cells differentiate into cardiomyocytes in a cell line-dependent manner. TSA induces myocardial differentiation in mouse iPS cells and might be useful to overcome cell line variation in the differentiation efficiency.

Kaitsuka, T. and K. Tomizawa (2015). "Cell-Penetrating Peptide as a Means of Directing the Differentiation of Induced-Pluripotent Stem Cells." Int J Mol Sci **16**(11): 26667-26676.

Protein transduction using cell-penetrating peptides (CPPs) is useful for the delivery of large protein molecules, including some transcription factors. This method is safer than gene transfection methods with a viral vector because there is no risk of genomic integration of the exogenous DNA. Recently, this method was reported as a means for the induction of induced pluripotent stem (iPS) cells, directing the differentiation into specific cell types and supporting gene editing/correction. Furthermore, we developed a direct differentiation method to obtain a pancreatic lineage from mouse and human pluripotent stem cells via the protein transduction of three transcription factors, Pdx1, NeuroD, and MafA. Here, we discuss the possibility of using CPPs as a means of directing the differentiation of iPS cells and other stem cell technologies.

Kajiwara, K., et al. (2017). "Fetal Therapy Model of Myelomeningocele with Three-Dimensional Skin Using Amniotic Fluid Cell-Derived Induced Pluripotent Stem Cells." Stem Cell Reports **8**(6): 1701-1713.

Myelomeningocele (MMC) is a congenital disease without genetic abnormalities. Neurological symptoms are irreversibly impaired after birth, and no effective treatment has been reported to date. Only surgical repairs have been reported so far. In this study, we performed antenatal treatment of MMC with an artificial skin using induced pluripotent stem cells (iPSCs) generated from a patient with Down syndrome (AF-T21-iPSCs) and twin-twin transfusion syndrome (AF-TTTS-iPSCs) to a rat model. We manufactured three-dimensional skin with epidermis generated from keratinocytes derived from AF-T21-iPSCs and AF-TTTS-iPSCs and dermis of human fibroblasts and collagen type I. For generation of epidermis, we developed a protocol using Y-27632 and epidermal growth factor. The artificial skin was successfully covered over MMC defect sites during pregnancy, implying a possible antenatal surgical treatment with iPSC technology.

Kakuda, H., et al. (1996). "A novel human leukaemic cell line, CTS, has a t(6;11) chromosomal translocation and characteristics of pluripotent stem cells." Br J Haematol **95**(2): 306-318.

A novel human leukaemic cell line, designated CTS, was established from the peripheral

blood of a 13-year-old girl suffering from acute myeloblastic leukaemia (AML) in relapse. CTS cells expressed CD7, CD13, CD33, CD34 and HLA-DR antigens, and showed ultrastructural myeloperoxidase activity. In addition, CTS cells showed DNA rearrangements of the immunoglobulin heavy chain gene and the light kappa chain gene, and deletions of the T-cell receptor delta 1 gene. Cytogenetic analysis revealed a human female diploid karyotype with a t(6;11)(q27;q23) chromosomal translocation. Molecular studies demonstrated a DNA rearrangement of the MLL gene, the expression of a truncated 11.0 kb MLL mRNA and the detection of the MLL/AF-6 fusion transcript in CTS cells. To our knowledge, this cell line is the first report of a human leukaemic cell line with a t(6;11) chromosomal translocation. CTS cells showed no significant proliferative response to the cytokines, IL-2, IL-3, IL-6, IL-11, GM-CSF, G-CSF, EPO, SCF, but were induced to differentiate to the T-cell, B-cell, erythroid or megakaryocytic lineage in the presence of particular cytokines. This CTS cell line may provide a useful tool in the study of the oncogenesis of mixed lineage leukaemia with 11q23 abnormalities and for the analysis of growth and differentiation of pluripotent stem cells.

Kamada, M., et al. (2014). "Tumorigenic risk of human induced pluripotent stem cell explants cultured on mouse SNL76/7 feeder cells." Biochem Biophys Res Commun **453**(3): 668-673.

The potential for tumor formation from transplanted human induced pluripotent stem cell (hiPSC) derivatives represents a high risk in their application to regenerative medicine. We examined the genetic origin and characteristics of tumors, that were formed when 13 hiPSC lines, established by ourselves, and 201B7 hiPSC from Kyoto University were transplanted into severe combined immune-deficient (SCID) mice. Though teratomas formed in 58% of mice, five angiosarcomas, one malignant solitary fibrous tumor and one undifferentiated pleomorphic sarcoma formed in the remaining mice. Three malignant cell lines were established from the tumors, which were derived from mitomycin C (MMC)-treated SNL76/7 (MMC-SNL) feeder cells, as tumor development from fusion cells between MMC-SNL and hiPSCs was negative by genetic analysis. While parent SNL76/7 cells produced malignant tumors, neither MMC-SNL nor MMC-treated mouse embryo fibroblast (MEF) produced malignant tumors. When MMC-SNL feeder cells were co-cultured with hiPSCs, growing cell lines were generated, that expressed genes similar to the parent SNL76/7 cells. Thus, hiPSCs grown on MMC-SNL feeder cells have a high risk of generating feeder-derived malignant tumors. The possible mechanism(s) of growth restoration and the formation of multiple tumor types are discussed with respect of the interactions between MMC-SNL and hiPSC.

Kamada, M., et al. (2016). "Reversible transformation and de-differentiation of human cells derived from induced pluripotent stem cell teratomas." Hum Cell **29**(1): 1-9.

We first aimed to generate transformed cell lines from a human induced pluripotent stem cell (hiPSC)-teratoma, and then examined the tumorigenic risks of the differentiated cells from hiPSC explant, because hiPSC-derivatives give rise to tumors in immune-deficient mice when transplanted. The colonies isolated from sparse cultures of hiPSC-teratoma cells expressed NANOG and OCT3/4 strongly, and telomerase reverse transcriptase (TERT) weakly. However, soft agar assay demonstrated that only one of them generated colonies in the gel, though hiPSCs, hTERT-transfected immortal cells, and its oncogene-transfected cells did not form any colonies. Furthermore, none of colonies isolated from the soft agar gel on primary culture (passage 0) of teratoma cells, expressed NANOG and OCT3/4 in the expanded cultures. The second soft agar assay on the colony-derived cells was unexpectedly negative. The cumulative growth curve, telomere shortening, and senescence-associated beta-galactosidase (SA beta-gal) staining confirmed the mortality of these cells, suggesting their reversible transformation. By using medium for embryonic stem cell (ESC medium) after MCDB 131 (MCDB) medium, the differentiated culture cells derived from hiPSC-teratoma converted into the cells expressing undifferentiated marker proteins, which lost afterwords even in ESC medium with feeder SNL76/7. The reversibility of transformation and de-differentiation suggest that tumorigenic risks of differentiated cells arise when they are exposed to suitable niches in vivo. Thus, removal of only the undifferentiated cells from iPSC-derivatives before transplantation does not solve the problem. Elucidation of mechanisms of reversibility and control of epigenetic changes is discussed as a safety bottleneck for hiPSC therapy.

Kambal, A., et al. (2011). "Generation of HIV-1 resistant and functional macrophages from hematopoietic stem cell-derived induced pluripotent stem cells." Mol Ther **19**(3): 584-593.

Induced pluripotent stem cells (iPSCs) have radically advanced the field of regenerative medicine by making possible the production of patient-specific pluripotent stem cells from adult individuals. By developing iPSCs to treat HIV, there is the potential for generating a continuous supply of therapeutic cells for transplantation into HIV-infected patients. In this study,

we have used human hematopoietic stem cells (HSCs) to generate anti-HIV gene expressing iPSCs for HIV gene therapy. HSCs were dedifferentiated into continuously growing iPSC lines with four reprogramming factors and a combination anti-HIV lentiviral vector containing a CCR5 short hairpin RNA (shRNA) and a human/rhesus chimeric TRIM5alpha gene. Upon directed differentiation of the anti-HIV iPSCs toward the hematopoietic lineage, a robust quantity of colony-forming CD133(+) HSCs were obtained. These cells were further differentiated into functional end-stage macrophages which displayed a normal phenotypic profile. Upon viral challenge, the anti-HIV iPSC-derived macrophages exhibited strong protection from HIV-1 infection. Here, we demonstrate the ability of iPSCs to develop into HIV-1 resistant immune cells and highlight the potential use of iPSCs for HIV gene and cellular therapies.

Kamei, K., et al. (2010). "Microfluidic image cytometry for quantitative single-cell profiling of human pluripotent stem cells in chemically defined conditions." Lab Chip **10**(9): 1113-1119.

Microfluidic image cytometry (MIC) has been developed to study phenotypes of various hPSC lines by screening several chemically defined serum/feeder-free conditions. A chemically defined hPSC culture was established using 20 ng mL(-1) of bFGF on 20 microg mL(-1) of Matrigel to grow hPSCs over a week in an undifferentiated state. Following hPSC culture, we conducted quantitative MIC to perform a single cell profiling of simultaneously detected protein expression (OCT4 and SSEA1). Using clustering analysis, we were able to systematically compare the characteristics of various hPSC lines in different conditions.

Kandasamy, K., et al. (2015). "Prediction of drug-induced nephrotoxicity and injury mechanisms with human induced pluripotent stem cell-derived cells and machine learning methods." Sci Rep **5**: 12337.

The renal proximal tubule is a main target for drug-induced toxicity. The prediction of proximal tubular toxicity during drug development remains difficult. Any in vitro methods based on induced pluripotent stem cell-derived renal cells had not been developed, so far. Here, we developed a rapid 1-step protocol for the differentiation of human induced pluripotent stem cells (hiPSC) into proximal tubular-like cells. These proximal tubular-like cells had a purity of >90% after 8 days of differentiation and could be directly applied for compound screening. The nephrotoxicity prediction performance of the cells was determined by evaluating their responses to 30 compounds. The results were automatically determined using a machine learning algorithm called random forest. In this way, proximal tubular toxicity in humans could be predicted with 99.8% training accuracy and 87.0% test accuracy. Further, we studied the underlying mechanisms of injury and drug-induced cellular pathways in these hiPSC-derived renal cells, and the results were in agreement with human and animal data. Our methods will enable the development of personalized or disease-specific hiPSC-based renal in vitro models for compound screening and nephrotoxicity prediction.

Kane, N. M., et al. (2011). "Pluripotent stem cell differentiation into vascular cells: a novel technology with promises for vascular re(generation)." Pharmacol Ther **129**(1): 29-49.

Several types of stem and progenitor cells are currently under investigation for their potential to accomplish vascular regeneration. This review focuses on embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). We will discuss the technologies allowing for their derivation, culture expansion and maintenance in a pluripotent status. Moreover, both ESCs and iPSCs can be differentiated in endothelial cells (ECs) and mural cell, including vascular smooth muscle cells (VSMCs). Here, we will describe the involvements of growth factors (vascular endothelial growth factors-VEGFs-, platet-derived growth factors-PDGFs-), Wnt and Notch signal pathways, reactive oxygen species (ROS), histone deacetylases (HDACs), and microRNAs (miRNAs) in vascular cell differentiation from pluripotent stem cells. We will additionally describe the therapeutic potential of stem cells for vascular medicine.

Kaneko, S. (2016). "In Vitro Generation of Antigen-Specific T Cells from Induced Pluripotent Stem Cells of Antigen-Specific T Cell Origin." Methods Mol Biol **1393**: 67-73.

Induced pluripotent stem (iPS) cells derived from T lymphocyte (T-iPS cells) preserve the T cell receptor (TCR) alpha and beta gene rearrangements identical to the original T cell clone. Re-differentiated CD8 single positive alphabeta T cells from the T-iPS cells exhibited antigen-specific cytotoxicity, improved proliferative response, and elongation of telomere indicating rejuvenation of antigen specific T cell immunity in vitro. To regenerate antigen specific cytotoxic T lymphocytes (CTL), first, we have optimized a method for reprogramming-resistant CD8 T cell clones into T-iPS cells by using sendaiviral vectors. Second, we have optimized stepwise differentiation methods for inducing hematopoietic progenitor cells, T cell progenitors, and functionally matured CD8 single positive CTL. These protocols provide useful in vitro tools and models both for research of antigen-specific T cell immunotherapy and for research of normal and pathological thymopoiesis.

Kao, D. I. and S. Chen (2012). "Pluripotent stem cell-derived pancreatic beta-cells: potential for regenerative medicine in diabetes." Regen Med **7**(4): 583-593.

Diabetes mellitus, which affects 346 million people, is one of the leading causes of death worldwide. Pancreatic beta-cells, existing in the islets of Langerhans, play central roles in the progression of diabetes. An efficient strategy to produce functional pancreatic beta-cells is important for both transplantation therapy and disease modeling of diabetes. Human pluripotent stem cells, including human embryonic stem cells and induced pluripotent stem cells, provide unlimited starting materials to generate differentiated cells for regenerative studies. Significant progress has been made in human embryonic/induced pluripotent stem cell differentiation in the last several years. However, efficient generation of mature pancreatic beta-cells with complete functional capabilities has not yet been accomplished. Here, we review recent successes as well as the technical and theoretical challenges in the use of pluripotent stem cell-derived pancreatic beta-cells for disease modeling and replacement therapy of diabetes.

Kardel, M. D. and C. J. Eaves (2012). "Modeling human hematopoietic cell development from pluripotent stem cells." Exp Hematol **40**(8): 601-611.

Understanding the steps and cues that allow hematopoietic cells to be generated during development holds great clinical as well as biological interest. Analysis of these events in mice has provided many important insights into the processes involved, but features that might be unique to humans remain challenging to elucidate because they cannot be studied directly in vivo. Human embryonic stem or induced pluripotent stem cells offer attractive in vitro alternatives to analyze the process. Here we review recent efforts to develop defined and quantitative systems to address outstanding developmental questions against a background of what we know about the development of hematopoietic cells in the fetus and derived from mouse embryonic stem cells.

Kasuda, S., et al. (2011). "Expression of coagulation factors from murine induced pluripotent stem cell-derived liver cells." Blood Coagul Fibrinolysis **22**(4): 271-279.

A protocol to differentiate liver cells from induced pluripotent stem (iPS) cells is being established. However, the ability of these differentiated iPS cells to express liver-specific proteins, such as coagulation cascade and related factors, has yet to be assessed. This study evaluated whether liver-like populations differentiated from murine iPS cells gain the ability to produce coagulation-related factors. Following differentiation of murine iPS cells into hematopoietic-like and liver-like embryoid bodies, we assessed gene expression profiles for coagulation-related markers, including fibrinogen, factors II, V, VII, VIII, IX, X, XI, XII, and XIIIbeta, protein C, protein S, antithrombin, plasminogen, von Willebrand factor, and ADAMTS13 by real-time reverse transcription PCR. Liver-like embryoid bodies demonstrated strong expression levels of nearly all the coagulation-related genes assessed, compared with undifferentiated iPS cells and hematopoietic-like embryoid bodies. We also confirmed efficient translation and secretion of fibrinogen and albumin (hepatocyte-specific marker proteins) into the conditioned medium by these differentiated cells, suggesting successful differentiation of iPS cells into the liver lineage. These findings suggest that iPS cells can be differentiated into liver-like populations that express coagulation-related factors. Liver-like embryoid bodies may provide a source for cell-based therapies directed toward liver diseases, including coagulation factor deficiencies in the future.

Kato, R., et al. (2016). "Parametric analysis of colony morphology of non-labelled live human pluripotent stem cells for cell quality control." Sci Rep **6**: 34009.

Given the difficulties inherent in maintaining human pluripotent stem cells (hPSCs) in a healthy state, hPSCs should be routinely characterized using several established standard criteria during expansion for research or therapeutic purposes. hPSC colony morphology is typically considered an important criterion, but it is not evaluated quantitatively. Thus, we designed an unbiased method to evaluate hPSC colony morphology. This method involves a combination of automated non-labelled live-cell imaging and the implementation of morphological colony analysis algorithms with multiple parameters. To validate the utility of the quantitative evaluation method, a parent cell line exhibiting typical embryonic stem cell (ESC)-like morphology and an aberrant hPSC subclone demonstrating unusual colony morphology were used as models. According to statistical colony classification based on morphological parameters, colonies containing readily discernible areas of differentiation constituted a major classification cluster and were distinguishable from typical ESC-like colonies; similar results were obtained via classification based on global gene expression profiles. Thus, the morphological features of hPSC colonies are closely associated with cellular characteristics. Our quantitative evaluation method provides a biological definition of 'hPSC colony morphology', permits the non-invasive monitoring of hPSC conditions and is particularly useful for detecting variations in hPSC heterogeneity.

Kaushik, A. and D. Bhartiya (2018). "Pluripotent Very Small Embryonic-Like Stem Cells in Adult Testes - An Alternate Premise to Explain Testicular Germ Cell Tumors." Stem Cell Rev.

Developmental exposure to endocrine disruptors has resulted in the increased incidence of infertility and testicular germ cell tumors (T2GCT) in young men residing in developed countries. Unlike T1GCT (infants and young children) and T3GCT (aged men), T2GCT arise from CIS/GCNIS that develops from pre-CIS. Pre-CIS represents undifferentiated, growth-arrested gonocytes that persist in fetal testes due to endocrine disruption. However, whether pre-CIS truly exist, do CIS develop into T2GCT, why no CIS in T1GCT/T3GCT, why germ cell tumors (GCT) also occur along midline at extra-gonadal sites, why T1GCT show partial erasure and T2GCT show complete erasure of genomic imprints are open questions that are awaiting answers. We propose that rather than pre-CIS, pluripotent, very small embryonic-like stem cells (VSELs) get affected by exposure to endocrine disruption. Since VSELs are developmentally equivalent to primordial germ cells (PGCs), T2GCT cells show complete erasure of genomic imprints and CIS represents growth-arrested clonally expanding stem/progenitor cells. PGCs/VSELs migrate along the midline to various organs and this explains why GCT occur along the midline, T1GCT show partial erasure of imprints as they develop from migrating PGCs. T3GCT possibly reflects effects of aging due to compromised differentiation and expansion of pre-meiotic spermatocytes. Absent spermatogenesis in pre-pubertal and aged testes explains absence of CIS in T1GCT and T3GCT. Endocrine disruptors possibly alter epigenetic state of VSELs and thus rather than maintaining normal tissue homeostasis, VSELs undergo increased proliferation and compromised differentiation resulting in reduced sperm count, infertility and TGCT. This newly emerging understanding offers alternate premise to explain TGCT and warrants further exploration.

Kauts, M. L., et al. (2018). "Rapid Mast Cell Generation from Gata2 Reporter Pluripotent Stem Cells." Stem Cell Reports **11**(4): 1009-1020.

Mast cells are tissue-resident immune cells. Their overgrowth/overactivation results in a range of common distressing, sometimes life-threatening disorders, including asthma, psoriasis, anaphylaxis, and mastocytosis. Currently, drug discovery is hampered by use of cancer-derived mast cell lines or primary cells. Cell lines provide low numbers of mature mast cells and are not representative of in vivo mast cells. Mast cell generation from blood/bone marrow gives poor reproducibility, requiring 8-12 weeks of culture. Here we report a method for the rapid/robust production of mast cells from pluripotent stem cells (PSCs). An advantageous Gata2Venus reporter enriches mast cells and progenitors as they differentiate from PSCs. Highly proliferative mouse mast cells and progenitors emerge after 2 weeks. This method is applicable for rapid human mast cell generation, and could enable the production of sufficient numbers of physiologically relevant human mast cells from patient induced PSCs for the study of mast cell-associated disorders and drug discovery.

Kawamura, T., et al. (2014). "N-glycans: phenotypic homology and structural differences between myocardial cells and induced pluripotent stem cell-derived cardiomyocytes." PLoS One **9**(10): e111064.

Cell surface glycans vary widely, depending on cell properties. We hypothesized that glycan expression on induced pluripotent stem cells (iPSCs) might change during cardiomyogenic differentiation toward the myocardial phenotype. N-glycans were isolated from iPSCs, iPSC-derived cardiomyocytes (iPSC-CM), and original C57BL/6 mouse myocardium (Heart). Their structures were analyzed by a mapping technique based on HPLC elution times and MALDI-TOF/MS spectra. Sixty-eight different N-glycans were isolated; the structures of 60 of these N-glycans were identified. The quantity of high-mannose type (immature) N-glycans on the iPSCs decreased with cardiomyogenic differentiation, but did not reach the low levels observed in the heart. We observed a similar reduction in neutral N-glycans and an increase in fucosylated or sialyl N-glycans. Some structural differences were detected between iPSC-CM and Heart. No N-glycolyl neuraminic acid (NeuGc) structures were detected in iPSC-CM, whereas the heart contained numerous NeuGc structures, corresponding to the expression of cytidine monophosphate-N-acetylneuraminic acid hydroxylase. Furthermore, several glycans containing Galalpha1-6 Gal, rarely identified in the other cells, were detected in the iPSC-CM. The expression of N-glycan on murine iPSCs changed toward the myocardial phenotype during cardiomyogenic differentiation, leaving the structural differences of NeuGc content or Galalpha1-6 Gal structures. Further studies will be warranted to reveal the meaning of the difference of N-glycans between the iPSC-CM and the myocardium.

Keller, K. C., et al. (2014). "Suspension culture of pluripotent stem cells: effect of shear on stem cell fate." Crit Rev Eukaryot Gene Expr **24**(1): 1-13.

Despite significant promise, the routine usage of suspension cell culture to manufacture stem cell-derived differentiated cells has progressed slowly. Suspension culture is an innovative way of either

expanding or differentiating cells and sometimes both are combined into a single bioprocess. Its advantages over static 2D culturing include a homogeneous and controllable culture environment and producing a large quantity of cells in a fraction of time. This feature makes suspension cell culture ideal for use in stem cell research and eventually ideal in the large-scale production of differentiated cells for regenerative medicine. Because of their tremendous differentiation capacities and unlimited growth properties, pluripotent stem cells (PSCs) in particular are considered potential sources for future cell-replacement therapies. Currently, expansion of PSCs is accomplished in 2D, which only permits a limited amount of cell growth per culture flask before cells need to be passaged. However, before stem cells can be applied clinically, several aspects of their expansion, such as directed growth, but also differentiation, need to be better controlled. This review will summarize recent advantages in suspension culture of PSCs, while at the same time highlighting current challenges.

Kempf, H., et al. (2016). "Bulk cell density and Wnt/TGFbeta signalling regulate mesendodermal patterning of human pluripotent stem cells." Nat Commun **7**: 13602.

In vitro differentiation of human pluripotent stem cells (hPSCs) recapitulates early aspects of human embryogenesis, but the underlying processes are poorly understood and controlled. Here we show that modulating the bulk cell density (BCD: cell number per culture volume) deterministically alters anteroposterior patterning of primitive streak (PS)-like priming. The BCD in conjunction with the chemical WNT pathway activator CHIR99021 results in distinct paracrine microenvironments codifying hPSCs towards definitive endoderm, precardiac or presomitic mesoderm within the first 24 h of differentiation, respectively. Global gene expression and secretome analysis reveals that TGFss superfamily members, antagonist of Nodal signalling LEFTY1 and CER1, are paracrine determinants restricting PS progression. These data result in a tangible model disclosing how hPSC-released factors deflect CHIR99021-induced lineage commitment over time. By demonstrating a decisive, functional role of the BCD, we show its utility as a method to control lineage-specific differentiation. Furthermore, these findings have profound consequences for inter-experimental comparability, reproducibility, bioprocess optimization and scale-up.

Kim, B. J., et al. (2017). "Modeling Group B Streptococcus and Blood-Brain Barrier Interaction by Using Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells." mSphere **2**(6).

Bacterial meningitis is a serious infection of the central nervous system (CNS) that occurs after bacteria interact with and penetrate the blood-brain barrier (BBB). The BBB is comprised of highly specialized brain microvascular endothelial cells (BMECs) that function to separate the circulation from the CNS and act as a formidable barrier for toxins and pathogens. Certain bacteria, such as Streptococcus agalactiae (group B Streptococcus [GBS]), possess the ability to interact with and penetrate the BBB to cause meningitis. Modeling bacterial interaction with the BBB in vitro has been limited to primary and immortalized BMEC culture. While useful, these cells often do not retain BBB-like properties, and human primary cells have limited availability. Recently, a human induced pluripotent stem cell (iPSC)-derived BMEC model has been established that is readily renewable and retains key BBB phenotypes. Here, we sought to evaluate whether the iPSC-derived BMECs were appropriate for modeling bacterial interaction with the BBB. Using GBS as a model meningeal pathogen, we demonstrate that wild-type GBS adhered to, invaded, and activated the iPSC-derived BMECs, while GBS mutants known to have diminished BBB interaction were attenuated in the iPSC-derived model. Furthermore, bacterial infection resulted in the disruption of tight junction components ZO-1, occludin, and claudin-5. Thus, we show for the first time that the iPSC-derived BBB model can be utilized to study BBB interaction with a bacterial CNS pathogen. IMPORTANCE Here for the first time, human iPSC-derived BMECs were used to model bacterial interaction with the BBB. Unlike models previously used to study these interactions, iPSC-derived BMECs possess robust BBB properties, such as the expression of complex tight junctions that are key components for the investigation of bacterial effects on the BBB. Here, we demonstrated that GBS interacts with the iPSC-derived BMECs and specifically disrupts these tight junctions. Thus, using this BBB model may allow researchers to uncover novel mechanisms of BBB disruption during meningitis that are inaccessible to immortalized or primary cell models that lack substantial tight junctions.

Kim, E. M., et al. (2017). "Induced pluripotent stem cell-derived gamete-associated proteins incite rejection of induced pluripotent stem cells in syngeneic mice." Immunology **151**(2): 191-197.

The safety of induced pluripotent stem cells (iPSCs) in autologous recipients has been questioned after iPSCs, but not embryonic stem cells (ESCs), were reported to be rejected in syngeneic mice. This important topic has remained controversial because there has not been a mechanistic explanation for this phenomenon. Here, we hypothesize that iPSCs, but not

ESCs, readily differentiate into gamete-forming cells that express meiotic antigens normally found in immune-privileged gonads. Because peripheral blood T cells are not tolerized to these antigens in the thymus, gamete-associated-proteins (GAPs) sensitize T cells leading to rejection. Here, we provide evidence that GAPs expressed in iPSC teratomas, but not in ESC teratomas, are responsible for the immunological rejection of iPSCs. Furthermore, silencing the expression of Stra8, 'the master regulator of meiosis', in iPSCs, using short hairpin RNA led to significant abrogation of the rejection of iPSCs, supporting our central hypothesis that GAPs expressed after initiation of meiosis in iPSCs were responsible for rejection. In contrast to iPSCs, iPSC-derivatives, such as haematopoietic progenitor cells, are able to engraft long-term into syngeneic recipients because they no longer express GAPs. Our findings, for the first time, provide a unifying explanation of why iPSCs, but not ESCs, are rejected in syngeneic recipients, ending the current controversy on the safety of iPSCs and their derivatives.

Kim, H. M., et al. (2016). "Xeno-sensing activity of the aryl hydrocarbon receptor in human pluripotent stem cell-derived hepatocyte-like cells." Sci Rep **6**: 21684.

Although hepatocyte-like cells derived from human pluripotent stem cells (hPSC-HLCs) are considered a promising model for predicting hepatotoxicity, their application has been restricted because of the low activity of drug metabolizing enzymes (DMEs). Here we found that the low expression of xenobiotic receptors (constitutive androstane receptor, CAR; and pregnane X receptor, PXR) contributes to the low activity of DMEs in hPSC-HLCs. Most CAR- and PXR-regulated DMEs and transporters were transcriptionally down-regulated in hPSC-HLC. Transcriptional expression of CAR and PXR was highly repressed in hPSC-HLCs, whereas mRNA levels of aryl hydrocarbon receptor (AHR) were comparable to those of adult liver. Furthermore, ligand-induced transcriptional activation was observed only at AHR in hPSC-HLCs. Bisulfite sequencing analysis demonstrated that promoter hypermethylation of CAR and PXR was associated with diminished transcriptional activity in hPSC-HLCs. Treatment with AHR-selective ligands increased the transcription of AHR-dependent target genes by direct AHR-DNA binding at the xenobiotic response element. In addition, an antagonist of AHR significantly inhibited AHR-dependent target gene expression. Thus, AHR may function intrinsically as a xenosensor as well as a ligand-dependent transcription factor in hPSC-HLCs. Our results indicate that hPSC-HLCs can be used to screen toxic substances related to AHR signaling and to identify potential AHR-targeted therapeutics.

Kim, H. S., et al. (2017). "Schwann Cell Precursors from Human Pluripotent Stem Cells as a Potential Therapeutic Target for Myelin Repair." Stem Cell Reports **8**(6): 1714-1726.

Schwann cells play a crucial role in successful nerve repair and regeneration by supporting both axonal growth and myelination. However, the sources of human Schwann cells are limited both for studies of Schwann cell development and biology and for the development of treatments for Schwann cell-associated diseases. Here, we provide a rapid and scalable method to produce self-renewing Schwann cell precursors (SCPs) from human pluripotent stem cells (hPSCs), using combined sequential treatment with inhibitors of the TGF-beta and GSK-3 signaling pathways, and with neuregulin-1 for 18 days under chemically defined conditions. Within 1 week, hPSC-derived SCPs could be differentiated into immature Schwann cells that were functionally confirmed by their secretion of neurotrophic factors and their myelination capacity in vitro and in vivo. We propose that hPSC-derived SCPs are a promising, unlimited source of functional Schwann cells for treating demyelination disorders and injuries to the peripheral nervous system.

Kim, I. G., et al. (2018). "Mechanotransduction of human pluripotent stem cells cultivated on tunable cell-derived extracellular matrix." Biomaterials **150**: 100-111.

Cell-derived matrices (CDM) are becoming an attractive alternative to conventional biological scaffolding platforms due to its unique ability to closely recapitulate a native extracellular matrix (ECM) de novo. Although cell-substrate interactions are recognized to be principal in regulating stem cell behavior, very few studies have documented the acclimation of human pluripotent stem cells (hPSCs) on pristine and altered cell-derived matrices. Here, we investigate crosslink-induced mechanotransduction of hPSCs cultivated on decellularized fibroblast-derived matrices (FDM) to explore cell adhesion, growth, migration, and pluripotency in various biological landscapes. The results showed either substrate-mediated induction or inhibition of the Epithelial-Mesenchymal-Transition (EMT) program, strongly suggesting that FDM stiffness can be a dominant factor in mediating hPSC plasticity. We further propose an optimal FDM substratum intended for long-term hPSC cultivation in a feeder-free niche-like microenvironment. This study carries significant implications for hPSC cultivation and encourages more in-depth studies towards the fundamentals of hPSC-CDM interactions.

Kim, J. H., et al. (2017). "Report of the International Stem Cell Banking Initiative Workshop Activity: Current Hurdles and Progress in Seed-Stock Banking of Human Pluripotent Stem Cells." Stem Cells Transl Med **6**(11): 1956-1962.

This article summarizes the recent activity of the International Stem Cell Banking Initiative (ISCBI) held at the California Institute for Regenerative Medicine (CIRM) in California (June 26, 2016) and the Korean National Institutes for Health in Korea (October 19-20, 2016). Through the workshops, ISCBI is endeavoring to support a new paradigm for human medicine using pluripotent stem cells (hPSC) for cell therapies. Priority considerations for ISCBI include ensuring the safety and efficacy of a final cell therapy product and quality assured source materials, such as stem cells and primary donor cells. To these ends, ISCBI aims to promote global harmonization on quality and safety control of stem cells for research and the development of starting materials for cell therapies, with regular workshops involving hPSC banking centers, biologists, and regulatory bodies. Here, we provide a brief overview of two such recent activities, with summaries of key issues raised. Stem Cells Translational Medicine 2017;6:1956-1962.

Kim, J. S., et al. (2014). "Conversion of partially reprogrammed cells to fully pluripotent stem cells is associated with further activation of stem cell maintenance- and gamete generation-related genes." Stem Cells Dev **23**(21): 2637-2648.

Somatic cells are reprogrammed to induced pluripotent stem cells (iPSCs) by overexpression of a combination of defined transcription factors. We generated iPSCs from mouse embryonic fibroblasts (with Oct4-GFP reporter) by transfection of pCX-OSK-2A (Oct4, Sox2, and Klf4) and pCX-cMyc vectors. We could generate partially reprogrammed cells (XiPS-7), which maintained more than 20 passages in a partially reprogrammed state; the cells expressed Nanog but were Oct4-GFP negative. When the cells were transferred to serum-free medium (with serum replacement and basic fibroblast growth factor), the XiPS-7 cells converted to Oct4-GFP-positive iPSCs (XiPS-7c, fully reprogrammed cells) with ESC-like properties. During the conversion of XiPS-7 to XiPS-7c, we found several clusters of slowly reprogrammed genes, which were activated at later stages of reprogramming. Our results suggest that partial reprogrammed cells can be induced to full reprogramming status by serum-free medium, in which stem cell maintenance- and gamete generation-related genes were upregulated. These long-term expandable partially reprogrammed cells can be used to verify the mechanism of reprogramming.

Kim, K., et al. (2011). "Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells." Nat Biotechnol **29**(12): 1117-1119.

We compared bona fide human induced pluripotent stem cells (iPSCs) derived from umbilical cord blood (CB) cells and neonatal keratinocytes (K). As a consequence of both incomplete erasure of tissue-specific methylation and aberrant de novo methylation, CB-iPSCs and K-iPSCs were distinct in genome-wide DNA methylation profiles and differentiation potential. Extended passage of some iPSC clones in culture did not improve their epigenetic resemblance to embryonic stem cells, implying that some human iPSCs retain a residual 'epigenetic memory' of their tissue of origin.

Kim, M. H. and M. Kino-oka (2014). "Switching between self-renewal and lineage commitment of human induced pluripotent stem cells via cell-substrate and cell-cell interactions on a dendrimer-immobilized surface." Biomaterials **35**(22): 5670-5678.

Understanding mechanisms that govern cell fate determination of human induced pluripotent stem cells (hiPSCs) could assist in maintenance of the undifferentiated state during cell expansion. We used polyamidoamine dendrimer surfaces with first-generation (G1), third-generation (G3) and fifth-generation (G5) of dendron structure in cultures of hiPSCs with SNL feeder cells. Cells on the G1 surface formed tightly packed colony with close cell-cell contacts during division and migration; those on the G3 surface exhibited loose or dispersed colony pattern by enhanced migration. On the G5 surface, formation of aggregated colony with ring-like structures occurred spontaneously. We found that the substrate-adsorbed fibronectin and feeder cell-secreted fibronectin appeared elevated levels with the varied generation numbers of dendrimer surfaces. This subsequently resulted in cell migration and in activation of paxillin of hiPSCs. Location-dependent expression of Rac1 induced rearrangement of E-cadherin-mediated cell-cell interactions on dendrimer surfaces, and was associated with alterations in the cell and colony morphology, and migratory behavior. Furthermore, caspase-3 occurred in apoptotic cells on dendrimer surfaces, concomitant with the loss of E-cadherin-mediated cell-cell interactions. Cells on the G1 surface were maintained in an undifferentiated state, while those on the G5 surface exhibited the early commitment to differentiation toward endodermal fates. We conclude that morphological changes associated with altered migration on the dendrimer surfaces were responsible for the coordinated regulation of balance between cell-cell and cell-substrate interactions, thereby switching their transition from self-renewal state to early endoderm differentiation in hiPSCs.

Kim, M. H. and M. Kino-oka (2015). "Maintenance of an undifferentiated state of human induced pluripotent stem cells through migration-dependent regulation of the balance between cell-cell and cell-substrate interactions." J Biosci Bioeng **119**(6): 617-622.

We present an outlook on the current strategies for maintaining and culturing human induced pluripotent stem cells (hiPSCs) in an undifferentiated state without affecting their pluripotency. First, cell structures and function are described in relation to interactions between hiPSCs and their surroundings. Second, the phenomenon of spontaneous deviation from undifferentiated hiPSCs in cultures with feeder cells is addressed, with a summary of current topics that are of particular interest to our studies. The key regulatory factors that can contribute to the growth and maintenance of undifferentiated hiPSCs are also discussed, with a summary of recent work toward a culture strategy to control hiPSC fate through balancing cell-cell and cell-substrate interactions. Finally, we discuss culture process design in our previous studies with respect to maintaining and expanding cultures of undifferentiated and pluripotent hiPSCs. We focus on the regulation of migration-dependent balance between cell-cell and cell-substrate interactions. This review offers novel insights into the regulation and processing of stem cells for research in regenerative medicine.

Kim, M. H., et al. (2018). "Role of cell-secreted extracellular matrix formation in aggregate formation and stability of human induced pluripotent stem cells in suspension culture." J Biosci Bioeng.

Clinical and industrial applications require large quantities of human induced pluripotent stem cells (hiPSCs); however, little is known regarding the mechanisms governing aggregate formation and stability in suspension culture. To address this, we determined differences in growth processes among hiPSC lines in suspension culture. Using an hiPSC aggregate suspension culture system, hiPSCs from different lines formed multicellular aggregates classified as large compact or small loose based on their size and morphology. Time-lapse observation of the growth processes of two different hiPSC lines revealed that the balance between cell division and the extent of subsequent cell death determined the final size and morphology of aggregates. Comparison of the cell survival and death of two hiPSC lines showed that the formation of small, loose aggregates was due to continued cell death during the exponential phase of growth, with apoptotic cells extruded from growing hiPSC aggregates by the concerted contraction of their neighbors. Western blot and immunofluorescent staining revealed that aggregate morphology and proliferative ability relied to a considerable extent upon secretion of the extracellular matrix (ECM). hiPSCs forming large compact and stable aggregates showed enhanced production of collagen type I in suspension culture at 120 h. Furthermore, these aggregates exhibited higher expression of E-cadherin and proliferation marker Ki-67 as compared with levels observed in small and loose aggregates at 120 h. These findings indicated that differences in both aggregate formation and stability in suspension culture among hiPSC lines were caused by differences in ECM secretion capacity.

Kim, N., et al. (2017). "Immobilized pH in culture reveals an optimal condition for somatic cell reprogramming and differentiation of pluripotent stem cells." Reprod Med Biol **16**(1): 58-66.

Aim: One of the parameters that greatly affects homeostasis in the body is the pH. Regarding reproductive biology, germ cells, such as oocytes or sperm, are exposed to severe changes in pH, resulting in dramatic changes in their characteristics. To date, the effect of the pH has not been investigated regarding the reprogramming of somatic cells and the maintenance and differentiation of pluripotent stem cells. Methods: In order to investigate the effects of the pH on cell culture, the methods to produce induced pluripotent stem cells (iPSCs) and to differentiate embryonic stem cells (ESCs) into mesendoderm and neuroectoderm were performed at each medium pH from 6.6 to 7.8. Using the cells of the Oct4-GFP (green fluorescent protein) carrying mouse, the effects of pH changes were examined on the timing and colony formation at cell reprogramming and on the cell morphology and direction of the differentiation of the ESCs. Results: The colony formation rate and timing of the reprogramming of the somatic cells varied depending on the pH of the culture medium. In addition, mesendodermal differentiation of the mouse ESCs was enhanced at the high pH level of 7.8. Conclusion: These results suggest that the pH in the culture medium is one of the key factors in the induction of the reprogramming of somatic cells and in the differentiation of pluripotent stem cells.

Kim, S., et al. (2018). "Exosomes Secreted from Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Accelerate Skin Cell Proliferation." Int J Mol Sci **19**(10).

Induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (iMSCs) serve as a unique source for cell therapy. We investigated whether exosomes from iMSCs promote the proliferation of human keratinocytes (HaCaT) and human dermal fibroblasts (HDFs). iPSCs were established from human Wharton's jelly MSCs and were allowed to

differentiate into iMSCs. Exosomes were collected from the culture supernatant of MSCs (MSC-exo) and iMSCs (iMSC-exo), and their characteristics were investigated. Both exosome types possessed basic characteristics of exosomes and were taken up by skin cells in vitro and in vivo. A significant increase in HaCaT proliferation was observed with iMSC-exo, although both exosomes increased the viability and cell cycle progression in HaCaT and HDFs. No significant difference was observed in the closure of wound scratch and the expression of reparative genes between cells treated with the two exosome types. Both exosomes enhanced the secretion of collagen in HaCaT and HDFs; however, an increase in fibronectin level was observed only in HaCaT, and this effect was better with iMSC-exo treatment. Only iMSC-exo increased the phosphorylation of extracellular signal-regulated kinase (ERK)-1/2. Our results indicate that iMSC-exo promote the proliferation of skin cells by stimulating ERK1/2 and highlight the application of iMSCs for producing exosomes.

Kim, Y. M., et al. (2017). "Technical note: Induction of pluripotent stem cell-like cells from chicken feather follicle cells." J Anim Sci **95**(8): 3479-3486.

Pluripotent stem cells including embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) are regarded as representative tools for conservation of animal genetic resources. Although ESC have been established from chicken, it is very difficult to obtain enough embryos for isolation of stem cells for avian conservation in most wild birds. Therefore, the high feasibility of obtaining the pluripotent cell is most important in avian conservation studies. In this study, we generated induced pluripotent stem cell-like cells (iPSLC) from avian Feather Follicular cells (FFC). Avian FFC are one of the most easily accessible cell sources in most avian species, and their reprogramming into pluripotent stem cells can be an alternative system for preservation of avian species. Intriguingly, FFC had mesenchymal stromal cells (MSC)-like characteristics with regard to gene expression, protein expression, and adipocyte differentiation. Subsequently, we attempted to generate iPSLC from FFC using retroviral vectors. The FFC-iPSLC can proliferate with the stem pluripotent property and differentiate into several types of cells in vitro. Our results suggest that chicken FFC are an alternative cell source for avian cell reprogramming into pluripotent stem cells. This experimental strategy should be useful for conservation and restoration of endangered or high-value avian species without sacrificing embryos.

Kimbrel, E. A., et al. (2014). "Mesenchymal stem cell population derived from human pluripotent stem cells displays potent immunomodulatory and therapeutic properties." Stem Cells Dev **23**(14): 1611-1624.

Mesenchymal stem cells (MSCs) are being tested in a wide range of human diseases; however, loss of potency and inconsistent quality severely limit their use. To overcome these issues, we have utilized a developmental precursor called the hemangioblast as an intermediate cell type in the derivation of a highly potent and replenishable population of MSCs from human embryonic stem cells (hESCs). This method circumvents the need for labor-intensive hand-picking, scraping, and sorting that other hESC-MSC derivation methods require. Moreover, unlike previous reports on hESC-MSCs, we have systematically evaluated their immunomodulatory properties and in vivo potency. As expected, they dynamically secrete a range of bioactive factors, display enzymatic activity, and suppress T-cell proliferation that is induced by either allogeneic cells or mitogenic stimuli. However, they also display unique immunophenotypic properties, as well as a smaller size and >30,000-fold proliferative capacity than bone marrow-derived MSCs. In addition, this is the first report which demonstrates that hESC-MSCs can inhibit CD83 up-regulation and IL-12p70 secretion from dendritic cells and enhance regulatory T-cell populations induced by interleukin 2 (IL-2). This is also the first report which shows that hESC-MSCs have therapeutic efficacy in two different autoimmune disorder models, including a marked increase in survival of lupus-prone mice and a reduction of symptoms in an autoimmune model of uveitis. Our data suggest that this novel and therapeutically active population of MSCs could overcome many of the obstacles that plague the use of MSCs in regenerative medicine and serve as a scalable alternative to current MSC sources.

Kimura, H., et al. (2018). "Stem cells purified from human induced pluripotent stem cell-derived neural crest-like cells promote peripheral nerve regeneration." Sci Rep **8**(1): 10071.

Strategies for therapeutic cell transplantation have been assessed for use in the treatment of massive peripheral nerve defects. To support safe and efficient cell transplantation, we have focused on the purification of cells using cell surface markers. Our group previously reported low-affinity nerve growth factor receptor (LNGFR)- and thymocyte antigen-1 (THY-1)-positive neural crest-like cells (LT-NCLCs), generated from human induced pluripotent stem cells (hiPSCs). In the present study, we investigated the efficacy of transplantation of hiPSC-derived LT-NCLCs in a murine massive peripheral nerve defect model. Animals with a sciatic nerve defect were treated with a bridging silicone tube prefilled with LT-NCLCs or medium in the transplantation (TP) and negative

control (NC) groups, respectively. The grafted LT-NCLCs survived and enhanced myelination and angiogenesis, as compared to the NC group. Behavioral analysis indicated that motor functional recovery in the TP group was superior to that in the NC group, and similar to that in the autograft (Auto) group. LT-NCLCs promoted axonal regrowth and remyelination by Schwann cells. Transplantation of LT-NCLCs is a promising approach for nerve regeneration treatment of massive peripheral nerve defects.

Kirshenbaum, A. S., et al. (1992). "Effect of IL-3 and stem cell factor on the appearance of human basophils and mast cells from CD34+ pluripotent progenitor cells." J Immunol **148**(3): 772-777.

Hemopoietic stem cell factor (SCF), which is the ligand for the proto-oncogene c-kit receptor (allelic with W locus) and the product of Sl locus of the mouse, has recently been cloned. The human homologue has also been cloned, and recombinant protein (human rSCF) expressed and purified to homogeneity. To determine the effect of human rSCF in the presence or absence of human rIL-3 on human bone marrow-derived mast cells and basophils, human CD34+ pluripotent progenitor cells, highly enriched (greater than 99%) from bone marrow mononuclear cells, were cultured over agarose surfaces (interphase cultures) in the presence of human rIL-3, human rIL-3 and increasing concentrations of human rSCF, or human rSCF alone. Over 3 to 4 wk, human rSCF acted synergistically with human rIL-3 at all concentrations, producing a three- to fivefold increase in total, mast cell, and basophil numbers over human rIL-3 alone when used at 100 ng/ml. The percentage of cell types in the human rIL-3 and human rIL-3 plus human rSCF cultures, however, remained the same, with basophils constituting 18 to 35% of the final cultured cells, and mast cells 3% or less of the final cell number. In the presence of human rSCF alone, the combined total percentage of mast cells and basophils was 0 to 1.0%, the majority of cells being macrophages. Mast cells cultured in human rIL-3 plus human rSCF, but not human rIL-3 alone, were berberine sulfate positive, suggesting the presence of heparin proteoglycans within granules. Electron microscopic examination of cultures supplemented with human rIL-3 and rSCF, but not human rIL-3 alone, revealed that after 3 wk in culture, mast cell granules contained tryptase and exhibited scroll, reticular, and homogeneous patterns as seen previously in CD34+/3T3 fibroblast cocultures. Thus, CD34+ cells cultured in the presence of both human rIL-3 and rSCF give rise to cultures containing increased numbers of basophils and mast cells, with the mast cells by ultrastructural studies showing evidence of maturation although the percentages of basophils and mast cells arising in these cultures remained unchanged.

Kitada, M., et al. (2012). "Muse cells and induced pluripotent stem cell: implication of the elite model." Cell Mol Life Sci **69**(22): 3739-3750.

Induced pluripotent stem (iPS) cells have attracted a great deal attention as a new pluripotent stem cell type that can be generated from somatic cells, such as fibroblasts, by introducing the transcription factors Oct3/4, Sox2, Klf4, and c-Myc. The mechanism of generation, however, is not fully understood. Two mechanistic theories have been proposed; the stochastic model purports that every cell type has the potential to be reprogrammed to become an iPS cell and the elite model proposes that iPS cell generation occurs only from a subset of cells. Some reports have provided theoretical support for the stochastic model, but a recent publication demonstrated findings that support the elite model, and thus the mechanism of iPS cell generation remains under debate. To enhance our understanding of iPS cells, it is necessary to clarify the properties of the original cell source, i.e., the components of the original populations and the potential of each population to become iPS cells. In this review, we discuss the two theories and their implications in iPS cell research.

Kitadani, J., et al. (2016). "[Cancer Immunotherapy Using Human Induced Pluripotent Stem Cell-Derived Dendritic Cells(iPSDCs)Expressing Carcinoembryonic Antigen]." Gan To Kagaku Ryoho **43**(9): 1071-1073.

The difficulty in obtaining a sufficient number of functional dendritic cells(DCs)is a well-known serious problem in DCbased immunotherapy. Therefore, we used induced pluripotent stem cell-derived DCs(iPSDCs). We have reported that mouse iPSDCs are equivalent to BMDCs, in terms of maturation and antigen presentation. In this study, the antitumor immune response of human iPSDCs expressing the carcinoembryonic antigen was examined, to determine its clinical application in gastrointestinal cancer. Human iPS cells were established from healthy human fibroblasts using a Sendai virus vector, and human iPSDCs were differentiated under a feeder-free culture. Additionally, the surface marker expression, cytokine production, and migratory capacity of human iPSDCs were equivalent to those of monocyte-derived DCs(MoDCs). After 3 cycles of stimulation of autologous PBMCs by genetically modified DCs, the 51Cr-release assay was performed. The lymphocytes stimulated by iPSDCs-CEA showed cytotoxic activity against LCL-CEA and CEA652-pulsed LCL, but showed no cytotoxicity against LCL-LacZ. In addition, they showed cytotoxic activity against CEA-positive human cancer cell lines,

MKN45 and HT29, but showed no cytotoxicity against CEA-negative human cancer cell line MKN1. In conclusion, CEA-specific CTLs responses could be induced by iPSDCs-CEA. This vaccination strategy may be useful in future clinical applications of cancer vaccines.

Klump, H., et al. (2013). "Development of patient-specific hematopoietic stem and progenitor cell grafts from pluripotent stem cells, in vitro." Curr Mol Med **13**(5): 815-820.

Pluripotent stem cells hold great promise for future applications in many areas of regenerative medicine. Their defining property of differentiation towards any of the three germ layers and all derivatives thereof, including somatic stem cells, explains the special interest of the biomedical community in this cell type. In this review, we focus on the current state of directed differentiation of pluripotent stem cells towards hematopoietic stem cells (HSCs). HSCs are especially interesting because they are the longest known and, thus, most intensively investigated somatic stem cells. They were the first stem cells successfully used for regenerative purposes in clinical human medicine, namely in bone marrow transplantation, and also the first stem cells to be genetically altered for the first successful gene therapy trial in humans. However, because of the technical difficulties associated with this rare type of cell, such as the current incapability of prospective isolation, in vitro expansion and gene repair by homologous recombination, there is great interest in using pluripotent stem cells, such as Embryonic Stem (ES-) cells, as a source for generating and genetically altering HSCs, ex vivo. This has been hampered by ethical concerns associated with the use of human ES-cells. However, since Shinya Yamanaka s successful attempts to reprogram somatic cells of mice and men to an ES-cell like state, so-called induced pluripotent stem (iPS) cells, this field of research has experienced a huge boost. In this brief review, we will reflect on the status quo of directed hematopoietic differentiation of human and mouse pluripotent stem cells.

Knorr, D. A. and D. S. Kaufman (2010). "Pluripotent stem cell-derived natural killer cells for cancer therapy." Transl Res **156**(3): 147-154.

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) provide an accessible, genetically tractable, and homogenous starting cell population to efficiently study human blood cell development. These cell populations provide platforms to develop new cell-based therapies to treat both malignant and nonmalignant hematological diseases. Our group previously demonstrated the ability of hESC-derived hematopoietic precursors to produce functional natural killer (NK) cells as well as an explanation of the underlying mechanism responsible for the inefficient development of T and B cells from hESCs. hESCs and iPSCs, which can be engineered reliably in vitro, provide an important new model system to study human lymphocyte development and produce enhanced cell-based therapies with the potential to serve as a "universal" source of antitumor lymphocytes. This review will focus on the application of hESC-derived NK cells with currently used and novel therapeutics for clinical trials, barriers to translation, and future applications through genetic engineering approaches.

Kobayashi, T. and H. Nakauchi (2011). "[From cell therapy to organ regeneration therapy: generation of functional organs from pluripotent stem cells]." Nihon Rinsho **69**(12): 2148-2155.

Current stem cell therapy mainly targets diseases that can be treated by cell transplantation. The complexity of organogenesis hinders in vitro generation of organs derived from patient's pluripotent stem cells (PSCs), an ultimate goal of regenerative medicine. To address this issue, we attempted in vivo generation of PSC-derived pancreas using Pdx1(-/-) blastocysts(pancreatogenesis-disabled) and blastocyst complementation technique. When wild type rat PSCs were injected into mouse Pdx1(-/-) blastocysts, defective cells were totally replaced and pancreas was formed almost entirely by injected rat PSC derived cells. Chimeric mice of Pdx1(-/-) genotype survived to adulthood without any sign of diabetes. Generation of organs using blastocyst complementation in vivo provides a new strategy for understanding organogenesis and a novel approach for organ supply.

Kobayashi, W., et al. (2018). "Culture Systems of Dissociated Mouse and Human Pluripotent Stem Cell-Derived Retinal Ganglion Cells Purified by Two-Step Immunopanning." Invest Ophthalmol Vis Sci **59**(2): 776-787.

Purpose: We aimed to establish purification and culture systems for retinal ganglion cells (RGCs) differentiated from mouse and human pluripotent stem cells (PSC) for in vitro and regenerative medicine studies. Methods: We used a two-step immunopanning method to purify RGCs from mouse and human PSC-derived three-dimensional (3D) retinal organoids. To assess the method, we purified RGCs from 3D retinal organoids derived from embryonic stem cells (ESCs) generated from Thy1-EGFP transgenic (TG) mice. In addition, 3D retinal organoids differentiated from human induced PSCs (iPSCs) were cultured for up to differentiation day (DD) 120, and RGCs were purified by immunopanning. RGC marker expressions were confirmed by immunostaining and reverse

transcription-quantitative PCR. The purified RGCs were cultured, and neurite outgrowth was measured and analyzed using an IncuCyte Zoom system. Results: Mouse RGCs purified from Thy1-EGFP TG mouse retinas and the ESC-derived 3D retinas could be maintained for approximately 2 to 3 weeks, expressing the markers BRN3B and SMI-312. Purified RGCs from human iPSC-derived retinal organoids expressed RGC markers and could be maintained for up to 4 weeks. The RGCs collected at DD 90 to 110 extended longer neurites than those collected at younger stages. Conclusions: We successfully purified RGCs from mouse and human PSC-derived 3D retinal organoids cultured for approximately 120 days. RGCs from older retinal organoids would be useful for neurite tracking. This method would be effective not only for studying the pathology of human RGC diseases but also for therapeutic drug studies and RGC transplantation.

Koch, L., et al. (2018). "Laser bioprinting of human induced pluripotent stem cells-the effect of printing and biomaterials on cell survival, pluripotency, and differentiation." Biofabrication **10**(3): 035005.

Research on human induced pluripotent stem cells (hiPSCs) is one of the fastest growing fields in biomedicine. Generated from patient's own somatic cells, hiPSCs can be differentiated towards all functional cell types and returned to the patient without immunological concerns. 3D printing of hiPSCs could enable the generation of functional organs for replacement therapies or realization of organ-on-chip systems for individualized medicine. Printing of living cells was demonstrated with immortalized cell lines, primary cells, and adult stem cells with different printing technologies and biomaterials. However, hiPSCs are more sensitive to handling procedures, in particular, when dissociated into single cells. Both pluripotency and directed differentiation are influenced by numerous environmental factors including culture media, biomaterials, and cell density. Notably, existing literature on the effect of applied biomaterials on pluripotency is rather ambiguous. In this study, laser bioprinting of undifferentiated hiPSCs in combination with different biomaterials was performed and the impact on cells' behavior, pluripotency, and differentiation was investigated. Our findings suggest that hiPSCs are indeed more sensitive to the applied biomaterials, but not to laser printing itself. With appropriate biomaterials, such as the hyaluronic acid based solutions applied in this study, hiPSCs can be successfully laser printed without losing their pluripotency.

Kodaka, Y., et al. (2017). "Skeletal Muscle Cell Induction from Pluripotent Stem Cells." Stem Cells Int **2017**: 1376151.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have the potential to differentiate into various types of cells including skeletal muscle cells. The approach of converting ESCs/iPSCs into skeletal muscle cells offers hope for patients afflicted with the skeletal muscle diseases such as the Duchenne muscular dystrophy (DMD). Patient-derived iPSCs are an especially ideal cell source to obtain an unlimited number of myogenic cells that escape immune rejection after engraftment. Currently, there are several approaches to induce differentiation of ESCs and iPSCs to skeletal muscle. A key to the generation of skeletal muscle cells from ESCs/iPSCs is the mimicking of embryonic mesodermal induction followed by myogenic induction. Thus, current approaches of skeletal muscle cell induction of ESCs/iPSCs utilize techniques including overexpression of myogenic transcription factors such as MyoD or Pax3, using small molecules to induce mesodermal cells followed by myogenic progenitor cells, and utilizing epigenetic myogenic memory existing in muscle cell-derived iPSCs. This review summarizes the current methods used in myogenic differentiation and highlights areas of recent improvement.

Kodama, H. A., et al. (1982). "A new preadipose cell line derived from newborn mouse calvaria can promote the proliferation of pluripotent hemopoietic stem cells in vitro." J Cell Physiol **112**(1): 89-95.

A clonal preadipose cell line MC3T3-G2/PA6, established from newborn mouse calvaria, responds to glucocorticoids and converts to adipose cells in a fashion similar to bone marrow preadipocytes. We investigated the effect of the cells on in vitro hemopoiesis of mouse bone marrow cells by cocultivation. When bone marrow cells were inoculated into confluent cultures of MC3T3-G2/PA6 cells (10(4)-10(6) cells/25-cm2 flask), the number of hemopoietic stem cells (CFU-S) significantly increased during 7-day cultivation in proportion to inoculum size. Under these conditions, active replication of CFU-S was maintained for several weeks until MC3T3-G2/PA6 cell layers detached from the substratum. This capacity of the MC3T3-G2/PA6 line was unique because other established cell lines, including the MTF preadipose line, failed to support CFU-S growth. When bone marrow cells were not allowed to contact the MC3T3-G2/PA6 cell layer, only a small number of CFU-S survived for 7 days. Moreover, MC3T3-G2/PA6 cell-conditioned medium did not show any growth-promoting activity for CFU-S. These results indicate that the MC3T3-G2/PA6 cell line has the ability to promote the proliferation of CFU-S through a short range cell-to-cell interaction by providing an in

vitro microenvironment probably similar to that for in vivo hemopoiesis.

Kojima, Y., et al. (2017). "Evolutionarily Distinctive Transcriptional and Signaling Programs Drive Human Germ Cell Lineage Specification from Pluripotent Stem Cells." Cell Stem Cell **21**(4): 517-532 e515.

Germline specification underlies human reproduction and evolution, but it has proven difficult to study in humans since it occurs shortly after blastocyst implantation. This process can be modeled with human induced pluripotent stem cells (hiPSCs) by differentiating them into primordial germ cell-like cells (hPGCLCs) through an incipient mesoderm-like cell (iMeLC) state. Here, we elucidate the key transcription factors and their interactions with important signaling pathways in driving hPGCLC differentiation from iPSCs. Germline competence of iMeLCs is dictated by the duration and dosage of WNT signaling, which induces expression of EOMES to activate SOX17, a key driver of hPGCLC specification. Upon hPGCLC induction, BMP signaling activates TFAP2C in a SOX17-independent manner. SOX17 and TFAP2C then cooperatively instate an hPGCLC transcriptional program, including BLIMP1 expression. This specification program diverges from its mouse counterpart regarding key transcription factors and their hierarchies, and it provides a foundation for further study of human germ cell development.

Konagaya, S., et al. (2015). "Long-term maintenance of human induced pluripotent stem cells by automated cell culture system." Sci Rep **5**: 16647.

Pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem (iPS) cells, are regarded as new sources for cell replacement therapy. These cells can unlimitedly expand under undifferentiated conditions and be differentiated into multiple cell types. Automated culture systems enable the large-scale production of cells. In addition to reducing the time and effort of researchers, an automated culture system improves the reproducibility of cell cultures. In the present study, we newly designed a fully automated cell culture system for human iPS maintenance. Using an automated culture system, hiPS cells maintained their undifferentiated state for 60 days. Automatically prepared hiPS cells had a potency of differentiation into three germ layer cells including dopaminergic neurons and pancreatic cells.

Kossack, N., et al. (2009). "Isolation and characterization of pluripotent human spermatogonial stem cell-derived cells." Stem Cells **27**(1): 138-149.

Several reports have documented the derivation of pluripotent cells (multipotent germline stem cells) from spermatogonial stem cells obtained from the adult mouse testis. These spermatogonia-derived stem cells express embryonic stem cell markers and differentiate to the three primary germ layers, as well as the germline. Data indicate that derivation may involve reprogramming of endogenous spermatogonia in culture. Here, we report the derivation of human multipotent germline stem cells (hMGSCs) from a testis biopsy. The cells express distinct markers of pluripotency, form embryoid bodies that contain derivatives of all three germ layers, maintain a normal XY karyotype, are hypomethylated at the H19 locus, and express high levels of telomerase. Teratoma assays indicate the presence of human cells 8 weeks post-transplantation but limited teratoma formation. Thus, these data suggest the potential to derive pluripotent cells from human testis biopsies but indicate a need for novel strategies to optimize hMGSC culture conditions and reprogramming.

Kotaka, M., et al. (2017). "Adrenergic receptor agonists induce the differentiation of pluripotent stem cell-derived hepatoblasts into hepatocyte-like cells." Sci Rep **7**(1): 16734.

Current induction methods of hepatocytes from human induced pluripotent stem cells (hiPSCs) are neither low cost nor stable. By screening a chemical library of 1,120 bioactive compounds and known drugs, we identified the alpha1-adrenergic receptor agonist methoxamine hydrochloride as a small molecule that promotes the differentiation of hiPSC-derived hepatoblasts into ALBUMIN(+) hepatocyte-like cells. Other alpha1-adrenergic receptor agonists also induced the differentiation of hepatocyte-like cells, and an alpha1-receptor antagonist blocked the hepatic-inducing activity of methoxamine hydrochloride and that of the combination of hepatocyte growth factor (HGF) and Oncostatin M (OsM), two growth factors often used for the induction of hepatoblasts into hepatocyte-like cells. We also confirmed that treatment with methoxamine hydrochloride activates the signal transducer and activator of transcription 3 (STAT3) pathway downstream of IL-6 family cytokines including OsM. These findings allowed us to establish hepatic differentiation protocols for both mouse embryonic stem cells (mESCs) and hiPSCs using small molecules at the step from hepatoblasts into hepatocyte-like cells. The results of the present study suggest that alpha1-adrenergic agonists induce hepatocyte-like cells by working downstream of HGF and OsM to activate STAT3.

Kuise, T., et al. (2014). "Establishment of a pancreatic stem cell line from fibroblast-derived induced pluripotent stem cells." Biomed Eng Online **13**: 64.

BACKGROUND: For cell therapies to treat diabetes, it is important to produce a sufficient number of pancreatic endocrine cells that function similarly to primary islets. Induced pluripotent stem (iPS) cells represent a potentially unlimited source of functional pancreatic endocrine cells. However, the use of iPS cells for laboratory studies and cell-based therapies is hampered by their high tumorigenic potential and limited ability to generate pure populations of differentiated cell types in vitro. The purpose of this study was to establish a pancreatic stem cell line from iPS cells derived from mouse fibroblasts. METHODS: Mouse iPS cells were induced to differentiate into insulin-producing cells by a multi-step differentiation protocol, which was conducted as described previously with minor modifications. Selection of the pancreatic stem cell was based on morphology and Pdx1 expression. The pancreatic potential of the pancreatic stem cells was evaluated using a reverse transcription PCR, real-time PCR, immunofluorescence, and a glucose challenge test. To assess potential tumorigenicity of the pancreatic stem cells, the cells were injected into the quadriceps femoris muscle of the left hindlimb of nude mice. RESULTS: The iPS-derived pancreatic stem cells expressed the transcription factor--Pdx1--a marker of pancreatic development, and continued to divide actively beyond passage 80. Endocrine cells derived from these pancreatic stem cells expressed insulin and pancreatic genes, and they released insulin in response to glucose stimulation. Mice injected with the pancreatic stem cells did not develop tumors, in contrast to mice injected with an equal number of iPS cells. CONCLUSION: This strategy provides a new approach for generation of insulin-producing cells that is more efficient and safer than using iPS cells. We believe that this approach will help to develop a patient-specific cell transplantation therapy for diabetes in the near future.

Kumar, N., et al. (2015). "Generation of an expandable intermediate mesoderm restricted progenitor cell line from human pluripotent stem cells." Elife **4**.

The field of tissue engineering entered a new era with the development of human pluripotent stem cells (hPSCs), which are capable of unlimited expansion whilst retaining the potential to differentiate into all mature cell populations. However, these cells harbor significant risks, including tumor formation upon transplantation. One way to mitigate this risk is to develop expandable progenitor cell populations with restricted differentiation potential. Here, we used a cellular microarray technology to identify a defined and optimized culture condition that supports the derivation and propagation of a cell population with mesodermal properties. This cell population, referred to as intermediate mesodermal progenitor (IMP) cells, is capable of unlimited expansion, lacks tumor formation potential, and, upon appropriate stimulation, readily acquires properties of a sub-population of kidney cells. Interestingly, IMP cells fail to differentiate into other mesodermally-derived tissues, including blood and heart, suggesting that these cells are restricted to an intermediate mesodermal fate.

Kumar, S. S., et al. (2014). "Recent developments in beta-cell differentiation of pluripotent stem cells induced by small and large molecules." Int J Mol Sci **15**(12): 23418-23447.

Human pluripotent stem cells, including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), hold promise as novel therapeutic tools for diabetes treatment because of their self-renewal capacity and ability to differentiate into beta (beta)-cells. Small and large molecules play important roles in each stage of beta-cell differentiation from both hESCs and hiPSCs. The small and large molecules that are described in this review have significantly advanced efforts to cure diabetic disease. Lately, effective protocols have been implemented to induce hESCs and human mesenchymal stem cells (hMSCs) to differentiate into functional beta-cells. Several small molecules, proteins, and growth factors promote pancreatic differentiation from hESCs and hMSCs. These small molecules (e.g., cyclopamine, wortmannin, retinoic acid, and sodium butyrate) and large molecules (e.g. activin A, betacellulin, bone morphogentic protein (BMP4), epidermal growth factor (EGF), fibroblast growth factor (FGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), noggin, transforming growth factor (TGF-alpha), and WNT3A) are thought to contribute from the initial stages of definitive endoderm formation to the final stages of maturation of functional endocrine cells. We discuss the importance of such small and large molecules in uniquely optimized protocols of beta-cell differentiation from stem cells. A global understanding of various small and large molecules and their functions will help to establish an efficient protocol for beta-cell differentiation.

Kurimoto, K. and M. Saitou (2018). "Epigenome regulation during germ cell specification and development from pluripotent stem cells." Curr Opin Genet Dev **52**: 57-64.

Germ cells undergo epigenome reprogramming for proper development of the next generation. The realization of germ cell derivation from human and mouse pluripotent stem cells offers unprecedented opportunity for investigation of germline development. Primordial germ cells

reconstituted in vitro (PGC-like cells [PGCLCs]) show progressive dilution of genomic DNA methylation, tightly linked with chromatin remodeling, during their specification. PGCLCs can be further expanded by plane culture, allowing maintenance of the gene-expression profiles of early PGCs and continuance of the DNA methylation erasure, thereby establishing an epigenetic `blank slate'. PGCLCs undergo further epigenome regulation to acquire the male or female fates. These findings will provide a foundation for basic germ cell biology and for in-depth evaluations of in vitro gametogenesis.

Kurokawa, Y. K., et al. (2017). "Human Induced Pluripotent Stem Cell-Derived Endothelial Cells for Three-Dimensional Microphysiological Systems." Tissue Eng Part C Methods **23**(8): 474-484.

Microphysiological systems (MPS), or "organ-on-a-chip" platforms, aim to recapitulate in vivo physiology using small-scale in vitro tissue models of human physiology. While significant efforts have been made to create vascularized tissues, most reports utilize primary endothelial cells that hinder reproducibility. In this study, we report the use of human induced pluripotent stem cell-derived endothelial cells (iPS-ECs) in developing three-dimensional (3D) microvascular networks. We established a CDH5-mCherry reporter iPS cell line, which expresses the vascular endothelial (VE)-cadherin fused to mCherry. The iPS-ECs demonstrate physiological functions characteristic of primary endothelial cells in a series of in vitro assays, including permeability, response to shear stress, and the expression of endothelial markers (CD31, von Willibrand factor, and endothelial nitric oxide synthase). The iPS-ECs form stable, perfusable microvessels over the course of 14 days when cultured within 3D microfluidic devices. We also demonstrate that inhibition of TGF-beta signaling improves vascular network formation by the iPS-ECs. We conclude that iPS-ECs can be a source of endothelial cells in MPS providing opportunities for human disease modeling and improving the reproducibility of 3D vascular networks.

Kusuma, S., et al. (2015). "Characterizing human pluripotent-stem-cell-derived vascular cells for tissue engineering applications." Stem Cells Dev **24**(4): 451-458.

Tissue-engineered constructs are rendered useless without a functional vasculature owing to a lack of nutrients and oxygen. Cell-based approaches to reconstruct blood vessels can yield structures that mimic native vasculature and aid transplantation. Vascular derivatives of human induced pluripotent stem cells (hiPSCs) offer opportunities to generate patient-specific therapies and potentially provide unlimited amounts of vascular cells. To be used in engineered vascular constructs and confer therapeutic benefit, vascular derivatives must exhibit additional key properties, including extracellular matrix (ECM) production to confer structural integrity and growth factor production to facilitate integration. In this study, we examine the hypothesis that vascular cells derived from hiPSCs exhibit these critical properties to facilitate their use in engineered tissues. hiPSCs were codifferentiated toward early vascular cells (EVCs), a bicellular population of endothelial cells (ECs) and pericytes, under varying low-oxygen differentiation conditions; subsequently, ECs were isolated and passaged. We found that EVCs differentiated under low-oxygen conditions produced copious amounts of collagen IV and fibronectin as well as vascular endothelial growth factor and angiopoietin 2. EVCs differentiated under atmospheric conditions did not demonstrate such abundant ECM expression, but exhibited greater expression of angiopoietin 1. Isolated ECs could proliferate up to three passages while maintaining the EC marker vascular endothelial cadherin. Isolated ECs demonstrated an increased propensity to produce ECM compared with their EVC correlates and took on an arterial-like fate. These findings illustrate that hiPSC vascular derivates hold great potential for therapeutic use and should continue to be a preferred cell source for vascular construction.

Kwon, D. J., et al. (2017). "Effects of Cell Cycle Regulators on the Cell Cycle Synchronization of Porcine induced Pluripotent Stem Cells." Dev Reprod **21**(1): 47-54.

Unlike mouse results, cloning efficiency of nuclear transfer from porcine induced pluripotent stem cells (piPSCs) is very low. The present study was performed to investigate the effect of cell cycle inhibitors on the cell cycle synchronization of piPSCs. piPSCs were generated using combination of six human transcriptional factors under stem cell culture condition. To examine the efficiency of cell cycle synchronization, piPSCs were cultured on a matrigel coated plate with stem cell media and they were treated with staurosporine (STA, 20 nM), daidzein (DAI, 100 muM), roscovitine (ROSC, 10 muM), or olomoucine (OLO, 200 muM) for 12 h. Flow Cytometry (FACs) data showed that piPSCs in control were in G1 (37.5+/-0.2%), S (34.0+/-0.6%) and G2/M (28.5+/-0.4%). The proportion of cells at G1 in DAI group was significantly higher than that in control, while STA, ROSC and OLO treatments could not block the cell cycle of piPSCs. Both of viability and apoptosis were affected by STA and ROSC treatment, but there were no significantly differences between control and DAI groups. Real-Time qPCR and FACs results revealed

that DAI treatment did not affect the expression of pluripotent gene, Oct4. In case of OLO, it did not affect both of viability and apoptosis, but Oct4 expression was significantly decreased. Our results suggest that DAI could be used for synchronizing piPSCs at G1 stage and has any deleterious effect on survival and pluripotency sustaining of piPSCs.

La Greca, A., et al. (2018). "Extracellular vesicles from pluripotent stem cell-derived mesenchymal stem cells acquire a stromal modulatory proteomic pattern during differentiation." Exp Mol Med **50**(9): 119.

Mesenchymal stem/stromal cells (MSCs) obtained from pluripotent stem cells (PSCs) constitute an interesting alternative to classical MSCs in regenerative medicine. Among their many mechanisms of action, MSC extracellular vesicles (EVs) are a potential suitable substitute for MSCs in future cell-free-based therapeutic approaches. Unlike cells, EVs do not elicit acute immune rejection, and they can be produced in large quantities and stored until ready to use. Although the therapeutic potential of MSC EVs has already been proven, a thorough characterization of MSC EVs is lacking. In this work, we used a label-free liquid chromatography tandem mass spectrometry proteomic approach to identify the most abundant proteins in EVs that are secreted from MSCs derived from PSCs (PD-MSCs) and from their parental induced PSCs (iPSCs). Next, we compared both datasets and found that while iPSC EVs enclose proteins that modulate RNA and microRNA stability and protein sorting, PD-MSC EVs are rich in proteins that organize extracellular matrix, regulate locomotion, and influence cell-substrate adhesion. Moreover, compared to their respective cells, iPSCs and iPSC EVs share a greater proportion of proteins, while the PD-MSC proteome appears to be more specific. Correlation and principal component analysis consistently aggregate iPSCs and iPSC EVs but segregate PD-MSC and their EVs. Altogether, these findings suggest that during differentiation, compared with their parental iPSC EVs, PD-MSC EVs acquire a more specific set of proteins; arguably, this difference might confer their therapeutic properties.

Lachmann, N., et al. (2015). "Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies." Stem Cell Reports **4**(2): 282-296.

Interleukin-3 (IL-3) is capable of supporting the proliferation of a broad range of hematopoietic cell types, whereas granulocyte colony-stimulating factor (G-CSF) and macrophage CSF (M-CSF) represent critical cytokines in myeloid differentiation. When this was investigated in a pluripotent-stem-cell-based hematopoietic differentiation model, IL-3/G-CSF or IL-3/M-CSF exposure resulted in the continuous generation of myeloid cells from an intermediate myeloid-cell-forming complex containing CD34(+) clonogenic progenitor cells for more than 2 months. Whereas IL-3/G-CSF directed differentiation toward CD45(+)CD11b(+)CD15(+)CD16(+)CD66b(+) granulocytic cells of various differentiation stages up to a segmented morphology displaying the capacity of cytokine-directed migration, respiratory burst response, and neutrophil-extracellular-trap formation, exposure to IL-3/M-CSF resulted in CD45(+)CD11b(+)CD14(+)CD163(+)CD68(+) monocyte/macrophage-type cells capable of phagocytosis and cytokine secretion. Hence, we show here that myeloid specification of human pluripotent stem cells by IL-3/G-CSF or IL-3/M-CSF allows for prolonged and large-scale production of myeloid cells, and thus is suited for cell-fate and disease-modeling studies as well as gene- and cell-therapy applications.

Lagerqvist, E. L., et al. (2015). "Comparing mouse and human pluripotent stem cell derived cardiac cells: Both systems have advantages for pharmacological and toxicological screening." J Pharmacol Toxicol Methods **74**: 17-25.

Pluripotent stem cells offer an unparalleled opportunity to investigate cardiac physiology, pharmacology, toxicology and pathophysiology. In this paper we describe the use of both mouse (Nkx2-5(eGFP/w)) and human (NKX2-5(eGFP/w)) pluripotent stem cell reporter lines, differentiated toward cardiac lineage, for live single cell high acquisition rate calcium imaging. We also assess the potential of NKX2-5(eGFP/w) cardiac lineage cells for use toxicological screening as well as establish their sensitivity to a shift between low and high oxygen environments. Differentiated mouse Nkx2-5(eGFP/w) cells demonstrated a wide range of spontaneous oscillation rates that could be reduced by ryanodine (10muM), thapsigargin (1muM) and ZD7288 (10muM). In contrast human NKX2-5(eGFP/w) cell activity was only reduced by thapsigargin (1muM). Human cell survival was sensitive to the addition of trastuzumab and doxorubicin, while the switch from a low to a high oxygen environment affected oscillation frequency. We suggest that the human NKX2-5(eGFP/w) cells are less suitable for studies of compounds affecting cardiac pacemaker activity than mouse Nkx2-5(eGFP/w) cells, but are very suitable for cardiac toxicity studies.

Lagrutta, A., et al. (2016). "Interaction between amiodarone and hepatitis-C virus nucleotide inhibitors in human induced pluripotent stem cell-derived cardiomyocytes and HEK-293 Cav1.2 over-expressing cells." Toxicol Appl Pharmacol **308**: 66-76.

Several clinical cases of severe bradyarrhythmias have been reported upon co-administration of the Hepatitis-C NS5B Nucleotide Polymerase Inhibitor (HCV-NI) direct-acting antiviral agent, sofosbuvir (SOF), and the Class-III anti-arrhythmic amiodarone (AMIO). We model the cardiac drug-drug interaction (DDI) between AMIO and SOF, and between AMIO and a closely-related SOF analog, MNI-1 (Merck Nucleotide Inhibitor #1), in functional assays of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), to provide mechanistic insights into recently reported clinical cases. AMIO co-applied with SOF or MNI-1 increased beating rate or field potential (FP) rate and decreased impedance (IMP) and Ca(2+) transient amplitudes in hiPSC-CM syncytia. This action resembled that of Ca(2+) channel blockers (CCBs) in the model, but CCBs did not substitute for AMIO in the DDI. AMIO analog dronedarone (DRON) did not substitute for, but competed with AMIO in the DDI. Ryanodine and thapsigargin, decreasing intracellular Ca(2+) stores, and SEA-0400, a Na(+)/Ca(2+) exchanger-1 (NCX1) inhibitor, partially antagonized or suppressed DDI effects. Other agents affecting FP rate only exerted additive or subtractive effects, commensurate with their individual effects. We also describe an interaction between AMIO and MNI-1 on Cav1.2 ion channels in an over-expressing HEK-293 cell line. MNI-1 enhanced Cav1.2 channel inhibition by AMIO, but did not affect inhibition of Cav1.2 by DRON, verapamil, nifedipine, or diltiazem. Our data in hiPSC-CMs indicate that HCV-NI agents such as SOF and MNI-1 interact with key intracellular Ca(2+)-handling mechanisms. Additional study in a Cav1.2 HEK-293 cell-line suggests that HCV-NIs potentiate the inhibitory action of AMIO on L-type Ca(2+) channels.

Lahlou, H., et al. (2018). "Modeling human early otic sensory cell development with induced pluripotent stem cells." PLoS One **13**(6): e0198954.

The inner ear represents a promising system to develop cell-based therapies from human induced pluripotent stem cells (hiPSCs). In the developing ear, Notch signaling plays multiple roles in otic region specification and for cell fate determination. Optimizing hiPSC induction for the generation of appropriate numbers of otic progenitors and derivatives, such as hair cells, may provide an unlimited supply of cells for research and cell-based therapy. In this study, we used monolayer cultures, otic-inducing agents, Notch modulation, and marker expression to track early and otic sensory lineages during hiPSC differentiation. Otic/placodal progenitors were derived from hiPSC cultures in medium supplemented with FGF3/FGF10 for 13 days. These progenitor cells were then treated for 7 days with retinoic acid (RA) and epidermal growth factor (EGF) or a Notch inhibitor. The differentiated cultures were analyzed in parallel by qPCR and immunocytochemistry. After the 13 day induction, hiPSC-derived cells displayed an upregulated expression of a panel of otic/placodal markers. Strikingly, a subset of these induced progenitor cells displayed key-otic sensory markers, the percentage of which was increased in cultures under Notch inhibition as compared to RA/EGF-treated cultures. Our results show that modulating Notch pathway during in vitro differentiation of hiPSC-derived otic/placodal progenitors is a valuable strategy to promote the expression of human otic sensory lineage genes.

Lai, F. P., et al. (2017). "Correction of Hirschsprung-Associated Mutations in Human Induced Pluripotent Stem Cells Via Clustered Regularly Interspaced Short Palindromic Repeats/Cas9, Restores Neural Crest Cell Function." Gastroenterology **153**(1): 139-153 e138.

BACKGROUND & AIMS: Hirschsprung disease is caused by failure of enteric neural crest cells (ENCCs) to fully colonize the bowel, leading to bowel obstruction and megacolon. Heterozygous mutations in the coding region of the RET gene cause a severe form of Hirschsprung disease (total colonic aganglionosis). However, 80% of HSCR patients have short-segment Hirschsprung disease (S-HSCR), which has not been associated with genetic factors. We sought to identify mutations associated with S-HSCR, and used the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing system to determine how mutations affect ENCC function. METHODS: We created induced pluripotent stem cell (iPSC) lines from 1 patient with total colonic aganglionosis (with the G731del mutation in RET) and from 2 patients with S-HSCR (without a RET mutation), as well as RET(+/-) and RET(-/-) iPSCs. IMR90-iPSC cells were used as the control cell line. Migration and differentiation capacities of iPSC-derived ENCCs were analyzed in differentiation and migration assays. We searched for mutation(s) associated with S-HSCR by combining genetic and transcriptome data from patient blood- and iPSC-derived ENCCs, respectively. Mutations in the iPSCs were corrected using the CRISPR/Cas9 system. RESULTS: ENCCs derived from all iPSC lines, but not control iPSCs, had defects in migration and neuronal lineage differentiation. RET mutations were associated with differentiation and migration defects of ENCCs in vitro. Genetic and transcriptome analyses associated a mutation in the vinculin gene (VCL M209L) with S-HSCR. CRISPR/Cas9 correction of the RET G731del and VCL M209L mutations in iPSCs restored the differentiation and migration capacities of ENCCs. CONCLUSIONS: We identified mutations in

VCL associated with S-HSCR. Correction of this mutation in iPSC using CRISPR/Cas9 editing, as well as the RET G731del mutation that causes Hirschsprung disease with total colonic aganglionosis, restored ENCC function. Our study demonstrates how human iPSCs can be used to identify disease-associated mutations and determine how they affect cell functions and contribute to pathogenesis.

Lanctot, C. (2015). "Single Cell Analysis Reveals Concomitant Transcription of Pluripotent and Lineage Markers During the Early Steps of Differentiation of Embryonic Stem Cells." Stem Cells **33**(10): 2949-2960.

The differentiation of embryonic stem cells is associated with extensive changes in gene expression. It is not yet clear whether these changes are the result of binary switch-like mechanisms or that of continuous and progressive variation. Here, I have used immunostaining and single molecule RNA fluorescence in situ hybridization (FISH) to assess changes in the expression of the well-known pluripotency-associated gene Pou5f1 (also known as Oct4) and early differentiation markers Sox1 and T-brachyury in single cells during the early steps of differentiation of mouse embryonic stem cells. I found extensive overlap between the expression of Pou5f1/Sox1 or Pou5f1/T-brachyury shortly after the initiation of differentiation towards either the neuronal or the mesendodermal lineage, but no evidence of correlation between their respective expression levels. Quantitative analysis of transcriptional output at the sites of nascent transcription revealed that Pou5f1 and Sox1 were transcribed in pulses and that embryonic stem cell differentiation was accompanied by changes in pulsing frequencies. The progressive induction of Sox1 was further associated with an increase in the average size of individual transcriptional bursts. Surprisingly, single cells that actively and simultaneously transcribe both the pluripotency- and the lineage-associated genes could easily be found in the differentiating population. The results presented here show for the first time that lineage priming can occur in cells that are actively transcribing a pluripotent marker. Furthermore, they suggest that this process is associated with changes in transcriptional dynamics.

Lang, J., et al. (2016). "Modeling Dengue Virus-Hepatic Cell Interactions Using Human Pluripotent Stem Cell-Derived Hepatocyte-like Cells." Stem Cell Reports **7**(3): 341-354.

The development of dengue antivirals and vaccine has been hampered by the incomplete understanding of molecular mechanisms of dengue virus (DENV) infection and pathology, partly due to the limited suitable cell culture or animal models that can capture the comprehensive cellular changes induced by DENV. In this study, we differentiated human pluripotent stem cells (hPSCs) into hepatocytes, one of the target cells of DENV, to investigate various aspects of DENV-hepatocyte interaction. hPSC-derived hepatocyte-like cells (HLCs) supported persistent and productive DENV infection. The activation of interferon pathways by DENV protected bystander cells from infection and protected the infected cells from massive apoptosis. Furthermore, DENV infection activated the NF-kappaB pathway, which led to production of proinflammatory cytokines and downregulated many liver-specific genes such as albumin and coagulation factor V. Our study demonstrates the utility of hPSC-derived hepatocytes as an in vitro model for DENV infection and reveals important aspects of DENV-host interactions.

Langer, K. B., et al. (2018). "Retinal Ganglion Cell Diversity and Subtype Specification from Human Pluripotent Stem Cells." Stem Cell Reports **10**(4): 1282-1293.

Retinal ganglion cells (RGCs) are the projection neurons of the retina and transmit visual information to postsynaptic targets in the brain. While this function is shared among nearly all RGCs, this class of cell is remarkably diverse, comprised of multiple subtypes. Previous efforts have identified numerous RGC subtypes in animal models, but less attention has been paid to human RGCs. Thus, efforts of this study examined the diversity of RGCs differentiated from human pluripotent stem cells (hPSCs) and characterized defined subtypes through the expression of subtype-specific markers. Further investigation of these subtypes was achieved using single-cell transcriptomics, confirming the combinatorial expression of molecular markers associated with these subtypes, and also provided insight into more subtype-specific markers. Thus, the results of this study describe the derivation of RGC subtypes from hPSCs and will support the future exploration of phenotypic and functional diversity within human RGCs.

Lapillonne, H., et al. (2010). "Red blood cell generation from human induced pluripotent stem cells: perspectives for transfusion medicine." Haematologica **95**(10): 1651-1659.

BACKGROUND: Ex vivo manufacture of red blood cells from stem cells is a potential means to ensure an adequate and safe supply of blood cell products. Advances in somatic cell reprogramming of human induced pluripotent stem cells have opened the door to generating specific cells for cell therapy. Human induced pluripotent stem cells represent a potentially unlimited source of stem cells for erythroid generation for transfusion medicine. DESIGN AND

METHODS: We characterized the erythroid differentiation and maturation of human induced pluripotent stem cell lines obtained from human fetal (IMR90) and adult fibroblasts (FD-136) compared to those of a human embryonic stem cell line (H1). Our protocol comprises two steps: (i) differentiation of human induced pluripotent stem cells by formation of embryoid bodies with indispensable conditioning in the presence of cytokines and human plasma to obtain early erythroid commitment, and (ii) differentiation/maturation to the stage of cultured red blood cells in the presence of cytokines. The protocol dispenses with major constraints such as an obligatory passage through a hematopoietic progenitor, co-culture on a cellular stroma and use of proteins of animal origin. RESULTS: We report for the first time the complete differentiation of human induced pluripotent stem cells into definitive erythrocytes capable of maturation up to enucleated red blood cells containing fetal hemoglobin in a functional tetrameric form. CONCLUSIONS: Red blood cells generated from human induced pluripotent stem cells pave the way for future development of allogeneic transfusion products. This could be done by banking a very limited number of red cell phenotype combinations enabling the safe transfusion of a great number of immunized patients.

Lee, E. X., et al. (2011). "Glioma gene therapy using induced pluripotent stem cell derived neural stem cells." Mol Pharm **8**(5): 1515-1524.

Using neural stem cells (NSCs) with tumor tropic migratory capacity to deliver therapeutic genes is an attractive strategy in eliminating metastatic or disseminated tumors. While different methods have been developed to isolate or generate NSCs, it has not been assessed whether induced pluripotent stem (iPS) cells, a type of pluripotent stem cells that hold great potential for regenerative medicine, can be used as a source for derivation of NSCs with tumor tropism. In this study, we used a conventional lentivirus transduction method to derive iPS cells from primary mouse embryonic fibroblasts and then generated NSCs from the iPS cells. To investigate whether the iPS cell derived NSCs can be used in the treatment of disseminated brain tumors, the cells were transduced with a baculoviral vector containing the herpes simplex virus thymidine kinase suicide gene and injected into the cerebral hemisphere contralateral to a tumor inoculation site in a mouse intracranial human glioma xenograft model. We observed that NSCs expressing the suicide gene were, in the presence of ganciclovir, effective in inhibiting the growth of the glioma xenografts and prolonging survival of tumor-bearing mice. Our findings provide evidence for the feasibility of using iPS cell derived NSCs as cellular vehicles for targeted anticancer gene therapy.

Lee, H. K., et al. (2016). "Peripheral blood mononuclear cell-converted induced pluripotent stem cells (iPSCs) from an early onset Alzheimer's patient." Stem Cell Res **16**(2): 213-215.

Improvement in transduction efficiency makes it possible to convert blood cells into induced pluripotent stem cells (iPSC). In this study, we generated an iPSC line from peripheral blood mononuclear cells (PBMC) donated by a patient who exhibited memory deficit at age 59; outcome of positron emission tomography scan is consistent with a diagnosis of Alzheimer's disease. Integration-free CytoTune-iPS Sendai Reprogramming factors which include Sendai virus particles of the four Yamanaka factors Oct4, Sox2, Klf4, and c-Myc were introduced to PBMC to convert them to iPSCs without retention of virus. Three germ layer differentiation was induced to demonstrate the pluripotency of these iPSCs.

Lee, J., et al. (2018). "Human induced pluripotent stem cell line with cytochrome P450 enzyme polymorphism (CYP2C19*2/CYP3A5*3C) generated from lymphoblastoid cells." Stem Cell Res **27**: 34-37.

Cytochrome P450 (CYP) comprises a superfamily of monooxygenase responsible for the metabolism of xenobiotics and approximately 75% of drugs in use today. Thus, genetic polymorphisms in CYP genes contribute to interindividual differences in hepatic metabolism of drugs, affecting on individual drug efficacy and may cause adverse effects. Here, we generated a human induced pluripotent stem cell (hiPSC) line with pharmacologically important traits (CYP2C19*2/CYP3A5*3C), which are highly polymorphic in Asian from lymphoblastoid cells. This hiPSC line could be a valuable source for predicting individual drug responses in the drug screening process that uses hiPSC-derived somatic cells, including hepatocytes.

Lee, S. J., et al. (2018). "Generation of Human Pluripotent Stem Cell-derived Endothelial Cells and Their Therapeutic Utility." Curr Cardiol Rep **20**(6): 45.

PURPOSE OF REVIEW: Human pluripotent stem cell-derived endothelial cells (hPSC-ECs) emerged as an important source of cells for cardiovascular regeneration. This review summarizes protocols for generating hPSC-ECs and provides an overview of the current state of the research in clinical application of hPSC-derived ECs. RECENT FINDINGS: Various systems were developed for differentiating hPSCs into the EC lineage. Stepwise two-dimensional systems are now well established, in which various growth factors, small molecules, and coating materials are used at specific developmental stages. Moreover, studies made significant advances in

clinical applicability of hPSC-ECs by removing undefined components from the differentiation system, improving the differentiation efficiency, and proving their direct vascular incorporating effects, which contrast with adult stem cells and their therapeutic effects in vivo. Finally, by using biomaterial-mediated delivery, investigators improved the survival of hPSC-ECs to more than 10 months in ischemic tissues and described long-term behavior and safety of in vivo transplanted hPSC-ECs at the histological level. hPSC-derived ECs can be as a critical source of cells for treating advanced cardiovascular diseases. Over the past two decades, substantial improvement has been made in the differentiation systems and their clinical compatibility. In the near future, establishment of fully defined differentiation systems and proof of the advantages of biomaterial-mediated cell delivery, with some additional pre-clinical studies, will move this therapy into a vital option for treating those diseases that cannot be managed by currently available therapies.

Lee, S. J., et al. (2017). "Enhanced Therapeutic and Long-Term Dynamic Vascularization Effects of Human Pluripotent Stem Cell-Derived Endothelial Cells Encapsulated in a Nanomatrix Gel." Circulation **136**(20): 1939-1954.

BACKGROUND: Human pluripotent stem cell (hPSC)-derived endothelial cells (ECs) have limited clinical utility because of undefined components in the differentiation system and poor cell survival in vivo. Here, we aimed to develop a fully defined and clinically compatible system to differentiate hPSCs into ECs. Furthermore, we aimed to enhance cell survival, vessel formation, and therapeutic potential by encapsulating hPSC-ECs with a peptide amphiphile (PA) nanomatrix gel. METHODS: We induced differentiation of hPSCs into the mesodermal lineage by culturing on collagen-coated plates with a glycogen synthase kinase 3beta inhibitor. Next, vascular endothelial growth factor, endothelial growth factor, and basic fibroblast growth factor were added for endothelial lineage differentiation, followed by sorting for CDH5 (VE-cadherin). We constructed an extracellular matrix-mimicking PA nanomatrix gel (PA-RGDS) by incorporating the cell adhesive ligand Arg-Gly-Asp-Ser (RGDS) and a matrix metalloproteinase-2-degradable sequence. We then evaluated whether the encapsulation of hPSC-CDH5(+) cells in PA-RGDS could enhance long-term cell survival and vascular regenerative effects in a hind-limb ischemia model with laser Doppler perfusion imaging, bioluminescence imaging, real-time reverse transcription-polymerase chain reaction, and histological analysis. RESULTS: The resultant hPSC-derived CDH5(+) cells (hPSC-ECs) showed highly enriched and genuine EC characteristics and proangiogenic activities. When injected into ischemic hind limbs, hPSC-ECs showed better perfusion recovery and higher vessel-forming capacity compared with media-, PA-RGDS-, or human umbilical vein EC-injected groups. However, the group receiving the PA-RGDS-encapsulated hPSC-ECs showed better perfusion recovery, more robust and longer cell survival (> 10 months), and higher and prolonged angiogenic and vascular incorporation capabilities than the bare hPSC-EC-injected group. Surprisingly, the engrafted hPSC-ECs demonstrated previously unknown sustained and dynamic vessel-forming behavior: initial perivascular concentration, a guiding role for new vessel formation, and progressive incorporation into the vessels over 10 months. CONCLUSIONS: We generated highly enriched hPSC-ECs via a clinically compatible system. Furthermore, this study demonstrated that a biocompatible PA-RGDS nanomatrix gel substantially improved long-term survival of hPSC-ECs in an ischemic environment and improved neovascularization effects of hPSC-ECs via prolonged and unique angiogenic and vessel-forming properties. This PA-RGDS-mediated transplantation of hPSC-ECs can serve as a novel platform for cell-based therapy and investigation of long-term behavior of hPSC-ECs.

Lee, T. J., et al. (2014). "Mesenchymal stem cell-conditioned medium enhances osteogenic and chondrogenic differentiation of human embryonic stem cells and human induced pluripotent stem cells by mesodermal lineage induction." Tissue Eng Part A **20**(7-8): 1306-1313.

Human mesenchymal stem cells (hMSCs) have the ability to differentiate into mesenchymal lineages. In this study, we hypothesized that treatment of embryoid bodies (EBs) composed of either human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) with a hMSC-conditioned medium (CM) can stimulate the induction of the mesodermal lineage and subsequent differentiation toward the osteogenic and chondrogenic lineage. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) analysis indicated that the hMSC-CM treatment increased gene expression related to the mesodermal lineage and decreased gene expression related to the endodermal and ectodermal lineage in EBs. Fourteen days after culturing the mesodermal lineage-induced EBs in the osteogenic or chondrogenic differentiation medium, we observed enhanced osteogenic and chondrogenic differentiation compared with untreated EBs, as evaluated using qRT-PCR, cytochemistry, immunocytochemistry, and flow cytometry. This method may be useful for enhancing the osteogenic or chondrogenic differentiation of hESCs or hiPSCs.

Leeper, N. J., et al. (2010). "Stem cell therapy for vascular regeneration: adult, embryonic, and induced pluripotent stem cells." Circulation **122**(5): 517-526.

Lehnen, D., et al. (2017). "IAP-Based Cell Sorting Results in Homogeneous Transplantable Dopaminergic Precursor Cells Derived from Human Pluripotent Stem Cells." Stem Cell Reports **9**(4): 1207-1220.

Human pluripotent stem cell (hPSC)-derived mesencephalic dopaminergic (mesDA) neurons can relieve motor deficits in animal models of Parkinson's disease (PD). Clinical translation of differentiation protocols requires standardization of production procedures, and surface-marker-based cell sorting is considered instrumental for reproducible generation of defined cell products. Here, we demonstrate that integrin-associated protein (IAP) is a cell surface marker suitable for enrichment of hPSC-derived mesDA progenitor cells. Immunomagnetically sorted IAP(+) mesDA progenitors showed increased expression of ventral midbrain floor plate markers, lacked expression of pluripotency markers, and differentiated into mature dopaminergic (DA) neurons in vitro. Intrastriatal transplantation of IAP(+) cells sorted at day 16 of differentiation in a rat model of PD resulted in functional recovery. Grafts from sorted IAP(+) mesDA progenitors were more homogeneous in size and DA neuron density. Thus, we suggest IAP-based sorting for reproducible prospective enrichment of mesDA progenitor cells in clinical cell replacement strategies.

Lengerke, C. and G. Q. Daley (2010). "Autologous blood cell therapies from pluripotent stem cells." Blood Rev **24**(1): 27-37.

The discovery of human embryonic stem cells (hESCs) raised promises for a universal resource for cell based therapies in regenerative medicine. Recently, fast-paced progress has been made towards the generation of pluripotent stem cells (PSCs) amenable for clinical applications, culminating in reprogramming of adult somatic cells to autologous PSCs that can be indefinitely expanded in vitro. However, besides the efficient generation of bona fide, clinically safe PSCs (e.g., without the use of oncoproteins and gene transfer based on viruses inserting randomly into the genome), a major challenge in the field remains how to efficiently differentiate PSCs to specific lineages and how to select cells that will function normally upon transplantation in adults. In this review, we analyse the in vitro differentiation potential of PSCs to the hematopoietic lineage by discussing blood cell types that can be currently obtained, limitations in derivation of adult-type HSCs and prospects for clinical application of PSCs-derived blood cells.

Lengerova, A., et al. (1973). "Analysis of deficient colony-forming performance of bone marrow cells in non-syngeneic cell milieu--the impact of non-immune interactions on the behaviour of pluripotent stem cells and the role of H-2 gene products." Transplant Rev **15**: 89-122.

Levy, A., et al. (2015). "Pluripotent stem cells as a cellular model for skin: relevance for physiopathology, cell/gene therapy and drug screening." Eur J Dermatol **25 Suppl 1**: 12-17.

The skin represents the largest tissue in the human body. Its external part, the epidermis, accomplishes vital functions such as barrier protection, thermoregulation and immune function. The mammalian skin epidermis has been for decades the paradigm for studying the molecular events that occur in tissue homeostasis and repair. Many genes and signaling pathways have been identified by the use of manipulated transgenic and KO mice. However, despite numerous elegant transgenic mice experiments, absence of an appropriate in vitro model system has hampered the molecular study of the early events responsible for epidermal and dermal commitments, stages at which congenital genetic alterations are responsible for hundreds of rare skin diseases. For most of them, etiology and treatment are still missing. Here we review the last decade of studies aimed at designing cellular models from pluripotent stem cells (PSC) that recapitulate in vitro the main molecular steps of skin formation. As described below, PSC-based models are powerful tools to (i) clarify early molecular events that occur during epithelial/mesenchymal interactions, (ii) produce in large amount skin cells that could become an alternative for cell/gene therapies and (iii) screen for therapeutic compounds to treat genodermatoses.

Lewandowski, J., et al. (2018). "The impact of in vitro cell culture duration on the maturation of human cardiomyocytes derived from induced pluripotent stem cells of myogenic origin." Cell Transplant **27**(7): 1047-1067.

Ischemic heart disease, also known as coronary artery disease (CAD), poses a challenge for regenerative medicine. iPSC technology might lead to a breakthrough due to the possibility of directed cell differentiation delivering a new powerful source of human autologous cardiomyocytes. One of the factors supporting proper cell maturation is in vitro culture duration. In this study, primary human skeletal muscle myoblasts were selected as a myogenic cell type reservoir for genetic iPSC reprogramming. Skeletal muscle myoblasts have similar ontogeny embryogenetic pathways (myoblasts vs. cardiomyocytes), and thus, a greater chance of

myocardial development might be expected, with maintenance of acquired myogenic cardiac cell characteristics, from the differentiation process when iPSCs of myoblastoid origin are obtained. Analyses of cell morphological and structural changes, gene expression (cardiac markers), and functional tests (intracellular calcium transients) performed at two in vitro culture time points spanning the early stages of cardiac development (day 20 versus 40 of cell in vitro culture) confirmed the ability of the obtained myogenic cells to acquire adult features of differentiated cardiomyocytes. Prolonged 40-day iPSC-derived cardiomyocytes (iPSC-CMs) revealed progressive cellular hypertrophy; a better-developed contractile apparatus; expression of marker genes similar to human myocardial ventricular cells, including a statistically significant CX43 increase, an MHC isoform switch, and a troponin I isoform transition; more efficient intercellular calcium handling; and a stronger response to beta-adrenergic stimulation.

Li, C., et al. (2013). "Arf tumor suppressor and miR-205 regulate cell adhesion and formation of extraembryonic endoderm from pluripotent stem cells." Proc Natl Acad Sci U S A **110**(12): E1112-1121.

Induction of the Arf tumor suppressor (encoded by the alternate reading frame of the Cdkn2a locus) following oncogene activation engages a p53-dependent transcriptional program that limits the expansion of incipient cancer cells. Although the p19(Arf) protein is not detected in most tissues of fetal or young adult mice, it is physiologically expressed in the fetal yolk sac, a tissue derived from the extraembryonic endoderm (ExEn). Expression of the mouse p19(Arf) protein marks late stages of ExEn differentiation in cultured embryoid bodies (EBs) derived from either embryonic stem cells or induced pluripotent stem cells. Arf inactivation delays differentiation of the ExEn lineage within EBs, but not the formation of other germ cell lineages from pluripotent progenitors. Arf is required for the timely induction of ExEn cells in response to Ras/Erk signaling and, in turn, acts through p53 to ensure the development, but not maintenance, of the ExEn lineage. Remarkably, a significant temporal delay in ExEn differentiation detected during the maturation of Arf-null EBs is rescued by enforced expression of mouse microRNA-205 (miR-205), a microRNA up-regulated by p19(Arf) and p53 that controls ExEn cell migration and adhesion. The noncanonical and canonical roles of Arf in ExEn development and tumor suppression, respectively, may be conceptually linked through mechanisms that govern cell attachment and migration.

Li, J., et al. (2014). "Advances in understanding the cell types and approaches used for generating induced pluripotent stem cells." J Hematol Oncol **7**: 50.

Successfully reprogramming somatic cells to a pluripotent state generates induced pluripotent stem (iPS) cells (or iPSCs), which have extensive self-renewal capacity like embryonic stem cells (ESCs). iPSCs can also generate daughter cells that can further undergo differentiation into various lineages or terminally differentiate to reach their final functional state. The discovery of how to produce iPSCs opened a new field of stem cell research with both intellectual and therapeutic benefits. The huge potential implications of disease-specific or patient-specific iPSCs have impelled scientists to solve problems hindering their applications in clinical medicine, especially the issues of convenience and safety. To determine the range of tissue types amenable to reprogramming as well as their particular characteristics, cells from three embryonic germ layers have been assessed, and the advantages that some tissue origins have over fibroblast origins concerning efficiency and accessibility have been elucidated. To provide safe iPSCs in an efficient and convenient way, the delivery systems and combinations of inducing factors as well as the chemicals used to generate iPSCs have also been significantly improved in addition to the efforts on finding better donor cells. Currently, iPSCs can be generated without c-Myc and Klf4 oncogenes, and non-viral delivery integration-free chemically mediated reprogramming methods have been successfully employed with relatively satisfactory efficiency. This paper will review recent advances in iPS technology by highlighting tissue origin and generation of iPSCs. The obstacles that need to be overcome for clinical applications of iPSCs are also discussed.

Li, L., et al. (2012). "Role of E-cadherin and other cell adhesion molecules in survival and differentiation of human pluripotent stem cells." Cell Adh Migr **6**(1): 59-70.

The survival, proliferation, self-renewal and differentiation of human pluripotent stem cells (hPSCs, including human embryonic stem cells and human induced pluripotent stem cells) involve a number of processes that require cell-cell and cell-matrix interactions. The cell adhesion molecules (CAMs), a group of cell surface proteins play a pivotal role in mediating such interactions. Recent studies have provided insights into the essential roles and mechanisms of CAMs in the regulation of hPSC fate decisions. Here, we review the latest research progress in this field and focus on how E-cadherin and several other important CAMs including classic cadherins, Ig-superfamily CAMs, integrins and heparin sulfate

proteoglycans control survival and differentiation of hPSCs.

Li, Q., et al. (2015). "Transplantation of induced pluripotent stem cell-derived renal stem cells improved acute kidney injury." Cell Biosci **5**: 45.

BACKGROUND: Acute kidney injury (AKI) is a severe disease with high morbidity and mortality. Methods that promote repair of the injured kidney have been extensively investigated. Cell-based therapy with mesenchymal stem cells or renal progenitor cells (RPCs) resident in the kidney has appeared to be an effective strategy for the treatment of AKI. Embryonic stem cells or induced pluripotent stem cells (iPSCs) are also utilized for AKI recovery. However, the therapeutic effect of iPSC-derived RPCs for AKI has yet to be determined. METHODS: In this study, we induced iPSCs differentiation into RPCs using a nephrogenic cocktail of factors combined with the renal epithelial cell growth medium. We then established the rat ischemia-reperfusion injury (IR) model and transplanted the iPSC-derived RPCs into the injured rats in combination with the hydrogel. Next, we examined the renal function-related markers and renal histology to assess the therapeutic effect of the injected cells. Moreover, we investigated the mechanism by which iPSC-derived RPCs affect AKI caused by IR. RESULTS: We showed that the differentiation efficiency of iPSCs to RPCs increased when cultured with renal epithelial cell growth medium after stimulation with a nephrogenic cocktail of factors. The transplantation of iPSC-derived RPCs decreased the levels of biomarkers indicative of renal injury and attenuated the necrosis and apoptosis of renal tissues, but resulted in the up-regulation of renal tubules formation, cell proliferation, and the expression of pro-renal factors. CONCLUSION: Our results revealed that iPSC-derived RPCs can protect AKI rat from renal function impairment and severe tubular injury by up-regulating the renal tubules formation, promoting cell proliferation, reducing apoptosis, and regulating the microenvironment in the injured kidney.

Li, W., et al. (2015). "Human induced pluripotent stem cells in Parkinson's disease: A novel cell source of cell therapy and disease modeling." Prog Neurobiol **134**: 161-177.

Human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) are two novel cell sources for studying neurodegenerative diseases. Dopaminergic neurons derived from hiPSCs/hESCs have been implicated to be very useful in Parkinson's disease (PD) research, including cell replacement therapy, disease modeling and drug screening. Recently, great efforts have been made to improve the application of hiPSCs/hESCs in PD research. Considerable advances have been made in recent years, including advanced reprogramming strategies without the use of viruses or using fewer transcriptional factors, optimized methods for generating highly homogeneous neural progenitors with a larger proportion of mature dopaminergic neurons and better survival and integration after transplantation. Here we outline the progress that has been made in these aspects in recent years, particularly during the last year, and also discuss existing issues that need to be addressed.

Li, W., et al. (2011). "Simple autogeneic feeder cell preparation for pluripotent stem cells." Stem Cell Res **6**(1): 83-89.

Mouse embryonic fibroblasts (MEFs) are the most commonly used feeder cells for pluripotent stem cells. However, autogeneic feeder (AF) cells have several advantages such as no xenogeneic risks and reduced costs. In this report, we demonstrate that common marmoset embryonic stem (cmES) cells can be maintained on common marmoset AF (cmAF) cells. These cmES cells were maintained on cmAF cells for 6 months, retaining their morphology, normal karyotype, and expression patterns for the pluripotent markers Oct-3/4, Nanog, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81, as well as their ability to differentiate into cardiac and neural cells. Antibody array analysis revealed equivalent protein expression profiles between cmES cells maintained on cmAF cells and MEFs. In addition, similarly prepared human embryonic stem (hES) and induced pluripotent stem (hiPS) cell-derived AF cells supported the growth of and maintained the morphology and pluripotent marker expressions of hES and hiPS cells, respectively. DNA microarray analysis revealed that these hES and hiPS cells had mRNA expression profiles similar to those of hES and hiPS cells maintained on MEFs, respectively. Taken together, these findings imply that AF cells can replace MEFs in the routine maintenance of primate pluripotent stem cells.

Li, X., et al. (2014). "Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells to airway epithelial cells attenuates cigarette smoke-induced damage." Am J Respir Cell Mol Biol **51**(3): 455-465.

Transplantation of mesenchymal stem cells (MSCs) holds great promise in the repair of cigarette smoke (CS)-induced lung damage in chronic obstructive pulmonary disease (COPD). Because CS leads to mitochondrial dysfunction, we aimed to investigate the potential benefit of mitochondrial transfer from human-induced pluripotent stem cell-derived MSCs (iPSC-MSCs) to CS-exposed airway epithelial cells in vitro and in vivo. Rats were exposed

to 4% CS for 1 hour daily for 56 days. At Days 29 and, human iPSC-MSCs or adult bone marrow-derived MSCs (BM-MSCs) were administered intravenously to CS-exposed rats. CS-exposed rats exhibited severe alveolar destruction with a higher mean linear intercept (Lm) than sham air-exposed rats ($P < 0.001$) that was attenuated in the presence of iPSC-MSCs or BM-MSCs ($P < 0.01$). The attenuation of Lm value and the severity of fibrosis was greater in the iPSC-MSC-treated group than in the BM-MSC-treated group ($P < 0.05$). This might have contributed to the novel observation of mitochondrial transfer from MSCs to rat airway epithelial cells in lung sections exposed to CS. In vitro studies further revealed that transfer of mitochondria from iPSC-MSCs to bronchial epithelial cells (BEAS-2B) was more effective than from BM-MSCs, with preservation of adenosine triphosphate contents. This distinct mitochondrial transfer occurred via the formation of tunneling nanotubes. Inhibition of tunneling nanotube formation blocked mitochondrial transfer. Our findings indicate a higher mitochondrial transfer capacity of iPSC-MSCs than BM-MSCs to rescue CS-induced mitochondrial damage. iPSC-MSCs may thus hold promise for the development of cell therapy in COPD.

Li, Y., et al. (2016). "Reprogramming of blood cells into induced pluripotent stem cells as a new cell source for cartilage repair." Stem Cell Res Ther **7**: 31.

BACKGROUND: An attempt was made to reprogram peripheral blood cells into human induced pluripotent stem cell (hiPSCs) as a new cell source for cartilage repair. METHODS: We generated chondrogenic lineage from human peripheral blood via hiPSCs using an integration-free method. Peripheral blood cells were either obtained from a human blood bank or freshly collected from volunteers. After transforming peripheral blood cells into iPSCs, the newly derived iPSCs were further characterized through karyotype analysis, pluripotency gene expression and cell differentiation ability. iPSCs were differentiated through multiple steps, including embryoid body formation, hiPSC-mesenchymal stem cell (MSC)-like cell expansion, and chondrogenic induction for 21 days. Chondrocyte phenotype was then assessed by morphological, histological and biochemical analysis, as well as the chondrogenic expression. RESULTS: hiPSCs derived from peripheral blood cells were successfully generated, and were characterized by fluorescent immunostaining of pluripotent markers and teratoma formation in vivo. Flow cytometric analysis showed that MSC markers CD73 and CD105 were present in monolayer cultured hiPSC-MSC-like cells. Both alcian blue and toluidine blue staining of hiPSC-MSC-chondrogenic pellets showed as positive. Immunohistochemistry of collagen II and X staining of the pellets were also positive. The sulfated glycosaminoglycan content was significantly increased, and the expression levels of the chondrogenic markers COL2, COL10, COL9 and AGGRECAN were significantly higher in chondrogenic pellets than in undifferentiated cells. These results indicated that peripheral blood cells could be a potential source for differentiation into chondrogenic lineage in vitro via generation of mesenchymal progenitor cells. CONCLUSIONS: This study supports the potential applications of utilizing peripheral blood cells in generating seed cells for cartilage regenerative medicine in a patient-specific and cost-effective approach.

Li, Y., et al. (2017). "Cell sex affects extracellular matrix protein expression and proliferation of smooth muscle progenitor cells derived from human pluripotent stem cells." Stem Cell Res Ther **8**(1): 156.

BACKGROUND: Smooth muscle progenitor cells (pSMCs) differentiated from human pluripotent stem cells (hPSCs) hold great promise for treating diseases or degenerative conditions involving smooth muscle pathologies. However, the therapeutic potential of pSMCs derived from men and women may be very different. Cell sex can exert a profound impact on the differentiation process of stem cells into somatic cells. In spite of advances in translation of stem cell technologies, the role of cell sex and the effect of sex hormones on the differentiation towards mesenchymal lineage pSMCs remain largely unexplored. METHODS: Using a standard differentiation protocol, two human embryonic stem cell lines (one male line and one female line) and three induced pluripotent stem cell lines (one male line and two female lines) were differentiated into pSMCs. We examined differences in the differentiation of male and female hPSCs into pSMCs, and investigated the effect of 17beta-estradiol (E2) on the extracellular matrix (ECM) metabolisms and cell proliferation rates of the pSMCs. Statistical analyses were performed by using Student's t test or two-way ANOVA, $p < 0.05$. RESULTS: Male and female hPSCs had similar differentiation efficiencies and generated morphologically comparable pSMCs under a standard differentiation protocol, but the derived pSMCs showed sex differences in expression of ECM proteins, such as MMP-2 and TIMP-1, and cell proliferation rates. E2 treatment induced the expression of myogenic gene markers and suppressed ECM degradation activities through reduction of MMP activity and increased expression of TIMP-1 in female pSMCs, but not in male pSMCs. CONCLUSIONS: hPSC-derived pSMCs from different sexes show differential expression of ECM proteins and proliferation rates. Estrogen appears to promote maturation and ECM protein expression in female

pSMCs, but not in male pSMCs. These data suggest that intrinsic cell-sex differences may influence progenitor cell biology.

Li, Z., et al. (2011). "Functional characterization and expression profiling of human induced pluripotent stem cell- and embryonic stem cell-derived endothelial cells." Stem Cells Dev **20**(10): 1701-1710.

With regard to human induced pluripotent stem cells (hiPSCs), in which adult cells are reprogrammed into embryonic-like cells using defined factors, their functional and transcriptional expression pattern during endothelial differentiation has yet to be characterized. In this study, hiPSCs and human embryonic stem cells (hESCs) were differentiated using the embryoid body method, and CD31(+) cells were sorted. Fluorescence activated cell sorting analysis of hiPSC-derived endothelial cells (hiPSC-ECs) and hESC-derived endothelial cells (hESC-ECs) demonstrated similar endothelial gene expression patterns. We showed functional vascular formation by hiPSC-ECs in a mouse Matrigel plug model. We compared the gene profiles of hiPSCs, hESCs, hiPSC-ECs, hESC-ECs, and human umbilical vein endothelial cells (HUVECs) using whole genome microarray. Our analysis demonstrates that gene expression variation of hiPSC-ECs and hESC-ECs contributes significantly to biological differences between hiPSC-ECs and hESC-ECs as well as to the "distances" among hiPSCs, hESCs, hiPSC-ECs, hESC-ECs, and HUVECs. We further conclude that hiPSCs can differentiate into functional endothelial cells, but with limited expansion potential compared with hESC-ECs; thus, extensive studies should be performed to explore the cause and extent of such differences before clinical application of hiPSC-ECs can begin.

Li, Z., et al. (2018). "Development of trophoblast cystic structures from human induced pluripotent stem cells in limited-area cell culture." Biochem Biophys Res Commun.

We developed a novel engineering technique to induce differentiation of human induced pluripotent stem cells (hiPSCs) into organoids mimicking the trophectoderm (TE). Here, hiPSCs were cultured on a limited area of 2-4mm in diameter. After 15-20 days, spherical cysts appeared on the surface of the limited area. Secretion of human chorionic gonadotrophin (hCG) began to increase after approximately 20 days and remained dramatically elevated over the next 20 days. Limited-area-cultured cysts exhibited expression of hCG, which was a result of epithelial differentiation. Low expression levels of pluripotent genes and high expression levels of trophoblast lineage-specific genes were detected in the cells of spherical cysts. Multinucleated syncytia trophoblast was observed in the reseeded cystic cells. We observed hiPSC-derived cysts that morphologically resembled trophectoderm in vivo. The limited-area cell culture induced a three-dimensional (3D) trophectoderm organoid, which has potential for use in the study of human trophoblast differentiation and placental morphogenesis.

Liao, J., et al. (2009). "Generation of induced pluripotent stem cell lines from adult rat cells." Cell Stem Cell **4**(1): 11-15.

Liao, Y. J., et al. (2018). "Porcine induced pluripotent stem cell-derived osteoblast-like cells prevent glucocorticoid-induced bone loss in Lanyu pigs." PLoS One **13**(8): e0202155.

The application of appropriate animal models and techniques for the study of osteoporosis is important. Lanyu pigs, a local miniature breed, have been widely used in various biomedical studies in Taiwan. This study aimed to induce bone loss in Lanyu pigs and to examine whether porcine induced pluripotent stem cell (piPSC)-derived osteoblast-like cells could recover bone mass of tibiae via local cell transplantation. piPSCs were directed to differentiate into osteoblast-like cells using osteogenic medium, and differentiated cells expressed osteogenic markers and phenotypes. Twenty mature female Lanyu pigs were divided into four groups, including control (C, 1% calcium diet), treatment 1 (T1, ovariectomy + 1% calcium diet), treatment 2 (T2, ovariectomy + 0.5% calcium diet), and treatment 3 (T3, ovariectomy + 0.5% calcium diet + 1 mg/kg of prednisolone) and were subjected to bone loss induction for twelve months. Micro-CT images revealed that the lowest trabecular bone parameters, such as trabecular bone volume, thickness, separation, number, and total porosity, were detected in the T3 group. The lowest proportions of cortical bone in the proximal metaphysis, proximal diaphysis, and distal diaphysis were also found in the T3 group. These results indicate that ovariectomy, calcium restriction, and prednisolone administration can be applied to induce proper bone loss in Lanyu pigs. After bone loss induction, pigs were subjected to cell transplantation in the left tibiae and were maintained for another six months. Results showed that transplanted piPSC-derived osteoblast-like cells significantly improved trabecular bone structures at transplanted sites and maintained cortical bone structures in the proximal metaphysis. In conclusion, the therapeutic potential of piPSC-derived osteoblast-like cells was confirmed via cell transplantation in the left tibiae of Lanyu pigs. These findings reveal the therapeutic potential of piPSCs for glucocorticoid-induced bone loss in pig models.

Liao, Y. J., et al. (2018). "Porcine-induced pluripotent stem cell-derived osteoblast-like cells ameliorate trabecular bone mass of osteoporotic rats." Regen Med **13**(6): 659-671.

AIM: We created rat models of osteoporosis and verified a novel idea to recover bone mass via local cell transplantation. MATERIALS & METHODS: The rats were treated with ovariectomy, 0.1% calcium diet or 3 mg/kg body weight/day of prednisolone and porcine-induced pluripotent stem cell (piPSC)-derived osteoblast-like cells were transplanted into the medullary cavity of the left femurs. RESULTS: The piPSC-derived osteoblast-like cells exerted therapeutic potential on prednisolone treatment group, which confirmed by improvements in trabecular bone volume (15.93 +/- 2.20%), bone surface/volume ratio (27.82 +/- 1.40 1/mm), thickness (1.40 +/- 0.01 mm), separation (0.99 +/- 0.10 mm), number (1.13 +/- 0.13 1/mm) and total porosity (84.06 +/- 2.20%). CONCLUSION: These results first uncovered therapeutic potential of xenotransplantation with piPSCs for glucocorticoid-induced osteoporosis treatment in the rat models.

Lieu, D. K., et al. (2012). "Engineered human pluripotent stem cell-derived cardiac cells and tissues for electrophysiological studies." Drug Discov Today Dis Models **9**(4): e209-e217.

Human cardiomyocytes (CMs) do not proliferate in culture and are difficult to obtain for practical reasons. As such, our understanding of the mechanisms that underlie the physiological and pathophysiological development of the human heart is mostly extrapolated from studies of the mouse and other animal models or heterologus expression of defective gene product(s) in non-human cells. Although these studies provided numerous important insights, much of the exact behavior in human cells remains unexplored given that significant species differences exist. With the derivation of human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSCs) from patients with underlying heart disease, a source of human CMs for disease modeling, cardiotoxicity screening and drug discovery is now available. In this review, we focus our discussion on the use of hESC/ iPSC-derived cardiac cells and tissues for studying various heart rhythm disorders and the associated pro-arrhythmogenic properties in relation to advancements in electrophysiology and tissue engineering.

Liew, C. G. (2010). "Generation of insulin-producing cells from pluripotent stem cells: from the selection of cell sources to the optimization of protocols." Rev Diabet Stud **7**(2): 82-92.

The pancreas arises from Pdx1-expressing progenitors in developing foregut endoderm in early embryo. Expression of Ngn3 and NeuroD1 commits the cells to form endocrine pancreas, and to differentiate into subsets of cells that constitute islets of Langerhans. beta-cells in the islets transcribe gene-encoding insulin, and subsequently process and secrete insulin, in response to circulating glucose. Dysfunction of beta-cells has profound metabolic consequences leading to hyperglycemia and diabetes mellitus. beta-cells are destroyed via autoimmune reaction in type 1 diabetes (T1D). Type 2 diabetes (T2D), characterized by impaired beta-cell functions and reduced insulin sensitivity, accounts for 90% of all diabetic patients. Islet transplantation is a promising treatment for T1D. Pluripotent stem cells provide an unlimited cell source to generate new beta-cells for patients with T1D. Furthermore, derivation of induced pluripotent stem cells (iPSCs) from patients captures "disease-in-a-dish" for autologous cell replacement therapy, disease modeling, and drug screening for both types of diabetes. This review highlights essential steps in pancreas development, and potential stem cell applications in cell regeneration therapy for diabetes mellitus.

Lim, W. F., et al. (2013). "Hematopoietic cell differentiation from embryonic and induced pluripotent stem cells." Stem Cell Res Ther **4**(3): 71.

Pluripotent stem cells, both embryonic stem cells and induced pluripotent stem cells, are undifferentiated cells that can self-renew and potentially differentiate into all hematopoietic lineages, such as hematopoietic stem cells (HSCs), hematopoietic progenitor cells and mature hematopoietic cells in the presence of a suitable culture system. Establishment of pluripotent stem cells provides a comprehensive model to study early hematopoietic development and has emerged as a powerful research tool to explore regenerative medicine. Nowadays, HSC transplantation and hematopoietic cell transfusion have successfully cured some patients, especially in malignant hematological diseases. Owing to a shortage of donors and a limited number of the cells, hematopoietic cell induction from pluripotent stem cells has been regarded as an alternative source of HSCs and mature hematopoietic cells for intended therapeutic purposes. Pluripotent stem cells are therefore extensively utilized to facilitate better understanding in hematopoietic development by recapitulating embryonic development in vivo, in which efficient strategies can be easily designed and deployed for the generation of hematopoietic lineages in vitro. We hereby review the current progress of hematopoietic cell induction from embryonic stem/induced pluripotent stem cells.

Lin, C. Y., et al. (2012). "Exacerbation of oxidative stress-induced cell death and differentiation in induced pluripotent stem cells lacking heme oxygenase-1." Stem Cells Dev **21**(10): 1675-1687.

Embryonic stem cells (ESCs) are promising donor sources in cell therapies for various diseases. Although low levels of reactive oxygen species (ROS) are necessary for the maintenance of stem cells, increased ROS levels initiate differentiation and cell damage. We and others have previously demonstrated that heme oxygenase (HO)-1, a stress response protein with antioxidative and anti-inflammatory properties, plays critical protective functions in cardiovascular and other diseases. However, the functions of HO-1 in ESCs remain to be elucidated. Our goal was to investigate the roles of HO-1 in ESC survival and differentiation. Due to the lack of HO-1-deficient ESCs, we used Oct3/4, Sox2, c-Myc, and Klf4 retroviruses to reprogram mouse embryonic fibroblasts into induced pluripotent stem (iPS) cells of different HO-1 genotypes. These iPS-HO-1 cells exhibited characteristics of mouse ESCs (mESCs) and formed teratomas that were composed of cell types of all 3 germ layers after injected into severe combined immunodeficiency mice. In response to oxidant stress, iPS-HO-1(-/-) cells accumulated higher levels of intracellular ROS compared with D3 mESCs or iPS-HO-1(+/+) cells and were more prone to oxidant-induced cell death. Spontaneous differentiation experiments revealed that Oct4 levels were significantly lower in iPS-HO-1(-/-) cells after leukemia inhibitory factor withdrawal and removal of feeders. Further, during the course of spontaneous differentiation, iPS-HO-1(-/-) cells had enhanced Erk1/2 phosphorylation, which has been linked to ESC differentiation. By the loss-of-function approach using iPS-HO-1(-/-) cells, our results demonstrate that a lack of HO-1 renders iPS cells more prone to oxidative stress-induced cell death and differentiation.

Lin, H., et al. (2018). "Hydrogel-Based Bioprocess for Scalable Manufacturing of Human Pluripotent Stem Cell-Derived Neural Stem Cells." ACS Appl Mater Interfaces **10**(35): 29238-29250.

Neural stem cells derived from human pluripotent stem cells (hPSC-NSCs) are of great value for modeling diseases, developing drugs, and treating neurological disorders. However, manufacturing high-quantity and -quality hPSC-NSCs, especially for clinical applications, remains a challenge. Here, we report a chemically defined, high-yield, and scalable bioprocess for manufacturing hPSC-NSCs. hPSCs are expanded and differentiated into NSCs in microscale tubes made with alginate hydrogels. The tubes are used to isolate cells from the hydrodynamic stresses in the culture vessel and limit the radial diameter of the cell mass to less than 400 mum to ensure efficient mass transport during the culture. The hydrogel tubes provide uniform, reproducible, and cell-friendly microspaces and microenvironments for cells. With this new technology, we showed that hPSC-NSCs could be produced in 12 days with high viability (approximately 95%), high purity (>90%), and high yield (approximately 5 x 10(8) cells/mL of microspace). The volumetric yield is about 250 times more than the current state-of-the-art. Whole transcriptome analysis and quantitative real-time polymerase chain reaction showed that hPSC-NSCs made by this process had a similar gene expression to hPSC-NSCs made by the conventional culture technology. The produced hPSC-NSCs could mature into both neurons and glial cells in vitro and in vivo. The process developed in this paper can be used to produce large numbers of hPSC-NSCs for various biomedical applications in the future.

Lin, H., et al. (2018). "A Scalable and Efficient Bioprocess for Manufacturing Human Pluripotent Stem Cell-Derived Endothelial Cells." Stem Cell Reports **11**(2): 454-469.

Endothelial cells (ECs) are of great value for cell therapy, tissue engineering, and drug discovery. Obtaining high-quantity and -quality ECs remains very challenging. Here, we report a method for the scalable manufacturing of ECs from human pluripotent stem cells (hPSCs). hPSCs are expanded and differentiated into ECs in a 3D thermoreversible PNIPAAm-PEG hydrogel. The hydrogel protects cells from hydrodynamic stresses in the culture vessel and prevents cells from excessive agglomeration, leading to high-culture efficiency including high-viability (>90%), high-purity (>80%), and high-volumetric yield (2.0 x 10(7) cells/mL). These ECs (i.e., 3D-ECs) had similar properties as ECs made using 2D culture systems (i.e., 2D-ECs). Genome-wide gene expression analysis showed that 3D-ECs had higher expression of genes related to vasculature development, extracellular matrix, and glycolysis, while 2D-ECs had higher expression of genes related to cell proliferation.

Lin, H., et al. (2017). "An Integrated Miniature Bioprocessing for Personalized Human Induced Pluripotent Stem Cell Expansion and Differentiation into Neural Stem Cells." Sci Rep **7**: 40191.

Human induced pluripotent stem cells (iPSCs) are ideal cell sources for personalized cell therapies since they can be expanded to generate large numbers of cells and differentiated into presumably all the cell types of the human body in vitro. In addition, patient specific iPSC-derived cells induce minimal or no immune response in vivo. However, with current cell culture technologies and bioprocessing, the cost for

biomanufacturing clinical-grade patient specific iPSCs and their derivatives are very high and not affordable for majority of patients. In this paper, we explored the use of closed and miniature cell culture device for biomanufacturing patient specific neural stem cells (NSCs) from iPSCs. We demonstrated that, with the assist of a thermoreversible hydrogel scaffold, the bioprocessing including iPSC expansion, iPSC differentiation into NSCs, the subsequent depletion of undifferentiated iPSCs from the NSCs, and concentrating and transporting the purified NSCs to the surgery room, could be integrated and completed within two closed 15 ml conical tubes.

Lin, H., et al. (2009). "Pluripotent hair follicle neural crest stem-cell-derived neurons and schwann cells functionally repair sciatic nerves in rats." Mol Neurobiol **40**(3): 216-223.

In this paper, we constructed a novel acellular nerve xenograft (ANX) seeded with neurons and Schwann cells to bridge long-distance gaps in rat sciatic nerves. The neurons and Schwann cells were induced from Sprague Dawley (SD) rat hair follicle neural crest stem cells with sonic hedgehog/retinoic acid and neuregulin 1, respectively. Fifty male SD rats were randomly divided into two groups (n = 25): ANX + cells group and ANX group. A 4-cm-long sciatic nerve defect was created on the right hind limb and bridged with cell-seeded ANX in ANX + cells group or ANX alone in ANX group. We found that the implanted neurons and Schwann cells could survive by 4 weeks and as far as 52 weeks posttransplantation. In implanted grafts, chemical synaptic structures were also found under transmission electron microscope and confirmed with immunostaining of synapsin 1, a synaptic marker. The number of regenerated axons in ANX + cells group was higher than that in ANX group ($P < 0.01$). This novel implantation of neurons and Schwann cells via acellular nerve graft may provide an alternative way for repairing peripheral nerve defect.

Lin, L., et al. (2016). "Towards Personalized Regenerative Cell Therapy: Mesenchymal Stem Cells Derived from Human Induced Pluripotent Stem Cells." Curr Stem Cell Res Ther **11**(2): 122-130.

Mesenchymal stem cells (MSCs) are adult stem cells with the capacity of self-renewal and multilineage differentiation, and can be isolated from several adult tissues. However, isolating MSCs from adult tissues for cell therapy is hampered by the invasive procedure, the rarity of the cells and their attenuated proliferation capacity when cultivated and expanded in vitro. Human MSCs derived from induced pluripotent stem cells (iPSC-MSCs) have now evolved as a promising alternative cell source for MSCs and regenerative medicine. Several groups, including ours, have reported successful derivation of functional iPSC-MSCs and applied these cells in MSC-based therapeutic testing. Still, the current experience and understanding of iPSC-MSCs with respect to production methods, safety and efficacy are primitive. In this review, we highlight the methodological progress in iPSC-MSC research, describing the importance of choosing the right sources of iPSCs, iPSC reprogramming methods, iPSC culture systems, embryoid body intermediates, pathway inhibitors, basal medium, serum, growth factors and culture surface coating. We also highlight some progress in the application of iPSC-MSCs in direct cell therapy, tissue engineering and gene therapy.

Lin, S. L., et al. (2010). "MicroRNA miR-302 inhibits the tumorigenecity of human pluripotent stem cells by coordinate suppression of the CDK2 and CDK4/6 cell cycle pathways." Cancer Res **70**(22): 9473-9482.

miR-302 is the major microRNA found in human embryonic stem cells and induced pluripotent stem cells, but its function has been unclear. In mice, there is evidence that miR-302 may silence p21Cip1 (CDKN1A) to promote cell proliferation, whereas studies in human reprogrammed pluripotent stem cells suggested that elevated miR-302 expression inhibited cell cycle transit. Here, we clarify this difference, reporting that in human cells, miR-302 simultaneously suppressed both the cyclin E-CDK2 and cyclin D-CDK4/6 pathways to block>70% of the G1-S cell cycle transition. Concurrent silencing of BMI-1, a cancer stem cell marker targeted by miR-302, further promoted tumor suppressor functions of p16Ink4a and p14/p19Arf directed against CDK4/6-mediated cell proliferation. Among all G1 phase checkpoint regulators, human p21Cip1 was found not to be a valid target of miR-302. Overall, our findings indicate that miR-302 inhibits human pluripotent stem cell tumorigenicity by enhancing multiple G1 phase arrest pathways rather than by silencing p21Cip1.

Lin, Y., et al. (2017). "Differentiation, Evaluation, and Application of Human Induced Pluripotent Stem Cell-Derived Endothelial Cells." Arterioscler Thromb Vasc Biol **37**(11): 2014-2025.

The emergence of induced pluripotent stem cell (iPSC) technology paves the way to generate large numbers of patient-specific endothelial cells (ECs) that can be potentially delivered for regenerative medicine in patients with cardiovascular disease. In the last decade, numerous protocols that differentiate EC from iPSC have been developed by many groups. In this review, we will discuss several common strategies that have been optimized for human iPSC-EC differentiation and subsequent studies that have evaluated the potential of human iPSC-EC as a cell

therapy or as a tool in disease modeling. In addition, we will emphasize the importance of using in vivo vessel-forming ability and in vitro clonogenic colony-forming potential as a gold standard with which to evaluate the quality of human iPSC-EC derived from various protocols.

Lindborg, B. A., et al. (2016). "Rapid Induction of Cerebral Organoids From Human Induced Pluripotent Stem Cells Using a Chemically Defined Hydrogel and Defined Cell Culture Medium." Stem Cells Transl Med **5**(7): 970-979.

UNLABELLED: Tissue organoids are a promising technology that may accelerate development of the societal and NIH mandate for precision medicine. Here we describe a robust and simple method for generating cerebral organoids (cOrgs) from human pluripotent stem cells by using a chemically defined hydrogel material and chemically defined culture medium. By using no additional neural induction components, cOrgs appeared on the hydrogel surface within 10-14 days, and under static culture conditions, they attained sizes up to 3 mm in greatest dimension by day 28. Histologically, the organoids showed neural rosette and neural tube-like structures and evidence of early corticogenesis. Immunostaining and quantitative reverse-transcription polymerase chain reaction demonstrated protein and gene expression representative of forebrain, midbrain, and hindbrain development. Physiologic studies showed responses to glutamate and depolarization in many cells, consistent with neural behavior. The method of cerebral organoid generation described here facilitates access to this technology, enables scalable applications, and provides a potential pathway to translational applications where defined components are desirable. SIGNIFICANCE: Tissue organoids are a promising technology with many potential applications, such as pharmaceutical screens and development of in vitro disease models, particularly for human polygenic conditions where animal models are insufficient. This work describes a robust and simple method for generating cerebral organoids from human induced pluripotent stem cells by using a chemically defined hydrogel material and chemically defined culture medium. This method, by virtue of its simplicity and use of defined materials, greatly facilitates access to cerebral organoid technology, enables scalable applications, and provides a potential pathway to translational applications where defined components are desirable.

Lipsitz, Y. Y., et al. (2018). "Modulating cell state to enhance suspension expansion of human pluripotent stem cells." Proc Natl Acad Sci U S A **115**(25): 6369-6374.

The development of cell-based therapies to replace missing or damaged tissues within the body or generate cells with a unique biological activity requires a reliable and accessible source of cells. Human pluripotent stem cells (hPSC) have emerged as a strong candidate cell source capable of extended propagation in vitro and differentiation to clinically relevant cell types. However, the application of hPSC in cell-based therapies requires overcoming yield limitations in large-scale hPSC manufacturing. We explored methods to convert hPSC to alternative states of pluripotency with advantageous bioprocessing properties, identifying a suspension-based small-molecule and cytokine combination that supports increased single-cell survival efficiency, faster growth rates, higher densities, and greater expansion than control hPSC cultures. ERK inhibition was found to be essential for conversion to this altered state, but once converted, ERK inhibition led to a loss of pluripotent phenotype in suspension. The resulting suspension medium formulation enabled hPSC suspension yields 5.7 +/- 0.2-fold greater than conventional hPSC in 6 d, for at least five passages. Treated cells remained pluripotent, karyotypically normal, and capable of differentiating into all germ layers. Treated cells could also be integrated into directed differentiated strategies as demonstrated by the generation of pancreatic progenitors (NKX6.1+/PDX1+ cells). Enhanced suspension-yield hPSC displayed higher oxidative metabolism and altered expression of adhesion-related genes. The enhanced bioprocess properties of this alternative pluripotent state provide a strategy to overcome cell manufacturing limitations of hPSC.

Liu, B., et al. (2018). "Cardiac recovery via extended cell-free delivery of extracellular vesicles secreted by cardiomyocytes derived from induced pluripotent stem cells." Nat Biomed Eng **2**(5): 293-303.

The ability of extracellular vesicles (EVs) to regulate a broad range of cellular processes has recently been exploited for the treatment of diseases. For example, EVs secreted by stem cells injected into infarcted hearts can induce recovery through the delivery of stem-cell-specific miRNAs. However, the retention of the EVs and the therapeutic effects are short-lived. Here, we show that an engineered hydrogel patch capable of slowly releasing EVs secreted from cardiomyocytes derived from induced pluripotent stem (iPS) cells reduced arrhythmic burden, promoted ejection-fraction recovery, decreased cardiomyocyte apoptosis 24 hours after infarction, and reduced infarct size and cell hypertrophy 4 weeks post-infarction when implanted onto infarcted rat hearts. We also show that the EVs are enriched with cardiac-specific miRNAs known to modulate cardiomyocyte-specific processes. The extended delivery of EVs secreted from iPS-cell-

derived cardiomyocytes into the heart may help understand heart recovery and treat heart injury.

Liu, J. (2013). "Induced pluripotent stem cell-derived neural stem cells: new hope for stroke?" Stem Cell Res Ther **4**(5): 115.

Human induced pluripotent stem cells (iPSCs) have attracted increasing interest in the field of ischemic stroke therapy, due to the lack of ethical concerns and reduced risk of immune rejection. However, the safety and efficiency of the donor iPSC derivates in the ischemic brain challenged the therapeutic efficacy of iPSC transplantation. Studies have focused on improving the methods to induce neural derivates from iPSCs and on trying to determine how these cells and the host ischemic environment influence each other. A recent study by Yuan and colleagues reported that neural stem cells induced from human iPSCs using retinoic acid and serum-free medium showed stable neural phenotype. After acute transplantation into the ischemic stroke model, these cells survived, migrated into the ischemic penumbra, differentiated into mature neural cells and showed beneficial effects on functional recovery. Their findings take a clear step towards the clinical application of human iPSCs for ischemic stroke. A wide range of careful studies may be raised from their work, from basic research to preclinic, to develop the best therapy for ischemic stroke.

Liu, J. T., et al. (2018). "Using Human Induced Pluripotent Stem Cell-derived Hepatocyte-like Cells for Drug Discovery." J Vis Exp(135).

The ability to differentiate human induced pluripotent stem cells (iPSCs) into hepatocyte-like cells (HLCs) provides new opportunities to study inborn errors in hepatic metabolism. However, to provide a platform that supports the identification of small molecules that can potentially be used to treat liver disease, the procedure requires a culture format that is compatible with screening thousands of compounds. Here, we describe a protocol using completely defined culture conditions, which allow the reproducible differentiation of human iPSCs to hepatocyte-like cells in 96-well tissue culture plates. We also provide an example of using the platform to screen compounds for their ability to lower Apolipoprotein B (APOB) produced from iPSC-derived hepatocytes generated from a familial hypercholesterolemia patient. The availability of a platform that is compatible with drug discovery should allow researchers to identify novel therapeutics for diseases that affect the liver.

Liu, L., et al. (2016). "Induced Pluripotent Stem Cells in Huntington's Disease: Disease Modeling and the Potential for Cell-Based Therapy." Mol Neurobiol **53**(10): 6698-6708.

Huntington's disease (HD) is an incurable neurodegenerative disorder that is characterized by motor dysfunction, cognitive impairment, and behavioral abnormalities. It is an autosomal dominant disorder caused by a CAG repeat expansion in the huntingtin gene, resulting in progressive neuronal loss predominately in the striatum and cortex. Despite the discovery of the causative gene in 1993, the exact mechanisms underlying HD pathogenesis have yet to be elucidated. Treatments that slow or halt the disease process are currently unavailable. Recent advances in induced pluripotent stem cell (iPSC) technologies have transformed our ability to study disease in human neural cells. Here, we firstly review the progress made to model HD in vitro using patient-derived iPSCs, which reveal unique insights into illuminating molecular mechanisms and provide a novel human cell-based platform for drug discovery. We then highlight the promises and challenges for pluripotent stem cells that might be used as a therapeutic source for cell replacement therapy of the lost neurons in HD brains.

Liu, Q., et al. (2016). "Muse Cells, a New Type of Pluripotent Stem Cell Derived from Human Fibroblasts." Cell Reprogram **18**(2): 67-77.

A new type of mesenchymal stem cells (MSCs) that expresses stage-specific embryonic antigen 3 (SSEA-3) and the mesenchymal cell marker CD105 are known as multilineage-differentiating stress-enduring (Muse) cells. Studies have shown that stem cells in suspension cultures are more likely to generate embryoid body-like stem cell spheres and maintain an undifferentiated phenotype and pluripotency. We separated Muse cells derived from human dermal fibroblasts by long-term trypsin incubation (LTT) through suspension cultures in methylcellulose. The Muse cells obtained expressed several pluripotency markers, including Nanog, Oct4, Sox2, and SSEA-3, and could differentiate in vitro into cells of the three germ layers, such as hepatocytes (endodermal), neural cells (ectodermal) and adipocytes, and osteocytes (mesodermal cells). These cells showed a low level of DNA methylation and a high nucleo-cytoplasmic ratio. Our study provides an innovative and exciting platform for exploring the potential cell-based therapy of various human diseases using Muse cells as well as their great possibility for regenerative medicine.

Liu, W., et al. (2018). "The Suppression of Medium Acidosis Improves the Maintenance and Differentiation of Human Pluripotent Stem Cells at High Density in

Defined Cell Culture Medium." Int J Biol Sci **14**(5): 485-496.

Cell density has profound impacts on the cell culture practices of human pluripotent stem cells. The regulation of cell growth, cell death, pluripotency and differentiation converge at high density, but it is largely unknown how different regulatory mechanisms act at this stage. We use a chemically defined medium to systemically examine cellular activities and the impact of medium components in high-density culture. We show that medium acidosis is the main factor that alters cell cycle, gene expression and cellular metabolism at high cell density. The low medium pH leads to inhibition of glucose consumption, cell cycle arrest, and subsequent cell death. At high cell density, the suppression of medium acidosis with sodium bicarbonate (NaHCO3) significantly increases culture capacity for stem cell survival, derivation, maintenance and differentiation. Our study provides a simple and effective tool to improve stem cell maintenance and applications.

Liu, X., et al. (2017). "Co-Seeding Human Endothelial Cells with Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells on Calcium Phosphate Scaffold Enhances Osteogenesis and Vascularization in Rats." Tissue Eng Part A **23**(11-12): 546-555.

A major challenge in repairing large bone defects with tissue-engineered constructs is the poor vascularization in the defect. The lack of vascular networks leads to insufficient oxygen and nutrients supply, which compromises the survival of seeded cells. To achieve favorable regenerative effects, prevascularization of tissue-engineered constructs by co-culturing of endothelial cells and bone cells is a promising strategy. The aim of this study was to investigate the effects of human-induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs) co-cultured with human umbilical vein endothelial cells (HUVECs) for prevascularization of calcium phosphate cement (CPC) scaffold on bone regeneration in vivo for the first time. HUVECs co-cultured with hiPSC-MSCs formed microcapillary-like structures in vitro. HUVECs promoted mineralization of hiPSC-MSCs on CPC scaffolds. Four groups were tested in a cranial bone defect model in nude rats: (1) CPC scaffold alone (CPC control); (2) HUVEC-seeded CPC (CPC-HUVEC); (3) hiPSC-MSC-seeded CPC (CPC-hiPSC-MSC); and (4) HUVECs co-cultured with hiPSC-MSCs on CPC scaffolds (co-culture group). After 12 weeks, the co-culture group achieved the greatest new bone area percentage of 46.38% +/- 3.8% among all groups ($p < 0.05$), which was more than four folds of the 10.61% +/- 1.43% of CPC control. In conclusion, HUVECs co-cultured with hiPSC-MSCs substantially promoted bone regeneration. The novel construct of HUVECs co-cultured with hiPSC-MSCs delivered via CPC scaffolds is promising to enhance bone and vascular regeneration in orthopedic applications.

Liu, X., et al. (2017). "Exosomes Secreted from Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Prevent Osteonecrosis of the Femoral Head by Promoting Angiogenesis." Int J Biol Sci **13**(2): 232-244.

Background: Local ischemia is the main pathological performance in osteonecrosis of the femoral head (ONFH). There is currently no effective therapy to promote angiogenesis in the femoral head. Recent studies revealed that exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells (iPS-MSC-Exos) have great therapeutic potential in ischemic tissues, but whether they could promote angiogenesis in ONFH has not been reported, and little is known regarding the underlying mechanism. Methods: iPS-MSC-Exos were intravenously injected to a steroid-induced rat osteonecrosis model. Samples of the femoral head were obtained 3 weeks after all the injections. The effects were assessed by measuring local angiogenesis and bone loss through histological and immunohistochemical (IHC) staining, micro-CT and three-dimensional microangiography. The effects of exosomes on endothelial cells were studied through evaluations of proliferation, migration and tube-forming analyses. The expression levels of angiogenic related PI3K/Akt signaling pathway of endothelial cells were evaluated following stimulation of iPS-MSC-Exos. The promoting effects of exosomes were re-evaluated following blockade of PI3K/Akt. Results: The in vivo study revealed that administration of iPS-MSC-Exos significantly prevented bone loss, and increased microvessel density in the femoral head compared with control group. We found that iPS-MSC-Exos significantly enhanced the proliferation, migration and tube-forming capacities of endothelial cells in vitro. iPS-MSC-Exos could activate PI3K/Akt signaling pathway in endothelial cells. Moreover, the promoting effects of iPS-MSC-Exos were abolished after blockade of PI3K/Akt on endothelial cells. Conclusions: Our findings suggest that transplantation of iPS-MSC-Exos exerts a preventative effect on ONFH by promoting local angiogenesis and preventing bone loss. The promoting effect might be attributed to activation of the PI3K/Akt signaling pathway on endothelial cells. The data provide the first evidence for the potential of iPS-MSC-Exos in treating ONFH.

Liu, Y., et al. (2012). "One-step derivation of mesenchymal stem cell (MSC)-like cells from human

pluripotent stem cells on a fibrillar collagen coating." PLoS One **7**(3): e33225.

Controlled differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) into cells that resemble adult mesenchymal stem cells (MSCs) is an attractive approach to obtain a readily available source of progenitor cells for tissue engineering. The present study reports a new method to rapidly derive MSC-like cells from hESCs and hiPSCs, in one step, based on culturing the cells on thin, fibrillar, type I collagen coatings that mimic the structure of physiological collagen. Human H9 ESCs and HDFa-YK26 iPSCs were singly dissociated in the presence of ROCK inhibitor Y-27632, plated onto fibrillar collagen coated plates and cultured in alpha minimum essential medium (alpha-MEM) supplemented with 10% fetal bovine serum, 50 uM magnesium L-ascorbic acid phosphate and 100 nM dexamethasone. While fewer cells attached on the collagen surface initially than standard tissue culture plastic, after culturing for 10 days, resilient colonies of homogenous spindle-shaped cells were obtained. Flow cytometric analysis showed that a high percentage of the derived cells expressed typical MSC surface markers including CD73, CD90, CD105, CD146 and CD166 and were negative as expected for hematopoietic markers CD34 and CD45. The MSC-like cells derived from pluripotent cells were successfully differentiated in vitro into three different lineages: osteogenic, chondrogenic, and adipogenic. Both H9 hES and YK26 iPS cells displayed similar morphological changes during the derivation process and yielded MSC-like cells with similar properties. In conclusion, this study demonstrates that bioimimetic, fibrillar, type I collagen coatings applied to cell culture plates can be used to guide a rapid, efficient derivation of MSC-like cells from both human ES and iPS cells.

Liu, Y., et al. (2013). "alpha-1,3-Galactosyltransferase knockout pig induced pluripotent stem cells: a cell source for the production of xenotransplant pigs." Cell Reprogram **15**(2): 107-116.

The shortage of human organs and tissues for transplant has led to significant interest in xenotransplantation of pig tissues for human patients. However, transplantation of pig organs results in an acute immune rejection, leading to death of the organ within minutes. The alpha-1,3-galactosyltransferase (GALT) gene has been knocked out in pigs to reduce rejection, yet additional genes need to be modified to ultimately make pig tissue immunocompatible with humans. The development of pig induced pluripotent stem cells (piPSCs) from GALT knockout (GALT-KO) tissue would provide an excellent cell source for complex genetic manipulations (e.g., gene targeting) that often require highly robust and proliferative cells. In this report, we generated GALT-KO piPSCs by the overexpression of POU5F1, SOX2, NANOG, LIN28, KLF-4, and C-MYC reprogramming genes. piPSCs showed classical stem cell morphology and characteristics, expressing integrated reprogramming genes in addition to the pluripotent markers AP, SSEA1, and SSEA4. GALT-KO piPSCs were highly proliferative and possessed doubling times and telomerase activity similar to human embryonic stem cells. These results demonstrated successful reprogramming of GALT-KO fibroblasts into GALT-KO piPSCs. GALT-KO piPSCs are potentially an excellent immortal cell source for the generation of pigs with complex genetic modifications for xenotransplantation, somatic cell nuclear transfer, or chimera formation.

Lopez Corrales, N. L., et al. (2012). "Comprehensive characterization of genomic instability in pluripotent stem cells and their derived neuroprogenitor cell lines." Appl Transl Genom **1**: 21-24.

The genomic integrity of two human pluripotent stem cells and their derived neuroprogenitor cell lines was studied, applying a combination of high-resolution genetic methodologies. The usefulness of combining array-comparative genomic hybridization (aCGH) and multiplex fluorescence in situ hybridization (M-FISH) techniques should be delineated to exclude/detect a maximum of possible genomic structural aberrations. Interestingly, in parts different genomic imbalances at chromosomal and subchromosomal levels were detected in pluripotent stem cells and their derivatives. Some of the copy number variations were inherited from the original cell line, whereas other modifications were presumably acquired during the differentiation and manipulation procedures. These results underline the necessity to study both pluripotent stem cells and their differentiated progeny by as many approaches as possible in order to assess their genomic stability before using them in clinical therapies.

Lopez, M., et al. (1991). "A low-molecular-weight factor extracted from human placenta inhibits the entry into cell cycle of murine pluripotent stem cells." Ann N Y Acad Sci **628**: 169-171.

Lopez, M., et al. (1991). "Human placental low molecular weight factors inhibit the entry into cell cycle of murine pluripotent stem cells." Leukemia **5**(3): 270-272.

In 1977, Frindel et al. reported the presence in fetal calf bone marrow of a low molecular factor, the tetrapeptide Ac-N-Ser-Asp-Lys-Pro (AcSDKP), capable of inhibiting in vivo the hematopoietic pluripotent stem cell (CFU-S) recruitment into DNA

synthesis and to increase the survival of mice which had received lethal doses of cytosine arabinoside (AraC), a phase-specific drug. Considering the potential clinical importance of CFU-S proliferation inhibitor during anticancer chemotherapy and the importance of monitoring the inhibitor by immunological methods, we tried to determine if a similar inhibitor is present in humans. Preliminary results indicate the presence in human placenta of an inhibitor coeluted with AcSDKP and which is effective in inhibiting murine CFU-S.

Lopez-Onieva, L., et al. (2016). "Corrigendum to "Generation of induced pluripotent stem cells (iPSCs) from a Bernard-Soulier syndrome patient carrying a W71R mutation in the GPIX gene" [Stem Cell Res. 16/3 (2016) 692-695]." Stem Cell Res **17**(2): 462.

Lu, M., et al. (2009). "Enhanced generation of hematopoietic cells from human hepatocarcinoma cell-stimulated human embryonic and induced pluripotent stem cells." Exp Hematol **37**(8): 924-936.

OBJECTIVE: Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) constitute unique sources of pluripotent cells, although the molecular mechanisms involved in their differentiation into specific lineages are just beginning to be defined. Here we evaluated the ability of MEDII (medium conditioned by HepG2 cells, a human hepatocarcinoma cell line) to selectively enhance generation of mesodermal derivatives, including hematopoietic cells, from hESCs and hiPSCs. MATERIALS AND METHODS: Test cells were exposed to MEDII prior to being placed in conditions that promote embryoid body (EB) formation. Hematopoietic activity was measured by clonogenic assays, flow cytometry, quantitative real-time polymerase chain reaction of specific transcript complementary DNAs and the ability of cells to repopulate sublethally irradiated nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice for almost 1 year. RESULTS: Exposure of both hESCs and hiPSCs to MEDII induced a rapid and preferential differentiation of hESCs into mesodermal elements. Subsequently produced EBs showed a further enhanced expression of transcripts characteristic of multiple mesodermal lineages, and a concurrent decrease in endodermal and ectodermal cell transcripts. Frequency of all types of clonogenic hematopoietic progenitors in subsequently derived EBs was also increased. In vivo assays of MEDII-treated hESC-derived EBs also showed they contained cells able to undertake low-level but longterm multilineage repopulation of primary and secondary nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice. CONCLUSIONS: MEDII treatment of hESCs and hiPSCs alike selectively enhances their differentiation into mesodermal cells and allows subsequent generation of detectable levels of hematopoietic progenitors with in vitro and in vivo differentiating activity.

Lu, T. Y., et al. (2013). "Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells." Nat Commun **4**: 2307.

Heart disease is the leading cause of death in the world. Heart tissue engineering holds a great promise for future heart disease therapy by building personalized heart tissues. Here we create heart constructs by repopulating decellularized mouse hearts with human induced pluripotent stem cell-derived multipotential cardiovascular progenitor cells. We show that the seeded multipotential cardiovascular progenitor cells migrate, proliferate and differentiate in situ into cardiomyocytes, smooth muscle cells and endothelial cells to reconstruct the decellularized hearts. After 20 days of perfusion, the engineered heart tissues exhibit spontaneous contractions, generate mechanical force and are responsive to drugs. In addition, we observe that heart extracellular matrix promoted cardiomyocyte proliferation, differentiation and myofilament formation from the repopulated human multipotential cardiovascular progenitor cells. Our novel strategy to engineer personalized heart constructs could benefit the study of early heart formation or may find application in preclinical testing.

Lu, Y., et al. (2013). "Differential responses to genotoxic agents between induced pluripotent stem cells and tumor cell lines." J Hematol Oncol **6**(1): 71.

Given potential values of induced pluripotent stem (iPS) cells in basic biomedical research and regenerative medicine, it is important to understand how these cells regulate their genome stability in response to environmental toxins and carcinogens. The present study characterized the effect of Cr(VI), a well-known genotoxic agent and environmental carcinogen, on major molecular components of DNA damage response pathways in human iPS cells. We compared the effect of Cr(VI) on human iPS cells with two established cell lines, Tera-1 (teratoma origin) and BEAS-2B (lung epithelial origin). We also studied the effect of hydrogen peroxide and doxorubicin on modulating DNA damage responses in these cell types. We demonstrated that ATM and p53 phosphorylation is differentially regulated in human iPS cells compared with Tera-1 and BEAS-2B cells after exposure to various genotoxic agents. Moreover, we observed that inhibition of CK2, but not p38, promotes phosphorylation of p53S392 in iPS cells. Combined,

our data reveal some unique features of DNA damage responses in human iPS cells.

Lui, K. O. (2014). "Advances in pluripotent stem cell-derived endothelial cells: from biomaterials to organ regeneration." Curr Stem Cell Res Ther **9**(5): 365.

Human embryonic stem cells (ESCs), by virtue of their capability to self-renew and differentiate into a variety of cell types, represent the first type of pluripotent stem cells (PSCs) to be used in clinical transplantation during recent phase-I trials; however, it is still unclear whether hESC-derived tissues can self-organize and form part of the vascularized, functional organ following transplantation. Recently, endothelial cells (ECs) or angiogenic factors such as VEGFA have been demonstrated to support development and regeneration of multiple organ systems, including the heart, pancreas, liver, lung and bone marrow. Therefore, co-transplantation of ECs derived from the same parental PSCs that differentiate into cell types of interest; or overexpression of the inductive angiogenic factors responsible for organ regeneration might be beneficial to support function of hPSC-derived tissues. In this special issue, we discuss how protein kinases (Ng and colleagues); DNA methylation and histone modification (Tsui and colleagues) regulate cellular pluripotency and cell-fate specification of PSCs. In addition, we discuss how ECs and angiogenic factors could contribute to repair and regeneration of organs such as the heart (Yuan and colleagues), the cardiovascular system (Tse and colleagues) and the pancreas (Lui). We also discuss the role of mesenchymal stem cells or paracrine factors secreted by them in tissue repair (Li and colleagues). Lastly, we discuss how to generate self-organized and vascularized tissues derived from PSCs in a 2- or 3-dimensional format by fusing tissue bioengineering approaches with stem cell technology (Chen).

Luo, Y., et al. (2016). "Targeted Inhibition of the miR-199a/214 Cluster by CRISPR Interference Augments the Tumor Tropism of Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells under Hypoxic Condition." Stem Cells Int **2016**: 3598542.

The human induced pluripotent stem cell (hiPSC) provides a breakthrough approach that helps overcoming ethical and allergenic challenges posed in application of neural stem cells (NSCs) in targeted cancer gene therapy. However, the tumor-tropic capacity of hiPSC-derived NSCs (hiPS-NSCs) still has much room to improve. Here we attempted to promote the tumor tropism of hiPS-NSCs by manipulating the activity of endogenous miR-199a/214 cluster that is involved in regulation of hypoxia-stimulated cell migration. We first developed a baculovirus-delivered CRISPR interference (CRISPRi) system that sterically blocked the E-box element in the promoter of the miR-199a/214 cluster with an RNA-guided catalytically dead Cas9 (dCas9). We then applied this CRISPRi system to hiPS-NSCs and successfully suppressed the expression of miR-199a-5p, miR-199a-3p, and miR-214 in the microRNA gene cluster. Meanwhile, the expression levels of their targets related to regulation of hypoxia-stimulated cell migration, such as HIF1A, MET, and MAPK1, were upregulated. Further migration assays demonstrated that the targeted inhibition of the miR-199a/214 cluster significantly enhanced the tumor tropism of hiPS-NSCs both in vitro and in vivo. These findings suggest a novel application of CRISPRi in NSC-based tumor-targeted gene therapy.

Lv, Y., et al. (2013). "Effects of low-intensity pulsed ultrasound on cell viability, proliferation and neural differentiation of induced pluripotent stem cells-derived neural crest stem cells." Biotechnol Lett **35**(12): 2201-2212.

Low-intensity pulsed ultrasound (LIPUS) acting on induced pluripotent stem cells-derived neural crest stem cells (iPSCs-NCSCs) is considered a promising therapy to improve the efficacy of injured peripheral nerve regeneration. Effects of LIPUS on cell viability, proliferation and neural differentiation of iPSCs-NCSCs were examined respectively in this study. LIPUS at 500 mW cm(-2) enhanced the viability and proliferation of iPSCs-NCSCs after 2 days and, after 4 days, up-regulated gene and protein expressions of NF-M, Tuj1, S100beta and GFAP in iPSCs-NCSCs whereas after 7 days expression of only NF-M, S100beta and GFAP were up-regulated. LIPUS treatment at an appropriate intensity can, therefore, be an efficient and cost-effective method to enhance cell viability, proliferation and neural differentiation of iPSCs-NCSCs in vitro for peripheral nerve tissue engineering.

Ma, H., et al. (2018). "Establishment of human pluripotent stem cell-derived pancreatic beta-like cells in the mouse pancreas." Proc Natl Acad Sci U S A **115**(15): 3924-3929.

Type 1 diabetes is characterized by autoimmune destruction of beta cells located in pancreatic islets. However, tractable in vivo models of human pancreatic beta cells have been limited. Here, we generated xenogeneic human pancreatic beta-like cells in the mouse pancreas by orthotopic transplantation of stem cell-derived beta (SC-beta) cells into the pancreas of neonatal mice. The engrafted beta-like cells expressed beta cell transcription factors and markers associated with functional maturity. Engrafted human cells recruited mouse endothelial cells, suggesting functional integration. Human insulin was detected in the blood circulation of transplanted mice

for months after transplantation and increased upon glucose stimulation. In addition to beta-like cells, human cells expressing markers for other endocrine pancreas cell types, acinar cells, and pancreatic ductal cells were identified in the pancreata of transplanted mice, indicating that this approach allows studying other human pancreatic cell types in the mouse pancreas. Our results demonstrate that orthotopic transplantation of human SC-beta cells into neonatal mice is an experimental platform that allows the generation of mice with human pancreatic beta-like cells in the endogenous niche.

Ma, M. S., et al. (2015). "Pluripotent stem cells for Schwann cell engineering." Stem Cell Rev **11**(2): 205-218.

Tissue engineering of Schwann cells (SCs) can serve a number of purposes, such as in vitro SC-related disease modeling, treatment of peripheral nerve diseases or peripheral nerve injury, and, potentially, treatment of CNS diseases. SCs can be generated from autologous stem cells in vitro by recapitulating the various stages of in vivo neural crest formation and SC differentiation. In this review, we survey the cellular and molecular mechanisms underlying these in vivo processes. We then focus on the current in vitro strategies for generating SCs from two sources of pluripotent stem cells, namely embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Different methods for SC engineering from ESCs and iPSCs are reviewed and suggestions are proposed for optimizing the existing protocols. Potential safety issues regarding the clinical application of iPSC-derived SCs are discussed as well. Lastly, we will address future aspects of SC engineering.

Ma, R., et al. (2015). "Thyroid cell differentiation from murine induced pluripotent stem cells." Front Endocrinol (Lausanne) **6**: 56.

BACKGROUND: Here, we demonstrate the successful differentiation of induced pluripotent stem (iPS) cells into functional thyroid cells indicating the therapeutic potential of this approach when applied to individuals with thyroid deficiency. RESEARCH DESIGN AND METHODS: Using embryonic murine fibroblasts, we generated iPS cells with a single lentiviral "stem cell cassette" vector and then differentiated these iPS cells into thyroid cells after transfection with PAX8 and NKX2-1 by Activin A and TSH stimulation. RESULTS: The generated iPS cells expressed pluripotent stem cell markers as assessed using both reverse transcription quantitative PCRs and immunofluorescence staining with ~0.5% reprograming efficiency. Compared to control cells, the expression of thyroid-specific genes NIS, TSHR, Tg, and TPO were greatly enhanced in PAX8(+)NKX2-1(+) iPS cells after differentiation. On stimulation with TSH, these differentiated iPS cells were also capable of dose-dependent cAMP generation and radioiodine uptake indicative of functional thyroid epithelial cells. Furthermore, the cells formed three-dimensional follicles in culture, and "thyroid organoids" formed after PAX8(+)NKX2-1(+) iPS cells transplanted into nude mice, and all expressed Tg protein as judged immunohistochemically. Taken together, thyroid epithelial cells differentiated from iPS cells, which were themselves derived from murine fibroblasts, exhibited very similar properties to thyroid cells previously developed from traditional murine embryonic stem cells. CONCLUSION: Thyroid cells differentiated from iPS cells offer the opportunity to examine the detailed transcriptional regulation of thyroid cell differentiation and may provide a useful future source for individualized regenerative cell therapy.

MacLean, G. A., et al. (2018). "Downregulation of Endothelin Receptor B Contributes to Defective B Cell Lymphopoiesis in Trisomy 21 Pluripotent Stem Cells." Sci Rep **8**(1): 8001.

Individuals with Trisomy 21 (T21) exhibit numerous hematological abnormalities, including reductions in numbers of circulating B and T lymphocytes. To elucidate molecular mechanisms underlying these phenotypes, we differentiated human isogenic disomic and trisomic pluripotent cells, and observed that trisomic cells showed defects in B cell, but not T cell differentiation. Global gene expression of differentiated, trisomic B cells revealed reduced expression of genes encoding endothelin signaling components, namely the Endothelin Receptor B (EDNRB), and its ligand Endothelin1 (EDN1). Depletion of EDNRB mRNA in cord blood-derived CD34(+) cells led to defective B cell differentiation, supporting a hypothesis that low EDNRB expression in T21 contributes to intrinsic lymphoid defects. Further evidence for the role of the EDNRB pathway in B cell differentiation was obtained through CRISPR/Cas9 gene targeting in disomic and trisomic iPS cells. Knockout of EDNRB in both cell backgrounds reduced the capacity for B cell differentiation. Collectively, this work identifies downregulation of EDNRB as a causative factor for impaired B lymphocyte generation in trisomic cells, which may contribute to defects in immune function associated with T21. Furthermore, a novel role for endothelin signaling in regulation of B cell development has been identified.

Magdy, T., et al. (2018). "Human Induced Pluripotent Stem Cell (hiPSC)-Derived Cells to Assess Drug Cardiotoxicity: Opportunities and Problems." Annu Rev Pharmacol Toxicol **58**: 83-103.

Billions of US dollars are invested every year by the pharmaceutical industry in drug development, with the aim of introducing new drugs that are effective and have minimal side effects. Thirty percent of in-pipeline drugs are excluded in an early phase of preclinical and clinical screening owing to cardiovascular safety concerns, and several lead molecules that pass the early safety screening make it to market but are later withdrawn owing to severe cardiac side effects. Although the current drug safety screening methodologies can identify some cardiotoxic drug candidates, they cannot accurately represent the human heart in many aspects, including genomics, transcriptomics, and patient- or population-specific cardiotoxicity. Despite some limitations, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a powerful and evolving technology that has been shown to recapitulate many attributes of human cardiomyocytes and their drug responses. In this review, we discuss the potential impact of the inclusion of the hiPSC-CM platform in premarket candidate drug screening.

Maguire, E. M., et al. (2017). "Differentiation and Application of Induced Pluripotent Stem Cell-Derived Vascular Smooth Muscle Cells." Arterioscler Thromb Vasc Biol **37**(11): 2026-2037.

Vascular smooth muscle cells (VSMCs) play a role in the development of vascular disease, for example, neointimal formation, arterial aneurysm, and Marfan syndrome caused by genetic mutations in VSMCs, but little is known about the mechanisms of the disease process. Advances in induced pluripotent stem cell technology have now made it possible to derive VSMCs from several different somatic cells using a selection of protocols. As such, researchers have set out to delineate key signaling processes involved in triggering VSMC gene expression to grasp the extent of gene regulatory networks involved in phenotype commitment. This technology has also paved the way for investigations into diseases affecting VSMC behavior and function, which may be treatable once an identifiable culprit molecule or gene has been repaired. Moreover, induced pluripotent stem cell-derived VSMCs are also being considered for their use in tissue-engineered blood vessels as they may prove more beneficial than using autologous vessels. Finally, while several issues remains to be clarified before induced pluripotent stem cell-derived VSMCs can become used in regenerative medicine, they do offer both clinicians and researchers hope for both treating and understanding vascular disease. In this review, we aim to update the recent progress on VSMC generation from stem cells and the underlying molecular mechanisms of VSMC differentiation. We will also explore how the use of induced pluripotent stem cell-derived VSMCs has changed the game for regenerative medicine by offering new therapeutic avenues to clinicians, as well as providing researchers with a new platform for modeling of vascular disease.

Makinen, M., et al. (2013). "Fluorescent probes as a tool for cell population tracking in spontaneously active neural networks derived from human pluripotent stem cells." J Neurosci Methods **215**(1): 88-96.

Applications such as 3D cultures and tissue modelling require cell tracking with non-invasive methods. In this work, the suitability of two fluorescent probes, CellTracker, CT, and long chain carbocyanine dye, DiD, was investigated for long-term culturing of labeled human pluripotent stem cell-derived neural cells. We found that these dyes did not affect the cell viability. However, proliferation was decreased in DiD labeled cell population. With both dyes the labeling was stable up to 4 weeks. CT and DiD labeled cells could be co-cultured and, importantly, these mixed populations had their normal ability to form spontaneous electrical network activity. In conclusion, human neural cells can be successfully labeled with these two fluorescent probes without significantly affecting the cell characteristics. These labeled cells could be utilized further in e.g. building controlled neuronal networks for neurotoxicity screening platforms, combining cells with biomaterials for 3D studies, and graft development.

Maldonado, M., et al. (2015). "The effects of electrospun substrate-mediated cell colony morphology on the self-renewal of human induced pluripotent stem cells." Biomaterials **50**: 10-19.

The development of xeno-free, chemically defined stem cell culture systems has been a primary focus in the field of regenerative medicine to enhance the clinical application of pluripotent stem cells (PSCs). In this regard, various electrospun substrates with diverse physiochemical properties were synthesized utilizing various polymer precursors and surface treatments. Human induced pluripotent stem cells (IPSCs) cultured on these substrates were characterized by their gene and protein expression to determine the effects of the substrate physiochemical properties on the cells' self-renewal, i.e., proliferation and the maintenance of pluripotency. The results showed that surface chemistry significantly affected cell colony formation via governing the colony edge propagation. More importantly, when surface chemistry of the substrates was uniformly controlled by collagen conjugation, the stiffness of substrate was inversely related to the sphericity, a degree of three dimensionality in colony morphology. The differences in sphericity subsequently affected spontaneous differentiation of IPSCs during a long-term culture,

implicating that the colony morphology is a deciding factor in the lineage commitment of PSCs. Overall, we show that the capability of controlling IPSC colony morphology by electrospun substrates provides a means to modulate IPSC self-renewal.

Malecki, M., et al. (2013). "Safeguarding Stem Cell-Based Regenerative Therapy against Iatrogenic Cancerogenesis: Transgenic Expression of DNASE1, DNASE1L3, DNASE2, DFFB Controlled By POLA1 Promoter in Proliferating and Directed Differentiation Resisting Human Autologous Pluripotent Induced Stem Cells Leads to their Death." J Stem Cell Res Ther **Suppl 9**(5).

INTRODUCTION: The worst possible complication of using stem cells for regenerative therapy is iatrogenic cancerogenesis. The ultimate goal of our work is to develop a self-triggering feedback mechanism aimed at causing death of all stem cells, which resist directed differentiation, keep proliferating, and can grow into tumors. SPECIFIC AIM: The specific aim was threefold: (1) to genetically engineer the DNA constructs for the human, recombinant DNASE1, DNASE1L3, DNASE2, DFFB controlled by POLA promoter; (2) to bioengineer anti-SSEA-4 antibody guided vectors delivering transgenes to human undifferentiated and proliferating pluripotent stem cells; (3) to cause death of proliferating and directed differentiation resisting stem cells by transgenic expression of the human recombinant the DNases (hrDNases). METHODS: The DNA constructs for the human, recombinant DNASE1, DNASE1L3, DNASE2, DFFB controlled by POLA promoter were genetically engineered. The vectors targeting specifically SSEA-4 expressing stem cells were bioengineered. The healthy volunteers' bone marrow mononuclear cells (BMMCs) were induced into human, autologous, pluripotent stem cells with non-integrating plasmids. Directed differentiation of the induced stem cells into endothelial cells was accomplished with EGF and BMP. The anti-SSEA 4 antibodies' guided DNA vectors delivered the transgenes for the human recombinant DNases' into proliferating stem cells. RESULTS: Differentiation of the pluripotent induced stem cells into the endothelial cells was verified by highlighting formation of tight and adherens junctions through transgenic expression of recombinant fluorescent fusion proteins: VE cadherin, claudin, zona occludens 1, and catenin. Proliferation of the stem cells was determined through highlighting transgenic expression of recombinant fluorescent proteins controlled by POLA promoter, while also reporting expression of the transgenes for the hrDNases. Expression of the transgenes for the DNases resulted in complete collapse of the chromatin architecture and degradation of the proliferating cells' genomic DNA. The proliferating stem cells, but not the differentiating ones, were effectively induced to die. CONCLUSION: Herein, we describe attaining the proof-of-concept for the strategy, whereby transgenic expression of the genetically engineered human recombinant DNases in proliferating and directed differentiation resisting stem cells leads to their death. This novel strategy reduces the risk of iatrogenic neoplasms in stem cell therapy.

Malik, N., et al. (2014). "Comparison of the gene expression profiles of human fetal cortical astrocytes with pluripotent stem cell derived neural stem cells identifies human astrocyte markers and signaling pathways and transcription factors active in human astrocytes." PLoS One **9**(5): e96139.

Astrocytes are the most abundant cell type in the central nervous system (CNS) and have a multitude of functions that include maintenance of CNS homeostasis, trophic support of neurons, detoxification, and immune surveillance. It has only recently been appreciated that astrocyte dysfunction is a primary cause of many neurological disorders. Despite their importance in disease very little is known about global gene expression for human astrocytes. We have performed a microarray expression analysis of human fetal astrocytes to identify genes and signaling pathways that are important for astrocyte development and maintenance. Our analysis confirmed that the fetal astrocytes express high levels of the core astrocyte marker GFAP and the transcription factors from the NFI family which have been shown to play important roles in astrocyte development. A group of novel markers were identified that distinguish fetal astrocytes from pluripotent stem cell-derived neural stem cells (NSCs) and NSC-derived neurons. As in murine astrocytes, the Notch signaling pathway appears to be particularly important for cell fate decisions between the astrocyte and neuronal lineages in human astrocytes. These findings unveil the repertoire of genes expressed in human astrocytes and serve as a basis for further studies to better understand astrocyte biology, especially as it relates to disease.

Manos, P. D., et al. (2011). "Live-cell immunofluorescence staining of human pluripotent stem cells." Curr Protoc Stem Cell Biol **Chapter 1**: Unit 1C 12.

Antibodies are instrumental tools in stem cell identification, purification, and analysis. Most commonly, cell samples are either dissociated to obtain a single-cell suspension suitable for FACS analysis or cell sorting, or fixed in situ for immunostaining and fluorescence microscopy imaging. This unit describes an alternative method in which live adherent cells are stained and imaged in situ without the need for cell dissociation, fixation, or fluorescent reporter genes.

This minimally invasive method is particularly useful for identification and distinction of fully and partially reprogrammed induced pluripotent stem cells (iPSCs). The unit also describes the use of mCD49e and hCD29 antibodies in live-cell (vital) imaging. mCD49e strongly stains mouse embryonic fibroblast (MEF) feeder cells in human pluripotent stem cell cultures, whereas hCD29 recognizes an antigen expressed on undifferentiated and many differentiated cells. A distinguishing feature of hCD29 in live-cell staining is that its antigen is precluded from detection wherever cells have formed tight epithelial junctions (e.g., in the center but not the periphery of pluripotent stem cell colonies) due to basolateral location. A non-fluorescent fixed-cell staining protocol is also provided for medium- to high-throughput quantification of stem cell experiments without an automated microscope. The discussion addresses technical limitations, pitfalls, troubleshooting, and potential applications, such as identification of emerging bona fide human iPSC colonies in reprogramming experiments.

Mao, D., et al. (2017). "A Synthetic Hybrid Molecule for the Selective Removal of Human Pluripotent Stem Cells from Cell Mixtures." Angew Chem Int Ed Engl **56**(7): 1765-1770.

A major hurdle in stem cell therapy is the tumorigenic risk of residual undifferentiated stem cells. This report describes the design and evaluation of synthetic hybrid molecules that efficiently reduce the number of human induced pluripotent stem cells (hiPSCs) in cell mixtures. The design takes advantage of Kyoto probe 1 (KP-1), a fluorescent chemical probe for hiPSCs, and clinically used anticancer drugs. Among the KP-1-drug conjugates we synthesized, we found an exceptionally selective, chemically tractable molecule that induced the death of hiPSCs. Mechanistic analysis suggested that the high selectivity originates from the synergistic combination of transporter-mediated efflux and the cytotoxicity mode of action. The present study offers a chemical and mechanistic rationale for designing selective, safe, and simple reagents for the preparation of non-tumorigenic clinical samples.

Marion, R. M., et al. (2009). "Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells." Cell Stem Cell **4**(2): 141-154.

Telomere shortening is associated with organismal aging. iPS cells have been recently derived from old patients; however, it is not known whether telomere chromatin acquires the same characteristics as in ES cells. We show here that telomeres are elongated in iPS cells compared to the parental differentiated cells both when using four (Oct3/4, Sox2, Klf4, cMyc) or three (Oct3/4, Sox2, Klf4) reprogramming factors and both from young and aged individuals. We demonstrate genetically that, during reprogramming, telomere elongation is usually mediated by telomerase and that iPS telomeres acquire the epigenetic marks of ES cells, including a low density of trimethylated histones H3K9 and H4K20 and increased abundance of telomere transcripts. Finally, reprogramming efficiency of cells derived from increasing generations of telomerase-deficient mice shows a dramatic decrease in iPS cell efficiency, a defect that is restored by telomerase reintroduction. Together, these results highlight the importance of telomere biology for iPS cell generation and functionality.

Martin, G. R. (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells." Proc Natl Acad Sci U S A **78**(12): 7634-7638.

This report describes the establishment directly from normal preimplantation mouse embryos of a cell line that forms teratocarcinomas when injected into mice. The pluripotency of these embryonic stem cells was demonstrated conclusively by the observation that subclonal cultures, derived from isolated single cells, can differentiate into a wide variety of cell types. Such embryonic stem cells were isolated from inner cell masses of late blastocysts cultured in medium conditioned by an established teratocarcinoma stem cell line. This suggests that such conditioned medium might contain a growth factor that stimulates the proliferation or inhibits the differentiation of normal pluripotent embryonic cells, or both. This method of obtaining embryonic stem cells makes feasible the isolation of pluripotent cells lines from various types of noninbred embryo, including those carrying mutant genes. The availability of such cell lines should made possible new approaches to the study of early mammalian development.

Mashimo, Y. and K. Kamei (2015). "Microfluidic Image Cytometry for Single-Cell Phenotyping of Human Pluripotent Stem Cells." Methods Mol Biol **1346**: 85-98.

A microfluidic human pluripotent stem cell (hPSC) array has been developed for robust and reproducible hPSC culture methods to assess chemically defined serum- and feeder-free culture conditions. This microfluidic platform, combined with image cytometry, enables the systematic analysis of multiple simultaneously detected marker expression in individual cells, for screening of various chemically defined media across hPSC lines, and the study of phenotypic responses.

Mathapati, S., et al. (2016). "Small-Molecule-Directed Hepatocyte-Like Cell Differentiation of Human

Pluripotent Stem Cells." Curr Protoc Stem Cell Biol **38**: 1G 6 1-1G 6 18.

Hepatocyte-like cells (HLCs) generated in vitro from human pluripotent stem cells (hPSCs) provide an invaluable resource for basic research, regenerative medicine, drug screening, toxicology, and modeling of liver disease and development. This unit describes a small-molecule-driven protocol for in vitro differentiation of hPSCs into HLCs without the use of growth factors. hPSCs are coaxed through a developmentally relevant route via the primitive streak to definitive endoderm (DE) using the small molecule CHIR99021 (a Wnt agonist), replacing the conventional growth factors Wnt3A and activin A. The small-molecule-derived DE is then differentiated to hepatoblast-like cells in the presence of dimethyl sulfoxide. The resulting hepatoblasts are then differentiated to HLCs with N-hexanoic-Tyr, Ile-6 aminohexanoic amide (Dihexa, a hepatocyte growth factor agonist) and dexamethasone. The protocol provides an efficient and reproducible procedure for differentiation of hPSCs into HLCs utilizing small molecules. (c) 2016 by John Wiley & Sons, Inc.

Matsumoto, T., et al. (2016). "Functional Neurons Generated from T Cell-Derived Induced Pluripotent Stem Cells for Neurological Disease Modeling." Stem Cell Reports **6**(3): 422-435.

Modeling of neurological diseases using induced pluripotent stem cells (iPSCs) derived from the somatic cells of patients has provided a means of elucidating pathogenic mechanisms and performing drug screening. T cells are an ideal source of patient-specific iPSCs because they can be easily obtained from samples. Recent studies indicated that iPSCs retain an epigenetic memory relating to their cell of origin that restricts their differentiation potential. The classical method of differentiation via embryoid body formation was not suitable for T cell-derived iPSCs (TiPSCs). We developed a neurosphere-based robust differentiation protocol, which enabled TiPSCs to differentiate into functional neurons, despite differences in global gene expression between TiPSCs and adult human dermal fibroblast-derived iPSCs. Furthermore, neurons derived from TiPSCs generated from a juvenile patient with Parkinson's disease exhibited several Parkinson's disease phenotypes. Therefore, we conclude that TiPSCs are a useful tool for modeling neurological diseases.

Matsumura, T., et al. (2014). "Single-cell cloning and expansion of human induced pluripotent stem cells by a microfluidic culture device." Biochem Biophys Res Commun **453**(1): 131-137.

The microenvironment of cells, which includes basement proteins, shear stress, and extracellular stimuli, should be taken into consideration when examining physiological cell behavior. Although microfluidic devices allow cellular responses to be analyzed with ease at the single-cell level, few have been designed to recover cells. We herein demonstrated that a newly developed microfluidic device helped to improve culture conditions and establish a clonality-validated human pluripotent stem cell line after tracing its growth at the single-cell level. The device will be a helpful tool for capturing various cell types in the human body that have not yet been established in vitro.

Matsuo, T., et al. (2015). "Efficient long-term survival of cell grafts after myocardial infarction with thick viable cardiac tissue entirely from pluripotent stem cells." Sci Rep **5**: 16842.

Poor engraftment of cells after transplantation to the heart is a common and unresolved problem in the cardiac cell therapies. We previously generated cardiovascular cell sheets entirely from pluripotent stem cells with cardiomyocytes, endothelial cells and vascular mural cells. Though sheet transplantation showed a better engraftment and improved cardiac function after myocardial infarction, stacking limitation (up to 3 sheets) by hypoxia hampered larger structure formation and long-term survival of the grafts. Here we report an efficient method to overcome the stacking limitation. Insertion of gelatin hydrogel microspheres (GHMs) between each cardiovascular cell sheet broke the viable limitation via appropriate spacing and fluid impregnation with GHMs. Fifteen sheets with GHMs (15-GHM construct; >1 mm thickness) were stacked within several hours and viable after 1 week in vitro. Transplantation of 5-GHM constructs (approximately 2 x 10(6) of total cells) to a rat myocardial infarction model showed rapid and sustained functional improvements. The grafts were efficiently engrafted as multiple layered cardiovascular cells accompanied by functional capillary networks. Large engrafted cardiac tissues (0.8 mm thickness with 40 cell layers) successfully survived 3 months after TX. We developed an efficient method to generate thicker viable tissue structures and achieve long-term survival of the cell graft to the heart.

Matsushita, N., et al. (2014). "[Establishment of induced pluripotent stem cells from adipose tissue-derived stem cells for dendritic cell-based cancer vaccines]." Gan To Kagaku Ryoho **41**(4): 467-470.

Recently, studies on regenerative stem cell therapy are being encouraged, and efforts to generate dendritic cells, which play important roles in cancer immunotherapy, from stem cells are being made in the field of tumor immunology. Therapeutic acquisition of stem cells has important clinical applications. Studies on induced pluripotent stem(iPS)cells generated from

somatic cells with pluripotent genes have advanced in recent years. Stem cells are reported to be found in adipose tissue (adipose-derived stem cells, ADSC). Our goal is to develop a new cancer vaccine by using dendritic cells generated from ADSC. In a preliminary study, we examined whether iPS cells can be generated from ADSC to serve as a source of dendritic cells.We introduced a plasmid with pluripotent genes(OCT3/4, KLF4, SOX2, L-MYC, LIN28, p53-shRNA)into an ADSC strain derived from adipose tissue by electroporation and subsequently cultured the cells for further examination. A colony sugges- tive of iPS cells from ADSC was observed. OCT3/4, KLF4, SOX2, L-MYC, and LIN28 mRNAs were expressed in the cultured cells, as confirmed by reverse transcriptase-polymerase chain reaction(RT-PCR). On the basis of these results, we confirmed that iPS cells were generated from ADSC. The method of inducing dendritic cells from iPS cells has already been reported, and the results of this study suggest that ADSC is a potential source of dendritic cells.

Matsuura, K., et al. (2015). "Elimination of remaining undifferentiated induced pluripotent stem cells in the process of human cardiac cell sheet fabrication using a methionine-free culture condition." Tissue Eng Part C Methods **21**(3): 330-338.

Cardiac tissue engineering is a promising method for regenerative medicine. Although we have developed human cardiac cell sheets by integration of cell sheet-based tissue engineering and scalable bioreactor culture, the risk of contamination by induced pluripotent stem (iPS) cells in cardiac cell sheets remains unresolved. In the present study, we established a novel culture method to fabricate human cardiac cell sheets with a decreased risk of iPS cell contamination while maintaining viabilities of iPS cell-derived cells, including cardiomyocytes and fibroblasts, using a methionine-free culture condition. When cultured in the methionine-free condition, human iPS cells did not survive without feeder cells and could not proliferate or form colonies on feeder cells or in coculture with cells for cardiac cell sheet fabrication. When iPS cell-derived cells after the cardiac differentiation were transiently cultured in the methionine-free condition, gene expression of OCT3/4 and NANOG was downregulated significantly compared with that in the standard culture condition. Furthermore, in fabricated cardiac cell sheets, spontaneous and synchronous beating was observed in the whole area while maintaining or upregulating the expression of various cardiac and extracellular matrix genes. These findings suggest that human iPS cells are methionine dependent and a methionine-free culture condition for cardiac cell sheet fabrication might reduce the risk of iPS cell contamination.

Matsuura, K., et al. (2012). "Creation of human cardiac cell sheets using pluripotent stem cells." Biochem Biophys Res Commun **425**(2): 321-327.

Although we previously reported the development of cell-dense thickened cardiac tissue by repeated transplantation-based vascularization of neonatal rat cardiac cell sheets, the cell sources for human cardiac cells sheets and their functions have not been fully elucidated. In this study, we developed a bioreactor to expand and induce cardiac differentiation of human induced pluripotent stem cells (hiPSCs). Bioreactor culture for 14 days produced around 8x10(7) cells/100 ml vessel and about 80% of cells were positive for cardiac troponin T. After cardiac differentiation, cardiomyocytes were cultured on temperature-responsive culture dishes and showed spontaneous and synchronous beating, even after cell sheets were detached from culture dishes. Furthermore, extracellular action potential propagation was observed between cell sheets when two cardiac cell sheets were partially overlaid. These findings suggest that cardiac cell sheets formed by hiPSC-derived cardiomyocytes might have sufficient properties for the creation of thickened cardiac tissue.

Mauritz, C., et al. (2011). "Induced pluripotent stem cell (iPSC)-derived Flk-1 progenitor cells engraft, differentiate, and improve heart function in a mouse model of acute myocardial infarction." Eur Heart J **32**(21): 2634-2641.

AIMS: Induced pluripotent stem cell (iPSC)-derived cardiovascular progenitor cells represent a suitable autologous cell source for myocardial regeneration as they have the capability to form myocardial cells and to contribute to revascularization. As a first proof of concept we evaluated the potential of a murine iPSC-derived cardiovascular progenitor population, which expresses the surface marker foetal liver kinase-1 (Flk-1), to restore myocardial tissue and improve cardiac function after acute myocardial infarction (MI) in mice. METHODS AND RESULTS: iPSC-derived Flk-1(pos) vs. Flk-1(neg) cells were selected by fluorescence activated cell sorting (FACS) and injected into the ischaemic myocardium of left anterior descending coronary artery (LAD)-ligated mice. Addressing safety aspects we used an octamer binding factor 4 (Oct4)-enhanced green fluorescent protein (eGFP) expressing iPSC clone from the transgenic Oct4-eGFP reporter mouse strain OG2 to enable FACS-based depletion of undifferentiated cells prior to transplantation. Infarcted animals were treated with placebo (phosphate-buffered saline, n = 13), Flk-1(neg) cells (n = 14), or Flk-1(pos) cells (n = 11; 5 x 10(5) cells each). Heart function was evaluated by magnetic resonance imaging and conductance catheter

analysis 2 weeks postoperatively. Cardiovascular in vitro and in vivo differentiations were investigated by immunofluorescence staining. Treatment with Flk-1(pos) and Flk-1(neg) cells resulted in a favourable myocardial remodelling and improved left ventricular function. Engraftment and functional benefits were superior after transplantation of Flk-1(pos) compared with Flk-1(neg) cells. Furthermore, Flk-1(pos) grafts contained considerably more vascular structures in relation to Flk-1(neg) grafts. CONCLUSION: iPSC-derived Flk-1(pos) progenitor cells differentiate into cardiovascular lineages in vitro and in vivo and improve cardiac function after acute MI. This proof of concept study paves the way for an autologous iPSC-based therapy of MI.

McLaren, D., et al. (2013). "Automated large-scale culture and medium-throughput chemical screen for modulators of proliferation and viability of human induced pluripotent stem cell-derived neuroepithelial-like stem cells." J Biomol Screen **18**(3): 258-268.

The aim of this study was to demonstrate proof-of-concept feasibility for the use of human neural stem cells (NSCs) for high-throughput screening (HTS) applications. For this study, an adherent human induced pluripotent stem (iPS) cell-derived long-term, self-renewing, neuroepithelial-like stem (lt-NES) cell line was selected as a representative NSC. Here, we describe the automated large-scale serum-free culture ("scale-up") of human lt-NES cells on the CompacT SelecT cell culture robotic platform, followed by their subsequent automated "scale-out" into a microwell plate format. We also report a medium-throughput screen of 1000 compounds to identify modulators of neural stem cell proliferation and/or survival. The screen was performed on two independent occasions using a cell viability assay with end-point reading resulting in the identification of 24 potential hit compounds, 5 of which were found to increase the proliferation and/or survival of human lt-NES on both occasions. Follow-up studies confirmed a dose-dependent effect of one of the hit compounds, which was a Cdk-2 modulator. This approach could be further developed as part of a strategy to screen compounds to either improve the procedures for the in vitro expansion of neural stem cells or to potentially modulate endogenous neural stem cell behavior in the diseased nervous system.

Medrano, J. V., et al. (2014). "Human germ cell differentiation from pluripotent embryonic stem cells and induced pluripotent stem cells." Methods Mol Biol **1154**: 563-578.

Although 10-15 % of couples are infertile, little is known of the diverse, underlying pathologies in men and women with poor germ cell production; furthermore, for those with few or no high-quality germ cells, there are few options available for treatment. Thus, over the last decade, concerted efforts have been aimed at developing a biological system to probe the fundamentals of human egg and sperm production via pluripotent stem cell cells with the hopes of informing clinical decisions and ultimately providing alternative methods for therapy which may include developing a source of germ cells ultimately for reproductive purposes.

Meneghini, V., et al. (2017). "Generation of Human Induced Pluripotent Stem Cell-Derived Bona Fide Neural Stem Cells for Ex Vivo Gene Therapy of Metachromatic Leukodystrophy." Stem Cells Transl Med **6**(2): 352-368.

Allogeneic fetal-derived human neural stem cells (hfNSCs) that are under clinical evaluation for several neurodegenerative diseases display a favorable safety profile, but require immunosuppression upon transplantation in patients. Neural progenitors derived from patient-specific induced pluripotent stem cells (iPSCs) may be relevant for autologous ex vivo gene-therapy applications to treat genetic diseases with unmet medical need. In this scenario, obtaining iPSC-derived neural stem cells (NSCs) showing a reliable "NSC signature" is mandatory. Here, we generated human iPSC (hiPSC) clones via reprogramming of skin fibroblasts derived from normal donors and patients affected by metachromatic leukodystrophy (MLD), a fatal neurodegenerative lysosomal storage disease caused by genetic defects of the arylsulfatase A (ARSA) enzyme. We differentiated hiPSCs into NSCs (hiPS-NSCs) sharing molecular, phenotypic, and functional identity with hfNSCs, which we used as a "gold standard" in a side-by-side comparison when validating the phenotype of hiPS-NSCs and predicting their performance after intracerebral transplantation. Using lentiviral vectors, we efficiently transduced MLD hiPSCs, achieving supraphysiological ARSA activity that further increased upon neural differentiation. Intracerebral transplantation of hiPS-NSCs into neonatal and adult immunodeficient MLD mice stably restored ARSA activity in the whole central nervous system. Importantly, we observed a significant decrease of sulfatide storage when ARSA-overexpressing cells were used, with a clear advantage in those mice receiving neonatal as compared with adult intervention. Thus, we generated a renewable source of ARSA-overexpressing iPSC-derived bona fide hNSCs with improved features compared with clinically approved hfNSCs. Patient-specific ARSA-overexpressing hiPS-NSCs may be used in autologous ex vivo gene therapy protocols to provide long-lasting enzymatic supply in MLD-affected brains. Stem Cells Translational Medicine 2017;6:352-368.

Meng, F. X. and W. Y. Guo (2010). "[Present status on studies of differentiation into retinal neurons and pigmented cell from induced pluripotent stem cells]." Zhonghua Yan Ke Za Zhi **46**(12): 1139-1142.

Somatic cells could be induced into pluripotent stem (iPS) cells through transferring special genes (Oct4, Sox2, c-myc and Klf4). This has brought a revolutionary change in stem cell study and application. The generation of iPS cells has great potential and enormous significance as it can resolve some insurmountable problems in stem cells research, such as ethical dilemma, immune rejection, etc. Because of these characteristics, it plays an important role in the repair of various tissues and organs. Rapid progress in this field during the past 3 years convinced us that iPS cells will be more and more applicable in tissue engineering. The present paper reviews the progress of pre-clinical study on iPS cells in the treatment of retinal and optic nerve diseases.

Mikhailova, A., et al. (2016). "Human pluripotent stem cell-derived limbal epithelial stem cells on bioengineered matrices for corneal reconstruction." Exp Eye Res **146**: 26-34.

Corneal epithelium is renewed by limbal epithelial stem cells (LESCs), a type of tissue-specific stem cells located in the limbal palisades of Vogt at the corneo-scleral junction. Acute trauma or inflammatory disorders of the ocular surface can destroy these stem cells, leading to limbal stem cell deficiency (LSCD) - a painful and vision-threatening condition. Treating these disorders is often challenging and complex, especially in bilateral cases with extensive damage. Human pluripotent stem cells (hPSCs) provide new opportunities for corneal reconstruction using cell-based therapy. Here, we investigated the use of hPSC-derived LESC-like cells on bioengineered collagen matrices in serum-free conditions, aiming for clinical applications to reconstruct the corneal epithelium and partially replace the damaged stroma. Differentiation of hPSCs towards LESC-like cells was directed using small-molecule induction followed by maturation in corneal epithelium culture medium. After four to five weeks of culture, differentiated cells were seeded onto bioengineered matrices fabricated as transparent membranes of uniform thickness, using medical-grade porcine collagen type I and a hybrid cross-linking technology. The bioengineered matrices were fully transparent, with high water content and swelling capacity, and parallel lamellar microstructure. Cell proliferation of hPSC-LESCs was significantly higher on bioengineered matrices than on collagen-coated control wells after two weeks of culture, and LESC markers p63 and cytokeratin 15, along with proliferation marker Ki67 were expressed even after 30 days in culture. Overall, hPSC-LESCs retained their capacity to self-renew and proliferate, but were also able to terminally differentiate upon stimulation, as suggested by protein expression of cytokeratins 3 and 12. We propose the use of bioengineered collagen matrices as carriers for the clinically-relevant hPSC-derived LESC-like cells, as a novel tissue engineering approach for corneal reconstruction.

Mikhailova, A., et al. (2014). "Small-molecule induction promotes corneal epithelial cell differentiation from human induced pluripotent stem cells." Stem Cell Reports **2**(2): 219-231.

Human induced pluripotent stem cells (hiPSCs) offer unique opportunities for developing novel cell-based therapies and disease modeling. In this study, we developed a directed differentiation method for hiPSCs toward corneal epithelial progenitor cells capable of terminal differentiation toward mature corneal epithelial-like cells. In order to improve the efficiency and reproducibility of our method, we replicated signaling cues active during ocular surface ectoderm development with the help of two small-molecule inhibitors in combination with basic fibroblast growth factor (bFGF) in serum-free and feeder-free conditions. First, small-molecule induction downregulated the expression of pluripotency markers while upregulating several transcription factors essential for normal eye development. Second, protein expression of the corneal epithelial progenitor marker p63 was greatly enhanced, with up to 95% of cells being p63 positive after 5 weeks of differentiation. Third, corneal epithelial-like cells were obtained upon further maturation.

Mikhailova, A., et al. (2015). "Comparative proteomics reveals human pluripotent stem cell-derived limbal epithelial stem cells are similar to native ocular surface epithelial cells." Sci Rep **5**: 14684.

Limbal epithelial stem cells (LESCs) are tissue-specific stem cells responsible for renewing the corneal epithelium. Acute trauma or chronic disease affecting LESCs may disrupt corneal epithelial renewal, causing vision threatening and painful ocular surface disorders, collectively referred to as LESC deficiency (LESCD). These disorders cannot be treated with traditional corneal transplantation and therefore alternative cell sources for successful cell-based therapy are needed. LESCs derived from human pluripotent stem cells (hPSCs) are a prospective source for ocular surface reconstruction, yet critical evaluation of these cells is crucial before considering clinical applications. In order to quantitatively evaluate hPSC-derived LESCs, we compared protein expression in native human corneal cells to that in hPSC-derived LESCs using isobaric tag for relative and absolute

quantitation (iTRAQ) technology. We identified 860 unique proteins present in all samples, including proteins involved in cell cycling, proliferation, differentiation and apoptosis, various LESC niche components, and limbal and corneal epithelial markers. Protein expression profiles were nearly identical in LESCs derived from two different hPSC lines, indicating that the differentiation protocol is reproducible, yielding homogeneous cell populations. Their protein expression profile suggests that hPSC-derived LESCs are similar to the human ocular surface epithelial cells, and possess LESC-like characteristics.

Milani, P., et al. (2016). "Cell freezing protocol suitable for ATAC-Seq on motor neurons derived from human induced pluripotent stem cells." Sci Rep **6**: 25474.

In recent years, the assay for transposase-accessible chromatin using sequencing (ATAC-Seq) has become a fundamental tool of epigenomic research. However, it is difficult to perform this technique on frozen samples because freezing cells before extracting nuclei can impair nuclear integrity and alter chromatin structure, especially in fragile cells such as neurons. Our aim was to develop a protocol for freezing neuronal cells that is compatible with ATAC-Seq; we focused on a disease-relevant cell type, namely motor neurons differentiated from induced pluripotent stem cells (iMNs) from a patient affected by spinal muscular atrophy. We found that while flash-frozen iMNs are not suitable for ATAC-Seq, the assay is successful with slow-cooled cryopreserved cells. Using this method, we were able to isolate high quality, intact nuclei, and we verified that epigenetic results from fresh and cryopreserved iMNs quantitatively agree.

Millette, K. and S. Georgia (2017). "Gene Editing and Human Pluripotent Stem Cells: Tools for Advancing Diabetes Disease Modeling and Beta-Cell Development." Curr Diab Rep **17**(11): 116.

PURPOSE OF REVIEW: This review will focus on the multiple approaches to gene editing and address the potential use of genetically modified human pluripotent stem cell-derived beta cells (SC-beta) as a tool to study human beta-cell development and model their function in diabetes. We will explore how new variations of CRISPR/Cas9 gene editing may accelerate our understanding of beta-cell developmental biology, elucidate novel mechanisms that establish and regulate beta-cell function, and assist in pioneering new therapeutic modalities for treating diabetes. RECENT FINDINGS: Improvements in CRISPR/Cas9 target specificity and homology-directed recombination continue to advance its use in engineering stem cells to model and potentially treat disease. We will review how CRISPR/Cas9 gene editing is informing our understanding of beta-cell development and expanding the therapeutic possibilities for treating diabetes and other diseases. Here we focus on the emerging use of gene editing technology, specifically CRISPR/Cas9, as a means of manipulating human gene expression to gain novel insights into the roles of key factors in beta-cell development and function. Taken together, the combined use of SC-beta cells and CRISPR/Cas9 gene editing will shed new light on human beta-cell development and function and accelerate our progress towards developing new therapies for patients with diabetes.

Millman, J. R. and F. W. Pagliuca (2017). "Autologous Pluripotent Stem Cell-Derived beta-Like Cells for Diabetes Cellular Therapy." Diabetes **66**(5): 1111-1120.

Development of stem cell technologies for cell replacement therapy has progressed rapidly in recent years. Diabetes has long been seen as one of the first applications for stem cell-derived cells because of the loss of only a single cell type-the insulin-producing beta-cell. Recent reports have detailed strategies that overcome prior hurdles to generate functional beta-like cells from human pluripotent stem cells in vitro, including from human induced pluripotent stem cells (hiPSCs). Even with this accomplishment, addressing immunological barriers to transplantation remains a major challenge for the field. The development of clinically relevant hiPSC derivation methods from patients and demonstration that these cells can be differentiated into beta-like cells presents a new opportunity to treat diabetes without immunosuppression or immunoprotective encapsulation or with only targeted protection from autoimmunity. This review focuses on the current status in generating and transplanting autologous beta-cells for diabetes cell therapy, highlighting the unique advantages and challenges of this approach.

Miranda, C. C., et al. (2015). "Spatial and temporal control of cell aggregation efficiently directs human pluripotent stem cells towards neural commitment." Biotechnol J **10**(10): 1612-1624.

3D suspension culture is generally considered a promising method to achieve efficient expansion and controlled differentiation of human pluripotent stem cells (hPSCs). In this work, we focused on developing an integrated culture platform for expansion and neural commitment of hPSCs into neural precursors using 3D suspension conditions and chemically-defined culture media. We evaluated different inoculation methodologies for hPSC expansion as 3D aggregates and characterized the resulting cultures in terms of aggregate size distribution. It was demonstrated that upon single-cell inoculation, after four days of culture,

3D aggregates were composed of homogenous populations of hPSC and were characterized by an average diameter of 139 +/- 26 mum, which was determined to be the optimal size to initiate neural commitment. Temporal analysis revealed that upon neural specification it is possible to maximize the percentage of neural precursor cells expressing the neural markers Sox1 and Pax6 after nine days of culture. These results highlight our ability to define a robust method for production of hPSC-derived neural precursors that minimizes processing steps and that constitutes a promising alternative to the traditional planar adherent culture system due to a high potential for scaling-up.

Mitsui, K., et al. (2015). "Conditionally replicating adenovirus prevents pluripotent stem cell-derived teratoma by specifically eliminating undifferentiated cells." Mol Ther Methods Clin Dev **2**: 15026.

Incomplete abolition of tumorigenicity creates potential safety concerns in clinical trials of regenerative medicine based on human pluripotent stem cells (hPSCs). Here, we demonstrate that conditionally replicating adenoviruses that specifically target cancers using multiple factors (m-CRAs), originally developed as anticancer drugs, may also be useful as novel antitumorigenic agents in hPSC-based therapy. The survivin promoter was more active in undifferentiated hPSCs than the telomerase reverse transcriptase (TERT) promoter, whereas both promoters were minimally active in differentiated normal cells. Accordingly, survivin-responsive m-CRA (Surv.m-CRA) killed undifferentiated hPSCs more efficiently than TERT-responsive m-CRAs (Tert.m-CRA); both m-CRAs exhibited efficient viral replication and cytotoxicity in undifferentiated hPSCs, but not in cocultured differentiated normal cells. Pre-infection of hPSCs with Surv.m-CRA or Tert.m-CRA abolished in vivo teratoma formation in a dose-dependent manner following hPSC implantation into mice. Thus, m-CRAs, and in particular Surv.m-CRAs, represent novel antitumorigenic agents that could facilitate safe clinical applications of hPSC-based regenerative medicine.

Miyagawa, S., et al. (2016). "Building A New Treatment For Heart Failure-Transplantation of Induced Pluripotent Stem Cell-derived Cells into the Heart." Curr Gene Ther **16**(1): 5-13.

Advanced cardiac failure is a progressive intractable disease and is the main cause of mortality and morbidity worldwide. Since this pathology is represented by a definite decrease in cardiomyocyte number, supplementation of functional cardiomyocytes into the heart would hypothetically be an ideal therapeutic option. Recently, unlimited in vitro production of human functional cardiomyocytes was established by using induced pluripotent stem cell (iPSC) technology, which avoids the use of human embryos. A number of basic studies including ours have shown that transplantation of iPSCderived cardiomyocytes (iPSC-CMs) into the damaged heart leads to recovery of cardiac function, thereby establishing "proof-of-concept" of this iPSC-transplantation therapy. However, considering clinical application of this therapy, its feasibility, safety, and therapeutic efficacy need to be further investigated in the pre-clinical stage. This review summarizes up-to-date important topics related to safety and efficacy of iPSC-CMs transplantation therapy for cardiac disease and discusses the prospects for this treatment in clinical studies.

Miyoshi, N., et al. (2016). "Erasure of DNA methylation, genomic imprints, and epimutations in a primordial germ-cell model derived from mouse pluripotent stem cells." Proc Natl Acad Sci U S A **113**(34): 9545-9550.

The genome-wide depletion of 5-methylcytosines (5meCs) caused by passive dilution through DNA synthesis without daughter strand methylation and active enzymatic processes resulting in replacement of 5meCs with unmethylated cytosines is a hallmark of primordial germ cells (PGCs). Although recent studies have shown that in vitro differentiation of pluripotent stem cells (PSCs) to PGC-like cells (PGCLCs) mimics the in vivo differentiation of epiblast cells to PGCs, how DNA methylation status of PGCLCs resembles the dynamics of 5meC erasure in embryonic PGCs remains controversial. Here, by differential detection of genome-wide 5meC and 5-hydroxymethylcytosine (5hmeC) distributions by deep sequencing, we show that PGCLCs derived from mouse PSCs recapitulated the process of genome-wide DNA demethylation in embryonic PGCs, including significant demethylation of imprint control regions (ICRs) associated with increased mRNA expression of the corresponding imprinted genes. Although 5hmeCs were also significantly diminished in PGCLCs, they retained greater amounts of 5hmeCs than intragonadal PGCs. The genomes of both PGCLCs and PGCs selectively retained both 5meCs and 5hmeCs at a small number of repeat sequences such as GSAT_MM, of which the significant retention of bisulfite-resistant cytosines was corroborated by reanalysis of previously published whole-genome bisulfite sequencing data for intragonadal PGCs. PSCs harboring abnormal hypermethylation at ICRs of the Dlk1-Gtl2-Dio3 imprinting cluster diminished these 5meCs upon differentiation to PGCLCs, resulting in transcriptional reactivation of the Gtl2 gene. These observations support the usefulness of PGCLCs in studying the

germline epigenetic erasure including imprinted genes, epimutations, and erasure-resistant loci, which may be involved in transgenerational epigenetic inheritance.

Mobarra, N., et al. (2014). "Efficient Differentiation of Human Induced Pluripotent Stem Cell (hiPSC) Derived Hepatocyte-Like Cells on hMSCs Feeder." Int J Hematol Oncol Stem Cell Res **8**(4): 20-29.

BACKGROUND: The use of stem cells is considered as an appropriate source in cell therapy and tissue engineering. Differentiation of human induced Pluripotent Stem Cells (hiPSCs) to Hepatocyte-like Cells (HLCs) on mouse embryonic fibroblasts (MEFs) feeders is confronted with several problems that hinder the clinical applications of these differentiated cells for the treatment of liver injuries. Safe appropriate cells for stem cell-based therapies could create new hopes for liver diseases. This work focused on the determination of a capacity/efficiency for the differentiation of the hiPSCs into Hepatocyte-like Cells on a novel human adult bone marrow mesenchymal stem cells (hMSCs) feeder. MATERIALS AND METHODS: Undifferentiated human iPSCs were cultured on mitotically inactivated human adult bone marrow mesenchymal stem cells. A three-step differentiation process has been performed in presence of activin A which added for 3 days to induce a definitive endoderm formation. In the second step, medium was exchanged for six days. Subsequently, cells were treated with oncostatin M plus dexamethasone for 9 days to generate hepatic cells. Endodermic and liver-specific genes were assessed via quantitative reverse transcription-polymerase chain reaction and RT-PCR, moreover, immunocytochemical staining for liver proteins including albumin and alpha-fetoprotein. In addition, functional tests for glycogen storage, oil red examination, urea production and alpha-fetoprotein synthesis, as well as, cells differentiated with a hepatocyte-like morphology was also performed. RESULTS: Our results show that inactivated human adult bone marrow mesenchymal stem cell feeders could support the efficient differentiation of hiPSCs into HLCs. This process induced differentiation of iPSCs into definitive endocrine cells that expressed sox17, foxa2 and expression of the specific genes profiles in hepatic-like cells. In addition, immunocytochemical analysis confirmed albumin and alpha-fetoprotein protein expression, as well as, the hiPSCs-derived Hepatocyte-like Cells on human feeder exhibited a typical morphology. CONCLUSIONS: we suggested a successful and efficient culture for differentiation and maturation of hepatocytes on an alternative human feeders; this is an important step to generate safe and functional hepatocytes that is vital for regenerative medicine and transplantation on the cell-based therapies.

Momcilovic, O., et al. (2009). "Ionizing radiation induces ataxia telangiectasia mutated-dependent checkpoint signaling and G(2) but not G(1) cell cycle arrest in pluripotent human embryonic stem cells." Stem Cells **27**(8): 1822-1835.

Human embryonic stem (ES) cells are highly sensitive to environmental insults including DNA damaging agents, responding with high levels of apoptosis. To understand the response of human ES cells to DNA damage, we investigated the function of the ataxia telangiectasia mutated (ATM) DNA damage signaling pathway in response to gamma-irradiation. Here, we demonstrate for the first time in human ES cells that ATM kinase is phosphorylated and properly localized to the sites of DNA double-strand breaks within 15 minutes of irradiation. Activation of ATM kinase resulted in phosphorylation of its downstream targets: Chk2, p53, and Nbs1. In contrast to murine ES cells, Chk2 and p53 were localized to the nucleus of irradiated human ES cells. We further show that irradiation resulted in a temporary arrest of the cell cycle at the G(2), but not G(1), phase. Human ES cells resumed cycling approximately 16 hours after irradiation, but had a fourfold higher incidence of aberrant mitotic figures compared to nonirradiated cells. Finally, we demonstrate an essential role of ATM in establishing G(2) arrest since inhibition with the ATM-specific inhibitor KU55933 resulted in abolishment of G(2) arrest, evidenced by an increase in the number of cycling cells 2 hours after irradiation. In summary, these results indicate that human ES cells activate the DNA damage checkpoint, resulting in an ATM-dependent G(2) arrest. However, these cells re-enter the cell cycle with prominent mitotic spindle defects.

Momcilovic, O., et al. (2011). "Cell cycle adaptations and maintenance of genomic integrity in embryonic stem cells and induced pluripotent stem cells." Results Probl Cell Differ **53**: 415-458.

Pluripotent stem cells have the capability to undergo unlimited self-renewal and differentiation into all somatic cell types. They have acquired specific adjustments in the cell cycle structure that allow them to rapidly proliferate, including cell cycle independent expression of cell cycle regulators and lax G(1) to S phase transition. However, due to the developmental role of embryonic stem cells (ES) it is essential to maintain genomic integrity and prevent acquisition of mutations that would be transmitted to multiple cell lineages. Several modifications in DNA damage response of ES cells accommodate dynamic cycling and preservation of genetic information. The absence of a G(1)/S cell cycle arrest promotes apoptotic response of damaged cells before DNA changes can be fixed in the form of mutation during the S phase, while

G(2)/M cell cycle arrest allows repair of damaged DNA following replication. Furthermore, ES cells express higher level of DNA repair proteins, and exhibit enhanced repair of multiple types of DNA damage. Similarly to ES cells, induced pluripotent stem (iPS) cells are poised to proliferate and exhibit lack of G(1)/S cell cycle arrest, extreme sensitivity to DNA damage, and high level of expression of DNA repair genes. The fundamental mechanisms by which the cell cycle regulates genomic integrity in ES cells and iPS cells are similar, though not identical.

Monette, F. C. (1979). "Antibodies against pluripotent stem cells: their use in studying stem cell function." Blood Cells **5**(2): 175-191.

The biologic characteristics and specificity of rabbit anti-mouse brain (RAMB) serum for pluripotent hemopoietic stem cells (CFU-s) is reviewed. The application of RAMB serum to the functional analysis of stem cell differentiation and self renewal characteristics is discussed. Preliminary data are presented which suggest the existence of two stem cell subcompartments. The majority of stem cells express membrane determinants that are detected by RAMB serum. A minor (5%-10%) stem cell subpopulation lacks the stem cell antigen and exhibits a greater self-renewal capacity than those cells expressing the antigen.

Mooney, B. M., et al. (2013). "Convergent mechanisms in pluripotent stem cells and cancer: implications for stem cell engineering." Biotechnol J **8**(4): 408-419.

Stem cells and cancer cells share certain characteristics, including the capacity to self-renew, differentiatie, and undergo epithelial-to-mesenchymal transition (EMT). The mechanisms underlying tumorigenesis retain similarities with processes in normal stem cell development. Comprehensive analysis and comparison of cancer cell and stem cell development will advance the study of cancer progression, enabling development of effective strategies for cancer treatment. In this review article, we first examine the convergence of outcome, cellular communication, and signaling pathways active in pluripotent stem cells and cancer cells. Next, we detail how stem cell engineering is able to mimic in vivo microenvironments. These efforts can help better identify stem cell-cancer cell interactions, elucidated dysregulated pluripotent signaling pathways occurring in cancer, revealed new factors that restrict tumorigenesis and metastasis potential, and reprogrammed cancer cells to a less aggressive phenotype. The potential of stem cell engineering to enhance cancer research is tremendous and may lead to alternative therapeutic options for aggressive cancers.

Morgani, S. M., et al. (2018). "Micropattern differentiation of mouse pluripotent stem cells recapitulates embryo regionalized cell fate patterning." Elife **7**.

During gastrulation epiblast cells exit pluripotency as they specify and spatially arrange the three germ layers of the embryo. Similarly, human pluripotent stem cells (PSCs) undergo spatially organized fate specification on micropatterned surfaces. Since in vivo validation is not possible for the human, we developed a mouse PSC micropattern system and, with direct comparisons to mouse embryos, reveal the robust specification of distinct regional identities. BMP, WNT, ACTIVIN and FGF directed mouse epiblast-like cells to undergo an epithelial-to-mesenchymal transition and radially pattern posterior mesoderm fates. Conversely, WNT, ACTIVIN and FGF patterned anterior identities, including definitive endoderm. By contrast, epiblast stem cells, a developmentally advanced state, only specified anterior identities, but without patterning. The mouse micropattern system offers a robust scalable method to generate regionalized cell types present in vivo, resolve how signals promote distinct identities and generate patterns, and compare mechanisms operating in vivo and in vitro and across species.

Moschidou, D., et al. (2013). "Human mid-trimester amniotic fluid stem cells cultured under embryonic stem cell conditions with valproic acid acquire pluripotent characteristics." Stem Cells Dev **22**(3): 444-458.

Human mid-trimester amniotic fluid stem cells (AFSC) have promising applications in regenerative medicine, being broadly multipotent with an intermediate phenotype between embryonic (ES) and mesenchymal stem cells (MSC). Despite this propluripotent phenotype, AFSC are usually cultured in adherence in a serum-based expansion medium, and how expansion in conditions sustaining pluripotency might affect their phenotype remains unknown. We recently showed that early AFSC from first trimester amniotic fluid, which endogenously express Sox2 and Klf4, can be reprogrammed to pluripotency without viral vectors using the histone deacetylase inhibitor valproic acid (VPA). Here, we show that mid-trimester AFSC cultured under MSC conditions contained a subset of cells endogenously expressing telomerase, CD24, OCT4, C-MYC, and SSEA4, but low/null levels of SOX2, NANOG, KLF4, SSEA3, TRA-1-60, and TRA-1-81, with cells unable to form embryoid bodies (EBs) or teratomas. In contrast, AFSC cultured under human ESC conditions were smaller in size, grew faster, formed colonies, upregulated OCT4 and C-MYC, and expressed KLF4 and SOX2, but not NANOG, SSEA3, TRA-1-60, and TRA-1-81.

Supplementation with VPA for 5 days further upregulated OCT4, KLF4, and SOX2, and induced expression of NANOG, SSEA3, TRA-1-60, and TRA-1-81, with cells now able to form EBs and teratomas. We conclude that human mid-trimester AFSC, which may be isolated autologously during pregnancy without ethics restriction, can acquire pluripotent characteristics without the use of ectopic factors. Our data suggest that this medium-dependant approach to pluripotent mid-trimester AFSC reflects true reprogramming and not the selection of prepluripotent cells.

Moslem, M., et al. (2015). "Mesenchymal Stem/Stromal Cells Derived from Induced Pluripotent Stem Cells Support CD34(pos) Hematopoietic Stem Cell Propagation and Suppress Inflammatory Reaction." Stem Cells Int **2015**: 843058.

Mesenchymal stem/stromal cells (MSCs) represent a promising cell source for research and therapeutic applications, but their restricted ex vivo propagation capabilities limit putative applications. Substantial self-renewing of stem cells can be achieved by reprogramming cells into induced pluripotent stem cells (iPSCs) that can be easily expanded as undifferentiated cells even in mass culture. Here, we investigated a differentiation protocol enabling the generation and selection of human iPSC-derived MSCs exhibiting relevant surface marker expression profiles (CD105 and CD73) and functional characteristics. We generated such iPSC-MSCs from fibroblasts and bone marrow MSCs utilizing two different reprogramming constructs. All such iPSC-MSCs exhibited the characteristics of normal bone marrow-derived (BM) MSCs. In direct comparison to BM-MSCs our iPSC-MSCs exhibited a similar surface marker expression profile but shorter doubling times without reaching senescence within 20 passages. Considering functional capabilities, iPSC-MSCs provided supportive feeder layer for CD34(+) hematopoietic stem cells' self-renewal and colony forming capacities. Furthermore, iPSC-MSCs gained immunomodulatory function to suppress CD4(+) cell proliferation, reduce proinflammatory cytokines in mixed lymphocyte reaction, and increase regulatory CD4(+)/CD69(+)/CD25(+) T-lymphocyte population. In conclusion, we generated fully functional MSCs from various iPSC lines irrespective of their starting cell source or reprogramming factor composition and we suggest that such iPSC-MSCs allow repetitive cell applications for advanced therapeutic approaches.

Moslem, M., et al. (2013). "Therapeutic potential of human induced pluripotent stem cell-derived mesenchymal stem cells in mice with lethal fulminant hepatic failure." Cell Transplant **22**(10): 1785-1799.

Large-scale production and noninvasive methods for harvesting mesenchymal stem cells (MSCs), particularly in elderly individuals, has prompted researchers to find new patient-specific sources for MSCs in regenerative medicine. This study aims to produce MSCs from human induced pluripotent stem cells (hiPSCs) and to evaluate their therapeutic effects in a CCl4-induced mouse model of fulminant hepatic failure (FHF). hiPSC-MSCs have shown MSC morphology, antigen profile and differentiation capabilities, and improved hepatic function in our model. hiPSC-MSC-transplanted animals provide significant benefit in terms of survival, serum LDH, total bilirubin, and lipid peroxidation. hiPSC-MSC therapy resulted in a one-third reduction of histologic activity index and a threefold increase in the number of proliferating hepatocytes. This was accompanied by a significant decrease in the expression levels of collagen type I, Mmp13, Mmp2, and Mmp9 genes and increase in Timp1 and Timp2 genes in transplanted groups. hiPSC-MSCs secreted hepatocyte growth factor (HGF) in vitro and also expressed HGF in evaluated liver sections. Similar results were observed with human bone marrow (hBM)-derived MSCs. In conclusion, our results have demonstrated that hiPSC-MSCs might be valuable appropriate alternatives for hBM-MSCs in FHF liver repair and support liver function by cell therapy with a large-scale production capacity, patient-specific nature, and no invasive MSC harvesting.

Mosley, M. C., et al. (2017). "Neurite extension and neuronal differentiation of human induced pluripotent stem cell derived neural stem cells on polyethylene glycol hydrogels containing a continuous Young's Modulus gradient." J Biomed Mater Res A **105**(3): 824-833.

Mechanotransduction in neural cells involves multiple signaling pathways that are not fully understood. Differences in lineage and maturation state are suggested causes for conflicting reports on neural cell mechanosensitivity. To optimize matrices for use in stem cell therapy treatments transplanting human induced pluripotent stem cell derived neural stem cells (hNSC) into lesions after spinal cord injury, the effects of Young's Modulus changes on hNSC behavior must be understood. The present study utilizes polyethylene glycol hydrogels containing a continuous gradient in Young's modulus to examine changes in the Young's Modulus of the culture substrate on hNSC neurite extension and neural differentiation. Changes in the Young's Modulus of the polyethylene glycol hydrogels was found to affect neurite extension and cellular organization on the matrices. hNSC cultured on 907 Pa hydrogels were found to extend longer neurites than hNSC cultured on other tested Young's Moduli

hydrogels. The gene expression of beta tubulin III and microtubule-associated protein 2 in hNSC was affected by changes in the Young's Modulus of the hydrogel. The combinatory method approach used in the present study demonstrates that hNSC are mechanosensitive and the matrix Young's Modulus should be a design consideration for hNSC transplant applications. (c) 2016 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 105A: 824-833, 2017.

Moura, R., et al. (2010). "Induced pluripotent stem (iPS) cells and endothelial cell generation: SIRT-ainly a good idea!" Atherosclerosis **212**(1): 36-39.

Muers, M. (2011). "Stem cells: Scorecards for pluripotent cell lines." Nat Rev Genet **12**(3): 153.

Muffat, J., et al. (2018). "Human induced pluripotent stem cell-derived glial cells and neural progenitors display divergent responses to Zika and dengue infections." Proc Natl Acad Sci U S A **115**(27): 7117-7122.

Maternal Zika virus (ZIKV) infection during pregnancy is recognized as the cause of an epidemic of microcephaly and other neurological anomalies in human fetuses. It remains unclear how ZIKV accesses the highly vulnerable population of neural progenitors of the fetal central nervous system (CNS), and which cell types of the CNS may be viral reservoirs. In contrast, the related dengue virus (DENV) does not elicit teratogenicity. To model viral interaction with cells of the fetal CNS in vitro, we investigated the tropism of ZIKV and DENV for different induced pluripotent stem cell-derived human cells, with a particular focus on microglia-like cells. We show that ZIKV infected isogenic neural progenitors, astrocytes, and microglia-like cells (pMGLs), but was only cytotoxic to neural progenitors. Infected glial cells propagated ZIKV and maintained ZIKV load over time, leading to viral spread to susceptible cells. DENV triggered stronger immune responses and could be cleared by neural and glial cells more efficiently. pMGLs, when cocultured with neural spheroids, invaded the tissue and, when infected with ZIKV, initiated neural infection. Since microglia derive from primitive macrophages originating in proximity to the maternal vasculature, they may act as a viral reservoir for ZIKV and establish infection of the fetal brain. Infection of immature neural stem cells by invading microglia may occur in the early stages of pregnancy, before angiogenesis in the brain rudiments. Our data are also consistent with ZIKV and DENV affecting the integrity of the blood-brain barrier, thus allowing infection of the brain later in life.

Mulyasasmita, W., et al. (2014). "Avidity-controlled hydrogels for injectable co-delivery of induced pluripotent stem cell-derived endothelial cells and growth factors." J Control Release **191**: 71-81.

To translate recent advances in induced pluripotent stem cell biology to clinical regenerative medicine therapies, new strategies to control the co-delivery of cells and growth factors are needed. Building on our previous work designing Mixing-Induced Two-Component Hydrogels (MITCHs) from engineered proteins, here we develop protein-polyethylene glycol (PEG) hybrid hydrogels, MITCH-PEG, which form physical gels upon mixing for cell and growth factor co-delivery. MITCH-PEG is a mixture of C7, which is a linear, engineered protein containing seven repeats of the CC43 WW peptide domain (C), and 8-arm star-shaped PEG conjugated with either one or two repeats of a proline-rich peptide to each arm (P1 or P2, respectively). Both 20kDa and 40kDa star-shaped PEG variants were investigated, and all four PEG-peptide variants were able to undergo a sol-gel phase transition when mixed with the linear C7 protein at constant physiological conditions due to noncovalent hetero-dimerization between the C and P domains. Due to the dynamic nature of the C-P physical crosslinks, all four gels were observed to be reversibly shear-thinning and self-healing. The P2 variants exhibited higher storage moduli than the P1 variants, demonstrating the ability to tune the hydrogel bulk properties through a biomimetic peptide-avidity strategy. The 20kDa PEG variants exhibited slower release of encapsulated vascular endothelial growth factor (VEGF), due to a decrease in hydrogel mesh size relative to the 40kDa variants. Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) adopted a well-spread morphology within three-dimensional MITCH-PEG cultures, and MITCH-PEG provided significant protection from cell damage during ejection through a fine-gauge syringe needle. In a mouse hindlimb ischemia model of peripheral arterial disease, MITCH-PEG co-delivery of hiPSC-ECs and VEGF was found to reduce inflammation and promote muscle tissue regeneration compared to a saline control.

Munsie, M. J., et al. (2000). "Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei." Curr Biol **10**(16): 989-992.

Pluripotent human stem cells isolated from early embryos represent a potentially unlimited source of many different cell types for cell-based gene and tissue therapies [1-3]. Nevertheless, if the full potential of cell lines derived from donor embryos is to be realised, the problem of donor-recipient tissue matching needs to be overcome. One approach, which avoids the problem of transplant rejection, would be to establish stem cell lines from the patient's own cells

through therapeutic cloning [3,4]. Recent studies have shown that it is possible to transfer the nucleus from an adult somatic cell to an unfertilised oocyte that is devoid of maternal chromosomes, and achieve embryonic development under the control of the transferred nucleus [5-7]. Stem cells isolated from such a cloned embryo would be genetically identical to the patient and pose no risk of immune rejection. Here, we report the isolation of pluripotent murine stem cells from reprogrammed adult somatic cell nuclei. Embryos were generated by direct injection of mechanically isolated cumulus cell nuclei into mature oocytes. Embryonic stem (ES) cells isolated from cumulus-cell-derived blastocysts displayed the characteristic morphology and marker expression of conventional ES cells and underwent extensive differentiation into all three embryonic germ layers (endoderm, mesoderm and ectoderm) in tumours and in chimaeric foetuses and pups. The ES cells were also shown to differentiate readily into neurons and muscle in culture. This study shows that pluripotent stem cells can be derived from nuclei of terminally differentiated adult somatic cells and offers a model system for the development of therapies that rely on autologous, human pluripotent stem cells.

Munst, S., et al. (2018). "In vitro segregation and isolation of human pluripotent stem cell-derived neural crest cells." Methods **133**: 65-80.

The neural crest (NC) is a transient embryonic cell population with remarkable characteristics. After delaminating from the neural tube, NC cells (NCCs) migrate extensively, populate nearly every tissue of the body and differentiate into highly diverse cell types such as peripheral neurons and glia, but also mesenchymal cells including chondrocytes, osteocytes, and adipocytes. While the NC has been extensively studied in several animal models, little is known about human NC development. A number of methods have been established to derive NCCs in vitro from human pluripotent stem cells (hPSC). Typically, these protocols comprise several cell culture steps to enrich for NCCs in the neural derivatives of the differentiating hPSCs. Here we report on a remarkable and hitherto unnoticed in vitro segregation phenomenon that enables direct extraction of virtually pure NCCs during the earliest stages of hPSC differentiation. Upon aggregation to embryoid bodies (EB) and replating, differentiating hPSCs give rise to a population of NCCs, which spontaneously segregate from the EB outgrowth to form conspicuous, macroscopically visible atoll-shaped clusters in the periphery of the EB outgrowth. Isolation of these NC clusters yields p75NTR(+)/SOXE(+) NCCs, which differentiate to peripheral neurons and glia as well as mesenchymal derivatives. Our data indicate that differentiating hPSC cultures recapitulate, in a simplified manner, the physical segregation of central nervous system (CNS) tissue and NCCs. This phenomenon may be exploited for NCC purification and for studying segregation and differentiation processes observed during early human NC development in vitro.

Nagamatsu, G. and T. Suda (2013). "Conversion of primordial germ cells to pluripotent stem cells: methods for cell tracking and culture conditions." Methods Mol Biol **1052**: 49-56.

Primordial germ cells (PGCs) are unipotent cells committed to germ lineage: PGCs can only differentiate into gametes in vivo. However, upon fertilization, germ cells acquire the capacity to differentiate into all cell types in the body, including germ cells. Therefore, germ cells are thought to have the potential for pluripotency. PGCs can convert to pluripotent stem cells in vitro when cultured under specific conditions that include bFGF, LIF, and the membrane-bound form of SCF (mSCF). Here, the culture conditions which efficiently convert PGCs to pluripotent embryonic germ (EG) cells are described, as well as methods used for identifying pluripotent candidate cells during culture.

Nagamoto, Y., et al. (2015). "Efficient Engraftment of Human Induced Pluripotent Stem Cell-Derived Hepatocyte-Like Cells in uPA/SCID Mice by Overexpression of FNK, a Bcl-xL Mutant Gene." Cell Transplant **24**(6): 1127-1138.

Human liver chimeric mice are expected to be applied for drug toxicity tests and human hepatitis virus research. Human induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs) are a highly attractive donor source for the generation of human liver chimeric mice because they can be produced on a large scale and established from an individual. Although these cells have been successfully used to generate human liver chimeric mice, there is still room for improvement in the repopulation efficiency. To enhance the repopulation efficacy, the human iPSC-HLCs were transduced with an adenovirus vector (Ad-FNK) expressing FNK, a hyperactive mutant gene from Bcl-xL, which was expected to inhibit apoptosis in the process of integration into liver parenchyma. We then transplanted Ad-FNK-transduced human iPSC-HLCs into urokinase-type plasminogen activator-transgenic severe combined immunodeficiency (uPA/SCID) mice (FNK mice) and evaluated the repopulation efficacy. The antiapoptotic effects of the human iPSC-HLCs were enhanced by FNK overexpression in vitro. Human albumin levels in the transplanted mice were significantly increased by transplantation of Ad-FNK-transduced human iPSC-HLCs (about 24,000 ng/ml). Immunohistochemical analysis with an anti-human

alphaAT antibody revealed greater repopulation efficacy in the livers of FNK mice than control mice. Interestingly, the expression levels of human hepatocyte-related genes in the human iPSC-HLCs of FNK mice were much higher than those in the human iPSC-HLCs before transplantation. We succeeded in improving the repopulation efficacy of human liver chimeric mice generated by transplanting the Ad-FNK-transduced human iPSC-HLCs into uPA/SCID mice. Our method using ectopic expression of FNK was useful for generating human chimeric mice with high chimerism.

Nagamoto, Y., et al. (2012). "The promotion of hepatic maturation of human pluripotent stem cells in 3D co-culture using type I collagen and Swiss 3T3 cell sheets." Biomaterials **33**(18): 4526-4534.

Hepatocyte-like cells differentiated from human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) are known to be a useful cell source for drug screening. We recently developed an efficient hepatic differentiation method from hESCs and hiPSCs by sequential transduction of FOXA2 and HNF1alpha. It is known that the combination of three-dimensional (3D) culture and co-culture, namely 3D co-culture, can maintain the functions of primary hepatocytes. However, hepatic maturation of hESC- or hiPSC-derived hepatocyte-like cells (hEHs or hiPHs, respectively) by 3D co-culture systems has not been examined. Therefore, we utilized a cell sheet engineering technology to promote hepatic maturation. The gene expression levels of hepatocyte-related markers (such as cytochrome P450 enzymes and conjugating enzymes) and the amount of albumin secretion in the hEHs or hiPHs, which were 3D co-cultured with the Swiss 3T3 cell sheet, were significantly up-regulated in comparison with those in the hEHs or hiPHs cultured in a monolayer. Furthermore, we found that type I collagen synthesized in Swiss 3T3 cells plays an important role in hepatic maturation. The hEHs or hiPHs that were 3D co-cultured with the Swiss 3T3 cell sheet would be powerful tools for medical applications, such as drug screening.

Nagasaka, R., et al. (2017). "Image-based cell quality evaluation to detect irregularities under same culture process of human induced pluripotent stem cells." J Biosci Bioeng **123**(5): 642-650.

To meet the growing demand for human induced pluripotent stem cells (iPSCs) for various applications, technologies that enable the manufacturing of iPSCs on a large scale should be developed. There are several technological challenges in iPSC manufacturing technology. Image-based cell quality evaluation technology for monitoring iPSC quality in culture enables the manufacture of intact cells for further applications. Although several studies have reported the effectiveness of image-based evaluation of iPSCs, it remains challenging to detect irregularities that may arise using the same processing operations during quality evaluation of automated processing. In this study, we investigated the evaluation performance of image-based cell quality analysis in detecting small differences that can result from human measurement, even when the same protocol is followed. To imitate such culture conditions, by image-analysis guided colony pickup, we changed the proportions of morphologically different subpopulations: "good morphology, regular morphology correlated with undifferentiation marker expression" and "bad morphology, irregular morphology correlated with loss of undifferentiation marker expression". In addition, comprehensive gene-expression and metabolomics analyses were carried out for the same samples to investigate performance differences. Our data shows an example of investigating the usefulness and sensitivity of quality evaluation methods for iPSC quality monitoring.

Nakamura, M. and H. Okano (2013). "Cell transplantation therapies for spinal cord injury focusing on induced pluripotent stem cells." Cell Res **23**(1): 70-80.

Stimulated by the 2012 Nobel Prize in Physiology or Medicine awarded for Shinya Yamanaka and Sir John Gurdon, there is an increasing interest in the induced pluripotent stem (iPS) cells and reprograming technologies in medical science. While iPS cells are expected to open a new era providing enormous opportunities in biomedical sciences in terms of cell therapies and regenerative medicine, safety-related concerns for iPS cell-based cell therapy should be resolved prior to the clinical application of iPS cells. In this review, the pre-clinical investigations of cell therapy for spinal cord injury (SCI) using neural stem/progenitor cells derived from iPS cells, and their safety issues in vivo, are outlined. We also wish to discuss the strategy for the first human trails of iPS cell-based cell therapy for SCI patients.

Nakamura, S., et al. (2014). "Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells." Cell Stem Cell **14**(4): 535-548.

The donor-dependent supply of platelets is frequently insufficient to meet transfusion needs. To address this issue, we developed a clinically applicable strategy for the derivation of functional platelets from human pluripotent stem cells (PSCs). This approach involves the establishment of stable immortalized megakaryocyte progenitor cell lines (imMKCLs) from

PSC-derived hematopoietic progenitors through the overexpression of BMI1 and BCL-XL to respectively suppress senescence and apoptosis and the constrained overexpression of c-MYC to promote proliferation. The resulting imMKCLs can be expanded in culture over extended periods (4-5 months), even after cryopreservation. Halting the overexpression of c-MYC, BMI1, and BCL-XL in growing imMKCLs led to the production of CD42b(+) platelets with functionality comparable to that of native platelets on the basis of a range of assays in vitro and in vivo. The combination of robust expansion capacity and efficient platelet production means that appropriately selected imMKCL clones represent a potentially inexhaustible source of hPSC-derived platelets for clinical application.

Nakane, T., et al. (2017). "Impact of Cell Composition and Geometry on Human Induced Pluripotent Stem Cells-Derived Engineered Cardiac Tissue." Sci Rep **7**: 45641.

The current study describes a scalable, porous large-format engineered cardiac tissue (LF-ECT) composed of human induced pluripotent stem cells (hiPSCs) derived multiple lineage cardiac cells with varied 3D geometries and cell densities developed towards the goal of scale-up for large animal pre-clinical studies. We explored multiple 15 x 15 mm ECT geometries using molds with rectangular internal staggered posts (mesh, ME), without posts (plain sheet, PS), or long parallel posts (multiple linear bundles, ML) and a gel matrix containing hiPSC-derived cardiomyocytes, endothelial, and vascular mural cells matured in vitro for 14 days. ME-ECTs displayed the lowest dead cell ratio ($p < 0.001$) and matured into 0.5 mm diameter myofiber bundles with greater 3D cell alignment and higher active stress than PS-ECTs. Increased initial ECT cell number beyond 6 M per construct resulted in reduced cell survival and lower active stress. The 6M-ME-ECTs implanted onto 1 week post-infarct immune tolerant rat hearts engrafted, displayed evidence for host vascular coupling, and recovered myocardial structure and function with reduced scar area. We generated a larger (30 x 30 mm) ME-ECT to confirm scalability. Thus, large-format ECTs generated from hiPSC-derived cardiac cells may be feasible for large animal preclinical cardiac regeneration paradigms.

Nakayama, C., et al. (2018). "The development of induced pluripotent stem cell-derived mesenchymal stem/stromal cells from normal human and RDEB epidermal keratinocytes." J Dermatol Sci **91**(3): 301-310.

BACKGROUND: Epidermolysis bullosa (EB) is a group of hereditary disorders caused by mutations in the genes encoding structural molecules of the dermal-epidermal junction (DEJ). Cell-based therapies such as allogeneic mesenchymal stem/stromal cell (MSC) transplantation have recently been explored for severe EB types, such as recessive dystrophic EB (RDEB). However, hurdles exist in current MSC-based therapies, such as limited proliferation from a single cell source and limited cell survival due to potential allogenic rejection. OBJECTIVES: We aimed to develop MSCs from keratinocyte-derived induced pluripotent stem cells (iPSCs). METHODS: Keratinocyte-derived iPSCs (KC-iPSCs) of a healthy human and an RDEB patient were cultured with activin A, 6-bromoindirubin-3'-oxime and bone morphogenetic protein 4 to induce mesodermal lineage formation. These induced cells were subjected to immunohistochemical analysis, flow cytometric analysis and RNA microarray analysis in vitro, and were injected subcutaneously and intravenously to wounded immunodeficient mice to assess their wound-healing efficacy. RESULTS: After their induction, KC-iPSC-induced cells were found to be compatible with MSCs. Furthermore, with the subcutaneous and intravenous injection of the KC-iPSC-induced cells into wounded immunodeficient mice, human type VII collagen was detected at the DEJ of epithelized areas. CONCLUSIONS: We successfully established iPSC-derived MSCs from keratinocytes (KC-iPSC-MSCs) of a normal human and an RDEB patient. KC-iPSC-MSCs may have potential in therapies for RDEB.

Nakayama, M. (2009). "Cell Therapy Using Induced Pluripotent Stem (iPS) Cells Meets Next-Next Generation DNA Sequencing Technology." Curr Genomics **10**(5): 303-305.

The recent development of induced pluripotent stem (iPS) cell technology brings cell and gene therapies to patients one large step closer to reality. Technical improvements in various research fields sometimes come together fortuitously, leading to approaches to treating disease. If iPS cell technology continues to progress smoothly as expected and is actually applied to patients, the next logical step to ensuring the success of iPS cell therapy is to make use of next-next generation DNA sequencing technology and bioinformatics of recipient genomes. Before a patient-derived iPS cell colony is used for clinical therapy in a patient, the colony should undergo whole-genome DNA sequencing, thus avoiding risks associated with spontaneously mutagenized iPS cells. Researchers participating in the Human Genome Project need to take full advantage of both technologies-iPS cell technology and DNA sequencing-as doing so will help us achieve the original long-term goal of the project: developing therapies that will benefit human health.

Narsinh, K. H., et al. (2011). "Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells." J Clin Invest **121**(3): 1217-1221.

Human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) are promising candidate cell sources for regenerative medicine. However, despite the common ability of hiPSCs and hESCs to differentiate into all 3 germ layers, their functional equivalence at the single cell level remains to be demonstrated. Moreover, single cell heterogeneity amongst stem cell populations may underlie important cell fate decisions. Here, we used single cell analysis to resolve the gene expression profiles of 362 hiPSCs and hESCs for an array of 42 genes that characterize the pluripotent and differentiated states. Comparison between single hESCs and single hiPSCs revealed markedly more heterogeneity in gene expression levels in the hiPSCs, suggesting that hiPSCs occupy an alternate, less stable pluripotent state. hiPSCs also displayed slower growth kinetics and impaired directed differentiation as compared with hESCs. Our results suggest that caution should be exercised before assuming that hiPSCs occupy a pluripotent state equivalent to that of hESCs, particularly when producing differentiated cells for regenerative medicine aims.

Natarajan, K. N., et al. (2017). "Single cell transcriptomics of pluripotent stem cells: reprogramming and differentiation." Curr Opin Genet Dev **46**: 66-76.

Single-cell transcriptomics serves as a powerful tool to identify cell states within populations of cells, and to dissect underlying heterogeneity at high resolution. Single-cell transcriptomics on pluripotent stem cells has provided new insights into cellular variation, subpopulation structures and the interplay of cell cycle with pluripotency. The single-cell perspective has helped to better understand gene regulation and regulatory networks during exit from pluripotency, cell-fate determination as well as molecular mechanisms driving cellular reprogramming of somatic cells to induced pluripotent stage. Here we review the recent progress and significant findings from application of single-cell technologies on pluripotent stem cells along with a brief outlook on new combinatorial single-cell approaches that further unravel pluripotent stem cell states.

Naujok, O. and S. Lenzen (2012). "[Pluripotent stem cells for cell replacement therapy of diabetes]." Dtsch Med Wochenschr **137**(20): 1062-1066.

The use of pluripotent stem cells (PSCs) harbours great potential for a future use in the cell replacement therapy of diabetes mellitus. The in vitro differentiation of human or mouse embryonic stem cells has yielded pancreatic progenitor cells, but not authentic insulin-producing beta cells. Induced pluripotent cells are a class of a pluripotent stem cells potentially suited as a cell source for cell replacement therapy. These patient specific pluripotent cells are generated by reprogramming but unfortunately accumulate genetic and epigenetic errors during reprogramming, precluding their use for therapeutic purposes in humans.

Navara, C. S., et al. (2013). "Derivation of induced pluripotent stem cells from the baboon: a nonhuman primate model for preclinical testing of stem cell therapies." Cell Reprogram **15**(6): 495-502.

Development of effective pluripotent stem cell-based therapies will require safety and efficacy testing in a clinically relevant preclinical model such as nonhuman primates (NHPs). Baboons and macaques are equally similar to humans genetically and both have been extensively used for biomedical research. Macaques are preferred for human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) research whereas baboons are preferred for transplantation studies because of the greater similarity of their anatomy and immunogenetic system to those of humans. We generated four induced pluripotent stem cell (iPSC) lines from skin cells of the olive baboon (Papio anubis). Each line shows the distinct morphology of primate pluripotent stem cells, including flat colonies with well-defined borders and a high nuclear/cytoplasm ratio. Each is positive for the pluripotency markers OCT4, SOX2, NANOG, and SSEA4. Pluripotency was confirmed in two lines by teratoma formation with representative tissues from each germ layer, whereas a third produced cells from all three germ layers following embryoid body differentiation. Three lines have a normal male karyotype and the fourth is missing the short arm of one copy of chromosome 18. This may serve as an in vitro model for the human developmental disorder 18p-, which impacts 1 in 50,000 births/year. These iPSC lines represent the first step toward establishing the baboon as a NHP model for developing stem cell-based therapies.

Nemade, H., et al. (2018). "Cell death mechanisms of the anti-cancer drug etoposide on human cardiomyocytes isolated from pluripotent stem cells." Arch Toxicol **92**(4): 1507-1524.

Etoposide (ETP) and anthracyclines are applied for wide anti-cancer treatments. However, the ETP-induced cardiotoxicity remains to be a major safety issue and the underlying cardiotoxic mechanisms are not well understood. This study is aiming to unravel

the cardiotoxicity profile of ETP in comparison to anthracyclines using physiologically relevant human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). Using xCELLigence real-time cell analyser (RTCA), we found that single high dose of ETP induces irreversible increase in hPSC-CMs beating rate and decrease in beating amplitude. We also identified 58 deregulated genes consisting of 33 upregulated and 25 downregulated genes in hPSC-CMs after ETP treatment. Gene ontology (GO) and pathway analysis showed that most upregulated genes are enriched in GO categories like positive regulation of apoptotic process, regulation of cell death, and mitochondria organization, whereas most downregulated genes were enriched in GO categories like cytoskeletal organization, muscle contraction, and Ca(2+) ion homeostasis. Moreover, we also found upregulation in 5 miRNAs (has-miR-486-3p, has-miR-34c-5p, has-miR-4423-3p, has-miR-182-5p, and has-miR-139-5p) which play role in muscle contraction, arginine and proline metabolism, and hypertrophic cardiomyopathy (HCM). Immunostaining and transmission electron microscopy also confirmed the cytoskeletal and mitochondrial damage in hPSC-CMs treated with ETP, as well as noticeable alterations in intracellular calcium handling and mitochondrial membrane potential were also observed. The apoptosis inhibitor, Pifithrin-alpha, found to protect hPSC-CMs from ETP-induced cardiotoxicity, whereas hPSC-CMs treated with ferroptosis inhibitor, Liproxstatin-1, showed significant recovery in hPSC-CMs functional properties like beating rate and amplitude after ETP treatment. We suggest that the damage to mitochondria is a major contributing factor involved in ETP-induced cardiotoxicity and the activation of the p53-mediated ferroptosis pathway by ETP is likely the critical pathway in ETP-induced cardiotoxicity. We also conclude that the genomic biomarkers identified in this study will significantly contribute to develop and predict potential cardiotoxic effects of novel anti-cancer drugs in vitro.

Ng, J., et al. (2016). "Immunomodulatory Properties of Induced Pluripotent Stem Cell-Derived Mesenchymal Cells." J Cell Biochem **117**(12): 2844-2853.

MSC-like populations derived from induced pluripotent stem cells (iPSC-MSC) serve as an alternative stem cell source due to their high proliferative capacity. In this study, we assessed the immunomodulatory potential of iPSC-MSC generated from periodontal ligament (PDL) and gingival (GF) tissue. The iPSC-MSC lines exhibited a similar level of suppression of mitogen-stimulated peripheral blood mononuclear cells (PBMNC) proliferation compared to their respective parental fibroblast populations in vitro. Moreover, iPSC-MSC demonstrated the ability to suppress T-cells effector cells, Th1/Th2/Th17 populations, and increase levels of Treg cells. In order to investigate the mechanisms involved, expression of common MSC-derived soluble factors known to supress lymphocyte proliferation were assessed in iPSC-MSC cultured with PBMNC with direct cell-cell contact or separated in transwells. Real-time PCR analysis of factors known to be involved in MSC mediated immune regulation, found a general trend of elevated IDO1 and IL6 transcript levels in iPSC-MSC lines and their respective primary cells co-cultured with activated PBMNC, with a wide range of gene expression levels between the different mesenchymal cell types. The results suggest that different iPSC-MSC may be useful as a potential alternative source of cells for future clinical use in therapeutic applications because of their potent immunosuppressive properties. J. Cell. Biochem. 117: 2844-2853, 2016. (c) 2016 Wiley Periodicals, Inc.

Nguyen, Q. H., et al. (2018). "Single-cell RNA-seq of human induced pluripotent stem cells reveals cellular heterogeneity and cell state transitions between subpopulations." Genome Res **28**(7): 1053-1066.

Heterogeneity of cell states represented in pluripotent cultures has not been described at the transcriptional level. Since gene expression is highly heterogeneous between cells, single-cell RNA sequencing can be used to identify how individual pluripotent cells function. Here, we present results from the analysis of single-cell RNA sequencing data from 18,787 individual WTC-CRISPRi human induced pluripotent stem cells. We developed an unsupervised clustering method and, through this, identified four subpopulations distinguishable on the basis of their pluripotent state, including a core pluripotent population (48.3%), proliferative (47.8%), early primed for differentiation (2.8%), and late primed for differentiation (1.1%). For each subpopulation, we were able to identify the genes and pathways that define differences in pluripotent cell states. Our method identified four transcriptionally distinct predictor gene sets composed of 165 unique genes that denote the specific pluripotency states; using these sets, we developed a multigenic machine learning prediction method to accurately classify single cells into each of the subpopulations. Compared against a set of established pluripotency markers, our method increases prediction accuracy by 10%, specificity by 20%, and explains a substantially larger proportion of deviance (up to threefold) from the prediction model. Finally, we developed an innovative method to predict cells transitioning between subpopulations and support our conclusions with results from two orthogonal pseudotime trajectory methods.

Ni, Z., et al. (2013). "Hematopoietic and nature killer cell development from human pluripotent stem cells." Methods Mol Biol **1029**: 33-41.

Natural killer (NK) cells are key effectors of the innate immune system, protecting the host from a variety of infections, as well as malignant cells. Recent advances in the field of NK cell biology have led to a better understanding of how NK cells develop. This progress has directly translated to improved outcomes in patients receiving hematopoietic stem cell transplants to treat potentially lethal malignancies. However, key differences between mouse and human NK cell development and biology limits the use of rodents to attain a more in depth understanding of NK cell development. Therefore, a readily accessible and genetically tractable cell source to study human NK cell development is warranted. Our lab has pioneered the development of lymphocytes, specifically NK cells, from human embryonic stem cells (hESCs) and more recently induced pluripotent stem cells (iPSCs). This chapter describes a reliable method to generate NK cells from hESCs and iPSCs using murine stromal cell lines. Additionally, we include an updated approach using a spin-embryoid body (spin-EB) differentiation system that allows for human NK cell development completely defined in vitro conditions.

Nii, T., et al. (2016). "Single-Cell-State Culture of Human Pluripotent Stem Cells Increases Transfection Efficiency." Biores Open Access **5**(1): 127-136.

Efficient gene transfer into human pluripotent stem cells (hPSCs) holds great promise for regenerative medicine and pharmaceutical development. In the past decade, various methods were developed for gene transfer into hPSCs; however, hPSCs form tightly packed colonies, making gene transfer difficult. In this study, we established a stable culture method of hPSCs at a single-cell state to reduce cell density and investigated gene transfection efficiency followed by gene editing efficiency. hPSCs cultured in a single-cell state were transfected using nonliposomal transfection reagents with plasmid DNA or mRNA encoding enhanced green fluorescent protein. We found that most cells (DNA > 90%; mRNA > 99%) were transfected without the loss of undifferentiated PSC marker expression or pluripotency. Moreover, we demonstrated an efficient gene editing method using transcription activator-like effector nucleases (TALENs) targeting the adenomatous polyposis coli (APC) gene. Our new method may improve hPSC gene transfer techniques, thus facilitating their use for human regenerative medicine.

Ninomiya, E., et al. (2014). "Glucocorticoids promote neural progenitor cell proliferation derived from human induced pluripotent stem cells." Springerplus **3**: 527.

Glucocorticoids (GCs) are frequently used for treating and preventing chronic lung disease and circulatory dysfunction in premature infants. However, there is growing concern about the detrimental effects of systemic GC administration on neurodevelopment. The first choice of GCs to minimize the adverse effects on the developing brain is still under debate. We investigated the effect of commonly used GCs such as dexamethasone (DEX), betamethasone (BET) and hydrocortisone (HDC) on the proliferation of human-induced pluripotent stem cell (iPSC)-derived neuronal progenitor cells (NPCs). In this study, NPCs were treated with various concentrations of GCs and subjected to cell proliferation assays. Furthermore, we quantified the number of microtubule-associated protein 2 (MAP2) positive neurons in NPCs by immunostaining. All GCs promoted NPC proliferation in a dose-dependent manner. We also confirmed that MAP2-positive neurons in NPCs increased upon GC treatment. However, differential effects of GCs on MAP2 positive neurons were observed when we treated NPCs with H2O2. The total numbers of NPCs increased upon any GC treatment even under oxidative conditions but the numbers of MAP2 positive neurons increased only by HDC treatment. GCs promoted human iPSCsaeuro"derived NPC proliferation and the differential effects of GCs became apparent under oxidative stress. Our results may support HDC as the preferred choice over DEX and BET to prevent adverse effects on the developing human brain.

Nishihara, S. (2018). "Glycans in stem cell regulation: from Drosophila tissue stem cells to mammalian pluripotent stem cells." FEBS Lett.

Cell surface glycans, which are tissue-specific and developmentally regulated, work as essential modulators in ligand-receptor interactions, binding to various signal ligands including Wnt, Hedgehog, fibroblast growth factors, epidermal growth factors, and bone morphogenetic proteins, as well as in cell-cell interactions and cell-extracellular matrix interactions. These signals are essential for the stemness and differentiation of various kinds of stem cells. In addition, the intracellular O-linked N-acetylglucosamine, a form of glycosylation found only on nuclear or cytoplasmic proteins, regulates core transcription factors of stemness and phosphorylation of downstream signal components. Therefore, various kinds of glycans regulate the stem cell status; the structures of many of which are evolutionarily conserved from Drosophila to mammals. Understanding the molecular mechanisms of glycans in stemness and differentiation is increasingly important for innovative clinical applications, as well as for basic research. This Review focuses on the roles of glycans

in Drosophila tissue stem cells and mammalian pluripotent stem cells.

Nishiyama, Y., et al. (2016). "Safe and efficient method for cryopreservation of human induced pluripotent stem cell-derived neural stem and progenitor cells by a programmed freezer with a magnetic field." Neurosci Res **107**: 20-29.

Stem cells represent a potential cellular resource in the development of regenerative medicine approaches to the treatment of pathologies in which specific cells are degenerated or damaged by genetic abnormality, disease, or injury. Securing sufficient supplies of cells suited to the demands of cell transplantation, however, remains challenging, and the establishment of safe and efficient cell banking procedures is an important goal. Cryopreservation allows the storage of stem cells for prolonged time periods while maintaining them in adequate condition for use in clinical settings. Conventional cryopreservation systems include slow-freezing and vitrification both have advantages and disadvantages in terms of cell viability and/or scalability. In the present study, we developed an advanced slow-freezing technique using a programmed freezer with a magnetic field called Cells Alive System (CAS) and examined its effectiveness on human induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs). This system significantly increased cell viability after thawing and had less impact on cellular proliferation and differentiation. We further found that frozen-thawed hiPSC-NS/PCs were comparable with non-frozen ones at the transcriptome level. Given these findings, we suggest that the CAS is useful for hiPSC-NS/PCs banking for clinical uses involving neural disorders and may open new avenues for future regenerative medicine.

Nissenbaum, J., et al. (2013). "Global indiscriminate methylation in cell-specific gene promoters following reprogramming into human induced pluripotent stem cells." Stem Cell Reports **1**(6): 509-517.

Molecular reprogramming of somatic cells into human induced pluripotent stem cells (iPSCs) is accompanied by extensive changes in gene expression patterns and epigenetic marks. To better understand the link between gene expression and DNA methylation, we have profiled human somatic cells from different embryonic cell types (endoderm, mesoderm, and parthenogenetic germ cells) and the iPSCs generated from them. We show that reprogramming is accompanied by extensive DNA methylation in CpG-poor promoters, sparing CpG-rich promoters. Intriguingly, methylation in CpG-poor promoters occurred not only in downregulated genes, but also in genes that are not expressed in the parental somatic cells or their respective iPSCs. These genes are predominantly tissue-specific genes of other cell types from different lineages. Our results suggest a role of DNA methylation in the silencing of the somatic cell identity by global nonspecific methylation of tissue-specific genes from all lineages, regardless of their expression in the parental somatic cells.

Niu, Z., et al. (2013). "Germ-like cell differentiation from induced pluripotent stem cells (iPSCs)." Cell Biochem Funct **31**(1): 12-19.

Historically, our understanding of molecular genetic aspects of germ cell development has been limited. Recently, results demonstrated that the derivation of pluripotent stem cells may provide the necessary genetic system to study germ cell development. Here, we characterized an induced pluripotent stem cell (iPSC) line, which can spontaneously differentiate into embryonic bodies (EBs) after 3 days of suspension culture, expressing specific markers of three germ layers. Then, we induced the iPSCs to differentiate into germ cells by culturing adherent EBs in retinoic acid (RA) and porcine follicular fluid (PFF) differentiation medium or seminiferous tubule transplantation. Our results indicated that RA and PFF were beneficial for the derivation of germ cells and oocyte-like cells from iPSCs, and iPSCs transplantation could make a contribution to repairing the testis of infertile mice. Our study offers an approach for further study on the development and the differentiation of germ cells derived from iPSCs.

No, J. G., et al. (2015). "Cell-free extract from porcine induced pluripotent stem cells can affect porcine somatic cell nuclear reprogramming." J Reprod Dev **61**(2): 90-98.

Pretreatment of somatic cells with undifferentiated cell extracts, such as embryonic stem cells and mammalian oocytes, is an attractive alternative method for reprogramming control. The properties of induced pluripotent stem cells (iPSCs) are similar to those of embryonic stem cells; however, no studies have reported somatic cell nuclear reprogramming using iPSC extracts. Therefore, this study aimed to evaluate the effects of porcine iPSC extracts treatment on porcine ear fibroblasts and early development of porcine cloned embryos produced from porcine ear skin fibroblasts pretreated with the porcine iPSC extracts. The Chariot(TM) reagent system was used to deliver the iPSC extracts into cultured porcine ear skin fibroblasts. The iPSC extracts-treated cells (iPSC-treated cells) were cultured for 3 days and used for analyzing histone modification and somatic cell nuclear transfer. Compared to the results for nontreated cells, the trimethylation status of histone H3 lysine

residue 9 (H3K9) in the iPSC-treated cells significantly decreased. The expression of Jmjd2b, the H3K9 trimethylation-specific demethylase gene, significantly increased in the iPSC-treated cells; conversely, the expression of the proapoptotic genes, Bax and p53, significantly decreased. When the iPSC-treated cells were transferred into enucleated porcine oocytes, no differences were observed in blastocyst development and total cell number in blastocysts compared with the results for control cells. However, H3K9 trimethylation of pronuclear-stage-cloned embryos significantly decreased in the iPSC-treated cells. Additionally, Bax and p53 gene expression in the blastocysts was significantly lower in iPSC-treated cells than in control cells. To our knowledge, this study is the first to show that an extracts of porcine iPSCs can affect histone modification and gene expression in porcine ear skin fibroblasts and cloned embryos.

Nomura, Y., et al. (2012). "Human periodontal ligament fibroblasts are the optimal cell source for induced pluripotent stem cells." Histochem Cell Biol **137**(6): 719-732.

Among the various kinds of fibroblasts existing in the human body, the periodontal ligament (PDL) fibroblasts have been suggested as multipotent cells. Periodontal ligament fibroblasts are characterized by rapid turnover, a high remodeling capacity and remarkable capacity for renewal and repair. They also differentiate into osteoblasts and cementoblasts. We established iPS cells from human PDL fibroblasts by introducing the ES cell markers Oct3/4, Sox2, Nanog, Klf4 and Lin28 by retrovirus transduction, even without the oncogene c-Myc. The iPS cells established in this study expressed the ES cell markers and formed teratomas in SCID mice. The c-Myc expression level in the PDL fibroblasts was higher than that in the iPS cells by quantitative RT-PCR. Therefore, we have concluded that PDL fibroblasts could be an optimal cell source for iPS cells.

Nong, K., et al. (2016). "Hepatoprotective effect of exosomes from human-induced pluripotent stem cell-derived mesenchymal stromal cells against hepatic ischemia-reperfusion injury in rats." Cytotherapy **18**(12): 1548-1559.

BACKGROUND: This study aimed to evaluate the effect of exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) on hepatic ischemia-reperfusion (I/R) injury. METHODS: Exosomes were isolated and concentrated from conditioned medium using ultracentrifugation and ultrafiltration. hiPSC-MSCs-Exo were injected systemically via the inferior vena cava in a rat model of 70% warm hepatic I/R injury, and the therapeutic effect was evaluated. The serum levels of transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were measured using an automatic analyzer. The expression of inflammatory factors was measured using enzyme-linked immunosorbent assay (ELISA). Histological changes indicated changes in pathology and inflammatory infiltration in liver tissue. Apoptosis of hepatic cells in liver tissue was measured using terminal-deoxynucleoitidyl transferase mediated nick end labeling (TUNEL) staining along with apoptotic markers. RESULTS: hiPSCs were efficiently induced into hiPSC-MSCs with typical MSC characteristics. hiPSC-MSCs-Exo had diameters ranging from 50 to 60 nm and expressed exosomal markers (CD9, CD63 and CD81). Hepatocyte necrosis and sinusoidal congestion were markedly suppressed with a lower Suzuki score after hiPSC-MSCs-Exo administration. The levels of the hepatocyte injury markers AST and ALT were significantly lower in the treated group than in the control group. Inflammatory markers, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and high mobility group box 1 (HMGB1), were significantly reduced after administration of hiPSC-MSCs-Exo, which suggests that the exosomes have a role in suppressing the inflammatory response. Additionally, in liver tissues from the experimental group, the levels of apoptotic markers, such as caspase-3 and bax, were significantly lower and the levels of oxidative markers, such as glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), were significantly higher than in the control group. These data point to an anti-apoptotic, anti-oxidative stress response role for hiPSC-MSCs-Exo. CONCLUSIONS: Our results demonstrated that hiPSC-MSCs-Exo alleviate hepatic I/R injury, possibly via suppression of inflammatory responses, attenuation of the oxidative stress response and inhibition of apoptosis.

Noormohammadi, A., et al. (2018). "Mechanisms of protein homeostasis (proteostasis) maintain stem cell identity in mammalian pluripotent stem cells." Cell Mol Life Sci **75**(2): 275-290.

Protein homeostasis, or proteostasis, is essential for cell function, development, and organismal viability. The composition of the proteome is adjusted to the specific requirements of a particular cell type and status. Moreover, multiple metabolic and environmental conditions challenge the integrity of the proteome. To maintain the quality of the proteome, the proteostasis network monitors proteins from their synthesis through their degradation. Whereas somatic stem cells lose their ability to maintain proteostasis with age, immortal pluripotent stem cells exhibit a stringent proteostasis network associated with their biological function and intrinsic characteristics.

Moreover, growing evidence indicates that enhanced proteostasis mechanisms play a central role in immortality and cell fate decisions of pluripotent stem cells. Here, we will review new insights into the melding fields of proteostasis and pluripotency and their implications for the understanding of organismal development and survival.

Normile, D. (2009). "Stem cell research. Recipe for induced pluripotent stem cells just got clearer." Science **325**(5942): 803.

Noto, F. K., et al. (2014). "Aneuploidy is permissive for hepatocyte-like cell differentiation from human induced pluripotent stem cells." BMC Res Notes **7**: 437.

BACKGROUND: The characterization of induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) routinely includes analyses of chromosomal integrity. The belief is that pluripotent stem cells best suited to the generation of differentiated derivatives should display a euploid karyotype; although, this does not appear to have been formally tested. While aneuploidy is commonly associated with cell transformation, several types of somatic cells, including hepatocytes, are frequently aneuploid and variation in chromosomal content does not contribute to a transformed phenotype. This insight has led to the proposal that dynamic changes in the chromosomal environment may be important to establish genetic diversity within the hepatocyte population and such diversity may facilitate an adaptive response by the liver to various insults. Such a positive contribution of aneuploidy to liver function raises the possibility that, in contrast to existing dogma, aneuploid iPSCs may be capable of generating hepatocyte-like cells that display hepatic activities. RESULTS: We examined whether a human iPSC line that had multiple chromosomal aberrations was competent to differentiate into hepatocytes and found that loss of normal chromosomal content had little impact on the production of hepatocyte-like cells from iPSCs. CONCLUSIONS: iPSCs that harbor an abnormal chromosomal content retain the capacity to generate hepatocyte-like cells with high efficiency.

Nsair, A., et al. (2012). "Characterization and therapeutic potential of induced pluripotent stem cell-derived cardiovascular progenitor cells." PLoS One **7**(10): e45603.

BACKGROUND: Cardiovascular progenitor cells (CPCs) have been identified within the developing mouse heart and differentiating pluripotent stem cells by intracellular transcription factors Nkx2.5 and Islet 1 (Isl1). Study of endogenous and induced pluripotent stem cell (iPSC)-derived CPCs has been limited due to the lack of specific cell surface markers to isolate them and conditions for their in vitro expansion that maintain their multipotency. METHODOLOGY/PRINCIPAL FINDINGS: We sought to identify specific cell surface markers that label endogenous embryonic CPCs and validated these markers in iPSC-derived Isl1(+)/Nkx2.5(+) CPCs. We developed conditions that allow propagation and characterization of endogenous and iPSC-derived Isl1(+)/Nkx2.5(+) CPCs and protocols for their clonal expansion in vitro and transplantation in vivo. Transcriptome analysis of CPCs from differentiating mouse embryonic stem cells identified a panel of surface markers. Comparison of these markers as well as previously described surface markers revealed the combination of Flt1(+)/Flt4(+) best identified and facilitated enrichment for Isl1(+)/Nkx2.5(+) CPCs from embryonic hearts and differentiating iPSCs. Endogenous mouse and iPSC-derived Flt1(+)/Flt4(+) CPCs differentiated into all three cardiovascular lineages in vitro. Flt1(+)/Flt4(+) CPCs transplanted into left ventricles demonstrated robust engraftment and differentiation into mature cardiomyocytes (CMs). CONCLUSION/SIGNIFICANCE: The cell surface marker combination of Flt1 and Flt4 specifically identify and enrich for an endogenous and iPSC-derived Isl1(+)/Nkx2.5(+) CPC with trilineage cardiovascular potential in vitro and robust ability for engraftment and differentiation into morphologically and electrophysiologically mature adult CMs in vivo post transplantation into adult hearts.

Nunez-Toldra, R., et al. (2017). "Dental pulp pluripotent-like stem cells (DPPSC), a new stem cell population with chromosomal stability and osteogenic capacity for biomaterials evaluation." BMC Cell Biol **18**(1): 21.

BACKGROUND: Biomaterials are widely used to regenerate or substitute bone tissue. In order to evaluate their potential use for clinical applications, these need to be tested and evaluated in vitro with cell culture models. Frequently, immortalized osteoblastic cell lines are used in these studies. However, their uncontrolled proliferation rate, phenotypic changes or aberrations in mitotic processes limits their use in long-term investigations. Recently, we described a new pluripotent-like subpopulation of dental pulp stem cells derived from the third molars (DPPSC) that shows genetic stability and shares some pluripotent characteristics with embryonic stem cells. In this study we aim to describe the use of DPPSC to test biomaterials, since we believe that the biomaterial cues will be more critical in order to enhance the differentiation of pluripotent stem cells. METHODS: The capacity of DPPSC to differentiate into osteogenic lineage was compared with human sarcoma osteogenic cell line (SAOS-2). Collagen and titanium were used to

assess the cell behavior in commonly used biomaterials. The analyses were performed by flow cytometry, alkaline phosphatase and mineralization stains, RT-PCR, immunohistochemistry, scanning electron microscopy, Western blot and enzymatic activity. Moreover, the genetic stability was evaluated and compared before and after differentiation by short-comparative genomic hybridization (sCGH). RESULTS: DPPSC showed excellent differentiation into osteogenic lineages expressing bone-related markers similar to SAOS-2. When cells were cultured on biomaterials, DPPSC showed higher initial adhesion levels. Nevertheless, their osteogenic differentiation showed similar trend among both cell types. Interestingly, only DPPSC maintained a normal chromosomal dosage before and after differentiation on 2D monolayer and on biomaterials. CONCLUSIONS: Taken together, these results promote the use of DPPSC as a new pluripotent-like cell model to evaluate the biocompatibility and the differentiation capacity of biomaterials used in bone regeneration.

Nurnberg, E., et al. (2018). "Basal glucocorticoid receptor activation induces proliferation and inhibits neuronal differentiation of human induced pluripotent stem cell-derived neuronal precursor cells." J Steroid Biochem Mol Biol **182**: 119-126.

Glucocorticoids (GC) have first been shown to originate from the adrenal glands where synthesis and release is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. Recently, it was shown that GC and other steroid hormones are also synthesized in the central nervous system, so-called neurosteroids. GC bind to specific GC receptors (GR) which function as ligand-activated transcription factors. GR are expressed in nearly all cell types in the brain, and therefore GC have a strong impact on neuronal development. Most knowledge of the influence of GC on neurodevelopment has been obtained from animal research. Recent advances in stem cell technology made it possible to generate neuronal precursor cells (NPCs) and neurons from human induced pluripotent stem cells (hiPSCs). To explore the cellular mechanism of GC affecting human neuronal development, we quantified the proliferation and differentiation of hiPSCs-derived NPCs in the absence and presence of the selective high-affinity GR agonist dexamethasone and the selective GR antagonist mifepristone, respectively. Our results show that inhibition of GR significantly reduced proliferation of NPCs and promoted differentiation whereas GR activation suppressed neuronal differentiation. This implies that neuronal GC must be present in NPCs for proliferation. Consequently we identified the presence of 11-beta-hydroxylase CYP11B1, which hydroxylates the respective steroid precursors to bioactive GC, in NPCs. We propose that hiPSC technology offers an ideal system to get more insight into the synthesising and regulatory pathways in steroidogenesis in human neurons and to differentiate between the mechanism by which adrenal GC and neuronal GC impact on neurodevelopment.

O'Brien, C. M., et al. (2017). "New Monoclonal Antibodies to Defined Cell Surface Proteins on Human Pluripotent Stem Cells." Stem Cells **35**(3): 626-640.

The study and application of human pluripotent stem cells (hPSCs) will be enhanced by the availability of well-characterized monoclonal antibodies (mAbs) detecting cell-surface epitopes. Here, we report generation of seven new mAbs that detect cell surface proteins present on live and fixed human ES cells (hESCs) and human iPS cells (hiPSCs), confirming our previous prediction that these proteins were present on the cell surface of hPSCs. The mAbs all show a high correlation with POU5F1 (OCT4) expression and other hPSC surface markers (TRA-160 and SSEA-4) in hPSC cultures and detect rare OCT4 positive cells in differentiated cell cultures. These mAbs are immunoreactive to cell surface protein epitopes on both primed and naive state hPSCs, providing useful research tools to investigate the cellular mechanisms underlying human pluripotency and states of cellular reprogramming. In addition, we report that subsets of the seven new mAbs are also immunoreactive to human bone marrow-derived mesenchymal stem cells (MSCs), normal human breast subsets and both normal and tumorigenic colorectal cell populations. The mAbs reported here should accelerate the investigation of the nature of pluripotency, and enable development of robust cell separation and tracing technologies to enrich or deplete for hPSCs and other human stem and somatic cell types. Stem Cells 2017;35:626-640.

Ohgushi, M., et al. (2010). "Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells." Cell Stem Cell **7**(2): 225-239.

Human embryonic stem cells (hESCs), unlike mouse ones (mESCs), are vulnerable to apoptosis upon dissociation. Here, we show that the apoptosis, which is of a nonanoikis type, is caused by ROCK-dependent hyperactivation of actomyosin and efficiently suppressed by the myosin inhibitor Blebbistatin. The actomyosin hyperactivation is triggered by the loss of E-cadherin-dependent intercellular contact and also observed in dissociated mouse epiblast-derived pluripotent cells but not in mESCs. We reveal that Abr, a unique Rho-GEF family factor containing a functional Rac-GAP domain, is an indispensable upstream regulator of the apoptosis and ROCK/myosin

hyperactivation. Rho activation coupled with Rac inhibition is induced in hESCs upon dissociation, but not in Abr-depleted hESCs or mESCs. Furthermore, artificial Rho or ROCK activation with Rac inhibition restores the vulnerability of Abr-depleted hESCs to dissociation-induced apoptosis. Thus, the Abr-dependent "Rho-high/Rac-low" state plays a decisive role in initiating the dissociation-induced actomyosin hyperactivation and apoptosis in hESCs.

Ohgushi, M. and Y. Sasai (2011). "Lonely death dance of human pluripotent stem cells: ROCKing between metastable cell states." Trends Cell Biol **21**(5): 274-282.

Two kinds of human pluripotent cells, human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), promise new avenues for medical innovation. These human cells share many similarities with mouse counterparts, including pluripotency, and they exhibit several unique properties. This review examines the diversity of mammalian pluripotent cells from a perspective of metastable pluripotency states. An intriguing phenomenon unique to human pluripotent stem cells is dissociation-induced apoptosis, which has been a technical problem for various cellular manipulations. The discovery that this apoptosis is suppressed by ROCK inhibitors brought revolutionary change to this troublesome situation. We discuss possible links of the metastable pluripotent state to ROCK-dependent human embryonic stem cell apoptosis and summarize recent progress in molecular understandings of this phenomenon.

Ohnishi, H., et al. (2015). "Limited hair cell induction from human induced pluripotent stem cells using a simple stepwise method." Neurosci Lett **599**: 49-54.

Disease-specific induced pluripotent stem cells (iPS) cells are expected to contribute to exploring useful tools for studying the pathophysiology of inner ear diseases and to drug discovery for treating inner ear diseases. For this purpose, stable induction methods for the differentiation of human iPS cells into inner ear hair cells are required. In the present study, we examined the efficacy of a simple induction method for inducing the differentiation of human iPS cells into hair cells. The induction of inner ear hair cell-like cells was performed using a stepwise method mimicking inner ear development. Human iPS cells were sequentially transformed into the preplacodal ectoderm, otic placode, and hair cell-like cells. As a first step, preplacodal ectoderm induction, human iPS cells were seeded on a Matrigel-coated plate and cultured in a serum free N2/B27 medium for 8 days according to a previous study that demonstrated spontaneous differentiation of human ES cells into the preplacodal ectoderm. As the second step, the cells after preplacodal ectoderm induction were treated with basic fibroblast growth factor (bFGF) for induction of differentiation into otic-placode-like cells for 15 days. As the final step, cultured cells were incubated in a serum free medium containing Matrigel for 48 days. After preplacodal ectoderm induction, over 90% of cultured cells expressed the genes that express in preplacodal ectoderm. By culture with bFGF, otic placode marker-positive cells were obtained, although their number was limited. Further 48-day culture in serum free media resulted in the induction of hair cell-like cells, which expressed a hair cell marker and had stereocilia bundle-like constructions on their apical surface. Our results indicate that hair cell-like cells are induced from human iPS cells using a simple stepwise method with only bFGF, without the use of xenogeneic cells.

Ohtani-Kaneko, R., et al. (2017). "Characterisation of human induced pluripotent stem cell-derived endothelial cells under shear stress using an easy-to-use microfluidic cell culture system." Biomed Microdevices **19**(4): 91.

Induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) can contribute to elucidating the pathogenesis of heart and vascular diseases and developing their treatments. Their precise characteristics in fluid flow however remain unclear. Therefore, the aim of the present study is to characterise these features. We cultured three types of ECs in a microfluidic culture system: commercially available human iPS-ECs, human umbilical vein endothelial cells (HUVECs) and human umbilical artery endothelial cells (HUAECs). We then examined the mRNA expression levels of endothelial marker gene cluster of differentiation 31 (CD31), fit-related receptor tyrosine kinase (Flk-1), and the smooth muscle marker gene smooth muscle alpha-actin, and investigated changes in plasminogen activator inhibitor-1 (PAI-1) secretion and intracellular F-actin arrangement following heat stress. We also compared expressions of the arterial and venous marker genes ephrinB2 and EphB4, and the endothelial gap junction genes connexin (Cx) 37, 40, and 43 under fluidic shear stress to determine their arterial or venous characteristics. We found that iPS-ECs had similar endothelial marker gene expressions and exhibited similar increases in PAI-1 secretion under heat stress as HUVECs and HUAECs. In addition, F-actin arrangement in iPSC-ECs also responded to heat stress, as previously reported. However, they had different expression patterns of arterial and venous marker genes and Cx genes under different fluidic shear stress levels, showing that iPSC-ECs exhibit different characteristics from arterial and venous ECs. This microfluidic culture system equipped with variable shear stress control will provide an easy-to-use assay tool to examine

characteristics of iPS-ECs generated by different protocols in various laboratories and contribute to basic and applied biomedical researches on iPS-ECs.

Okada, A., et al. (2016). "Selective Differentiation into Hematopoietic and Cardiac Cells from Pluripotent Stem Cells Based on the Expression of Cell Surface Markers." Methods Mol Biol **1341**: 181-195.

Flk1-expressing (+) mesodermal cells are useful source for the generation of hematopoietic cells and cardiomyocytes from pluripotent stem cells (PSCs). However, they have been reported as a heterogenous population that includes hematopoietic and cardiac progenitors. Therefore, to provide a method for a highly efficient production of hematopoietic cells and cardiomyocytes, cell surface markers are often used for separating these progenitors in Flk1(+) cells. Our recent study has shown that the expression of coxsackievirus and adenovirus receptor (CAR), a tight junction component molecule, could divide mouse and human PSC- and mouse embryo-derived Flk1(+) cells into Flk1(+)CAR(-) and Flk1(+)CAR(+) cells. Flk1(+)CAR(-) and Flk1(+)CAR(+) cells efficiently differentiated into hematopoietic cells and cardiomyocytes, respectively. These results indicate that CAR is a novel cell surface marker for separating PSC-derived Flk1(+) mesodermal cells into hematopoietic and cardiac progenitors. We herein describe a differentiation method from PSCs into hematopoietic cells and cardiomyocytes based on CAR expression.

Okano, H., et al. (2013). "Steps toward safe cell therapy using induced pluripotent stem cells." Circ Res **112**(3): 523-533.

The enthusiasm for producing patient-specific human embryonic stem cells using somatic nuclear transfer has somewhat abated in recent years because of ethical, technical, and political concerns. However, the interest in generating induced pluripotent stem cells (iPSCs), in which pluripotency can be obtained by transcription factor transduction of various somatic cells, has rapidly increased. Human iPSCs are anticipated to open enormous opportunities in the biomedical sciences in terms of cell therapies for regenerative medicine and stem cell modeling of human disease. On the other hand, recent reports have emphasized the pitfalls of iPSC technology, including the potential for genetic and epigenetic abnormalities, tumorigenicity, and immunogenicity of transplanted cells. These constitute serious safety-related concerns for iPSC-based cell therapy. However, preclinical data supporting the safety and efficacy of iPSCs are also accumulating. In this Review, recent achievements and future tasks for safe iPSC-based cell therapy are summarized, using regenerative medicine for repair strategies in the damaged central nervous system (CNS) as a model. Insights on safety and preclinical use of iPSCs in cardiovascular repair model are also discussed.

Okeyo, K. O., et al. (2015). "Cell Adhesion Minimization by a Novel Mesh Culture Method Mechanically Directs Trophoblast Differentiation and Self-Assembly Organization of Human Pluripotent Stem Cells." Tissue Eng Part C Methods **21**(10): 1105-1115.

Mechanical methods for inducing differentiation and directing lineage specification will be instrumental in the application of pluripotent stem cells. Here, we demonstrate that minimization of cell-substrate adhesion can initiate and direct the differentiation of human pluripotent stem cells (hiPSCs) into cyst-forming trophoblast lineage cells (TLCs) without stimulation with cytokines or small molecules. To precisely control cell-substrate adhesion area, we developed a novel culture method where cells are cultured on microstructured mesh sheets suspended in a culture medium such that cells on mesh are completely out of contact with the culture dish. We used microfabricated mesh sheets that consisted of open meshes (100 approximately 200 mum in pitch) with narrow mesh strands (3-5 mum in width) to provide support for initial cell attachment and growth. We demonstrate that minimization of cell adhesion area achieved by this culture method can trigger a sequence of morphogenetic transformations that begin with individual hiPSCs attached on the mesh strands proliferating to form cell sheets by self-assembly organization and ultimately differentiating after 10-15 days of mesh culture to generate spherical cysts that secreted human chorionic gonadotropin (hCG) hormone and expressed caudal-related homeobox 2 factor (CDX2), a specific marker of trophoblast lineage. Thus, this study demonstrates a simple and direct mechanical approach to induce trophoblast differentiation and generate cysts for application in the study of early human embryogenesis and drug development and screening.

Onuma, Y., et al. (2013). "rBC2LCN, a new probe for live cell imaging of human pluripotent stem cells." Biochem Biophys Res Commun **431**(3): 524-529.

Cell surface biomarkers have been applied to discriminate pluripotent human embryonic stem cells and induced pluripotent stem cells from differentiated cells. Here, we demonstrate that a recombinant lectin probe, rBC2LCN, a new tool for fluorescence-based imaging and flow cytometry analysis of pluripotent stem cells, is an alternative to conventional pluripotent maker antibodies. Live or fixed colonies of both human embryonic stem cells and induced pluripotent stem cells were visualized in culture medium containing

fluorescent dye-labeled rBC2LCN. Fluorescent dye-labeled rBC2LCN was also successfully used to separate live pluripotent stem cells from a mixed cell population by flow cytometry.

Oshima, K., et al. (2010). "Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells." Cell **141**(4): 704-716.

Mechanosensitive sensory hair cells are the linchpin of our senses of hearing and balance. The inability of the mammalian inner ear to regenerate lost hair cells is the major reason for the permanence of hearing loss and certain balance disorders. Here, we present a stepwise guidance protocol starting with mouse embryonic stem and induced pluripotent stem cells, which were directed toward becoming ectoderm capable of responding to otic-inducing growth factors. The resulting otic progenitor cells were subjected to varying differentiation conditions, one of which promoted the organization of the cells into epithelial clusters displaying hair cell-like cells with stereociliary bundles. Bundle-bearing cells in these clusters responded to mechanical stimulation with currents that were reminiscent of immature hair cell transduction currents.

Otsu, M., et al. (2014). "Pluripotent stem cell-derived neural stem cells: From basic research to applications." World J Stem Cells **6**(5): 651-657.

Basic research on pluripotent stem cells is designed to enhance understanding of embryogenesis, whereas applied research is designed to develop novel therapies and prevent diseases. Attainment of these goals has been enhanced by the establishment of embryonic stem cell lines, the technological development of genomic reprogramming to generate induced-pluripotent stem cells, and improvements in vitro techniques to manipulate stem cells. This review summarizes the techniques required to generate neural cells from pluripotent stem cells. In particular, this review describes current research applications of a simple neural differentiation method, the neural stem sphere method, which we developed.

Ouchi, T., et al. (2016). "LNGFR(+)THY-1(+) human pluripotent stem cell-derived neural crest-like cells have the potential to develop into mesenchymal stem cells." Differentiation **92**(5): 270-280.

Mesenchymal stem cells (MSCs) are defined as non-hematopoietic, plastic-adherent, self-renewing cells that are capable of tri-lineage differentiation into bone, cartilage or fat in vitro. Thus, MSCs are promising candidates for cell-based medicine. However, classifications of MSCs have been defined retrospectively; moreover, this conventional criterion may be inaccurate due to contamination with other hematopoietic lineage cells. Human MSCs can be enriched by selection for LNGFR and THY-1, and this population may be analogous to murine PDGFRalpha(+)Sca-1(+) cells, which are developmentally derived from neural crest cells (NCCs). Murine NCCs were labeled by fluorescence, which provided definitive proof of neural crest lineage, however, technical considerations prevent the use of a similar approach to determine the origin of human LNGFR(+)THY-1(+) MSCs. To further clarify the origin of human MSCs, human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) were used in this study. Under culture conditions required for the induction of neural crest cells, human ESCs and iPSCs-derived cells highly expressed LNGFR and THY-1. These LNGFR(+)THY-1(+) neural crest-like cells, designated as LT-NCLCs, showed a strong potential to differentiate into both mesenchymal and neural crest lineages. LT-NCLCs proliferated to form colonies and actively migrated in response to serum concentration. Furthermore, we transplanted LT-NCLCs into chick embryos, and traced their potential for survival, migration and differentiation in the host environment. These results suggest that LNGFR(+)THY-1(+) cells identified following NCLC induction from ESCs/iPSCs shared similar potentials with multipotent MSCs.

Ould-Brahim, F., et al. (2018). "Metformin Preconditioning of Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells Promotes Their Engraftment and Improves Post-Stroke Regeneration and Recovery." Stem Cells Dev **27**(16): 1085-1096.

While transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) shows therapeutic potential in animal stroke models, major concerns for translating hiPSC therapy to the clinic are efficacy and safety. Therefore, there is a demand to develop an optimal strategy to enhance the engraftment and regenerative capacity of transplanted hiPSC-NSCs to produce fully differentiated neural cells to replace lost brain tissues. Metformin, an FDA-approved drug, is an optimal neuroregenerative agent that not only promotes NSC proliferation but also drives NSCs toward differentiation. In this regard, we hypothesize that preconditioning of hiPSC-NSCs with metformin before transplantation into the stroke-damaged brain will improve engraftment and regenerative capabilities of hiPSC-NSCs, ultimately enhancing functional recovery. In this study, we show that pretreatment of hiPSC-NSCs with metformin enhances the proliferation and differentiation of hiPSC-NSCs in culture. Furthermore, metformin-preconditioned hiPSC-NSCs show increased engraftment 1 week post-transplantation in a rat endothelin-1 focal ischemic stroke model. In addition,

metformin-preconditioned cell grafts exhibit increased survival compared to naive cell grafts at 7 weeks post-transplantation. Analysis of the grafts demonstrates that metformin preconditioning enhances the differentiation of hiPSC-NSCs at the expense of their proliferation. As an outcome, rats receiving metformin-preconditioned cells display accelerated gross motor recovery and reduced infarct volume. These studies represent a vital step forward in the optimization of hiPSC-NSC-based transplantation to promote post-stroke recovery.

Ovchinnikov, D. A., et al. (2014). "Transgenic human ES and iPS reporter cell lines for identification and selection of pluripotent stem cells in vitro." Stem Cell Res **13**(2): 251-261.

Optimization of pluripotent stem cell expansion and differentiation is facilitated by biological tools that permit non-invasive and dynamic monitoring of pluripotency, and the ability to select for an undifferentiated input cell population. Here we report on the generation and characterisation of clonal human embryonic stem (HES3, H9) and human induced pluripotent stem cell lines (UQEW01i-epifibC11) that have been stably modified with an artificial EOS(C3+) promoter driving expression of EGFP and puromycin resistance-conferring proteins. We show that EGFP expression faithfully reports on the pluripotency status of the cells in these lines and that antibiotic selection allows for an efficient elimination of differentiated cells from the cultures. We demonstrate that the extinction of the expression of the pluripotency reporter during differentiation closely correlates with the decrease in expression of conventional pluripotency markers, such as OCT4 (POU5F1), TRA-1-60 and SSEA4 when screening across conditions with various levels of pluripotency-maintaining or differentiation-inducing signals. We further illustrate the utility of these lines for real-time monitoring of pluripotency in embryoid bodies and microfluidic bioreactors.

Ozawa, M., et al. (2014). "Development of FGF2-dependent pluripotent stem cells showing naive state characteristics from murine preimplantation inner cell mass." Stem Cell Res **13**(1): 75-87.

Two distinct types of embryonic pluripotent stem cells can be established from either the inner cell mass (ICM) of preimplantation blastocyst (leukemia inhibitory factor (LIF)-dependent embryonic stem cell, ESC, called naive state) or the epiblast of postimplantation fetuses (fibroblast growth factor 2 (FGF2)-dependent epiblast stem cells, EpiSC, called primed state). Here, we report that naive pluripotent stem cell was established from the ICM, but maintained its self-renewal by treatment with FGF2 and mouse embryonic fibroblasts (MEFs) when they were exposed FGF2 during establishment. This cell line is competent to contribute to chimeric animals, including germ cells, at high efficiency. The ERK1/2, SMAD2/3, and JAK/STAT3 pathways are essential to maintain self-renewal. Inhibition of ERK1/2 or SMAD2/3 initiates transition to a naive state ESC-like state, whereas inhibition of JAK/STAT3 promotes a primed EpiSC-like character. Our present results could provide novel insights into understanding the growth factor environment and ICM plasticity, and mechanisms which orchestrate the pluripotency of embryonic stem cells and the capacity for chimeric contributions.

Ozeki, N., et al. (2017). "Gelatin scaffold combined with bone morphogenetic protein-4 induces odontoblast-like cell differentiation involving integrin profile changes, autophagy-related gene 10, and Wnt5 sequentially in human induced pluripotent stem cells." Differentiation **93**: 1-14.

While human induced pluripotent stem (hiPS) cells have potential use in regenerative medicine, there are no reports on odontoblastic differentiation of hiPS cells. In the current study, to examine integrin profiles and explore the early signaling cascade of odontoblastic differentiation in hiPS cells, we investigated the regulation of autophagy-related gene (Atg) and wingless/int1 (Wnt) signaling in gelatin scaffold (GS) combined with bone morphogenetic protein (BMP)-4 (GS/BMP-4)-mediated odontoblastic differentiation. Following GS/BMP-4 treatment, there was a dramatic loss of alpha3 and alpha6 integrins, and reciprocal strong induction of alpha1 integrin expression in the differentiated cells. GS/BMP-4 increased the mRNA and protein levels of Atg10, Lrp5/Fzd9 (an Atg10 receptor), and Wnt5 together with the amount of autophagosomes and autophagic fluxes. Treatment with siRNAs against Atg10 and Wnt5a individually suppressed the GS/BMP-4-induced increase in odontoblastic differentiation. The odontoblastic phenotype was inhibited by chloroquine, but increased after treatment with rapamycin (an autophagy enhancer). Taken together with our previous findings, we have replicated our results from the rodent system in a novel human system. We have revealed a unique sequential cascade involving Atg10, Wnt5a, alpha1 integrin, and matrix metalloproteinase-3 in GS/BMP-4-induced differentiation of hiPS cells into odontoblast-like cells at a relatively early stage.

Ozeki, N., et al. (2017). "MicroRNA-211 and autophagy-related gene 14 signaling regulate osteoblast-like cell differentiation of human induced pluripotent stem cells." Exp Cell Res **352**(1): 63-74.

MicroRNAs (miRNAs) have been the subject of recent attention as key regulatory factors in cell

differentiation. In the current study, to explore the early signaling cascade of osteogenic differentiation of human induced pluripotent stem (hiPS) cells, we investigated miR-211 regulation and autophagy-related gene (Atg) signaling in osteogenic differentiation. In addition to reciprocal strong induction of miR-211 expression in differentiated cells following osteogenic differentiation, we found abundant Argonaute 3 bound to miR-211. There were also dramatic increases in the mRNA and protein levels of Atg14 together with increases in the amount of autophagosomes as well as autophagic fluxes. While transfection of a miR-211 inhibitor abrogated the induction of Atg14, autophagy events, osteoblast differentiation markers, and induction of calcification were suppressed markedly. Treatment with small interfering RNAs against Atg14 also suppressed the osteogenic differentiation medium (ODM)-induced increase in osteogenic differentiation. The osteogenic phenotype was inhibited by chloroquine (an autophagy inhibitor), but increased after treatment with rapamycin (an autophagy inducer). Taken together with our previous findings, we have revealed a unique sequential cascade involving miR-211 and Atg14 in ODM-induced differentiation of hiPS cells into osteoblast-like cells at a relatively early stage.

Paik, D. T., et al. (2018). "Large-Scale Single-Cell RNA-Seq Reveals Molecular Signatures of Heterogeneous Populations of Human Induced Pluripotent Stem Cell-Derived Endothelial Cells." Circ Res.

Rationale: Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) have risen as a useful tool in cardiovascular research, offering a wide gamut of translational and clinical applications. However, inefficiency of the currently available iPSC-EC differentiation protocol and underlying heterogeneity of derived iPSC-ECs remain as major limitations of iPSC-EC technology. Objective: Here we performed droplet-based single-cell RNA-sequencing (scRNA-seq) of the human iPSCs following iPSC-EC differentiation. Droplet-based scRNA-seq enables analysis of thousands of cells in parallel, allowing comprehensive analysis of transcriptional heterogeneity. Methods and Results: Bona fide iPSC-EC cluster was identified by scRNA-seq, which expressed high levels of endothelial-specific genes. iPSC-ECs, sorted by CD144 antibody-conjugated magnetic sorting, exhibited standard endothelial morphology and function including tube formation, response to inflammatory signals, and production of nitric oxide. Non-endothelial cell populations resulting from the differentiation protocol were identified, which included immature and atrial-like cardiomyocytes, hepatic-like cells, and vascular smooth muscle cells. Furthermore, scRNA-seq analysis of purified iPSC-ECs revealed transcriptional heterogeneity with four major subpopulations, marked by robust enrichment of CLDN5, APLNR, GJA5, and ESM1 genes respectively. Conclusions: Massively parallel, droplet-based scRNA-seq allowed meticulous analysis of thousands of human iPSCs subjected to iPSC-EC differentiation. Results showed inefficiency of the differentiation technique, which can be improved with further studies based on identification of molecular signatures that inhibit expansion of non-endothelial cell types. Subtypes of bona fide human iPSC-ECs were also identified, allowing us to sort for iPSC-ECs with specific biological function and identity.

Paik, I., et al. (2012). "Rapid micropatterning of cell lines and human pluripotent stem cells on elastomeric membranes." Biotechnol Bioeng **109**(10): 2630-2641.

Tissue function during development and in regenerative medicine completely relies on correct cell organization and patterning at micro and macro scales. We describe a rapid method for patterning mammalian cells including human embryonic stem cells (HESCs) and induced pluripotent stem cells (iPSCs) on elastomeric membranes such that micron-scale control of cell position can be achieved over centimeter-length scales. Our method employs surface engineering of hydrophobic polydimethylsiloxane (PDMS) membranes by plasma polymerization of allylamine. Deposition of plasma polymerized allylamine (ppAAm) using our methods may be spatially restricted using a micro-stencil leaving faithful hydrophilic ppAAm patterns. We employed airbrushing to create aerosols which deposit extracellular matrix (ECM) proteins (such as fibronectin and Matrigel) onto the same patterned ppAAm rich regions. Cell patterns were created with a variety of well characterized cell lines (e.g., NIH-3T3, C2C12, HL1, BJ6, HESC line HUES7, and HiPSC line IPS2). Individual and multiple cell line patterning were also achieved. Patterning remains faithful for several days and cells are viable and proliferate. To demonstrate the utility of our technique we have patterned cells in a variety of configurations. The ability to rapidly pattern cells at high resolution over macro scales should aid future tissue engineering efforts for regenerative medicine applications and in creating in vitro stem cell niches.

Pal, R., et al. (2016). "Editorial: Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells: Ushering of a New Era in Personalized Cell Therapies." Curr Stem Cell Res Ther **11**(2): 97-98.

Pallotta, I., et al. (2017). "BMP protein-mediated crosstalk between inflammatory cells and human pluripotent stem cell-derived cardiomyocytes." J Tissue Eng Regen Med **11**(5): 1466-1478.

Following cardiac injury, the ischaemic heart tissue is characterized by the invasion of pro-inflammatory (M1) and pro-healing (M2) macrophages. Any engineered cardiac tissue will inevitably interact with the inflammatory environment found at the site of myocardial infarction at the time of implantation. However, the interactions between the inflammatory and the cardiac repair cells remain poorly understood. Here we recapitulated in vitro some of the important cellular events found at the site of myocardial injury, such as macrophage recruitment and their effect on cardiac differentiation and maturation, by taking into account the involvement of paracrine-mediated signalling. By using a 3D inverted invasion assay, we found that cardiomyocyte (CM) conditioned medium can trigger the recruitment of pro-inflammatory (M1) macrophages, through a mechanism that involves, in part, CM-derived BMP4. Pro-inflammatory (M1) macrophages were also found to affect CM proliferation and differentiation potential, in part due to BMP molecules secreted by macrophages. These effects involved the activation of the canonical outside-in signalling pathways, such as SMAD1,5,8, which are known to be activated during myocardial injury in vivo. In the present study we propose a new role for CM- and macrophage-derived BMP proteins during the recruitment of macrophage subtypes and the maturation of repair cells, representing an important step towards creating a functional cardiac patch with superior therapeutic properties. Copyright (c) 2015 John Wiley & Sons, Ltd.

Panopoulos, A. D., et al. (2012). "The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming." Cell Res **22**(1): 168-177.

Metabolism is vital to every aspect of cell function, yet the metabolome of induced pluripotent stem cells (iPSCs) remains largely unexplored. Here we report, using an untargeted metabolomics approach, that human iPSCs share a pluripotent metabolomic signature with embryonic stem cells (ESCs) that is distinct from their parental cells, and that is characterized by changes in metabolites involved in cellular respiration. Examination of cellular bioenergetics corroborated with our metabolomic analysis, and demonstrated that somatic cells convert from an oxidative state to a glycolytic state in pluripotency. Interestingly, the bioenergetics of various somatic cells correlated with their reprogramming efficiencies. We further identified metabolites that differ between iPSCs and ESCs, which revealed novel metabolic pathways that play a critical role in regulating somatic cell reprogramming. Our findings are the first to globally analyze the metabolome of iPSCs, and provide mechanistic insight into a new layer of regulation involved in inducing pluripotency, and in evaluating iPSC and ESC equivalence.

Panula, S., et al. (2011). "Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells." Hum Mol Genet **20**(4): 752-762.

Historically, our understanding of molecular genetic aspects of human germ cell development has been limited, at least in part due to inaccessibility of early stages of human development to experimentation. However, the derivation of pluripotent stem cells may provide the necessary human genetic system to study germ cell development. In this study, we compared the potential of human induced pluripotent stem cells (iPSCs), derived from adult and fetal somatic cells to form primordial and meiotic germ cells, relative to human embryonic stem cells. We found that approximately 5% of human iPSCs differentiated to primordial germ cells (PGCs) following induction with bone morphogenetic proteins. Furthermore, we observed that PGCs expressed green fluorescent protein from a germ cell-specific reporter and were enriched for the expression of endogenous germ cell-specific proteins and mRNAs. In response to the overexpression of intrinsic regulators, we also observed that iPSCs formed meiotic cells with extensive synaptonemal complexes and post-meiotic haploid cells with a similar pattern of ACROSIN staining as observed in human spermatids. These results indicate that human iPSCs derived from reprogramming of adult somatic cells can form germline cells. This system may provide a useful model for molecular genetic studies of human germline formation and pathology and a novel platform for clinical studies and potential therapeutical applications.

Papapetrou, E. P. (2017). "Gene and Cell Therapy for beta-Thalassemia and Sickle Cell Disease with Induced Pluripotent Stem Cells (iPSCs): The Next Frontier." Adv Exp Med Biol **1013**: 219-240.

In recent years, breakthroughs in human pluripotent stem cell (hPSC) research, namely cellular reprogramming and the emergence of sophisticated genetic engineering technologies, have opened new frontiers for cell and gene therapy. The prospect of using hPSCs, either autologous or histocompatible, as targets of genetic modification and their differentiated progeny as cell products for transplantation, presents a new paradigm of regenerative medicine of potential tremendous value for the treatment of blood disorders, including beta-thalassemia (BT) and sickle cell disease (SCD). Despite advances at a remarkable pace and great promise, many roadblocks remain before clinical translation can be realistically considered. Here we discuss the theoretical advantages of cell therapies

utilizing hPSC derivatives, recent proof-of-principle studies and the main challenges towards realizing the potential of hPSC therapies in the clinic.

Parameswaran, V., et al. (2007). "Development of a pluripotent ES-like cell line from Asian sea bass (Lates calcarifer)--an oviparous stem cell line mimicking viviparous ES cells." Mar Biotechnol (NY) **9**(6): 766-775.

We report a pluripotent embryonic stem cell-like cell line designated as SBES from blastula stage embryos of Asian sea bass (Lates calcarifer), which is an economically important cultivable and edible marine fish species in India. The SBES cells were cultured at 28 degrees C in Leibovitz L-15 medium supplemented with 20% fetal bovine serum without a feeder layer. The ES-like cells were round or polygonal and grew exponentially in culture. The SBES cells exhibited an intense alkaline phosphatase activity and expression of transcription factor Oct 4. The undifferentiated state of these cells was maintained at low seeding densities and the cells formed embryoid bodies when seeded in bacteriological plates. On treatment with all-trans retinoic acid, these cells differentiated into neuron-like cells, muscle cells, and beating cardiomyocytes, indicating their pluripotency. This embryonic ES-like cell line derived from an oviparous fish blastula conserved several peculiar features of viviparous mammalian embryonic stem cell lines. The present study highlights the importance and potential of piscine ES-like cell line for stem cell research without evoking ethical issues and invasive interventions sparing mammalian embryos.

Park, J., et al. (2018). "DSG2 Is a Functional Cell Surface Marker for Identification and Isolation of Human Pluripotent Stem Cells." Stem Cell Reports **11**(1): 115-127.

Pluripotent stem cells (PSCs) represent the most promising clinical source for regenerative medicine. However, given the cellular heterogeneity within cultivation and safety concerns, the development of specific and efficient tools to isolate a pure population and eliminate all residual undifferentiated PSCs from differentiated derivatives is a prerequisite for clinical applications. In this study, we raised a monoclonal antibody and identified its target antigen as desmoglein-2 (DSG2). DSG2 co-localized with human PSC (hPSC)-specific cell surface markers, and its expression was rapidly downregulated upon differentiation. The depletion of DSG2 markedly decreased hPSC proliferation and pluripotency marker expression. In addition, DSG2-negative population in hPSCs exhibited a notable suppression in embryonic body and teratoma formation. The actions of DSG2 in regulating the self-renewal and pluripotency of hPSCs were predominantly exerted through the regulation of beta-catenin/Slug-mediated epithelial-to-mesenchymal transition. Our results demonstrate that DSG2 is a valuable PSC surface marker that is essential for the maintenance of PSC self-renewal.

Park, J. A., et al. (2012). "High sensitivity of embryonic stem cells to proteasome inhibitors correlates with low expression of heat shock protein and decrease of pluripotent cell marker expression." BMB Rep **45**(5): 299-304.

The ubiquitin-proteasome system is a major proteolytic system for nonlysosomal degradation of cellular proteins. Here, we investigated the response of mouse embryonic stem (ES) cells under proteotoxic stress. Proteasome inhibitors induced expression of heat shock protein 70 (HSP70) in a concentration- and time-dependent manner, and also induced apoptosis of ES cells. Importantly, more apoptotic cells were observed in ES cells compared with other somatic cells. To understand this phenomenon, we further investigated the expression of HSP70 and pluripotent cell markers. HSP70 expression was more significantly increased in somatic cells than in ES cells, and expression levels of pluripotent cell markers such as Oct4 and Nanog were decreased in ES cells. These results suggest that higher sensitivity of ES cells to proteotoxic stress may be related with lower capacity of HSP70 expression and decreased pluripotent cell marker expression, which is essential for the survival of ES cells.

Park, S., et al. (2017). "A Comprehensive, Ethnically Diverse Library of Sickle Cell Disease-Specific Induced Pluripotent Stem Cells." Stem Cell Reports **8**(4): 1076-1085.

Sickle cell anemia affects millions of people worldwide and is an emerging global health burden. As part of a large NIH-funded NextGen Consortium, we generated a diverse, comprehensive, and fully characterized library of sickle-cell-disease-specific induced pluripotent stem cells (iPSCs) from patients of different ethnicities, beta-globin gene (HBB) haplotypes, and fetal hemoglobin (HbF) levels. iPSCs stand to revolutionize the way we study human development, model disease, and perhaps eventually, treat patients. Here, we describe this unique resource for the study of sickle cell disease, including novel haplotype-specific polymorphisms that affect disease severity, as well as for the development of patient-specific therapeutics for this phenotypically diverse disorder. As a complement to this library, and as proof of principle for future cell- and gene-based therapies, we also designed and employed CRISPR/Cas gene editing tools to correct the sickle hemoglobin (HbS) mutation.

Park, S. W., et al. (2014). "Variable allelic expression of imprinted genes in human pluripotent stem cells during differentiation into specialized cell types in vitro." Biochem Biophys Res Commun **446**(2): 493-498.

Genomic imprinting is an epigenetic phenomenon by which a subset of genes is asymmetrically expressed in a parent-of-origin manner. However, little is known regarding the epigenetic behaviors of imprinted genes during human development. Here, we show dynamic epigenetic changes in imprinted genes in hESCs during in vitro differentiation into specialized cell types. Out of 9 imprinted genes with single nucleotide polymorphisms, mono-allelic expression for three imprinted genes (H19, KCNQ1OT1, and IPW), and bi- or partial-allelic expression for three imprinted genes (OSBPL5, PPP1R9A, and RTL1) were stably retained in H9-hESCs throughout differentiation, representing imprinting stability. Three imprinted genes (KCNK9, ATP10A, and SLC22A3) showed a loss and a gain of imprinting in a lineage-specific manner during differentiation. Changes in allelic expression of imprinted genes were observed in another hESC line during in vitro differentiation. These findings indicate that the allelic expression of imprinted genes may be vulnerable in a lineage-specific manner in human pluripotent stem cells during differentiation.

Parmar, V. M., et al. (2018). "A2E-associated cell death and inflammation in retinal pigmented epithelial cells from human induced pluripotent stem cells." Stem Cell Res **27**: 95-104.

Accumulation of lipofuscin in the retinal pigmented epithelium (RPE) is observed in retinal degenerative diseases including Stargardt disease and age-related macular degeneration. Bis-retinoid N-retinyl-N-retinylidene ethanolamine (A2E) is a major component of lipofuscin. A2E has been implicated in RPE atrophy and retinal inflammation; however, mice with A2E accumulation display only a mild retinal phenotype. In the current study, human iPSC-RPE (hiPSC-RPE) cells were generated from healthy individuals to examine effects of A2E in human RPE cells. hiPSC-RPE cells displayed RPE-specific features, which include expression of RPE-specific genes, tight junction formation and ability to carry out phagocytosis. hiPSC-RPE cells demonstrated cell death and increased VEGF-A production in a time-dependent manner when they were cocultured with 10muM of A2E. PCR array analyses revealed upregulation of 26 and 12 pro-inflammatory cytokines upon A2E and H2O2 exposure respectively, indicating that A2E and H2O2 can cause inflammation in human retinas. Notably, identified gene profiles were different between A2E- and H2O2-treated hiPSC-RPE cells. A2E caused inflammatory changes observed in retinal degenerative diseases more closely as compared to H2O2. Collectively, these data obtained with hiPSC-RPE cells provide evidence that A2E plays an important role in pathogenesis of retinal degenerative diseases in humans.

Parsons, X. H. (2013). "Constraining the Pluripotent Fate of Human Embryonic Stem Cells for Tissue Engineering and Cell Therapy - The Turning Point of Cell-Based Regenerative Medicine." Br Biotechnol J **3**(4): 424-457.

To date, the lack of a clinically-suitable source of engraftable human stem/progenitor cells with adequate neurogenic potential has been the major setback in developing safe and effective cell-based therapies for regenerating the damaged or lost CNS structure and circuitry in a wide range of neurological disorders. Similarly, the lack of a clinically-suitable human cardiomyocyte source with adequate myocardium regenerative potential has been the major setback in regenerating the damaged human heart. Given the limited capacity of the CNS and heart for self-repair, there is a large unmet healthcare need to develop stem cell therapies to provide optimal regeneration and reconstruction treatment options to restore normal tissues and function. Derivation of human embryonic stem cells (hESCs) provides a powerful in vitro model system to investigate molecular controls in human embryogenesis as well as an unlimited source to generate the diversity of human somatic cell types for regenerative medicine. However, realizing the developmental and therapeutic potential of hESC derivatives has been hindered by the inefficiency and instability of generating clinically-relevant functional cells from pluripotent cells through conventional uncontrollable and incomplete multi-lineage differentiation. Recent advances and breakthroughs in hESC research have overcome some major obstacles in bringing hESC therapy derivatives towards clinical applications, including establishing defined culture systems for de novo derivation and maintenance of clinical-grade pluripotent hESCs and lineage-specific differentiation of pluripotent hESCs by small molecule induction. Retinoic acid was identified as sufficient to induce the specification of neuroectoderm direct from the pluripotent state of hESCs and trigger a cascade of neuronal lineage-specific progression to human neuronal progenitors and neurons of the developing CNS in high efficiency, purity, and neuronal lineage specificity by promoting nuclear translocation of the neuronal specific transcription factor Nurr-1. Similarly, nicotinamide was rendered sufficient to induce the specification of cardiomesoderm direct from the pluripotent state of hESCs by promoting the expression of the earliest

cardiac-specific transcription factor Csx/Nkx2.5 and triggering progression to cardiac precursors and beating cardiomyocytes with high efficiency. This technology breakthrough enables direct conversion of pluripotent hESCs into a large supply of high purity neuronal cells or heart muscle cells with adequate capacity to regenerate CNS neurons and contractile heart muscles for developing safe and effective stem cell therapies. Transforming pluripotent hESCs into fate-restricted therapy derivatives dramatically increases the clinical efficacy of graft-dependent repair and safety of hESC-derived cellular products. Such milestone advances and medical innovations in hESC research allow generation of a large supply of clinical-grade hESC therapy derivatives targeting for major health problems, bringing cell-based regenerative medicine to a turning point.

Parsons, X. H. (2013). "Human Stem Cell Derivatives Retain More Open Epigenomic Landscape When Derived from Pluripotent Cells than from Tissues." J Regen Med **1**(2).

The growing number of identified stem cell derivatives and escalating concerns for safety and efficacy of these cells towards clinical applications have made it increasingly crucial to be able to assess the relative risk-benefit ratio of a given stem cell from a given source for a particular disease. Discerning the intrinsic plasticity and regenerative potential of human stem cell populations might reside in chromatin modifications that shape the respective epigenomes of their derivation routes. Previously, we have generated engraftable human neuronal progenitors direct from pluripotent human embryonic stem cells (hESCs) by small molecule induction (hESC-I hNuPs). Unlike the prototypical neuroepithelial-like nestin-positive human neural stem cells (hNSCs), these in vitro neuroectoderm-derived Nurr1-positive hESC-I hNuPs are a more neuronal lineage-specific and plastic hESC derivative. In this study, the global chromatin landscape changes in pluripotent hESCs and their neuronal lineage-specific derivative hESC-I hNuPs were profiled using genome-wide mapping and compared to CNS tissue-derived hNSCs. This study found that the broad potential of pluripotent hESCs is defined by an epigenome constituted of open conformation of chromatin mediated by a pattern of Oct-4 global distribution that corresponds closely with those of acetylated nucleosomes genome-wide. The epigenomic transition from pluripotency to restriction in lineage choices is characterized by genome-wide increases in histone H3K9 methylation that mediates global chromatin-silencing and somatic identity. Tissue-resident CNS-derived hNSCs have acquired a substantial number of additional histone H3K9 methylation, therefore, more silenced chromatin. These data suggest that the intrinsic plasticity and regenerative potential of human stem cell derivatives can be differentiated by their epigenomic landscape features, and that human stem cell derivatives retain more open epigenomic landscape, therefore, more developmental potential for scale-up regeneration, when derived from the hESCs in vitro than from the CNS tissue in vivo.

Parsons, X. H. (2016). "Direct Conversion of Pluripotent Human Embryonic Stem Cells Under Defined Culture Conditions into Human Neuronal or Cardiomyocyte Cell Therapy Derivatives." Methods Mol Biol **1307**: 299-318.

Developing novel strategies for well-controlled efficiently directing pluripotent human embryonic stem cells (hESCs) exclusively and uniformly towards clinically relevant cell types in a lineage-specific manner is not only crucial for unveiling the molecular and cellular cues that direct human embryogenesis but also vital to harnessing the power of hESC biology for tissue engineering and cell-based therapies. Conventional hESC differentiation methods require uncontrollable simultaneous multi-lineage differentiation of pluripotent cells, which yield embryoid bodies (EB) or aggregates consisting of a mixed population of cell types of three embryonic germ layers, among which only a very small fraction of cells display targeted differentiation, impractical for commercial and clinical applications. Here, a protocol for lineage-specific differentiation of hESCs, maintained under defined culture systems, direct from the pluripotent stage using small-molecule induction exclusively and uniformly to a neural or a cardiac lineage is described. Lineage-specific differentiation of pluripotent hESCs by small-molecule induction enables well-controlled highly efficient direct conversion of nonfunctional pluripotent hESCs into a large supply of high-purity functional human neuronal or cardiomyocyte cell therapy derivatives for commercial and therapeutic uses.

Pascoal, J. F., et al. (2018). "Three-Dimensional Cell-Based Microarrays: Printing Pluripotent Stem Cells into 3D Microenvironments." Methods Mol Biol **1771**: 69-81.

Cell-based microarrays are valuable platforms for the study of cytotoxicity and cellular microenvironment because they enable high-throughput screening of large sets of conditions at reduced reagent consumption. However, most of the described microarray technologies have been applied to two-dimensional cultures, which do not accurately emulate the in vivo three-dimensional (3D) cell-cell and cell-extracellular matrix interactions.Herein, we describe the methodology for production of alginate-

and Matrigel-based 3-D cell microarrays for the study of mouse and human pluripotent stem cells on two different chip-based platforms. We further provide protocols for on-chip proliferation/viability analysis and the assessment of protein expression by immunofluorescence.

Pellett, S., et al. (2015). "Human Induced Pluripotent Stem Cell Derived Neuronal Cells Cultured on Chemically-Defined Hydrogels for Sensitive In Vitro Detection of Botulinum Neurotoxin." Sci Rep **5**: 14566.

Botulinum neurotoxin (BoNT) detection provides a useful model for validating cell-based neurotoxicity screening approaches, as sensitivity is dependent on functionally competent neurons and clear quantitative endpoints are available for correlating results to approved animal testing protocols. Here, human induced pluripotent stem cell (iPSC)-derived neuronal cells were cultured on chemically-defined poly(ethylene glycol) (PEG) hydrogels formed by "thiol-ene" photopolymerization and tested as a cell-based neurotoxicity assay by determining sensitivity to active BoNT/A1. BoNT/A1 sensitivity was comparable to the approved in vivo mouse bioassay for human iPSC-derived neurons and neural stem cells (iPSC-NSCs) cultured on PEG hydrogels or treated tissue culture polystyrene (TCP) surfaces. However, maximum sensitivity for BoNT detection was achieved two weeks earlier for iPSC-NSCs that were differentiated and matured on PEG hydrogels compared to TCP. Therefore, chemically-defined synthetic hydrogels offer benefits over standard platforms when optimizing culture conditions for cell-based screening and achieve sensitivities comparable to an approved animal testing protocol.

Peng, K. Y., et al. (2016). "Human pluripotent stem cell (PSC)-derived mesenchymal stem cells (MSCs) show potent neurogenic capacity which is enhanced with cytoskeletal rearrangement." Oncotarget **7**(28): 43949-43959.

Mesenchymal stem cells (MSCs) are paraxial mesodermal progenitors with potent immunomodulatory properties. Reports also indicate that MSCs can undergo neural-like differentiation, offering hope for use in neurodegenerative diseases. However, ex vivo expansion of these rare somatic stem cells for clinical use leads to cellular senescence. A newer source of MSCs derived from human pluripotent stem cells (PSC) can offer the 'best-of-both-worlds' scenario, abrogating the concern of teratoma formation while preserving PSC proliferative capacity. PSC-derived MSCs (PSC-MSCs) also represent MSCs at the earliest developmental stage, and we found that these MSCs harbor stronger neuro-differentiation capacity than post-natal MSCs. PSC-MSCs express higher levels of neural stem cell (NSC)-related genes and transcription factors than adult bone marrow MSCs at baseline, and rapidly differentiate into neural-like cells when cultured in either standard neurogenic differentiation medium (NDM) or when the cytoskeletal modulator RhoA kinase (ROCK) is inhibited. Interestingly, when NDM is combined with ROCK inhibition, PSC-MSCs undergo further commitment, acquiring characteristics of post-mitotic neurons including nuclear condensation, extensive dendritic growth, and neuron-restricted marker expression including NeuN, beta-III-tubulin and Doublecortin. Our data demonstrates that PSC-MSCs have potent capacity to undergo neural differentiation and also implicate the important role of the cytoskeleton in neural lineage commitment.

Pera, R. A. (2013). "Status of human germ cell differentiation from pluripotent stem cells." Reprod Fertil Dev **25**(2): 396-404.

Historically, the quality of life of infertile couples has been greatly diminished by the loss of opportunity to conceive. However, beginning with the advent of IVF in the late 1970s, novel clinical interventions have greatly changed the outlook for those with severe forms of infertility. Yet, in cases in which the quality and quantity of germ cells are most compromised, there are few options. In the present paper, the current status of germ cell development from stem cells is reviewed in light of potential utility for basic science and clinical applications.

Peters, D. T., et al. (2016). "Asialoglycoprotein receptor 1 is a specific cell-surface marker for isolating hepatocytes derived from human pluripotent stem cells." Development **143**(9): 1475-1481.

Hepatocyte-like cells (HLCs) are derived from human pluripotent stem cells (hPSCs) in vitro, but differentiation protocols commonly give rise to a heterogeneous mixture of cells. This variability confounds the evaluation of in vitro functional assays performed using HLCs. Increased differentiation efficiency and more accurate approximation of the in vivo hepatocyte gene expression profile would improve the utility of hPSCs. Towards this goal, we demonstrate the purification of a subpopulation of functional HLCs using the hepatocyte surface marker asialoglycoprotein receptor 1 (ASGR1). We analyzed the expression profile of ASGR1-positive cells by microarray, and tested their ability to perform mature hepatocyte functions (albumin and urea secretion, cytochrome activity). By these measures, ASGR1-positive HLCs are enriched for the gene expression profile and functional characteristics of primary hepatocytes compared with unsorted HLCs. We have demonstrated that ASGR1-positive sorting isolates a functional

subpopulation of HLCs from among the heterogeneous cellular population produced by directed differentiation.

Pettinato, G., et al. (2014). "Formation of well-defined embryoid bodies from dissociated human induced pluripotent stem cells using microfabricated cell-repellent microwell arrays." Sci Rep **4**: 7402.

A simple, scalable, and reproducible technology that allows direct formation of large numbers of homogeneous and synchronized embryoid bodies (EBs) of defined sizes from dissociated human induced pluripotent stem cells (hiPSCs) was developed. Non-cell-adhesive hydrogels were used to create round-bottom microwells to host dissociated hiPSCs. No Rho-associated kinase inhibitor (ROCK-i), or centrifugation was needed and the side effects of ROCK-i can be avoided. The key requirement for the successful EB formation in addition to the non-cell-adhesive round-bottom microwells is the input cell density per microwell. Too few or too many cells loaded into the microwells will compromise the EB formation process. In parallel, we have tested our microwell-based system for homogeneous hEB formation from dissociated human embryonic stem cells (hESCs). Successful production of homogeneous hEBs from dissociated hESCs in the absence of ROCK-i and centrifugation was achieved within an optimal range of input cell density per microwell. Both the hiPSC- and hESC-derived hEBs expressed key proteins characteristic of all the three developmental germ layers, confirming their EB identity. This novel EB production technology may represent a versatile platform for the production of homogeneous EBs from dissociated human pluripotent stem cells (hPSCs).

Peyrard, T., et al. (2011). "Banking of pluripotent adult stem cells as an unlimited source for red blood cell production: potential applications for alloimmunized patients and rare blood challenges." Transfus Med Rev **25**(3): 206-216.

The transfusion of red blood cells (RBCs) is now considered a well-settled and essential therapy. However, some difficulties and constraints still occur, such as long-term blood product shortage, blood donor population aging, known and yet unknown transfusion-transmitted infectious agents, growing cost of the transfusion supply chain management, and the inescapable blood group polymorphism barrier. Red blood cells can be now cultured in vitro from human hematopoietic, human embryonic, or human-induced pluripotent stem cells (hiPSCs). The highly promising hiPSC technology represents a potentially unlimited source of RBCs and opens the door to the revolutionary development of a new generation of allogeneic transfusion products. Assuming that in vitro large-scale cultured RBC production efficiently operates in the near future, we draw here some futuristic but realistic scenarios regarding potential applications for alloimmunized patients and those with a rare blood group. We retrospectively studied a cohort of 16,486 consecutive alloimmunized patients (10-year period), showing 1 to 7 alloantibodies with 361 different antibody combinations. We showed that only 3 hiPSC clones would be sufficient to match more than 99% of the 16,486 patients in need of RBC transfusions. The study of the French National Registry of People with a Rare Blood Phenotype/Genotype (10-year period) shows that 15 hiPSC clones would cover 100% of the needs in patients of white ancestry. In addition, one single hiPSC clone would meet 73% of the needs in alloimmunized patients with sickle cell disease for whom rare cryopreserved RBC units were required. As a result, we consider that a very limited number of RBC clones would be able to not only provide for the need for most alloimmunized patients and those with a rare blood group but also efficiently allow for a policy for alloimmunization prevention in multiply transfused patients.

Phanthong, P., et al. (2017). "Enhancement of beta-Globin Gene Expression in Thalassemic IVS2-654 Induced Pluripotent Stem Cell-Derived Erythroid Cells by Modified U7 snRNA." Stem Cells Transl Med **6**(4): 1059-1069.

The therapeutic use of patient-specific induced pluripotent stem cells (iPSCs) is emerging as a potential treatment of beta-thalassemia. Ideally, patient-specific iPSCs would be genetically corrected by various approaches to treat beta-thalassemia including lentiviral gene transfer, lentivirus-delivered shRNA, and gene editing. These corrected iPSCs would be subsequently differentiated into hematopoietic stem cells and transplanted back into the same patient. In this article, we present a proof of principle study for disease modeling and screening using iPSCs to test the potential use of the modified U7 small nuclear (sn) RNA to correct a splice defect in IVS2-654 beta-thalassemia. In this case, the aberration results from a mutation in the human beta-globin intron 2 causing an aberrant splicing of beta-globin pre-mRNA and preventing synthesis of functional beta-globin protein. The iPSCs (derived from mesenchymal stromal cells from a patient with IVS2-654 beta-thalassemia/hemoglobin (Hb) E) were transduced with a lentivirus carrying a modified U7 snRNA targeting an IVS2-654 beta-globin pre-mRNA in order to restore the correct splicing. Erythroblasts differentiated from the transduced iPSCs expressed high level of correctly spliced beta-globin mRNA suggesting that the modified U7 snRNA was expressed and mediated splicing correction of IVS2-654 beta-globin pre-mRNA in these cells. Moreover, a less active apoptosis cascade

process was observed in the corrected cells at transcription level. This study demonstrated the potential use of a genetically modified U7 snRNA with patient-specific iPSCs for the partial restoration of the aberrant splicing process of beta-thalassemia. Stem Cells Translational Medicine 2017;6:1059-1069.

Picanco-Castro, V., et al. (2014). "Can pluripotent stem cells be used in cell-based therapy?" Cell Reprogram **16**(2): 98-107.

Pluripotent stem cells, both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have the ability to differentiate into several cell types that can be used in drug testing and also in the study and treatment of diseases. These cells can be differentiated by in vitro systems, which may serve as models for human diseases and for cell transplantation. In this review, we address the pluripotent cell types, how to obtain and characterize these cells, and differentiation assays. We also focus on the potential of these cells in clinical trials, and we describe the clinical trials that are underway.

Polinati, P. P., et al. (2015). "Patient-Specific Induced Pluripotent Stem Cell-Derived RPE Cells: Understanding the Pathogenesis of Retinopathy in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency." Invest Ophthalmol Vis Sci **56**(5): 3371-3382.

PURPOSE: Retinopathy is an important manifestation of trifunctional protein (TFP) deficiencies but not of other defects of fatty acid oxidation. The common homozygous mutation in the TFP alpha-subunit gene HADHA (hydroxyacyl-CoA dehydrogenase), c.1528G>C, affects the long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity of TFP and blindness in infancy. The pathogenesis of the retinopathy is unknown. This study aimed to utilize human induced pluripotent stem cell (hiPSC) technology to create a disease model for the disorder, and to derive clues for retinopathy pathogenesis. METHODS: We implemented hiPSC technology to generate LCHAD deficiency (LCHADD) patient-specific retinal pigment epithelial (RPE) monolayers. These patient and control RPEs were extensively characterized for function and structure, as well as for lipid composition by mass spectrometry. RESULTS: The hiPSC-derived RPE monolayers of patients and controls were functional, as they both were able to phagocytose the photoreceptor outer segments in vitro. Interestingly, the patient RPEs had intense cytoplasmic neutral lipid accumulation, and lipidomic analysis revealed an increased triglyceride accumulation. Further, patient RPEs were small and irregular in shape, and their tight junctions were disorganized. Their ultrastructure showed decreased pigmentation, few melanosomes, and more melanolysosomes. CONCLUSIONS: We demonstrate that the RPE cell model reveals novel early pathogenic changes in LCHADD retinopathy, with robust lipid accumulation, inefficient pigmentation that is evident soon after differentiation, and a defect in forming tight junctions inducing apoptosis. We propose that LCHADD-RPEs are an important model for mitochondrial TFP retinopathy, and that their early pathogenic changes contribute to infantile blindness of LCHADD.

Polo, J. M., et al. (2010). "Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells." Nat Biotechnol **28**(8): 848-855.

Induced pluripotent stem cells (iPSCs) have been derived from various somatic cell populations through ectopic expression of defined factors. It remains unclear whether iPSCs generated from different cell types are molecularly and functionally similar. Here we show that iPSCs obtained from mouse fibroblasts, hematopoietic and myogenic cells exhibit distinct transcriptional and epigenetic patterns. Moreover, we demonstrate that cellular origin influences the in vitro differentiation potentials of iPSCs into embryoid bodies and different hematopoietic cell types. Notably, continuous passaging of iPSCs largely attenuates these differences. Our results suggest that early-passage iPSCs retain a transient epigenetic memory of their somatic cells of origin, which manifests as differential gene expression and altered differentiation capacity. These observations may influence ongoing attempts to use iPSCs for disease modeling and could also be exploited in potential therapeutic applications to enhance differentiation into desired cell lineages.

Pomeshchik, Y., et al. (2015). "Transplanted Human Induced Pluripotent Stem Cell-Derived Neural Progenitor Cells Do Not Promote Functional Recovery of Pharmacologically Immunosuppressed Mice With Contusion Spinal Cord Injury." Cell Transplant **24**(9): 1799-1812.

Improved functional recovery after spinal cord injury by transplantation of induced pluripotent stem cell-derived neural stem/progenitor cells (iPSC-NPCs) has been reported. However, beneficial effects of iPSC-based therapy have so far been produced mostly using genetically immunodeficient rodents. Because of the long time required for generation and characterization of iPSCs, the use of autologous iPSCs for treating patients with acute spinal cord injury (SCI) is not feasible. Therefore, it is of utmost importance to investigate the effect of iPSC-based therapy on functional recovery after SCI using pharmacologically immunosuppressed, immunocompetent animal models.

Here we studied the functional outcome following subacute transplantation of human iPSC-derived NPCs into contused mouse spinal cord when tacrolimus was used as an immunosuppressive agent. We show that human iPSC-derived NPCs transplanted into pharmacologically immunosuppressed C57BL/6J mice exhibited poor long-term survival and failed to improve functional recovery after SCI as measured by Basso Mouse Scale (BMS) for locomotion and CatWalk gait analysis when compared to vehicle-treated animals. The scarce effect of iPSC-based therapy observed in the current study may be attributable to insufficient immunosuppressive effect, provided by monotherapy with tacrolimus in combination with immunogenicity of transplanted cells and complex microenvironment of the injured spinal cord. Our results highlight the importance of extensive preclinical studies of transplanted cells before the clinical application of iPSC-based cell therapy is achieved.

Pradelles, P., et al. (1990). "Negative regulator of pluripotent hematopoietic stem cell proliferation in human white blood cells and plasma as analysed by enzyme immunoassay." Biochem Biophys Res Commun **170**(3): 986-993.

This paper describes the analysis, by a highly sensitive and specific enzyme immunoassay (EIA), of AcSDKP, a tetrapeptide recently isolated from fetal calf bone marrow and subsequently purified and identified which substantially inhibits entry into cycle of hematopoietic pluripotent stem cells (CFU-S). This molecule has a marked protective effect in mice during anticancer chemotherapy with phase-specific drugs and plays an essential role in maintaining CFU-S out of cycle in normal mice. Using acetylcholinesterase-AcSDKP conjugate as tracer, rabbit specific antiserum and 96-well microtiter plates coated with a mouse monoclonal anti-rabbit IgG antibody, this EIA allows detection of AcSDKP at 15 fmol levels with a coefficient of variation less than 10% in the 50-500 fmol range. When combined with high-performance liquid chromatography, this assay clearly reveals the presence of this peptide in normal human white blood cells whereas in supernatant from cultured lymphocytes and in plasma the immunoreactive material is distinct from standard AcSDKP.

Prelle, K., et al. (2002). "Pluripotent stem cells--model of embryonic development, tool for gene targeting, and basis of cell therapy." Anat Histol Embryol **31**(3): 169-186.

Embryonic stem (ES) cells are pluripotent cell lines with the capacity of self-renewal and a broad differentiation plasticity. They are derived from pre-implantation embryos and can be propagated as a homogeneous, uncommitted cell population for an almost unlimited period of time without losing their pluripotency and their stable karyotype. Murine ES cells are able to reintegrate fully into embryogenesis when returned into an early embryo, even after extensive genetic manipulation. In the resulting chimeric offspring produced by blastocyst injection or morula aggregation, ES cell descendants are represented among all cell types, including functional gametes. Therefore, mouse ES cells represent an important tool for genetic engineering, in particular via homologous recombination, to introduce gene knock-outs and other precise genomic modifications into the mouse germ line. Because of these properties ES cell technology is of high interest for other model organisms and for livestock species like cattle and pigs. However, in spite of tremendous research activities, no proven ES cells colonizing the germ line have yet been established for vertebrate species other than the mouse (Evans and Kaufman, 1981; Martin, 1981) and chicken (Pain et al., 1996). The in vitro differentiation capacity of ES cells provides unique opportunities for experimental analysis of gene regulation and function during cell commitment and differentiation in early embryogenesis. Recently, pluripotent stem cells were established from human embryos (Thomson et al., 1998) and early fetuses (Shamblott et al., 1998), opening new scenarios both for research in human developmental biology and for medical applications, i.e. cell replacement strategies. At about the same time, research activities focused on characteristics and differentiation potential of somatic stem cells, unravelling an unexpected plasticity of these cell types. Somatic stem cells are found in differentiated tissues and can renew themselves in addition to generating the specialized cell types of the tissue from which they originate. Additional to discoveries of somatic stem cells in tissues that were previously not thought to contain these kinds of cells, they also appear to be capable of developing into cell types of other tissues, but have a reduced differentiation potential as compared to embryo-derived stem cells. Therefore, somatic stem cells are referred to as multipotent rather than pluripotent. This review summarizes characteristics of pluripotent stem cells in the mouse and in selected livestock species, explains their use for genetic engineering and basic research on embryonic development, and evaluates their potential for cell therapy as compared to somatic stem cells.

Prigione, A., et al. (2011). "Human induced pluripotent stem cells harbor homoplasmic and heteroplasmic mitochondrial DNA mutations while maintaining human embryonic stem cell-like metabolic reprogramming." Stem Cells **29**(9): 1338-1348.

Human induced pluripotent stem cells (iPSCs) have been recently found to harbor genomic alterations.

However, the integrity of mitochondrial DNA (mtDNA) within reprogrammed cells has yet to be investigated. mtDNA mutations occur at a high rate and contribute to the pathology of a number of human disorders. Furthermore, the lack of mtDNA integrity may alter cellular bioenergetics and limit efficient differentiation. We demonstrated previously that the derivation of iPSCs is associated with mitochondrial remodeling and a metabolic switch towards glycolysis. Here, we have discovered that alterations of mtDNA can occur upon the induction of pluripotency. Massively parallel pyrosequencing of mtDNA revealed that human iPSCs derived from young healthy donors harbored single base mtDNA mutations (substitutions, insertions, and deletions), both homoplasmic (in all mtDNA molecules) and heteroplasmic (in a fraction of mtDNAs), not present in the parental cells. mtDNA modifications were mostly common variants and not disease related. Moreover, iPSC lines bearing different mtDNA mutational loads maintained a consistent human embryonic stem cell-like reprogramming of energy metabolism. This involved the upregulation of glycolytic enzymes, increased glucose-6-phosphate levels, and the over-expression of pyruvate dehydrogenase kinase 1 protein, which reroutes the bioenergetic flux toward glycolysis. Hence, mtDNA mutations within iPSCs may not necessarily impair the correct establishment of pluripotency and the associated metabolic reprogramming. Nonetheless, the occurrence of pathogenic mtDNA modifications might be an important aspect to monitor when characterizing iPSC lines. Finally, we speculate that this random rearrangement of mtDNA molecules might prove beneficial for the derivation of mutation-free iPSCs from patients with mtDNA disorders.

Pryzhkova, M. V., et al. (2013). "Patterning Pluripotent Stem Cells at a Single Cell Level." J Biomater Tissue Eng **3**(4): 461-471.

Studies of cell-extracellular matrix (ECM) interactions at a single cell level have drawn interest from scientists around the world. Subcellular ECM micropatterning techniques allow researchers to control cell shape, migration, and spindle orientation during mitosis potentially influencing the stem cell fate. Generally these studies have been limited to somatic cells rather than human pluripotent stem cells (hPSCs) which are capable of enormous differentiation potential. hPSCs require a defined ECM for attachment and express characteristic integrins mediating cell-substrate interactions. hPSCs also rely on cell-cell contacts for survival and to maintain self-renewal properties, but these circumstances also significantly limit hPSC observation at a single cell level. In addition, currently available methods for ECM micropatterning generally require a facility with trained personnel and intricate equipment to produce protein micropatterns. To overcome this problem, we have developed a protocol for vitronectin micropatterning using simple UV/ozone modification of polystyrene. Single hPSCs were able to attach and form characteristic stress fibers and focal adhesions similar to somatic cell types which demonstrate hPSC responsiveness to extracellular adhesive cues. Micropatterned hPSCs were able to be cultured for up to 48 hours while maintaining expression of pluripotency-associated transcription factor OCT4. Although further studies are necessary, the results of our investigation will potentially have a large impact on cell regenerative medicine and tissue engineering.

Puceat, M. (2012). "[Pluripotent stem cells: a cell model for early cardiac development]." Biol Aujourdhui **206**(1): 25-29.

Mouse embryonic stem cell lines were derived three decades ago and allow the process of transgenesis and in turn the generation of transgenic mice. In the past and still nowadays, these mice as well as more primitive organisms have provided models to study the first cell decisions in the embryo. Derivation of human embryonic stem cells more than a decade ago has provided a similar cell model for human early embryonic development, an issue that could not be addressed for obvious ethical reasons which limit research on human embryos. These cells allow investigating the genetic and epigenetic mechanisms underlying the first cell decisions in the human embryo. Herein, we use cardiogenesis as an example to reveal the potential of these cells to better understand the first steps of cardiac development.

Qi, X., et al. (2016). "Exosomes Secreted by Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Repair Critical-Sized Bone Defects through Enhanced Angiogenesis and Osteogenesis in Osteoporotic Rats." Int J Biol Sci **12**(7): 836-849.

Bone defects caused by trauma, severe infection, tumor resection and skeletal abnormalities are common osteoporotic conditions and major challenges in orthopedic surgery, and there is still no effective solution to this problem. Consequently, new treatments are needed to develop regeneration procedures without side effects. Exosomes secreted by mesenchymal stem cells (MSCs) derived from human induced pluripotent stem cells (hiPSCs, hiPSC-MSC-Exos) incorporate the advantages of both MSCs and iPSCs with no immunogenicity. However, there are no reports on the application of hiPSC-MSC-Exos to enhance angiogenesis and osteogenesis under osteoporotic conditions. HiPSC-MSC-Exos were isolated and identified before use. The effect of hiPSC-MSC-Exos on the proliferation and osteogenic

differentiation of bone marrow MSCs derived from ovariectomized (OVX) rats (rBMSCs-OVX) in vitro were investigated. In vivo, hiPSC-MSC-Exos were implanted into critical size bone defects in ovariectomized rats, and bone regeneration and angiogenesis were examined by microcomputed tomography (micro-CT), sequential fluorescent labeling analysis, microfil perfusion and histological and immunohistochemical analysis. The results in vitro showed that hiPSC-MSC-Exos enhanced cell proliferation and alkaline phosphatase (ALP) activity, and up-regulated mRNA and protein expression of osteoblast-related genes in rBMSCs-OVX. In vivo experiments revealed that hiPSC-MSC-Exos dramatically stimulated bone regeneration and angiogenesis in critical-sized calvarial defects in ovariectomized rats. The effect of hiPSC-MSC-Exos increased with increasing concentration. In this study, we showed that hiPSC-MSC-Exos effectively stimulate the proliferation and osteogenic differentiation of rBMSCs-OVX, with the effect increasing with increasing exosome concentration. Further analysis demonstrated that the application of hiPSC-MSC-Exos+beta-TCP scaffolds promoted bone regeneration in critical-sized calvarial defects by enhancing angiogenesis and osteogenesis in an ovariectomized rat model.

Qin, J., et al. (2014). "Cell fusion enhances mesendodermal differentiation of human induced pluripotent stem cells." Stem Cells Dev **23**(23): 2875-2882.

Human induced pluripotent stem cells (iPS cells) resemble embryonic stem cells and can differentiate into cell derivatives of all three germ layers. However, frequently the differentiation efficiency of iPS cells into some lineages is rather poor. Here, we found that fusion of iPS cells with human hematopoietic stem cells (HSCs) enhances iPS cell differentiation. Such iPS hybrids showed a prominent differentiation bias toward hematopoietic lineages but also toward other mesendodermal lineages. Additionally, during differentiation of iPS hybrids, expression of early mesendodermal markers-Brachyury (T), MIX1 Homeobox-Like Protein 1 (MIXL1), and Goosecoid (GSC)-appeared with faster kinetics than in parental iPS cells. Following iPS hybrid differentiation there was a prominent induction of NODAL and inhibition of NODAL signaling blunted mesendodermal differentiation. This indicates that NODAL signaling is critically involved in mesendodermal bias of iPS hybrid differentiation. In summary, we demonstrate that iPS cell fusion with HSCs prominently enhances iPS cell differentiation.

Qin, Y., et al. (2016). "Potential Cancer Prevention and Treatment by Silencing the Killer Cell Immunoglobulin-like Receptor Gene in Natural Killer Cells Derived from Induced Pluripotent Stem Cells." Enliven J Stem Cell Res Regen Med **3**(1).

Cancer immunosurveillance is an important host protection process, monitoring the presence of irregular cells that could potentially transform into tumor cells, effectively clearing the body of transformed tumor cells at their earliest stages, and thus maintaining regular cellular homeostasis. Natural killer (NK) cells are effector lymphocytes of the innate immune system, playing a critical role in surveillance for tumor cells, while also eliminating virally infected cells. The significance of the anti-tumor role of NK cells was recently further verified by findings that immunosuppression in most cancer patients is not perceptible until late stages. NK cells express the low-affinity Fc-activating receptor, CD16, and the inhibitory receptor, killer cell immunoglobulin-like receptor (KIR). Consequently, activation of NK cells is determined by the balance of inhibitory and activating receptor stimulation. Here, we propose establishing an induced pluripotent stem cell (iPSC)-derived NK cell line with KIR gene knockout or knockdown as a possible regimen to treat and prevent cancer. We further postulate that an optimal mixture of NK iPSCs with and without KIR gene knockout, would reach a maximum antitumor activity, with minimal side effects. We also discuss the possible advantages of KIR-knockout NK iPSCs for adoptive immunotherapy in patients with cancer.

Quang, T., et al. (2014). "Dosage and cell line dependent inhibitory effect of bFGF supplement in human pluripotent stem cell culture on inactivated human mesenchymal stem cells." PLoS One **9**(1): e86031.

Many different culture systems have been developed for expanding human pluripotent stem cells (hESCs and hiPSCs). In general, 4-10 ng/ml of bFGF is supplemented in culture media in feeder-dependent systems regardless of feeder cell types, whereas in feeder-free systems, up to 100 ng/ml of bFGF is required for maintaining long-term culture on various substrates. The amount of bFGF required in native hESCs growth niche is unclear. Here we report using inactivated adipose-derived human mesenchymal stem cells as feeder cells to examine long-term parallel cultures of two hESCs lines (H1 and H9) and one hiPSCs line (DF19-9-7T) in media supplemented with 0, 0.4 or 4 ng/ml of bFGF for up to 23 passages, as well as parallel cultures of H9 and DF19 in media supplemented with 4, 20 or 100 ng/ml bFGF for up to 13 passages for comparison. Across all cell lines tested, bFGF supplement demonstrated inhibitory effect over

growth expansion, single cell colonization and recovery from freezing in a dosage dependent manner. In addition, bFGF exerted differential effects on different cell lines, inducing H1 and DF19 differentiation at 4 ng/ml or higher, while permitting long-term culture of H9 at the same concentrations with no apparent dosage effect. Pluripotency was confirmed for all cell lines cultured in 0, 0.4 or 4 ng/ml bFGF excluding H1-4 ng, as well as H9 cultured in 4, 20 and 100 ng/ml bFGF. However, DF19 demonstrated similar karyotypic abnormality in both 0 and 4 ng/ml bFGF media while H1 and H9 were karyotypically normal in 0 ng/ml bFGF after long-term culture. Our results indicate that exogenous bFGF exerts dosage and cell line dependent effect on human pluripotent stem cells cultured on mesenchymal stem cells, and implies optimal use of bFGF in hESCs/hiPSCs culture should be based on specific cell line and its culture system.

Quintana-Bustamante, O. and J. C. Segovia (2016). "Generation of Patient-Specific induced Pluripotent Stem Cell from Peripheral Blood Mononuclear Cells by Sendai Reprogramming Vectors." Methods Mol Biol **1353**: 1-11.

Induced pluripotent stem cells (iPSC) technology has changed preclinical research since their generation was described by Shinya Yamanaka in 2006. iPSCs are derived from somatic cells after being reprogrammed back to an embryonic state by specific combination of reprogramming factors. These reprogrammed cells resemble all the characteristic of embryonic stem cells (ESC). The reprogramming technology is even more valuable to research diseases biology and treatment by opening gene and cell therapies in own patient's iPSC. Patient-specific iPSC can be generated from a large variety of patient cells by any of the myriad of reprogramming platforms described. Here, we describe the generation of patient-specific iPSC from patient peripheral blood mononuclear cells by Sendai Reprogramming vectors.

Rahman, N., et al. (2010). "The use of vascular endothelial growth factor functionalized agarose to guide pluripotent stem cell aggregates toward blood progenitor cells." Biomaterials **31**(32): 8262-8270.

The developmental potential of pluripotent stem cells is influenced by their local cellular microenvironment. To better understand the role of vascular endothelial growth factor (VEGFA) in the embryonic cellular microenvironment, we synthesized an artificial stem cell niche wherein VEGFA was immobilized in an agarose hydrogel. Agarose was first modified with coumarin-protected thiols. Upon exposure to ultra-violet excitation, the coumarin groups were cleaved leaving reactive thiols to couple with maleimide-activated VEGFA. Mouse embryonic stem cells (ESC) aggregates were encapsulated in VEGFA immobilized agarose and cultured for 7 days as free-floating aggregates under serum-free conditions. Encapsulated aggregates were assessed for their capacity to give rise to blood progenitor cells. In the presence of bone morphogenetic protein-4 (BMP-4), cells exposed to immobilized VEGFA upregulated mesodermal markers, brachyury and VEGF receptor 2 (T+VEGFR2+) by day 4, and expressed CD34 and CD41 (CD34+CD41+) on day 7. It was found that immobilized VEGFA treatment was more efficient at inducing blood progenitors (including colony forming cells) on a per molecule basis than soluble VEGFA. This work demonstrates the use of functionalized hydrogels to guide encapsulated ESCs toward blood progenitor cells and introduces a tool capable of recapitulating aspects of the embryonic microenvironment.

Rajaei, B., et al. (2017). "Pancreatic Endoderm-Derived From Diabetic Patient-Specific Induced Pluripotent Stem Cell Generates Glucose-Responsive Insulin-Secreting Cells." J Cell Physiol **232**(10): 2616-2625.

Human-induced pluripotent stem cells (hiPSCs) can potentially serve as an invaluable source for cell replacement therapy and allow the creation of patient- and disease-specific stem cells without the controversial use of embryos and avoids any immunological incompatibility. The generation of insulin-producing pancreatic beta-cells from pluripotent stem cells in vitro provides an unprecedented cell source for personal drug discovery and cell transplantation therapy in diabetes. A new five-step protocol was introduced in this study, effectively induced hiPSCs to differentiate into glucose-responsive insulin-producing cells. This process mimics in vivo pancreatic organogenesis by directing cells through stages resembling definitive endoderm, primitive gut-tube endoderm, posterior foregut, pancreatic endoderm, and endocrine precursor. Each stage of differentiation were characterized by stage-specific markers. The produced cells exhibited many properties of functional beta-cells, including expression of critical beta-cells transcription factors, the potency to secrete C-peptide in response to high levels of glucose and the presence of mature endocrine secretory granules. This high efficient differentiation protocol, established in this study, yielded 79.18% insulin-secreting cells which were responsive to glucose five times higher than the basal level. These hiPSCs-derived glucose-responsive insulin-secreting cells might provide a promising approach for the treatment of type I diabetes mellitus. J. Cell. Physiol. 232: 2616-2625, 2017. (c) 2016 Wiley Periodicals, Inc.

Rajaei, B., et al. (2017). "In Vitro Generation of Glucose-Responsive Insulin-Secreting Cells from Pancreatic and Duodenal Homeobox 1-Overexpressing Human-Induced Pluripotent Stem Cell Derived from Diabetic Patient." ASAIO J.

Pancreatic and duodenal homeobox 1 (PDX1), a member of the homeodomain-containing transcription factor family, is a key transcription factor for pancreas development and mature beta-cell function. In this study, induced overexpression of PDX1 resulted in producing susceptible cells for pancreatic differentiation and was well beneficial to enhance beta-cell production, maturation, function, and survival. Induced PDX1 overexpression in harmony with a set of signaling molecules involves in guiding the signaling pathways toward pancreas development, leaded to high-efficient in vitro generation of ectopic insulin-producing cells (IPCs) with the effectively reduced number of polyhormonal cells and increased number of INS single-positive cells. This strategy yielded 85.61% glucose-responsive insulin-positive cells in vitro, which was seven times higher than the basal level, and electron microscopy images revealed the presence of mature beta-cell secretory granules. The generation of glucose-responsive insulin-secreting beta-like cells from human-induced pluripotent stem cells (hiPSCs) in vitro would provide a promising approach to produce an unprecedented cell source for cell transplantation therapy in diabetes without the ethical obstacle of embryonic stem cells and would bypass immune rejection. These cells are an invaluable source for disease modeling, drug discovery, and pharmacogenomics studies as well.

Rajesh, D., et al. (2011). "Human lymphoblastoid B-cell lines reprogrammed to EBV-free induced pluripotent stem cells." Blood **118**(7): 1797-1800.

Generation of patient-specific induced pluripotent cells (iPSCs) holds great promise for regenerative medicine. Epstein-Barr virus immortalized lymphoblastoid B-cell lines (LCLs) can be generated from a minimal amount of blood and are banked worldwide as cellular reference material for immunologic or genetic analysis of pedigreed study populations. We report the generation of iPSCs from 2 LCLs (LCL-iPSCs) via a feeder-free episomal method using a cocktail of transcription factors and small molecules. LCL-derived iPSCs exhibited normal karyotype, expressed pluripotency markers, lost oriP/EBNA-1 episomal vectors, generated teratomas, retained donor identity, and differentiated in vitro into hematopoietic, cardiac, neural, and hepatocyte-like lineages. Significantly, although the parental LCLs express viral EBNA-1 and other Epstein-Barr virus latency-related elements for their survival, their presence was not detectable in LCL-iPSCs. Thus, reprogramming LCLs could offer an unlimited source for patient-specific iPSCs.

Ramasamy, T. S., et al. (2018). "Stem Cells Derived from Amniotic Fluid: A Potential Pluripotent-Like Cell Source for Cellular Therapy?" Curr Stem Cell Res Ther **13**(4): 252-264.

BACKGROUND: Regenerative medicine aims to provide therapeutic treatment for disease or injury, and cell-based therapy is a newer therapeutic approach different from conventional medicine. Ethical issues that rose by the utilisation of human embryonic stem cells (hESC) and the limited capacity of adult stem cells, however, hinder the application of these stem cells in regenerative medicine. Recently, isolation and characterisation of c-kit positive cells from human amniotic fluid, which possess intermediate characteristics between hESCs and adult stem cells, provided a new approach towards realising their promise for fetal and adult regenerative medicine. Despite the number of studies that have been initiated to characterize their molecular signature, research on developing approaches to maintain and enhance their regenerative potential is urgently needed and must be developed. AIM: Thus, this review is focused on understanding their potential uses and factors influencing their pluripotent status in vitro. CONCLUSION: In short, this cell source could be an ideal cellular resource for pluripotent cells for potential applications in allogeneic cellular replacement therapies, fetal tissue engineering, pharmaceutical screening, and in disease modelling.

Ramirez, J. M., et al. (2010). "Human pluripotent stem cells: from biology to cell therapy." World J Stem Cells **2**(2): 24-33.

Human pluripotent stem cells (PSCs), encompassing embryonic stem cells and induced pluripotent stem cells, proliferate extensively and differentiate into virtually any desired cell type. PSCs endow regenerative medicine with an unlimited source of replacement cells suitable for human therapy. Several hurdles must be carefully addressed in PSC research before these theoretical possibilities are translated into clinical applications. These obstacles are: (1) cell proliferation; (2) cell differentiation; (3) genetic integrity; (4) allogenicity; and (5) ethical issues. We discuss these issues and underline the fact that the answers to these questions lie in a better understanding of the biology of PSCs. To contribute to this aim, we have developed a free online expression atlas, Amazonia!, that displays for each human gene a virtual northern blot for PSC samples and adult tissues (http://www.amazonia.transcriptome.eu).

Ramos-Ibeas, P., et al. (2015). "Characterisation of the deleted in azoospermia like (Dazl)-green fluorescent protein mouse model generated by a two-step embryonic stem cell-based strategy to identify pluripotent and germ cells." Reprod Fertil Dev.

The deleted in azoospermia like (Dazl) gene is preferentially expressed in germ cells; however, recent studies indicate that it may have pluripotency-related functions. We generated Dazl-green fluorescent protein (GFP) transgenic mice and assayed the ability of Dazl-driven GFP to mark preimplantation embryo development, fetal, neonatal and adult tissues, and in vitro differentiation from embryonic stem cells (ESCs) to embryoid bodies (EBs) and to primordial germ cell (PGC)-like cells. The Dazl-GFP mice were generated by a two-step ESC-based strategy, which enabled primary and secondary screening of stably transfected clones before embryo injection. During preimplantation embryo stages, GFP was detected from the zygote to blastocyst stage. At Embryonic Day (E) 12.5, GFP was expressed in gonadal ridges and in neonatal gonads of both sexes. In adult mice, GFP expression was found during spermatogenesis from spermatogonia to elongating spermatids and in the cytoplasm of oocytes. However, GFP mRNA was also detected in other tissues harbouring multipotent cells, such as the intestine and bone marrow. Fluorescence was maintained along in vitro Dazl-GFP ESC differentiation to EBs, and in PGC-like cells. In addition to its largely known function in germ cell development, Dazl could have an additional role in pluripotency, supporting these transgenic mice as a valuable tool for the prospective identification of stem cells from several tissues.

Rasmussen, M. L., et al. (2018). "Wnt Signaling and Its Impact on Mitochondrial and Cell Cycle Dynamics in Pluripotent Stem Cells." Genes (Basel) **9**(2).

The core transcriptional network regulating stem cell self-renewal and pluripotency remains an intense area of research. Increasing evidence indicates that modified regulation of basic cellular processes such as mitochondrial dynamics, apoptosis, and cell cycle are also essential for pluripotent stem cell identity and fate decisions. Here, we review evidence for Wnt regulation of pluripotency and self-renewal, and its connections to emerging features of pluripotent stem cells, including (1) increased mitochondrial fragmentation, (2) increased sensitivity to cell death, and (3) shortened cell cycle. We provide a general overview of the stem cell-specific mechanisms involved in the maintenance of these uncharacterized hallmarks of pluripotency and highlight potential links to the Wnt signaling pathway. Given the physiological importance of stem cells and their enormous potential for regenerative medicine, understanding fundamental mechanisms mediating the crosstalk between Wnt, organelle-dynamics, apoptosis, and cell cycle will be crucial to gain insight into the regulation of stemness.

Ratajczak, M. Z., et al. (2016). "Stem cells and clinical practice: new advances and challenges at the time of emerging problems with induced pluripotent stem cell therapies." Pol Arch Med Wewn **126**(11): 879-890.

Humans, like other species that reproduce sexually, originate from a fertilized oocyte (zygote), which is a totipotent stem cell giving rise to an adult organism. During the process of embryogenesis, stem cells at different levels of the developmental hierarchy establish all 3 germ layers and give rise to tissuecommitted stem cells, which are responsible for rejuvenation of a given tissue or organ. The robustness of the stem cell compartment is one of the major factors that directly impact life quality as well as lifespan. Stem cells continuously replace cells and tissues that are used up during life; however, this replacement occurs at a different pace in various organs. The rapidly developing field of regenerative medicine is taking advantage of these physiological properties of stem cells and is attempting to employ them in clinical settings to regenerate damaged organs (eg, the heart, liver or bone). For this purpose, the stem cells most successfully employed so far are adult tissue-derived stem cells isolated mainly from bone marrow, mobilized peripheral blood, umbilical cord blood, fat tissue, and even myocardial biopsies. At the same time, attempts to employ embryonic stem cells and induced pluripotent stem cells in the clinic have failed due to their genomic instability and the risk of tumor formation. In this review, we will discuss the various potential sources of stem cells that are currently employed in regenerative medicine and the mechanisms that explain their beneficial effects. We will also highlight the preliminary results of clinical trials as well as the emerging problems relating to stem cell therapies in cardiology.

Reboun, M., et al. (2016). "X-Chromosome Inactivation Analysis in Different Cell Types and Induced Pluripotent Stem Cells Elucidates the Disease Mechanism in a Rare Case of Mucopolysaccharidosis Type II in a Female." Folia Biol (Praha) **62**(2): 82-89.

Mucopolysaccharidosis type II (MPS II) is an X-linked lysosomal storage disorder resulting from deficiency of iduronate-2-sulphatase activity. The disease manifests almost exclusively in males; only 16 symptomatic heterozygote girls have been reported so far. We describe the results of X-chromosome inactivation analysis in a 5-year-old girl with clinically severe disease and heterozygous mutation p.Arg468Gln in the IDS gene. X inactivation analysed at three X-chromosome loci showed extreme skewing (96/4 to

99/1) in two patient's cell types. This finding correlated with exclusive expression of the mutated allele. Induced pluripotent stem cells (iPSC) generated from the patient's peripheral blood demonstrated characteristic pluripotency markers, deficiency of enzyme activity, and mutation in the IDS gene. These cells were capable of differentiation into other cell types (cardiomyocytes, neurons). In MPS II iPSC clones, the X inactivation ratio remained highly skewed in culture conditions that led to partial X inactivation reset in Fabry disease iPSC clones. Our data, in accordance with the literature, suggest that extremely skewed X inactivation favouring the mutated allele is a crucial condition for manifestation of MPS II in females. This suggests that the X inactivation status and enzyme activity have a prognostic value and should be used to evaluate MPS II in females. For the first time, we show generation of iPSC from a symptomatic MPS II female patient that can serve as a cellular model for further research of the pathogenesis and treatment of this disease.

Reddy, G. P., et al. (1997). "Cell cycle analysis and synchronization of pluripotent hematopoietic progenitor stem cells." Blood **90**(6): 2293-2299.

Hematopoietic stem cells purified from mouse bone marrow are quiescent with less than 2% of Lin-Hoechst(low)/Rhodamine(low) (Lin-Ho(low)/Rho(low)) and 10% to 15% of Lin-/Sca+ cells in S phase. These cells enter proliferative cycle and progress through G1 and into S phase in the presence of cytokines and 5% heat-inactivated fetal calf serum (HI-FCS). Cytokine-stimulated Lin- Ho(low)/Rho(low) cells took 36 to 40 hours to complete first division and only 12 hours to complete each of 5 subsequent divisions. These cells require 16 to 18 hours to transit through G0/G1 period and 28 to 30 hours to enter into mid-S phase during the first cycle. Up to 56% of Lin-Rho(low)/Ho(low) cells are high-proliferative potential (7 factor-responsive) colony-forming cells (HPP-CFC). At isolation, HPP-CFC are quiescent, but after 28 to 30 hours of culture, greater than 60% are in S phase. Isoleucine-deprivation of Lin- Ho(low)/Rho(low) cells in S phase of first cycle reversibly blocked them from entering into second cycle. After the release from isoleucine-block, these cells exhibited a G1 period of less than 2 hours and entered into mid-S phase by 12 hours. Thus, the duration of G1 phase of the cells in second cycle is 4 to 5 times shorter than that observed in their first cycle. Similar cell cycle kinetics are observed with Lin-/Sca+ population of bone marrow cells. Stem cell factor (SCF) alone, in the presence of HI-FCS, is as effective as a cocktail of 2 to 7 cytokines in inducing quiescent Lin-/Sca+ cells to enter into proliferative cycle. Aphidicolin treatment reversibly blocked cytokine-stimulated Lin-/Sca+ cells at G1/S boundary, allowing their tight synchrony as they progress through first S phase and enter into second G1. For these cells also, SCF alone is sufficient for their progression through S phase. These studies indicate a very short G1 phase for stem cells induced to proliferate and offer experimental approaches to synchronize murine hematopoietic stem cells.

Rezanejad, H. and M. M. Matin (2012). "Induced pluripotent stem cells: progress and future perspectives in the stem cell world." Cell Reprogram **14**(6): 459-470.

Pluripotent stem cells (PSCs) have the potential to differentiate into many cell types and therefore can be a valuable source for cell therapy. Embryonic stem cells (ESCs), which are derived from the inner cell mass (ICM) of the blastocyst, are representative of PSCs. However, use of these cells has some limitations, especially ethical restrictions and immune response. As a result, researchers have been looking for other cell sources or strategies to overcome these limitations. One kind of cellular reprogramming is the process of guiding mature cells into a state of gene expression similar to PSCs. It has been demonstrated that somatic cells can be reprogrammed by various methods, including somatic cell nuclear transfer (SCNT) and cell fusion with ESCs or treatment with their extracts. This implies that some factors in oocytes and ESCs are able to initiate the reprogramming process. Accordingly, induced pluripotent stem cells (iPSCs) have been derived from somatic cells by ectopic expression of some transcription factors. This discovery has resulted in raising several important questions about the mechanisms by which these factors influence the reprogramming and epigenetic status of the cells. iPSCs hold great promise for regenerative medicine, developmental biology, and drug discovery because they circumvent problems associated with both ethical issues and immunological rejection. Here we review the experiments involved in the discovery of iPSCs, important factors in their reprogramming, and their future perspectives in cell therapy.

Rim, Y. A., et al. (2016). "Induced Pluripotent Stem Cell Generation from Blood Cells Using Sendai Virus and Centrifugation." J Vis Exp(118).

The recent development of human induced pluripotent stem cells (hiPSCs) proved that mature somatic cells can return to an undifferentiated, pluripotent state. Now, reprogramming is done with various types of adult somatic cells: keratinocytes, urine cells, fibroblasts, etc. Early experiments were usually done with dermal fibroblasts. However, this required an invasive surgical procedure to obtain fibroblasts from the patients. Therefore, suspension cells, such as blood and urine cells, were considered

ideal for reprogramming because of the convenience of obtaining the primary cells. Here, we report an efficient protocol for iPSC generation from peripheral blood mononuclear cells (PBMCs). By plating the transduced PBMCs serially to a new, matrix-coated plate using centrifugation, this protocol can easily provide iPSC colonies. This method is also applicable to umbilical cord blood mononuclear cells (CBMCs). This study presents a simple and efficient protocol for the reprogramming of PBMCs and CBMCs.

Roese-Koerner, B., et al. (2013). "Pluripotent stem cell-derived somatic stem cells as tool to study the role of microRNAs in early human neural development." Curr Mol Med **13**(5): 707-722.

The in vitro differentiation of human pluripotent stem cells represents a convenient approach to generate large numbers of neural cells for basic and translational research. We recently described the derivation of homogeneous populations of long-term self-renewing neuroepithelial-like stem cells from human pluripotent stem cells (lt-NES(R) cells). These cells constitute a suitable source of neural stem cells for in vitro modelling of early human neural development. Recent evidence demonstrates that microRNAs are important regulators of stem cells and nervous system development. Studies in several model organisms suggest that microRNAs contribute to different stages of neurogenesis - from progenitor self-renewal to survival and function of differentiated neurons. However, the understanding of the impact of microRNA-based regulation in human neural development is still at its dawn. Here, we give an overview on the current state of microRNA biology in stem cells and neural development and examine the role of the neural-associated miR-124, miR- 125b and miR-9/9* in human lt-NES(R) cells. We show that overexpression of miR-124, as well as overexpression of miR-125b, impair lt-NES(R) cell self-renewal and induce differentiation into neurons. Overexpression of the miR-9/9* locus also impairs self-renewal of lt-NES(R) cells and supports their commitment to neuronal differentiation. A detailed examination revealed that overexpression of miR-9 promotes differentiation, while overexpression of miR-9* affects both proliferation and differentiation of lt-NES(R) cells. This work provides insights into the regulation of early human neuroepithelial cells by microRNAs and highlights the potential of controlling differentiation of human stem cells by modulating the expression of selected microRNAs.

Ronaghi, M., et al. (2010). "Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells?" Stem Cells **28**(1): 93-99.

Spinal cord injury (SCI) causes myelopathy, damage to white matter, and myelinated fiber tracts that carry sensation and motor signals to and from the brain. The gray matter damage causes segmental losses of interneurons and motoneurons and restricts therapeutic options. Recent advances in stem cell biology, neural injury, and repair, and the progress toward development of neuroprotective and regenerative interventions are the basis for increased optimism. This review summarizes the pathophysiological mechanisms following SCI and compares human embryonic, adult neural, and the induced pluripotent stem cell-based therapeutic strategies for SCI.

Rossbach, B., et al. (2016). "Generation of a human induced pluripotent stem cell line from urinary cells of a healthy donor using an integration free vector." Stem Cell Res **16**(2): 314-317.

We have generated a human induced pluripotent stem cell (iPSC) line derived from urinary cells of a 30 year old healthy female donor. The cells were reprogrammed using a non-integrating viral vector and have shown full differentiation potential. Together with the iPSC-line, the donor provided blood cells for the study of immunological effects of the iPSC line and its derivatives in autologous and allogeneic settings. The line is available and registered in the human pluripotent stem cell registry as BCRTi004-A.

Rossbach, B., et al. (2016). "WITHDRAWN: Generation of a human induced pluripotent stem cell line from urinary cells of a healthy donor using integration free Sendai technology." Stem Cell Res **16**(1): 133-136.

The Publisher regrets that this article is an accidental duplication of an article that has already been published in Stem Cell Res., 16 (2016) 133-136, http://dx.doi.org/10.1016/j.scr.2015.12.021. The duplicate article has therefore been withdrawn. The full Elsevier Policy on Article Withdrawal can be found at http://www.elsevier.com/locate/withdrawalpolicy.

Rossbach, B., et al. (2017). "Generation of a human induced pluripotent stem cell line from urinary cells of a healthy donor using integration free Sendai virus technology." Stem Cell Res **21**: 167-170.

We have generated a human induced pluripotent stem cell (iPSC) line derived from urinary cells of a 28year old healthy female donor. The cells were reprogrammed using a non-integrating viral vector and have shown full differentiation potential. Together with the iPSC line, the donor provided blood cells for the study of immunological effects of the iPSC line and its derivatives in autologous and allogeneic

settings. The line is available and registered in the human pluripotent stem cell registry as BCRTi005-A.

Rouhani, F. J., et al. (2016). "Mutational History of a Human Cell Lineage from Somatic to Induced Pluripotent Stem Cells." PLoS Genet **12**(4): e1005932.

The accuracy of replicating the genetic code is fundamental. DNA repair mechanisms protect the fidelity of the genome ensuring a low error rate between generations. This sustains the similarity of individuals whilst providing a repertoire of variants for evolution. The mutation rate in the human genome has recently been measured to be 50-70 de novo single nucleotide variants (SNVs) between generations. During development mutations accumulate in somatic cells so that an organism is a mosaic. However, variation within a tissue and between tissues has not been analysed. By reprogramming somatic cells into induced pluripotent stem cells (iPSCs), their genomes and the associated mutational history are captured. By sequencing the genomes of polyclonal and monoclonal somatic cells and derived iPSCs we have determined the mutation rates and show how the patterns change from a somatic lineage in vivo through to iPSCs. Somatic cells have a mutation rate of 14 SNVs per cell per generation while iPSCs exhibited a ten-fold lower rate. Analyses of mutational signatures suggested that deamination of methylated cytosine may be the major mutagenic source in vivo, whilst oxidative DNA damage becomes dominant in vitro. Our results provide insights for better understanding of mutational processes and lineage relationships between human somatic cells. Furthermore it provides a foundation for interpretation of elevated mutation rates and patterns in cancer.

Rubach, M., et al. (2014). "Mesenchymal stem cells and their conditioned medium improve integration of purified induced pluripotent stem cell-derived cardiomyocyte clusters into myocardial tissue." Stem Cells Dev **23**(6): 643-653.

Induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) might become therapeutically relevant to regenerate myocardial damage. Purified iPS-CMs exhibit poor functional integration into myocardial tissue. The aim of this study was to investigate whether murine mesenchymal stem cells (MSCs) or their conditioned medium (MScond) improves the integration of murine iPS-CMs into myocardial tissue. Vital or nonvital embryonic murine ventricular tissue slices were cocultured with purified clusters of iPS-CMs in combination with murine embryonic fibroblasts (MEFs), MSCs, or MScond. Morphological integration was assessed by visual scoring and functional integration by isometric force and field potential measurements. We observed a moderate morphological integration of iPS-CM clusters into vital, but a poor integration into nonvital, slices. MEFs and MSCs but not MScond improved morphological integration of CMs into nonvital slices and enabled purified iPS-CMs to confer force. Coculture of vital slices with iPS-CMs and MEFs or MSCs resulted in an improved electrical integration. A comparable improvement of electrical coupling was achieved with the cell-free MScond, indicating that soluble factors secreted by MSCs were involved in electrical coupling. We conclude that cells such as MSCs support the engraftment and adhesion of CMs, and confer force to noncontractile tissue. Furthermore, soluble factors secreted by MSCs mediate electrical coupling of purified iPS-CM clusters to myocardial tissue. These data suggest that MSCs may increase the functional engraftment and therapeutic efficacy of transplanted iPS-CMs into infarcted myocardium.

Rufaihah, A. J., et al. (2013). "Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity." Am J Transl Res **5**(1): 21-35.

Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) are promising for treatment of vascular diseases. However, hiPSC-ECs purified based on CD31 expression are comprised of arterial, venous, and lymphatic subtypes. It is unclear whether hiPSC-ECs are heterogeneous in nature, and whether there may be functional benefits of enriching for specific subtypes. Therefore, we sought to characterize the hiPSC-ECs and enrich for each subtype, and demonstrate whether such enrichment would have functional significance. The hiPSC-ECs were generated from differentiation of hiPSCs using vascular endothelial growth factor (VEGF)-A and bone morphogenetic protein-4. The hiPSC-ECs were purified based on positive expression of CD31. Subsequently, we sought to enrich for each subtype. Arterial hiPSC-ECs were induced using higher concentrations of VEGF-A and 8-bromoadenosine-3':5'-cyclic monophosphate in the media, whereas lower concentrations of VEGF-A favored venous subtype. VEGF-C and angiopoietin-1 promoted the expression of lymphatic phenotype. Upon FACS purification based on CD31+ expression, the hiPSC-EC population was observed to display typical endothelial surface markers and functions. However, the hiPSC-EC population was heterogeneous in that they displayed arterial, venous, and to a lesser degree, lymphatic lineage markers. Upon comparing vascular formation in matrigel plugs in vivo, we observed that arterial enriched hiPSC-ECs formed a more extensive capillary network in this model, by comparison to a heterogeneous population of hiPSC-ECs. This study demonstrates that FACS purification of CD31+ hiPSC-ECs produces a diverse population of ECs. Refining

the differentiation methods can enrich for subtype-specific hiPSC-ECs with functional benefits of enhancing neovascularization.

Ruiz, S., et al. (2015). "Limiting replication stress during somatic cell reprogramming reduces genomic instability in induced pluripotent stem cells." Nat Commun **6**: 8036.

The generation of induced pluripotent stem cells (iPSC) from adult somatic cells is one of the most remarkable discoveries in recent decades. However, several works have reported evidence of genomic instability in iPSC, raising concerns on their biomedical use. The reasons behind the genomic instability observed in iPSC remain mostly unknown. Here we show that, similar to the phenomenon of oncogene-induced replication stress, the expression of reprogramming factors induces replication stress. Increasing the levels of the checkpoint kinase 1 (CHK1) reduces reprogramming-induced replication stress and increases the efficiency of iPSC generation. Similarly, nucleoside supplementation during reprogramming reduces the load of DNA damage and genomic rearrangements on iPSC. Our data reveal that lowering replication stress during reprogramming, genetically or chemically, provides a simple strategy to reduce genomic instability on mouse and human iPSC.

Rungsiwiwut, R., et al. (2016). "Human Umbilical Cord Blood-Derived Serum for Culturing the Supportive Feeder Cells of Human Pluripotent Stem Cell Lines." Stem Cells Int **2016**: 4626048.

Although human pluripotent stem cells (hPSCs) can proliferate robustly on the feeder-free culture system, genetic instability of hPSCs has been reported in such environment. Alternatively, feeder cells enable hPSCs to maintain their pluripotency. The feeder cells are usually grown in a culture medium containing fetal bovine serum (FBS) prior to coculture with hPSCs. The use of FBS might limit the clinical application of hPSCs. Recently, human cord blood-derived serum (hUCS) showed a positive effect on culture of mesenchymal stem cells. It is interesting to test whether hUCS can be used for culture of feeder cells of hPSCs. This study was aimed to replace FBS with hUCS for culturing the human foreskin fibroblasts (HFFs) prior to feeder cell preparation. The results showed that HFFs cultured in hUCS-containing medium (HFF-hUCS) displayed fibroblastic features, high proliferation rates, short population doubling times, and normal karyotypes after prolonged culture. Inactivated HFF-hUCS expressed important genes, including Activin A, FGF2, and TGFbeta1, which have been implicated in the maintenance of hPSC pluripotency. Moreover, hPSC lines maintained pluripotency, differentiation capacities, and karyotypic stability after being cocultured for extended period with inactivated HFF-hUCS. Therefore, the results demonstrated the benefit of hUCS for hPSCs culture system.

Saadai, P., et al. (2013). "Human induced pluripotent stem cell-derived neural crest stem cells integrate into the injured spinal cord in the fetal lamb model of myelomeningocele." J Pediatr Surg **48**(1): 158-163.

BACKGROUND/PURPOSE: Neurological function in patients with myelomeningocele (MMC) is limited even after prenatal repair. Neural crest stem cells (NCSCs) can improve neurological function in models of spinal cord injury. We aimed to evaluate the survival, integration, and differentiation of human NCSCs derived from induced pluripotent stem cells (iPSC-NCSCs) in the fetal lamb model of MMC. METHODS: Human iPSCs derived from skin fibroblasts were differentiated into NCSCs in vitro, mixed with hydrogel, and seeded on nanofibrous scaffolds for surgical transplantation. Fetal lambs (n=2) underwent surgical MMC creation and repair with iPSC-NCSC seeded scaffolds. Gross necropsy and immunohistochemistry were performed at term. RESULTS: IPSC-NCSCs expressed NCSC markers, maintained > 95% viability, and demonstrated neuronal differentiation in vitro. Immunohistochemical analysis of repaired spinal cords thirty days after transplantation demonstrated the co-localization of human nuclear mitotic apparatus protein (NuMA) and Neurofilament M subunit (NFM) in the area of spinal cord injury. No gross tumors were identified. CONCLUSIONS: Human iPSC-NCSCs survived, integrated, and differentiated into neuronal lineage in the fetal lamb model of MMC. This is the first description of human stem cell engraftment in a model of fetal MMC and supports the concept of using NCSCs to address spinal cord damage in MMC.

Sakiyama, R., et al. (2017). "Clinical translation of bioartificial liver support systems with human pluripotent stem cell-derived hepatic cells." World J Gastroenterol **23**(11): 1974-1979.

There is currently a pressing need for alternative therapies to liver transplantation. The number of patients waiting for a liver transplant is substantially higher than the number of transplantable donor livers, resulting in a long waiting time and a high waiting list mortality. An extracorporeal liver support system is one possible approach to overcome this problem. However, the ideal cell source for developing bioartificial liver (BAL) support systems has yet to be determined. Recent advancements in stem cell technology allow researchers to generate highly functional hepatocyte-like cells from human pluripotent stem cells (hPSCs). In this mini-review, we

summarize previous clinical trials with different BAL systems, and discuss advantages of and potential obstacles to utilizing hPSC-derived hepatic cells in clinical-scale BAL systems.

Sakurai, F., et al. (2017). "Human induced-pluripotent stem cell-derived hepatocyte-like cells as an in vitro model of human hepatitis B virus infection." Sci Rep **7**: 45698.

In order to understand the life cycle of hepatitis B virus (HBV) and to develop efficient anti-HBV drugs, a useful in vitro cell culture system which allows HBV infection and recapitulates virus-host interactions is essential; however, pre-existing in vitro HBV infection models are often problematic. Here, we examined the potential of human induced-pluripotent stem (iPS) cell-derived hepatocyte-like cells (iPS-HLCs) as an in vitro HBV infection model. Expression levels of several genes involved in HBV infection, including the sodium taurocholate cotransporting polypeptide (NTCP) gene, were gradually elevated as the differentiation status of human iPS cells proceeded to iPS-HLCs. The mRNA levels of these genes were comparable between primary human hepatocytes (PHHs) and iPS-HLCs. Following inoculation with HBV, we found significant production of HBV proteins and viral RNAs in iPS-HLCs. The three major forms of the HBV genome were detected in iPS-HLCs by Southern blotting analysis. Anti-HBV agents entecavir and Myrcludex-B, which are a nucleoside analogue reverse transcriptase inhibitor and a synthetic pre-S1 peptide, respectively, significantly inhibited HBV infection in iPS-HLCs. These data demonstrate that iPS-HLCs can be used as a promising in vitro HBV infection model.

Salewski, R. P., et al. (2010). "Are induced pluripotent stem cells the future of cell-based regenerative therapies for spinal cord injury?" J Cell Physiol **222**(3): 515-521.

Despite advances in medical and surgical care, current clinical therapies for spinal cord injury (SCI) are limited. During the last two decades, the search for new therapies has been revolutionized by the discovery of stem cells, inspiring scientists and clinicians to search for stem cell-based reparative approaches for many disorders, including neurotrauma. Cell-based therapies using embryonic and adult stem cells in animal models of these disorders have provided positive outcome results. However, the availability of clinically suitable cell sources for human application has been hindered by both technical and ethical issues. The recent discovery of induced pluripotent stem (iPS) cells holds the potential to revolutionize the field of regenerative medicine by offering the option of autologous transplantation, thus eliminating the issue of host rejection. Herein, we will provide the rationale for the use of iPS cells in SCI therapies. In this review, we will evaluate the recent advancements in the field of iPS cells including their capacity for differentiation toward neural lineages that may allow iPS cells transplantation in cell-based therapy for spinal cord repair.

Salewski, R. P., et al. (2015). "Transplantation of Induced Pluripotent Stem Cell-Derived Neural Stem Cells Mediate Functional Recovery Following Thoracic Spinal Cord Injury Through Remyelination of Axons." Stem Cells Transl Med **4**(7): 743-754.

UNLABELLED: : Neural stem cells (NSCs) from embryonic or fetal/adult tissue sources have shown considerable promise in regenerative strategies for traumatic spinal cord injury (SCI). However, there are limitations with their use related to the availability, immunogenicity, and uncertainty of the mechanisms involved. To address these issues, definitive NSCs derived from induced pluripotent stem (iPS) cells generated using a nonviral, piggyBac transposon approach, were investigated. Committed NSCs were generated from iPS cells using a free-floating neurosphere methodology previously described by our laboratory. To delineate the mechanism of action, specifically the role of exogenous myelination, NSCs derived from wildtype (wt) and nonmyelinating Shiverer (shi) iPS cell lines were used following thoracic SCI with subacute intraspinal transplantation. Behavioral, histological, and electrophysiological outcomes were analyzed to assess the effectiveness of this treatment. The wt- and shi-iPS-NSCs were validated and shown to be equivalent except in myelination capacity. Both iPS-NSC lines successfully integrated into the injured spinal cord and predominantly differentiated to oligodendrocytes, but only the wt-iPS-NSC treatment resulted in a functional benefit. The wt-iPS-dNSCs, which exhibited the capacity for remyelination, significantly improved neurobehavioral function (Basso Mouse Scale and CatWalk), histological outcomes, and electrophysiological measures of axonal function (sucrose gap analysis) compared with the nonmyelinating iPS-dNSCs and cell-free controls. In summary, we demonstrated that iPS cells can generate translationally relevant NSCs for applications in SCI. Although NSCs have a diverse range of functions in the injured spinal cord, remyelination is the predominant mechanism of recovery following thoracic SCI. SIGNIFICANCE: Gain-of-function/loss-of-function techniques were used to examine the mechanistic importance of graft-derived remyelination following thoracic spinal cord injury (SCI). The novel findings of this study include the first use of neural stem cells (NSCs) from induced pluripotent stem cells

(iPSCs) derived using the clonal neurosphere expansion conditions, for the treatment of SCI, the first characterization and in vivo application of iPSCs from Shiverer mouse fibroblasts, and the first evidence of the importance of remyelination by pluripotent-sourced NSCs for SCI repair and regeneration.

Salomonis, N., et al. (2016). "Integrated Genomic Analysis of Diverse Induced Pluripotent Stem Cells from the Progenitor Cell Biology Consortium." Stem Cell Reports **7**(1): 110-125.

The rigorous characterization of distinct induced pluripotent stem cells (iPSC) derived from multiple reprogramming technologies, somatic sources, and donors is required to understand potential sources of variability and downstream potential. To achieve this goal, the Progenitor Cell Biology Consortium performed comprehensive experimental and genomic analyses of 58 iPSC from ten laboratories generated using a variety of reprogramming genes, vectors, and cells. Associated global molecular characterization studies identified functionally informative correlations in gene expression, DNA methylation, and/or copy-number variation among key developmental and oncogenic regulators as a result of donor, sex, line stability, reprogramming technology, and cell of origin. Furthermore, X-chromosome inactivation in PSC produced highly correlated differences in teratoma-lineage staining and regulator expression upon differentiation. All experimental results, and raw, processed, and metadata from these analyses, including powerful tools, are interactively accessible from a new online portal at https://www.synapse.org to serve as a reusable resource for the stem cell community.

Sambathkumar, R., et al. (2018). "Pluripotent Stem Cell-Derived Pancreatic Progenitors and beta-Like Cells for Type 1 Diabetes Treatment." Physiology (Bethesda) **33**(6): 394-402.

In this review, we focus on the processes guiding human pancreas development and provide an update on methods to efficiently generate pancreatic progenitors (PPs) and beta-like cells in vitro from human pluripotent stem cells (hPSCs). Furthermore, we assess the strengths and weaknesses of using PPs and beta-like cell for cell replacement therapy for the treatment of Type 1 diabetes with respect to cell manufacturing, engrafting, functionality, and safety. Finally, we discuss the identification and use of specific cell surface markers to generate safer populations of PPs for clinical translation and to study the development of PPs in vivo and in vitro.

Sanchez-Freire, V., et al. (2014). "Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells." J Am Coll Cardiol **64**(5): 436-448.

BACKGROUND: Human-induced pluripotent stem cells (iPSCs) are a potentially unlimited source for generation of cardiomyocytes (iPSC-CMs). However, current protocols for iPSC-CM derivation face several challenges, including variability in somatic cell sources and inconsistencies in cardiac differentiation efficiency. OBJECTIVES: This study aimed to assess the effect of epigenetic memory on differentiation and function of iPSC-CMs generated from somatic cell sources of cardiac versus noncardiac origins. METHODS: Cardiac progenitor cells (CPCs) and skin fibroblasts from the same donors were reprogrammed into iPSCs and differentiated into iPSC-CMs via embryoid body and monolayer-based differentiation protocols. RESULTS: Differentiation efficiency was found to be higher in CPC-derived iPSC-CMs (CPC-iPSC-CMs) than in fibroblast-derived iPSC-CMs (Fib-iPSC-CMs). Gene expression analysis during cardiac differentiation demonstrated up-regulation of cardiac transcription factors in CPC-iPSC-CMs, including NKX2-5, MESP1, ISL1, HAND2, MYOCD, MEF2C, and GATA4. Epigenetic assessment revealed higher methylation in the promoter region of NKX2-5 in Fib-iPSC-CMs compared with CPC-iPSC-CMs. Epigenetic differences were found to dissipate with increased cell passaging, and a battery of in vitro assays revealed no significant differences in their morphological and electrophysiological properties at early passage. Finally, cell delivery into a small animal myocardial infarction model indicated that CPC-iPSC-CMs and Fib-iPSC-CMs possess comparable therapeutic capabilities in improving functional recovery in vivo. CONCLUSIONS: This is the first study to compare differentiation of iPSC-CMs from human CPCs versus human fibroblasts from the same donors. The authors demonstrate that although epigenetic memory improves differentiation efficiency of cardiac versus noncardiac somatic cell sources in vitro, it does not contribute to improved functional outcome in vivo.

Santosa, M. M., et al. (2015). "Knowledge Gaps in Rodent Pancreas Biology: Taking Human Pluripotent Stem Cell-Derived Pancreatic Beta Cells into Our Own Hands." Front Endocrinol (Lausanne) **6**: 194.

In the field of stem cell biology and diabetes, we and others seek to derive mature and functional human pancreatic beta cells for disease modeling and cell replacement therapy. Traditionally, knowledge gathered from rodents is extended to human pancreas developmental biology research involving human pluripotent stem cells (hPSCs). While much has been learnt from rodent pancreas biology in the early steps toward Pdx1(+) pancreatic progenitors, much less is

known about the transition toward Ngn3(+) pancreatic endocrine progenitors. Essentially, the later steps of pancreatic beta cell development and maturation remain elusive to date. As a result, the most recent advances in the stem cell and diabetes field have relied upon combinatorial testing of numerous growth factors and chemical compounds in an arbitrary trial-and-error fashion to derive mature and functional human pancreatic beta cells from hPSCs. Although this hit-or-miss approach appears to have made some headway in maturing human pancreatic beta cells in vitro, its underlying biology is vaguely understood. Therefore, in this mini-review, we discuss some of these late-stage signaling pathways that are involved in human pancreatic beta cell differentiation and highlight our current understanding of their relevance in rodent pancreas biology. Our efforts here unravel several novel signaling pathways that can be further studied to shed light on unexplored aspects of rodent pancreas biology. New investigations into these signaling pathways are expected to advance our knowledge in human pancreas developmental biology and to aid in the translation of stem cell biology in the context of diabetes treatments.

Sarafian, R., et al. (2018). "Monitoring cell line identity in collections of human induced pluripotent stem cells." Stem Cell Res **28**: 66-70.

The ability to reprogram somatic cells into induced pluripotent stem cells (hiPSCs) has led to the generation of large collections of cell lines from thousands of individuals with specific phenotypes, many of which will be shared among different research groups as invaluable tools for biomedical research. As hiPSC-based research involves extensive culture of many cell lines, the issue periodic cell line identification is particularly important to ensure that cell line identity remains accurate. Here we analyzed the different commercially available genotyping methods considering ease of in-house genotyping, cost and informativeness, and applied one of them in our workflow for hiPSC generation. We show that the chosen STR method was able to establish a unique DNA profile for each of the 35 individuals/hiPSC lines at the examined sites, as well as identify two discrepancies resulting from inadvertently exchanged samples. Our results highlight the importance of hiPSC line genotyping by an in-house method that allows periodic cell line identification and demonstrate that STR is a useful approach to supplement less frequent karyotyping and epigenetic evaluations.

Sasaki, K., et al. (2015). "Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells." Cell Stem Cell **17**(2): 178-194.

Mechanisms underlying human germ cell development are unclear, partly due to difficulties in studying human embryos and lack of suitable experimental systems. Here, we show that human induced pluripotent stem cells (hiPSCs) differentiate into incipient mesoderm-like cells (iMeLCs), which robustly generate human primordial germ cell-like cells (hPGCLCs) that can be purified using the surface markers EpCAM and INTEGRINalpha6. The transcriptomes of hPGCLCs and primordial germ cells (PGCs) isolated from non-human primates are similar, and although specification of hPGCLCs and mouse PGCs rely on similar signaling pathways, hPGCLC specification transcriptionally activates germline fate without transiently inducing eminent somatic programs. This includes genes important for naive pluripotency and repression of key epigenetic modifiers, concomitant with epigenetic reprogramming. Accordingly, BLIMP1, which represses somatic programs in mice, activates and stabilizes a germline transcriptional circuit and represses a default neuronal differentiation program. Together, these findings provide a foundation for understanding and reconstituting human germ cell development in vitro.

Sato, M., et al. (2017). "Regeneration of cervical reserve cell-like cells from human induced pluripotent stem cells (iPSCs): A new approach to finding targets for cervical cancer stem cell treatment." Oncotarget **8**(25): 40935-40945.

Cervical reserve cells are epithelial progenitor cells that are pathologically evident as the origin of cervical cancer. Thus, investigating the characteristics of cervical reserve cells could yield insight into the features of cervical cancer stem cells (CSCs). In this study, we established a method for the regeneration of cervical reserve cell-like properties from human induced pluripotent stem cells (iPSCs) and named these cells induced reserve cell-like cells (iRCs). Approximately 70% of iRCs were positive for the reserve cell markers p63, CK5 and CK8. iRCs also expressed the SC junction markers CK7, AGR2, CD63, MMP7 and GDA. While iRCs expressed neither ERalpha nor ERbeta, they expressed CA125. These data indicated that iRCs possessed characteristics of cervical epithelial progenitor cells. iRCs secreted higher levels of several inflammatory cytokines such as macrophage migration inhibitory factor (MIF), soluble intercellular adhesion molecule 1 (sICAM-1) and C-X-C motif ligand 10 (CXCL-10) compared with normal cervical epithelial cells. iRCs also expressed human leukocyte antigen-G (HLA-G), which is an important cell-surface antigen for immune tolerance and carcinogenesis. Together with the fact that cervical CSCs can originate from reserve cells, our data suggested that iRCs were potent immune modulators

that might favor cervical cancer cell survival. In conclusion, by generating reserve cell-like properties from iPSCs, we provide a new approach that may yield new insight into cervical cancer stem cells and help find new oncogenic targets.

Schmeckebier, S., et al. (2013). "Keratinocyte growth factor and dexamethasone plus elevated cAMP levels synergistically support pluripotent stem cell differentiation into alveolar epithelial type II cells." Tissue Eng Part A **19**(7-8): 938-951.

Alveolar epithelial type II (ATII)-like cells can be generated from murine embryonic stem cells (ESCs), although to date, no robust protocols applying specific differentiation factors are established. We hypothesized that the keratinocyte growth factor (KGF), an important mediator of lung organogenesis and primary ATII cell maturation and proliferation, together with dexamethasone, 8-bromoadenosine-cAMP, and isobutylmethylxanthine (DCI), which induce maturation of primary fetal ATII cells, also support the alveolar differentiation of murine ESCs. Here we demonstrate that the above stimuli synergistically potentiate the alveolar differentiation of ESCs as indicated by increased expression of the surfactant proteins (SP-) C and SP-B. This effect is most profound if KGF is supplied not only in the late stage, but at least also during the intermediate stage of differentiation. Our results indicate that KGF most likely does not enhance the generation of (mes)endodermal or NK2 homeobox 1 (Nkx2.1) expressing progenitor cells but rather, supported by DCI, accelerates further differentiation/maturation of respiratory progeny in the intermediate phase and maturation/proliferation of emerging ATII cells in the late stage of differentiation. Ultrastructural analyses confirmed the presence of ATII-like cells with intracellular composite and lamellar bodies. Finally, induced pluripotent stem cells (iPSCs) were generated from transgenic mice with ATII cell-specific lacZ reporter expression. Again, KGF and DCI synergistically increased SP-C and SP-B expression in iPSC cultures, and lacZ expressing ATII-like cells developed. In conclusion, ATII cell-specific reporter expression enabled the first reliable proof for the generation of murine iPSC-derived ATII cells. In addition, we have shown KGF and DCI to synergistically support the generation of ATII-like cells from ESCs and iPSCs. Combined application of these factors will facilitate more efficient generation of stem cell-derived ATII cells for future basic research and potential therapeutic application.

Schneider, R. P., et al. (2013). "TRF1 is a stem cell marker and is essential for the generation of induced pluripotent stem cells." Nat Commun **4**: 1946.

TRF1 is a component of the shelterin complex that protects chromosome ends. TRF1 deficiency leads to early embryonic lethality and to severe organ atrophy when deleted in adult tissues. Here we generate a reporter mouse carrying a knock-in eGFP-TRF1 fusion allele to study the role of TRF1 in stem cell biology and tissue homeostasis. We find that eGFP-TRF1 expression in mice is maximal in known adult stem cell compartments and show that TRF1 ensures their functionality. eGFP-TRF1 is highly expressed in induced pluripotent stem cells, uncoupled from the telomere elongation associated with reprogramming. Selection of eGFP-TRF1-high induced pluripotent stem cells correlates with higher pluripotency as indicated by their ability to form teratomas and chimeras. We further show that TRF1 is necessary for both induction and maintenance of pluripotency, and that TRF1 is a direct transcriptional target of Oct3/4.

Schopperle, W. M., et al. (2010). "The human cancer and stem cell marker podocalyxin interacts with the glucose-3-transporter in malignant pluripotent stem cells." Biochem Biophys Res Commun **398**(3): 372-376.

Podocalyxin, an integral plasma membrane cell-adhesion glycoprotein, is a marker of human pluripotent and multipotent stem cells. Podocalyxin is also a marker of many types of cancers and its expression correlates with an aggressive and poor-prognosis tumor phenotype. The function of podocalyxin in stem cells and malignant cells is unknown. Protein sequence data obtained from purified podocalyxin protein isolated from embryonal carcinoma cancer stem cells reveals peptide sequence data for the glucose-3-transporter. Protein-precipitation experiments of embryonal carcinoma protein extracts identify a podocalyxin/glucose-3-transporter protein complex. Cell imaging studies demonstrate co-localization of podocalyxin and glucose-3-transporter and confirm the interaction in vivo. Finally, siRNA podocalyxin-knockdown experiments show decreased expression levels of the glucose-3-transporter. These findings suggest a novel interaction of the glucose-3-transporter and the cell-adhesion protein podocalyxin. In pluripotent stem cells and in human cancer disease, podocalyxin may function in part to regulate and maintain the cell surface expression of the glucose-3-transporter.

Schwartz, R. E., et al. (2016). "Pluripotent Stem Cell-Derived Hepatocyte-like Cells: A Tool to Study Infectious Disease." Curr Pathobiol Rep **4**(3): 147-156.

Purpose of Review: Liver disease is an important clinical and global problem and is the 16th leading cause of death worldwide and responsible for 1 million deaths worldwide each year. Infectious disease

is a major cause of liver disease specifically and overall is even a greater cause of patient morbidity and mortality. Tools to study human liver disease and infectious disease have been lacking which has significantly hampered the study of liver disease generally and hepatotropic pathogens more specifically. Historically, hepatoma cell lines have been used for in vitro cell culture models to study infectious disease. Significant differences between human hepatoma cell lines and the human hepatocyte has hampered our understanding of hepatocyte pathogen infection and hepatocyte--pathogen interactions. Recent Findings: Despite these limitations, great progress was made in the understanding of specific aspects of the life cycle of the canonical hepatocyte viral pathogen, Hepatitis C Virus. Over time various specific drugs targeting various proteins of the HCV virion or aspects of the HCV viral life cycle have been created that enable almost complete elimination of the virus in vitro and clinically. These drugs, direct-acting antivirals have enabled achieving sustained virologic response in over 90-95 percent of patients. Summary: Despite the development of direct-acting antivirals and the extreme success in achieving sustained virologic response, there has only been limited success elucidating host-pathogen interactions largely due to the poor nature of the hepatoma platform. Alternative approaches are needed. Pluripotent stem cells are renewable, can be derived from a single donor and can be efficiently and reproducibly differentiated towards many cell types including ectodermal-, endodermal-, and mesodermal-derived lineages. The development of pluripotent stem cell-derived hepatocyte-like cells (iHLCS) changes the paradigm as robust cells with the phenotype and function of hepatocytes can be readily created on demand with a variety of genetic background or alterations. iHLCs are readily used as models to study human drug metabolism, human liver disease, and human hepatotropic infectious disease. In this review, we discuss the biology of the HCV virus, the use of iHLCs as models to study human liver disease, and review the current work on using iHLCs to study HCV infection.

Schwartz, R. E., et al. (2014). "Pluripotent stem cell-derived hepatocyte-like cells." Biotechnol Adv **32**(2): 504-513.

Liver disease is an important clinical problem, impacting over 30 million Americans and over 600 million people worldwide. It is the 12th leading cause of death in the United States and the 16th worldwide. Due to a paucity of donor organs, several thousand Americans die yearly while waiting for liver transplantation. Unfortunately, alternative tissue sources such as fetal hepatocytes and hepatic cell lines are unreliable, difficult to reproduce, and do not fully recapitulate hepatocyte phenotype and functions. As a consequence, alternative cell sources that do not have these limitations have been sought. Human embryonic stem (hES) cell- and induced pluripotent stem (iPS) cell-derived hepatocyte-like cells may enable cell based therapeutics, the study of the mechanisms of human disease and human development, and provide a platform for screening the efficacy and toxicity of pharmaceuticals. iPS cells can be differentiated in a step-wise fashion with high efficiency and reproducibility into hepatocyte-like cells that exhibit morphologic and phenotypic characteristics of hepatocytes. In addition, iPS-derived hepatocyte-like cells (iHLCs) possess some functional hepatic activity as they secrete urea, alpha-1-antitrypsin, and albumin. However, the combined phenotypic and functional traits exhibited by iHLCs resemble a relatively immature hepatic phenotype that more closely resembles that of fetal hepatocytes rather than adult hepatocytes. Specifically, iHLCs express fetal markers such as alpha-fetoprotein and lack key mature hepatocyte functions, as reflected by drastically reduced activity (~0.1%) of important detoxification enzymes (i.e. CYP2A6, CYP3A4). These key differences between iHLCs and primary adult human hepatocytes have limited the use of stem cells as a renewable source of functional adult hepatocytes for in vitro and in vivo applications. Unfortunately, the developmental pathways that control hepatocyte maturation from a fetal into an adult hepatocyte are poorly understood, which has hampered the field in its efforts to induce further maturation of iPS-derived hepatic lineage cells. This review analyzes recent developments in the derivation of hepatocyte-like cells, and proposes important points to consider and assays to perform during their characterization. In the future, we envision that iHLCs will be used as in vitro models of human disease, and in the longer term, provide an alternative cell source for drug testing and clinical therapy.

Schweizer, P. A., et al. (2017). "Subtype-specific differentiation of cardiac pacemaker cell clusters from human induced pluripotent stem cells." Stem Cell Res Ther **8**(1): 229.

BACKGROUND: Human induced pluripotent stem cells (hiPSC) harbor the potential to differentiate into diverse cardiac cell types. Previous experimental efforts were primarily directed at the generation of hiPSC-derived cells with ventricular cardiomyocyte characteristics. Aiming at a straightforward approach for pacemaker cell modeling and replacement, we sought to selectively differentiate cells with nodal-type properties. METHODS: hiPSC were differentiated into spontaneously beating clusters by co-culturing with visceral endoderm-like cells in a serum-free medium.

Subsequent culturing in a specified fetal bovine serum (FBS)-enriched cell medium produced a pacemaker-type phenotype that was studied in detail using quantitative real-time polymerase chain reaction (qRT-PCR), immunocytochemistry, and patch-clamp electrophysiology. Further investigations comprised pharmacological stimulations and co-culturing with neonatal cardiomyocytes. RESULTS: hiPSC co-cultured in a serum-free medium with the visceral endoderm-like cell line END-2 produced spontaneously beating clusters after 10-12 days of culture. The pacemaker-specific genes HCN4, TBX3, and TBX18 were abundantly expressed at this early developmental stage, while levels of sarcomeric gene products remained low. We observed that working-type cardiomyogenic differentiation can be suppressed by transfer of early clusters into a FBS-enriched cell medium immediately after beating onset. After 6 weeks under these conditions, sinoatrial node (SAN) hallmark genes remained at high levels, while working-type myocardial transcripts (NKX2.5, TBX5) were low. Clusters were characterized by regular activity and robust beating rates (70-90 beats/min) and were triggered by spontaneous Ca(2+) transients recapitulating calcium clock properties of genuine pacemaker cells. They were responsive to adrenergic/cholinergic stimulation and able to pace neonatal rat ventricular myocytes in co-culture experiments. Action potential (AP) measurements of cells individualized from clusters exhibited nodal-type (63.4%) and atrial-type (36.6%) AP morphologies, while ventricular AP configurations were not observed. CONCLUSION: We provide a novel culture media-based, transgene-free approach for targeted generation of hiPSC-derived pacemaker-type cells that grow in clusters and offer the potential for disease modeling, drug testing, and individualized cell-based replacement therapy of the SAN.

Sebastiano, V., et al. (2011). "In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases." Stem Cells **29**(11): 1717-1726.

The combination of induced pluripotent stem cell (iPSC) technology and targeted gene modification by homologous recombination (HR) represents a promising new approach to generate genetically corrected, patient-derived cells that could be used for autologous transplantation therapies. This strategy has several potential advantages over conventional gene therapy including eliminating the need for immunosuppression, avoiding the risk of insertional mutagenesis by therapeutic vectors, and maintaining expression of the corrected gene by endogenous control elements rather than a constitutive promoter. However, gene targeting in human pluripotent cells has remained challenging and inefficient. Recently, engineered zinc finger nucleases (ZFNs) have been shown to substantially increase HR frequencies in human iPSCs, raising the prospect of using this technology to correct disease causing mutations. Here, we describe the generation of iPSC lines from sickle cell anemia patients and in situ correction of the disease causing mutation using three ZFN pairs made by the publicly available oligomerized pool engineering method (OPEN). Gene-corrected cells retained full pluripotency and a normal karyotype following removal of reprogramming factor and drug-resistance genes. By testing various conditions, we also demonstrated that HR events in human iPSCs can occur as far as 82 bps from a ZFN-induced break. Our approach delineates a roadmap for using ZFNs made by an open-source method to achieve efficient, transgene-free correction of monogenic disease mutations in patient-derived iPSCs. Our results provide an important proof of principle that ZFNs can be used to produce gene-corrected human iPSCs that could be used for therapeutic applications.

Secher, J. O., et al. (2016). "Initial embryology and pluripotent stem cells in the pig--The quest for establishing the pig as a model for cell therapy." Theriogenology **85**(1): 162-171.

The quest for porcine pluripotent stem cells (PSCs) was initiated in the early 90s. Initially, it was the intention to benefit from these cells for production of genetically modified pigs using homologous recombination followed by derivation of chimeric offspring; a technology that has been used to produce genetically modified mice since the mid-80s. However, no convincing reports on the generation of bona fide porcine embryonic stem cells or embryonic germ cells resulted from these activities, and with the advent of somatic cell nuclear transfer during the late 90s, alternative methods for creating genetically modified pigs emerged. Over the past years, renewed interest in porcine PSCs has sparked activities in deriving in particular porcine induced pluripotent stem cells to develop the pig as a faithful model for studying the potentials and risks associated with induced pluripotent stem cell-based human therapy. Here, we review the recent data on establishment of porcine PSCs and the differences in embryonic development between pig and mouse, which may be underlying factors for the continuing challenge to culture and maintain porcine PSCs.

Seki, T., et al. (2014). "Generation and characterization of functional cardiomyocytes derived from human T cell-derived induced pluripotent stem cells." PLoS One **9**(1): e85645.

Induced pluripotent stem cells (iPSCs) have been proposed as novel cell sources for genetic disease models and revolutionary clinical therapies. Accordingly, human iPSC-derived cardiomyocytes are potential cell sources for cardiomyocyte transplantation therapy. We previously developed a novel generation method for human peripheral T cell-derived iPSCs (TiPSCs) that uses a minimally invasive approach to obtain patient cells. However, it remained unknown whether TiPSCs with genomic rearrangements in the T cell receptor (TCR) gene could differentiate into functional cardiomyocyte in vitro. To address this issue, we investigated the morphology, gene expression pattern, and electrophysiological properties of TiPSC-derived cardiomyocytes differentiated by floating culture. RT-PCR analysis and immunohistochemistry showed that the TiPSC-derived cardiomyocytes properly express cardiomyocyte markers and ion channels, and show the typical cardiomyocyte morphology. Multiple electrode arrays with application of ion channel inhibitors also revealed normal electrophysiological responses in the TiPSC-derived cardiomyocytes in terms of beating rate and the field potential waveform. In this report, we showed that TiPSCs successfully differentiated into cardiomyocytes with morphology, gene expression patterns, and electrophysiological features typical of native cardiomyocytes. TiPSCs-derived cardiomyocytes obtained from patients by a minimally invasive technique could therefore become disease models for understanding the mechanisms of cardiac disease and cell sources for revolutionary cardiomyocyte therapies.

Selekman, J. A., et al. (2016). "Generation of Epithelial Cell Populations from Human Pluripotent Stem Cells Using a Small-Molecule Inhibitor of Src Family Kinases." Methods Mol Biol **1307**: 319-327.

Human pluripotent stem cells (hPSCs), under the right conditions, can be engineered to generate populations of any somatic cell type. Knowledge of what mechanisms govern differentiation towards a particular lineage is often quite useful for efficiently producing somatic cell populations from hPSCs. Here, we have outlined a strategy for deriving populations of simple epithelial cells, as well as more mature epidermal keratinocyte progenitors, from hPSCs by exploiting a mechanism previously shown to direct epithelial differentiation of hPSCs. Specifically, we describe how to direct epithelial differentiation of hPSCs using an Src family kinase inhibitor, SU6656, which has been shown to modulate beta-catenin translocation to the cell membrane and thus promote epithelial differentiation. The differentiation platform outlined here produces cells with the ability to terminally differentiate to epidermal keratinocytes in culture through a stable simple epithelial cell intermediate that can be expanded in culture for numerous (>10) passages.

Selvaraj, V., et al. (2010). "Switching cell fate: the remarkable rise of induced pluripotent stem cells and lineage reprogramming technologies." Trends Biotechnol **28**(4): 214-223.

Cell reprogramming, in which a differentiated cell is made to switch its fate, is an emerging field with revolutionary prospects in biotechnology and medicine. The recent discovery of induced pluripotency by means of in vitro reprogramming has made way for unprecedented approaches for regenerative medicine, understanding human disease and drug discovery. Moreover, recent studies on regeneration and repair by direct lineage reprogramming in vivo offer an attractive novel alternative to cell therapy. Although we continue to push the limits of current knowledge in the field of cell reprogramming, the mechanistic elements that underlie these processes remain largely elusive. This article reviews landmark developments in cell reprogramming, current knowledge, and technological developments now on the horizon with significant promise for biomedical applications.

Senju, S., et al. (2011). "Generation of dendritic cells and macrophages from human induced pluripotent stem cells aiming at cell therapy." Gene Ther **18**(9): 874-883.

This report describes generation of dendritic cells (DCs) and macrophages from human induced pluripotent stem (iPS) cells. iPS cell-derived DC (iPS-DC) exhibited the morphology of typical DC and function of T-cell stimulation and antigen presentation. iPS-DC loaded with cytomegalovirus (CMV) peptide induced vigorous expansion of CMV-specific autologous CD8+ T cells. Macrophages (iPS-MP) with activity of zymosan phagocytosis and C5a-induced chemotaxis were also generated from iPS cells. Genetically modified iPS-MPs were generated by the introduction of expression vectors into undifferentiated iPS cells, isolation of transfectant iPS cell clone and subsequent differentiation. By this procedure, we generated iPS-MP expressing a membrane-bound form of single chain antibody (scFv) specific to amyloid beta (Abeta), the causal protein of Alzheimer's disease. The scFv-transfectant iPS-MP exhibited efficient Abeta-specific phagocytosis activity. iPS-MP expressing CD20-specific scFv engulfed and killed BALL-1 B-cell leukemia cells. Anti-BALL-1 effect of iPS-MP in vivo was demonstrated in a xeno-transplantation model using severe combined immunodeficient mice. In addition, we established a xeno-free culture protocol to generate iPS-DC and iPS-MP. Collectively, we demonstrated the possibility of application of iPS-DC and macrophages to cell therapy.

Senju, S., et al. (2010). "Pluripotent stem cell-derived dendritic cells for immunotherapy." Front Biosci (Elite Ed) **2**: 1520-1527.

Dendritic cell (DC) is regarded as a powerful means for anti-cancer immunotherapy. Clinical trials of cancer therapy with DC loaded with cancer antigens, such as tumor cell-lysates or HLA class I-binding antigenic peptides, have been conducted. Antigen-specific negative manipulation of the immune response by DC is a potential treatment for autoimmune diseases and also for control of allo-reactive immune responses in transplantation medicine. Currently, DC for clinical use are generated from peripheral blood monocytes of the patients. However, the number of monocytes obtained from the patients is limited and the potential of monocytes to differentiate into DC varies depending on the blood donor. Thus, the issue of limited cells is a serious obstacle for DC therapy. ES cells and iPS cells have pluripotency and unlimited propagation capacity and may be an ideal cell source for DC-therapy. Several groups, including us, have developed methods to generate DC from ES cells or iPS cells. This review introduces the studies on generation, characterization, and genetic modification of DC derived from ES cells or iPS cells.

Senju, S., et al. (2011). "Immunotherapy with pluripotent stem cell-derived dendritic cells." Semin Immunopathol **33**(6): 603-612.

In vivo transfer of dendritic cells (DC) has proven efficient in the priming of T cells and is regarded as a powerful means of providing anti-cancer immunotherapy. Clinical trials of anti-cancer therapy with DC pulsed with peptide antigens have been carried out in many institutions, although dramatic therapeutic effect has not been observed in most of the trials. Negative regulation of the immune response by DC might be applicable to treatment of autoimmune diseases and transplantation medicine. Currently, the DC used for anti-cancer vaccine therapy are generated from the peripheral blood monocytes of the patients. However, there is a limitation in the number of available monocytes and the potential of monocytes to differentiate into DC varies depending on the individual blood donors. To resolve the issue of the cell source for DC therapy, several groups have developed methods to generate DC from pluripotent stem cells. This review introduces methods to generate functional DC from pluripotent stem cells of mouse and human.

Sequiera, G. L., et al. (2017). "Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells as an Individual-Specific and Renewable Source of Adult Stem Cells." Methods Mol Biol **1553**: 183-190.

This chapter deals with the employment of human-induced pluripotent stem cells (hiPSCs) as a candidate to differentiate into mesenchymal stem cells (MSCs). This would enable to help establish a regular source of human MSCs with the aim of avoiding the problems associated with procuring the MSCs either from different healthy individuals or patients, limited extraction potentials, batch-to-batch variations or from diverse sources such as bone marrow or adipose tissue. The procedures described herein allow for a guided and ensured approach for the regular maintenance of hiPSCs and their subsequent differentiation into MSCs using the prescribed medium. Subsequently, an easy protocol for the successive isolation and purification of the hiPSC-differentiated MSCs is outlined, which is carried out through passaging and can be further sorted through flow cytometry. Further, the maintenance and expansion of the resultant hiPSC-differentiated MSCs using appropriate characterization techniques, i.e., Reverse-transcription PCR and immunostaining is also elaborated. The course of action has been deliberated keeping in mind the awareness and the requisites available to even beginner researchers who mostly have access to regular consumables and medium components found in the general laboratory.

Shaer, A., et al. (2014). "Differentiation of human induced pluripotent stem cells into insulin-like cell clusters with miR-186 and miR-375 by using chemical transfection." Appl Biochem Biotechnol **174**(1): 242-258.

Diabetes mellitus is characterized by either the inability to produce insulin or insensitivity to insulin secreted by the body. Islet cell replacement is an effective approach for diabetes treatment; however, it is not sufficient for all the diabetic patients. MicroRNAs (miRNAs) are a class of small noncoding RNAs that play an important role in mediating a broad and expanding range of biological activities, such as pancreas development. The present study aimed to develop a protocol to efficiently differentiate human induced pluripotent stem (iPS) cells into islet-like cell clusters (ILCs) in vitro by using miR-186 and miR-375. The human iPS colonies were transfected with hsa-miR-186 and hsa-miR-375 by using siPORT NeoFX Transfection Agent, and the differentiation was compared to controls. Total RNA was extracted 24 and 48 h after transfection. The gene expressions of insulin, NGN3, GLUT2, PAX4, PAX6, KIR6.2, NKX6.1, PDX1, Glucagon, and OCT4 were then evaluated through real-time qPCR. On the third day, the potency of the clusters was assessed in response to high glucose levels. Dithizone (DTZ) was used to identify the existence of the beta-cells. Besides, the presence of insulin and NGN3 proteins was investigated by immunocytochemistry. Morphological changes were

observed on the first day after the chemical transfection, and cell clusters were formed on the third day. The expression of pancreatic specific transcription factors was increased on the first day and significantly increased on the second day. The ILCs were positive for insulin and NGN3 proteins in the immunocytochemistry. Besides, the clusters were stained with DTZ and secreted insulin in glucose challenge test. Overexpression of miR-186 and miR-375 can be an alternative strategy for producing ILCs from the iPS cells in a short time. This work provides a new approach by using patient-specific iPSCs for beta-cell replacement therapy in diabetic patients.

Shafa, M., et al. (2018). "Human induced pluripotent stem cell-derived lung progenitor and alveolar epithelial cells attenuate hyperoxia-induced lung injury." Cytotherapy **20**(1): 108-125.

BACKGROUND AIMS: Bronchopulmonary dysplasia (BPD), a chronic lung disease characterized by disrupted lung growth, is the most common complication in extreme premature infants. BPD leads to persistent pulmonary disease later in life. Alveolar epithelial type 2 cells (AEC2s), a subset of which represent distal lung progenitor cells (LPCs), promote normal lung growth and repair. AEC2 depletion may contribute to persistent lung injury in BPD. We hypothesized that induced pluripotent stem cell (iPSC)-derived AECs prevent lung damage in experimental oxygen-induced BPD. METHODS: Mouse AECs (mAECs), miPSCs/mouse embryonic stem sells, human umbilical cord mesenchymal stromal cells (hUCMSCs), human (h)iPSCs, hiPSC-derived LPCs and hiPSC-derived AECs were delivered intratracheally to hyperoxia-exposed newborn mice. Cells were pre-labeled with a red fluorescent dye for in vivo tracking. RESULTS: Airway delivery of primary mAECs and undifferentiated murine pluripotent cells prevented hyperoxia-induced impairment in lung function and alveolar growth in neonatal mice. Similar to hUCMSC therapy, undifferentiated hiPSCs also preserved lung function and alveolar growth in hyperoxia-exposed neonatal NOD/SCID mice. Long-term assessment of hiPSC administration revealed local teratoma formation and cellular infiltration in various organs. To develop a clinically relevant cell therapy, we used a highly efficient method to differentiate hiPSCs into a homogenous population of AEC2s. Airway delivery of hiPSC-derived AEC2s and hiPSC-derived LPCs, improved lung function and structure and resulted in long-term engraftment without evidence of tumor formation. CONCLUSIONS: hiPSC-derived AEC2 therapy appears effective and safe in this model and warrants further exploration as a therapeutic option for BPD and other lung diseases characterized by AEC injury.

Shah, S. N., et al. (2012). "HMGA1 reprograms somatic cells into pluripotent stem cells by inducing stem cell transcriptional networks." PLoS One **7**(11): e48533.

BACKGROUND: Although recent studies have identified genes expressed in human embryonic stem cells (hESCs) that induce pluripotency, the molecular underpinnings of normal stem cell function remain poorly understood. The high mobility group A1 (HMGA1) gene is highly expressed in hESCs and poorly differentiated, stem-like cancers; however, its role in these settings has been unclear. METHODS/PRINCIPAL FINDINGS: We show that HMGA1 is highly expressed in fully reprogrammed iPSCs and hESCs, with intermediate levels in ECCs and low levels in fibroblasts. When hESCs are induced to differentiate, HMGA1 decreases and parallels that of other pluripotency factors. Conversely, forced expression of HMGA1 blocks differentiation of hESCs. We also discovered that HMGA1 enhances cellular reprogramming of somatic cells to iPSCs together with the Yamanaka factors (OCT4, SOX2, KLF4, cMYC - OSKM). HMGA1 increases the number and size of iPSC colonies compared to OSKM controls. Surprisingly, there was normal differentiation in vitro and benign teratoma formation in vivo of the HMGA1-derived iPSCs. During the reprogramming process, HMGA1 induces the expression of pluripotency genes, including SOX2, LIN28, and cMYC, while knockdown of HMGA1 in hESCs results in the repression of these genes. Chromatin immunoprecipitation shows that HMGA1 binds to the promoters of these pluripotency genes in vivo. In addition, interfering with HMGA1 function using a short hairpin RNA or a dominant-negative construct blocks cellular reprogramming to a pluripotent state. CONCLUSIONS: Our findings demonstrate for the first time that HMGA1 enhances cellular reprogramming from a somatic cell to a fully pluripotent stem cell. These findings identify a novel role for HMGA1 as a key regulator of the stem cell state by inducing transcriptional networks that drive pluripotency. Although further studies are needed, these HMGA1 pathways could be exploited in regenerative medicine or as novel therapeutic targets for poorly differentiated, stem-like cancers.

Shi, Y. (2009). "Induced pluripotent stem cells, new tools for drug discovery and new hope for stem cell therapies." Curr Mol Pharmacol **2**(1): 15-18.

Somatic cell nuclear transfer or therapeutic cloning has provided great hope for stem cell-based therapies. However, therapeutic cloning has been experiencing both ethical and technical difficulties. Recent breakthrough studies using a combination of four factors to reprogram human somatic cells into

pluripotent stem cells without using embryos or eggs have led to an important revolution in stem cell research. Comparative analysis of human induced pluripotent stem cells and human embryonic stem cells using assays for morphology, cell surface marker expression, gene expression profiling, epigenetic status, and differentiation potential have revealed a remarkable degree of similarity between these two pluripotent stem cell types. This mini-review summarizes these ground-breaking studies. These advances in reprogramming will enable the creation of patient-specific stem cell lines to study various disease mechanisms. The cellular models created will provide valuable tools for drug discovery. Furthermore, this reprogramming system provides great potential to design customized patient-specific stem cell therapies with economic feasibility.

Shi, Z., et al. (2012). "Induced pluripotent stem cell-related genes influence biological behavior and 5-fluorouracil sensitivity of colorectal cancer cells." J Zhejiang Univ Sci B **13**(1): 11-19.

OBJECTIVE: We aimed to perform a preliminary study of the association between induced pluripotent stem cell (iPS)-related genes and biological behavior of human colorectal cancer (CRC) cells, and the potential for developing anti-cancer drugs targeting these genes. METHODS: We used real-time reverse transcriptase polymerase chain reaction (RT-PCR) to evaluate the transcript levels of iPS-related genes NANOG, OCT4, SOX2, C-MYC and KLF4 in CRC cell lines and cancer stem cells (CSCs)-enriched tumor spheres. NANOG was knockdowned in CRC cell line SW620 by lentiviral transduction. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, plate colony formation, and a mouse xenograft model were used to evaluate alterations in biological behavior in NANOG-knockdown SW620 cells. Also, mock-knockdown and NANOG-knockdown cells were treated with 5-fluorouracil (5-FU) and survival rate was measured by MTT assay to evaluate drug sensitivity. RESULTS: A significant difference in the transcript levels of iPS-related genes between tumor spheres and their parental bulky cells was observed. NANOG knockdown suppressed proliferation, colony formation, and in vivo tumorigenicity but increased the sensitivity to 5-FU of SW620 cells. 5-FU treatment greatly inhibited the expression of the major stemness-associated genes NANOG, OCT4, and SOX2. CONCLUSIONS: These results collectively suggest an overlap between iPS-related genes and CSCs in CRC. Quenching a certain gene NANOG may truncate the aggressiveness of CRC cells.

Shimba, K., et al. (2016). "Cell-cycle-dependent Ca(2+) transients in human induced pluripotent stem cells revealed by a simultaneous imaging of cell nuclei and intracellular Ca(2+) level." Integr Biol (Camb) **8**(9): 985-990.

Cell cycle phase and [Ca(2+)]i are key determinants of self-renewal and differentiation in pluripotent stem cells. However, little is known about their relationship in human pluripotent stem cells owing to the lack of an effective method. Here, we applied an imaging-based approach for evaluating the relationship between the cell cycle and Ca(2+) transients in human induced pluripotent stem (iPS) cells. Ca imaging and DNA staining was simultaneously performed at the same site. Then, individual cells were recognized and the cell cycle phase was estimated from the image of nuclei. We found that 18 +/- 4% of human iPS cells exhibited spontaneous Ca(2+) transients and their inter-transient interval was 119 +/- 19 s. Ca wave events were observed in 64% of the sample and the [Ca(2+)]i elevation propagated among 47 +/- 30 cells with a duration of 57 +/- 22 s. With the imaging-based approach, we demonstrated that the ratio of cells exhibiting Ca(2+) transients significantly decreased during cell cycle progression, suggesting that the relationship previously described in mouse cells holds true in the human context as well. These results suggest that our method is suitable for evaluating Ca(2+) transients, the cell cycle phase, and their relationship with densely cultured cells.

Shirane, K., et al. (2016). "Global Landscape and Regulatory Principles of DNA Methylation Reprogramming for Germ Cell Specification by Mouse Pluripotent Stem Cells." Dev Cell **39**(1): 87-103.

Specification of primordial germ cells (PGCs) activates epigenetic reprogramming for totipotency, the elucidation of which remains a fundamental challenge. Here, we uncover regulatory principles for DNA methylation reprogramming during in vitro PGC specification, in which mouse embryonic stem cells (ESCs) are induced into epiblast-like cells (EpiLCs) and then PGC-like cells (PGCLCs). While ESCs reorganize their methylome to form EpiLCs, PGCLCs essentially dilute the EpiLC methylome at constant, yet different, rates between unique sequence regions and repeats. ESCs form hypomethylated domains around pluripotency regulators for their activation, whereas PGCLCs create demethylation-sensitive domains around developmental regulators by accumulating abundant H3K27me3 for their repression. Loss of PRDM14 globally upregulates methylation and diminishes the hypomethylated domains, but it preserves demethylation-sensitive domains. Notably, female ESCs form hypomethylated lamina-associated

domains, while female PGCLCs effectively reverse such states into a more normal configuration. Our findings illuminate the unique orchestration of DNA methylation and histone modification reprogramming during PGC specification.

Shofuda, T., et al. (2013). "Human Decidua-Derived Mesenchymal Cells Are a Promising Source for the Generation and Cell Banking of Human Induced Pluripotent Stem Cells." Cell Med **4**(3): 125-147.

Placental tissue is a biomaterial with remarkable potential for use in regenerative medicine. It has a three-layer structure derived from the fetus (amnion and chorion) and the mother (decidua), and it contains huge numbers of cells. Moreover, placental tissue can be collected without any physical danger to the donor and can be matched with a variety of HLA types. The decidua-derived mesenchymal cells (DMCs) are highly proliferative fibroblast-like cells that express a similar pattern of CD antigens as bone marrow-derived mesenchymal cells (BM-MSCs). Here we demonstrated that induced pluripotent stem (iPS) cells could be efficiently generated from DMCs by retroviral transfer of reprogramming factor genes. DMC-hiPS cells showed equivalent characteristics to human embryonic stem cells (hESCs) in colony morphology, global gene expression profile (including human pluripotent stem cell markers), DNA methylation status of the OCT3/4 and NANOG promoters, and ability to differentiate into components of the three germ layers in vitro and in vivo. The RNA expression of XIST and the methylation status of its promoter region suggested that DMC-iPSCs, when maintained undifferentiated and pluripotent, had three distinct states: (1) complete X-chromosome reactivation, (2) one inactive X-chromosome, or (3) an epigenetic aberration. Because DMCs are derived from the maternal portion of the placenta, they can be collected with the full consent of the adult donor and have considerable ethical advantages for cell banking and the subsequent generation of human iPS cells for regenerative applications.

Shtrichman, R., et al. (2013). "Induced pluripotent stem cells (iPSCs) derived from different cell sources and their potential for regenerative and personalized medicine." Curr Mol Med **13**(5): 792-805.

Human induced pluripotent stem cells (hiPSCs) have great potential as a robust source of progenitors for regenerative medicine. The novel technology also enables the derivation of patient-specific cells for applications to personalized medicine, such as for personal drug screening and toxicology. However, the biological characteristics of iPSCs are not yet fully understood and their similarity to human embryonic stem cells (hESCs) is still unresolved. Variations among iPSCs, resulting from their original tissue or cell source, and from the experimental protocols used for their derivation, significantly affect epigenetic properties and differentiation potential. Here we review the potential of iPSCs for regenerative and personalized medicine, and assess their expression pattern, epigenetic memory and differentiation capabilities in relation to their parental tissue source. We also summarize the patient-specific iPSCs that have been derived for applications in biological research and drug discovery; and review risks that must be overcome in order to use iPSC technology for clinical applications.

Shu, T., et al. (2015). "Protective Effects and Mechanisms of Salvianolic Acid B Against H(2)O(2)-Induced Injury in Induced Pluripotent Stem Cell-Derived Neural Stem Cells." Neurochem Res **40**(6): 1133-1143.

Induced pluripotent stem cells (iPSCs) have the potential to differentiate into neural lineages. Salvianolic acid B (Sal B) is a commonly used, traditional Chinese medicine for enhancing neuroprotective effects, and has antioxidant, anti-inflammatory, and antiapoptotic properties. Here, we explore the potential mechanism of Sal B in protecting iPSC-derived neural stem cells (NSCs) against H2O2-induced injury. iPSCs were induced into NSCs, iPSC-derived NSCs were treated with 50 muM Sal B for 24.5 h and 500 muM H2O2 for 24 h. The resulting effects were examined by flow cytometry analysis, quantitative reverse-transcription polymerase chain reaction, and western blotting. Upon H2O2 exposure, Sal B significantly promoted cell viability and stabilization of the mitochondrial membrane potential. Sal B also visibly decreased the cell apoptotic ratio. In addition, Sal B markedly reduced expression of matrix metalloproteinase (MMP)-2 and -9, and phosphospecific signal transducer and activator of transcription 3 (p-STAT3), and increased the level of tissue inhibitor of metalloproteinase (TIMP)-2 in iPSC-derived NSCs induced by H2O2. These results suggest that Sal B protects iPSC-derived NSCs against H2O2-induced oxidative stress. The mechanisms of this stress tolerance may be attributed to modulation of the MMP/TIMP system and inhibition of the STAT3 signaling pathway.

Silk, K. M., et al. (2012). "Cross-presentation of tumour antigens by human induced pluripotent stem cell-derived CD141(+)XCR1+ dendritic cells." Gene Ther **19**(10): 1035-1040.

Monocyte-derived dendritic cells (moDC) have been widely used in cancer immunotherapy but show significant donor-to-donor variability and low capacity for the cross-presentation of tumour-

associated antigens (TAA) to CD8(+) T cells, greatly limiting the success of this approach. Given recent developments in induced pluripotency and the relative ease with which induced pluripotent stem (iPS) cell lines may be generated from individuals, we have succeeded in differentiating dendritic cells (DC) from human leukocyte antigen (HLA)-A(*)0201(+) iPS cells (iPS cell-derived DC (ipDC)), using protocols compliant with their subsequent clinical application. Unlike moDC, a subset of ipDC was found to coexpress CD141 and XCR1 that have been shown previously to define the human equivalent of mouse CD8alpha(+) DC, in which the capacity for cross-presentation has been shown to reside. Accordingly, ipDC were able to cross-present the TAA, Melan A, to a CD8(+) T-cell clone and stimulate primary Melan A-specific responses among naive T cells from an HLA-A(*)0201(+) donor. Given that CD141(+)XCR1(+) DC are present in peripheral blood in trace numbers that preclude their clinical application, the ability to generate a potentially unlimited source from iPS cells offers the possibility of harnessing their capacity for cross-priming of cytotoxic T lymphocytes for the induction of tumour-specific immune responses.

Simsek, S., et al. (2016). "Modeling Cystic Fibrosis Using Pluripotent Stem Cell-Derived Human Pancreatic Ductal Epithelial Cells." Stem Cells Transl Med **5**(5): 572-579.

UNLABELLED: We established an efficient strategy to direct human pluripotent stem cells, including human embryonic stem cells (hESCs) and an induced pluripotent stem cell (iPSC) line derived from patients with cystic fibrosis, to differentiate into pancreatic ductal epithelial cells (PDECs). After purification, more than 98% of hESC-derived PDECs expressed functional cystic fibrosis transmembrane conductance regulator (CFTR) protein. In addition, iPSC lines were derived from a patient with CF carrying compound frameshift and mRNA splicing mutations and were differentiated to PDECs. PDECs derived from Weill Cornell cystic fibrosis (WCCF)-iPSCs showed defective expression of mature CFTR protein and impaired chloride ion channel activity, recapitulating functional defects of patients with CF at the cellular level. These studies provide a new methodology to derive pure PDECs expressing CFTR and establish a "disease in a dish" platform to identify drug candidates to rescue the pancreatic defects of patients with CF. SIGNIFICANCE: An efficient strategy was established to direct human pluripotent stem cells, including human embryonic stem cells (hESCs) and an induced pluripotent stem cell line derived from patients with cystic fibrosis (CF-iPSCs), to differentiate into pancreatic ductal epithelial cells (PDECs). After purification, more than 98% of hESC-PDECs derived from CF-iPSCs showed defective expression of mature cystic fibrosis transmembrane conductance regulator (CFTR) protein and impaired chloride ion channel activity, recapitulating functional pancreatic defects of patients with CF at the cellular level. These studies provide a new methodology for deriving pure PDECs expressing CFTR, and they establish a "disease-in-a-dish" platform for identifying drug candidates to rescue the pancreatic defects of these patients.

Sirenko, O., et al. (2014). "High-content assays for hepatotoxicity using induced pluripotent stem cell-derived cells." Assay Drug Dev Technol **12**(1): 43-54.

Development of predictive in vitro assays for early toxicity evaluation is extremely important for improving the drug development process and reducing drug attrition rates during clinical development. High-content imaging-based in vitro toxicity assays are emerging as efficient tools for safety and efficacy testing to improve drug development efficiency. In this report we have used an induced pluripotent stem cell (iPSC)-derived hepatocyte cell model having a primary tissue-like phenotype, unlimited availability, and the potential to compare cells from different individuals. We examined a number of assays and phenotypic markers and developed automated screening methods for assessing multiparameter readouts of general and mechanism-specific hepatotoxicity. Endpoints assessed were cell viability, nuclear shape, average and integrated cell area, mitochondrial membrane potential, phospholipid accumulation, cytoskeleton integrity, and apoptosis. We assayed compounds with known mechanisms of toxicity and also evaluated a diverse hepatotoxicity library of 240 compounds. We conclude that high-content automated screening assays using iPSC-derived hepatocytes are feasible, provide information about mechanisms of toxicity, and can facilitate the safety assessment of drugs and chemicals.

Sivalingam, J., et al. (2016). "Superior Red Blood Cell Generation from Human Pluripotent Stem Cells Through a Novel Microcarrier-Based Embryoid Body Platform." Tissue Eng Part C Methods **22**(8): 765-780.

In vitro generation of red blood cells (RBCs) from human embryonic stem cells and human induced pluripotent stem cells appears to be a promising alternate approach to circumvent shortages in donor-derived blood supplies for clinical applications. Conventional methods for hematopoietic differentiation of human pluripotent stem cells (hPSC) rely on embryoid body (EB) formation and/or coculture with xenogeneic cell lines. However, most current methods for hPSC expansion and EB formation are not amenable for scale-up to levels required for large-scale RBC generation. Moreover, differentiation methods

that rely on xenogenic cell lines would face obstacles for future clinical translation. In this study, we report the development of a serum-free and chemically defined microcarrier-based suspension culture platform for scalable hPSC expansion and EB formation. Improved survival and better quality EBs generated with the microcarrier-based method resulted in significantly improved mesoderm induction and, when combined with hematopoietic differentiation, resulted in at least a 6-fold improvement in hematopoietic precursor expansion, potentially culminating in a 80-fold improvement in the yield of RBC generation compared to a conventional EB-based differentiation method. In addition, we report efficient terminal maturation and generation of mature enucleated RBCs using a coculture system that comprised primary human mesenchymal stromal cells. The microcarrier-based platform could prove to be an appealing strategy for future scale-up of hPSC culture, EB generation, and large-scale generation of RBCs under defined and xeno-free conditions.

Smith, M. J., et al. (2015). "In Vitro T-Cell Generation From Adult, Embryonic, and Induced Pluripotent Stem Cells: Many Roads to One Destination." Stem Cells **33**(11): 3174-3180.

T lymphocytes are critical mediators of the adaptive immune system and have the capacity to serve as therapeutic agents in the areas of transplant and cancer immunotherapy. While T cells can be isolated and expanded from patients, T cells derived in vitro from both hematopoietic stem/progenitor cells (HSPCs) and human pluripotent stem cells (hPSCs) offer great potential advantages in generating a self-renewing source of T cells that can be readily genetically modified. T-cell differentiation in vivo is a complex process requiring tightly regulated signals; providing the correct signals in vitro to induce T-cell lineage commitment followed by their development into mature, functional, single positive T cells, is similarly complex. In this review, we discuss current methods for the in vitro derivation of T cells from murine and human HSPCs and hPSCs that use feeder-cell and feeder-cell-free systems. Furthermore, we explore their potential for adoption for use in T-cell-based therapies.

Solari, C., et al. (2011). "Induced pluripotent stem cells' self-renewal and pluripotency is maintained by a bovine granulosa cell line-conditioned medium." Biochem Biophys Res Commun **410**(2): 252-257.

Induced pluripotent stem cells (iPSCs) are a promising type of stem cells, comparable to embryonic stem cells (ESCs) in terms of self-renew and pluripotency, generated by reprogramming somatic cells. These cells are an attractive approach to supply patient-specific pluripotent cells, for producing in vitro models of disease, drug discovery, toxicology and potentially treating degenerative disease circumventing immune rejection. In spite of the great advance since iPSCs' establishment, their obtention and propagation is an increasing area of great interest. In a recent work, we have shown that the conditioned medium from a bovine granulosa cell line (BGC-CM) is able to preserve the basic properties of mESCs. Therefore, based on our previous results and the reported resemblance between iPSCs and ESCs, we hypothesized that BGC-CM could provide a favorable context to culturing iPSCs. In this work, we have reprogrammed mouse embryonic fibroblasts obtaining iPSC lines, and showed that they can be propagated in BGC-CM while maintaining self-renewal and pluripotency, evidenced by expression of specific gene markers and capability of in vitro and in vivo differentiation to cell types from the three germ layers. We believe that these findings may provide a novel context to propagate iPSCs to study the molecular mechanisms involved in self-renewal and pluripotency.

Solozobova, V., et al. (2012). "Lessons from the embryonic neural stem cell niche for neural lineage differentiation of pluripotent stem cells." Stem Cell Rev **8**(3): 813-829.

Pluripotent stem cells offer an abundant and malleable source for the generation of differentiated cells for transplantation as well as for in vitro screens. Patterning and differentiation protocols have been developed to generate neural progeny from human embryonic or induced pluripotent stem cells. However, continued refinement is required to enhance efficiency and to prevent the generation of unwanted cell types. We summarize and interpret insights gained from studies of embryonic neuroepithelium. A multitude of factors including soluble molecules, interactions with the extracellular matrix and neighboring cells cooperate to control neural stem cell self-renewal versus differentiation. Applying these findings and concepts to human stem cell systems in vitro may yield more appropriately patterned cell types for biomedical applications.

Somers, A., et al. (2010). "Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette." Stem Cells **28**(10): 1728-1740.

The development of methods to achieve efficient reprogramming of human cells while avoiding the permanent presence of reprogramming transgenes represents a critical step toward the use of induced pluripotent stem cells (iPSC) for clinical purposes, such as disease modeling or reconstituting therapies. Although several methods exist for generating iPSC free of reprogramming transgenes from mouse cells or

neonatal normal human tissues, a sufficiently efficient reprogramming system is still needed to achieve the widespread derivation of disease-specific iPSC from humans with inherited or degenerative diseases. Here, we report the use of a humanized version of a single lentiviral "stem cell cassette" vector to accomplish efficient reprogramming of normal or diseased skin fibroblasts obtained from humans of virtually any age. Simultaneous transfer of either three or four reprogramming factors into human target cells using this single vector allows derivation of human iPSC containing a single excisable viral integration that on removal generates human iPSC free of integrated transgenes. As a proof of principle, here we apply this strategy to generate >100 lung disease-specific iPSC lines from individuals with a variety of diseases affecting the epithelial, endothelial, or interstitial compartments of the lung, including cystic fibrosis, alpha-1 antitrypsin deficiency-related emphysema, scleroderma, and sickle-cell disease. Moreover, we demonstrate that human iPSC generated with this approach have the ability to robustly differentiate into definitive endoderm in vitro, the developmental precursor tissue of lung epithelia.

Somoza, R. A. and F. J. Rubio (2012). "Cell therapy using induced pluripotent stem cells or somatic stem cells: this is the question." Curr Stem Cell Res Ther **7**(3): 191-196.

A lot of effort has been developed to bypass the use of embryonic stem cells (ES) in human therapies, because of several concerns and ethical issues. Some unsolved problems of using stem cells for human therapies, excluding the human embryonic origin, are: how to regulate cell plasticity and proliferation, immunological compatibility, potential adverse side-effects when stem cells are systemically administrated, and the in vivo signals to rule out a specific cell fate after transplantation. Currently, it is known that almost all tissues of an adult organism have somatic stem cells (SSC). Whereas ES are primary involved in the genesis of new tissues and organs, SSC are involved in regeneration processes, immuno-regulatory and homeostasis mechanisms. Although the differentiating potential of ES is higher than SSC, several studies suggest that some types of SSC, such as mesenchymal stem cells (MSC), can be induced epigenetically to differentiate into tissue-specific cells of different lineages. This unexpected pluripotency and the variety of sources that they come from, can make MSC-like cells suitable for the treatment of diverse pathologies and injuries. New hopes for cell therapy came from somatic/mature cells and the discovery that could be reprogrammed to a pluripotent stage similar to ES, thus generating induced pluripotent stem cells (iPS). For this, it is necessary to overexpress four main reprogramming factors, Sox2, Oct4, Klf4 and c-Myc. The aim of this review is to analyze the potential and requirements of cellular based tools in human therapy strategies, focusing on the advantage of using MSC over iPS.

Song, H., et al. (2010). "Interrogating functional integration between injected pluripotent stem cell-derived cells and surrogate cardiac tissue." Proc Natl Acad Sci U S A **107**(8): 3329-3334.

Myocardial infarction resulting in irreversible loss of cardiomyocytes (CMs) remains a leading cause of heart failure. Although cell transplantation has modestly improved cardiac function, major challenges including increasing cell survival, engraftment, and functional integration with host tissue, remain. Embryonic stem cells (ESCs), which can be differentiated into cardiac progenitors (CPs) and CMs, represent a candidate cell source for cardiac cell therapy. However, it is not known what specific cell type or condition is the most appropriate for transplantation. This problem is exasperated by the lack of efficient and predictive strategies to screen the large numbers of parameters that may impact cell transplantation. We used a cardiac tissue model, engineered heart tissue (EHT), and quantitative molecular and electrophysiological analyses, to test transplantation conditions and specific cell populations for their potential to functionally integrate with the host tissue. In this study, we validated our analytical platform using contractile mouse neonatal CMs (nCMs) and noncontractile cardiac fibroblasts (cFBs), and screened for the integration potential of ESC-derived CMs and CPs (ESC-CMs and -CPs). Consistent with previous in vivo studies, cFB injection interfered with electrical signal propagation, whereas injected nCMs improved tissue function. Purified bioreactor-generated ESC-CMs exhibited a diminished capacity for electrophysiological integration; a result correlated with lower (compared with nCMs) connexin 43 expression. ESC-CPs, however, appeared able to appropriately mature and integrate into EHT, enhancing the amplitude of tissue contraction. Our results support the use of EHT as a model system to accelerate development of cardiac cell therapy strategies.

Song, M. J. and K. Bharti (2016). "Looking into the future: Using induced pluripotent stem cells to build two and three dimensional ocular tissue for cell therapy and disease modeling." Brain Res **1638**(Pt A): 2-14.

Retinal degenerative diseases are the leading cause of irreversible vision loss in developed countries. In many cases the diseases originate in the homeostatic unit in the back of the eye that contains the retina, retinal pigment epithelium (RPE) and the

choriocapillaris. RPE is a central and a critical component of this homeostatic unit, maintaining photoreceptor function and survival on the apical side and choriocapillaris health on the basal side. In diseases like age-related macular degeneration (AMD), it is thought that RPE dysfunctions cause disease-initiating events and as the RPE degenerates photoreceptors begin to die and patients start loosing vision. Patient-specific induced pluripotent stem (iPS) cell-derived RPE provides direct access to a patient's genetics and allow the possibility of identifying the initiating events of RPE-associated degenerative diseases. Furthermore, iPS cell-derived RPE cells are being tested as a potential cell replacement in disease stages with RPE atrophy. In this article we summarize the recent progress in the field of iPS cell-derived RPE "disease modeling" and cell therapies and also discuss the possibilities of developing a model of the entire homeostatic unit to aid in studying disease processes in the future. This article is part of a Special Issue entitled SI: PSC and the brain.

Sorkio, A. E., et al. (2015). "Biomimetic collagen I and IV double layer Langmuir-Schaefer films as microenvironment for human pluripotent stem cell derived retinal pigment epithelial cells." Biomaterials **51**: 257-269.

The environmental cues received by the cells from synthetic substrates in vitro are very different from those they receive in vivo. In this study, we applied the Langmuir-Schaefer (LS) deposition, a variant of Langmuir-Blodgett technique, to fabricate a biomimetic microenvironment mimicking the structure and organization of native Bruch's membrane for the production of the functional human embryonic stem cell derived retinal pigment epithelial (hESC-RPE) cells. Surface pressure-area isotherms were measured simultaneously with Brewster angle microscopy to investigate the self-assembly of human collagens type I and IV on air-subphase interface. Furthermore, the structure of the prepared collagen LS films was characterized with scanning electron microscopy, atomic force microscopy, surface plasmon resonance measurements and immunofluorescent staining. The integrity of hESC-RPE on double layer LS films was investigated by measuring transepithelial resistance and permeability of small molecular weight substance. Maturation and functionality of hESC-RPE cells on double layer collagen LS films was further assessed by RPE-specific gene and protein expression, growth factor secretion, and phagocytic activity. Here, we demonstrated that the prepared collagen LS films have layered structure with oriented fibers corresponding to architecture of the uppermost layers of Bruch's membrane and result in increased barrier properties and functionality of hESC-RPE cells as compared to the commonly used dip-coated controls.

Sotthibundhu, A., et al. (2016). "Rapamycin regulates autophagy and cell adhesion in induced pluripotent stem cells." Stem Cell Res Ther **7**(1): 166.

BACKGROUND: Cellular reprogramming is a stressful process, which requires cells to engulf somatic features and produce and maintain stemness machineries. Autophagy is a process to degrade unwanted proteins and is required for the derivation of induced pluripotent stem cells (iPSCs). However, the role of autophagy during iPSC maintenance remains undefined. METHODS: Human iPSCs were investigated by microscopy, immunofluorescence, and immunoblotting to detect autophagy machinery. Cells were treated with rapamycin to activate autophagy and with bafilomycin to block autophagy during iPSC maintenance. High concentrations of rapamycin treatment unexpectedly resulted in spontaneous formation of round floating spheres of uniform size, which were analyzed for differentiation into three germ layers. Mass spectrometry was deployed to reveal altered protein expression and pathways associated with rapamycin treatment. RESULTS: We demonstrate that human iPSCs express high basal levels of autophagy, including key components of APMKalpha, ULK1/2, BECLIN-1, ATG13, ATG101, ATG12, ATG3, ATG5, and LC3B. Block of autophagy by bafilomycin induces iPSC death and rapamycin attenuates the bafilomycin effect. Rapamycin treatment upregulates autophagy in iPSCs in a dose/time-dependent manner. High concentration of rapamycin reduces NANOG expression and induces spontaneous formation of round and uniformly sized embryoid bodies (EBs) with accelerated differentiation into three germ layers. Mass spectrometry analysis identifies actin cytoskeleton and adherens junctions as the major targets of rapamycin in mediating iPSC detachment and differentiation. CONCLUSIONS: High levels of basal autophagy activity are present during iPSC derivation and maintenance. Rapamycin alters expression of actin cytoskeleton and adherens junctions, induces uniform EB formation, and accelerates differentiation. IPSCs are sensitive to enzyme dissociation and require a lengthy differentiation time. The shape and size of EBs also play a role in the heterogeneity of end cell products. This research therefore highlights the potential of rapamycin in producing uniform EBs and in shortening iPSC differentiation duration.

Sougawa, N., et al. (2018). "Immunologic targeting of CD30 eliminates tumourigenic human pluripotent stem cells, allowing safer clinical application of hiPSC-based cell therapy." Sci Rep **8**(1): 3726.

Induced pluripotent stem cells (iPSCs) are promising candidate cells for cardiomyogenesis in the failing heart. However, teratoma/tumour formation originating from undifferentiated iPSCs contaminating the graft is a critical concern for clinical application. Here, we hypothesized that brentuximab vedotin, which targets CD30, induces apoptosis in tumourigenic cells, thus increasing the safety of iPSC therapy for heart failure. Flow cytometry analysis identified consistent expression of CD30 in undifferentiated human iPSCs. Addition of brentuximab vedotin in vitro for 72 h efficiently induced cell death in human iPSCs, associated with a significant increase in G2/M phase cells. Brentuximab vedotin significantly reduced Lin28 expression in cardiomyogenically differentiated human iPSCs. Transplantation of human iPSC-derived cardiomyocytes (CMs) without treatment into NOG mice consistently induced teratoma/tumour formation, with a substantial number of Ki-67-positive cells in the graft at 4 months post-transplant, whereas iPSC-derived CMs treated with brentuximab vedotin prior to the transplantation did not show teratoma/tumour formation, which was associated with absence of Ki-67-positive cells in the graft over the same period. These findings suggest that in vitro treatment with brentuximab vedotin, targeting the CD30-positive iPSC fraction, reduced tumourigenicity in human iPSC-derived CMs, potentially providing enhanced safety for iPSC-based cardiomyogenesis therapy in clinical scenarios.

Sperger, J. M., et al. (2003). "Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors." Proc Natl Acad Sci U S A **100**(23): 13350-13355.

Remarkably little is known about the transcriptional profiles of human embryonic stem (ES) cells or the molecular mechanisms that underlie their pluripotency. To identify commonalties among the transcriptional profiles of different human pluripotent cells and to search for clues into the genesis of human germ cell tumors, we compared the expression profiles of human ES cell lines, human germ cell tumor cell lines and tumor samples, somatic cell lines, and testicular tissue samples by using cDNA microarray analysis. Hierarchical cluster analysis of gene expression profiles showed that the five independent human ES cell lines clustered tightly together, reflecting highly similar expression profiles. The gene expression patterns of human ES cell lines showed many similarities with the human embryonal carcinoma cell samples and more distantly with the seminoma samples. We identified 895 genes that were expressed at significantly greater levels in human ES and embryonal carcinoma cell lines than in control samples. These genes are candidates for involvement in the maintenance of a pluripotent, undifferentiated phenotype.

Spitzhorn, L. S., et al. (2018). "Transplanted human pluripotent stem cell-derived mesenchymal stem cells support liver regeneration in Gunn rats." Stem Cells Dev.

Gunn rats bear a mutation within the uridine diphosphate glucuronosyltransferase-1A1 (Ugt1a1) gene resulting in high serum bilirubin levels as seen in Crigler-Najjar syndrome. In the present study, the Gunn rat was used as an animal model for heritable liver dysfunction. Human mesenchymal stem cells (iMSC) derived from embryonic stem cells (H1) and induced pluripotent stem cells were transplanted into Gunn rats after partial hepatectomy. The iMSCs engrafted and survived in the liver for up to 2 months. The transplanted iMSCs differentiated into functional hepatocytes as evidenced by partially suppressed hyperbilirubinemia and expression of multiple human-specific hepatocyte markers such as Albumin, hepatocyte nuclear factor 4alpha, UGT1A1, Cytokeratin 18, bile salt export pump, multidrug resistance protein 2, Na/taurocholate-cotransporting polypeptide and alpha-Fetoprotein. These findings imply that transplanted human iMSCs can contribute to liver regeneration in vivo and thus represent a promising tool for the treatment of inherited liver diseases.

Srinivasan, G., et al. (2018). "An integrated biomanufacturing platform for the large-scale expansion and neuronal differentiation of human pluripotent stem cell-derived neural progenitor cells." Acta Biomater **74**: 168-179.

Human pluripotent stem cell derived neural progenitor cells (hNPCs) have the unique properties of long-term in vitro expansion as well as differentiation into the various neurons and supporting cell types of the central nervous system (CNS). Because of these characteristics, hNPCs have tremendous potential in the modeling and treatment of various CNS diseases and disorders. However, expansion and neuronal differentiation of hNPCs in quantities necessary for these applications is not possible with current two dimensional (2-D) approaches. Here, we used a fully defined peptide substrate as the basis for a microcarrier (MC)-based suspension culture system. Several independently derived hNPC lines were cultured on MCs for multiple passages as well as efficiently differentiated to neurons. Finally, this MC-based system was used in conjunction with a low shear rotating wall vessel (RWV) bioreactor for the integrated, large-scale expansion and neuronal differentiation of hNPCs. Overall, this fully defined and scalable biomanufacturing system will facilitate the

generation of hNPCs and their neuronal derivatives in quantities necessary for basic and translational applications. STATEMENT OF SIGNIFICANCE: In this work, we developed a microcarrier (MC)-based culture system that allows for the expansion and neuronal differentiation of human pluripotent stem cell-derived neural progenitor cells (hNPCs) under defined conditions. In turn, this MC approach was implemented in a rotating wall vessel (RWV) bioreactor for the large-scale expansion and neuronal differentiation of hNPCs. This work is of significance as it overcomes current limitations of conventional two dimensional (2-D) culture systems to enable the generation of hNPCs and their neuronal derivatives in quantities required for downstream applications in disease modeling, drug screening, and regenerative medicine.

Stebbins, M. J., et al. (2018). "Activation of RARalpha, RARgamma, or RXRalpha Increases Barrier Tightness in Human Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells." Biotechnol J **13**(2).

The blood-brain barrier (BBB) is critical to central nervous system (CNS) health. Brain microvascular endothelial cells (BMECs) are often used as in vitro BBB models for studying BBB dysfunction and therapeutic screening applications. Human pluripotent stem cells (hPSCs) can be differentiated to cells having key BMEC barrier and transporter properties, offering a renewable, scalable source of human BMECs. hPSC-derived BMECs have previously been shown to respond to all-trans retinoic acid (RA), and the goal of this study was to identify the stages at which differentiating human induced pluripotent stem cells (iPSCs) respond to activation of RA receptors (RARs) to impart BBB phenotypes. Here the authors identified that RA application to iPSC-derived BMECs at days 6-8 of differentiation led to a substantial elevation in transendothelial electrical resistance and induction of VE-cadherin expression. Specific RAR agonists identified RARalpha, RARgamma, and RXRalpha as receptors capable of inducing barrier phenotypes. Moreover, RAR/RXRalpha costimulation elevated VE-cadherin expression and improved barrier fidelity to levels that recapitulated the effects of RA. This study elucidates the roles of RA signaling in iPSC-derived BMEC differentiation, and identifies directed agonist approaches that can improve BMEC fidelity for drug screening studies while also distinguishing potential nuclear receptor targets to explore in BBB dysfunction and therapy.

Stebbins, M. J., et al. (2016). "Differentiation and characterization of human pluripotent stem cell-derived brain microvascular endothelial cells." Methods **101**: 93-102.

The blood-brain barrier (BBB) is a critical component of the central nervous system (CNS) that regulates the flux of material between the blood and the brain. Because of its barrier properties, the BBB creates a bottleneck to CNS drug delivery. Human in vitro BBB models offer a potential tool to screen pharmaceutical libraries for CNS penetration as well as for BBB modulators in development and disease, yet primary and immortalized models respectively lack scalability and robust phenotypes. Recently, in vitro BBB models derived from human pluripotent stem cells (hPSCs) have helped overcome these challenges by providing a scalable and renewable source of human brain microvascular endothelial cells (BMECs). We have demonstrated that hPSC-derived BMECs exhibit robust structural and functional characteristics reminiscent of the in vivo BBB. Here, we provide a detailed description of the methods required to differentiate and functionally characterize hPSC-derived BMECs to facilitate their widespread use in downstream applications.

Stover, A. E., et al. (2016). "Culturing Human Pluripotent and Neural Stem Cells in an Enclosed Cell Culture System for Basic and Preclinical Research." J Vis Exp(112).

This paper describes how to use a custom manufactured, commercially available enclosed cell culture system for basic and preclinical research. Biosafety cabinets (BSCs) and incubators have long been the standard for culturing and expanding cell lines for basic and preclinical research. However, as the focus of many stem cell laboratories shifts from basic research to clinical translation, additional requirements are needed of the cell culturing system. All processes must be well documented and have exceptional requirements for sterility and reproducibility. In traditional incubators, gas concentrations and temperatures widely fluctuate anytime the cells are removed for feeding, passaging, or other manipulations. Such interruptions contribute to an environment that is not the standard for cGMP and GLP guidelines. These interruptions must be minimized especially when cells are utilized for therapeutic purposes. The motivation to move from the standard BSC and incubator system to a closed system is that such interruptions can be made negligible. Closed systems provide a work space to feed and manipulate cell cultures and maintain them in a controlled environment where temperature and gas concentrations are consistent. This way, pluripotent and multipotent stem cells can be maintained at optimum health from the moment of their derivation all the way to their eventual use in therapy.

Stover, A. E. and P. H. Schwartz (2011). "Adaptation of human pluripotent stem cells to feeder-free

conditions in chemically defined medium with enzymatic single-cell passaging." Methods Mol Biol **767**: 137-146.

This protocol describes the culture of human pluripotent stem cells (PSCs) under feeder-free conditions in a commercially available, chemically defined, growth medium, using Matrigel as a substrate and the enzyme solution Accutase for single-cell passaging. This system is strikingly different from traditional PSC culture, where the cells are co-cultured with feeder cells and in medium containing serum replacement. PSCs cultured in this new system have a different morphology than those cultured on feeder cells but retain their characteristic pluripotency. This feeder-free PSC culture system is conceptually similar to feeder-free systems that use mouse embryonic fibroblast (MEF)-conditioned medium (MEF-CM) and Matrigel substratum. Instead of MEF-CM, a very complex and undefined medium, this new system uses StemPro SFM, a chemically defined medium that permits enzymatic passaging with Accutase to disaggregate the colonies into single cells. Accutase passaging has been used in conjunction with Stempro in our hands for 20+ passages without detectable karyotypic abnormalities. We will also review techniques for adapting cultures previously grown on MEFs, routine passaging of the cells, and cryopreservation.

Sugawara, T., et al. (2012). "Investigating cellular identity and manipulating cell fate using induced pluripotent stem cells." Stem Cell Res Ther **3**(2): 8.

Induced pluripotent stem (iPS) cells, obtained from reprogramming somatic cells by ectopic expression of a defined set of transcription factors or chemicals, are expected to be used as differentiated cells for drug screening or evaluations of drug toxicity and cell replacement therapies. As pluripotent stem cells, iPS cells are similar to embryonic stem (ES) cells in morphology and marker expression. Several types of iPS cells have been generated using combinations of reprogramming molecules and/or small chemical compounds from different types of tissues. A comprehensive approach, such as global gene or microRNA expression analysis and whole genomic DNA methylation profiling, has demonstrated that iPS cells are similar to their embryonic counterparts. Considering the substantial variation among iPS cell lines reported to date, the safety and therapeutic implications of these differences should be thoroughly evaluated before they are used in cell therapies. Here, we review recent research defining the concept of standardization for iPS cells, their ability to differentiate and the identity of the differentiated cells.

Sugita, S., et al. (2015). "Inhibition of T-cell activation by retinal pigment epithelial cells derived from induced pluripotent stem cells." Invest Ophthalmol Vis Sci **56**(2): 1051-1062.

PURPOSE: The purpose of this study was to determine whether human retinal pigment epithelial (RPE) cells from induced pluripotent stem (iPS) cells could inhibit T-cell activation in vitro. METHODS: Cultured iPS-derived RPE (iPS-RPE) cells were established from fresh skin tissues or dental pulp cells obtained from healthy donors or a retinal patient after informed consent was obtained. To confirm expression of the specific markers on iPS and iPS-RPE cells, immunohistochemistry, quantitative RT-PCR (qRT-PCR), and flow cytometry were performed. Target T cells were obtained from peripheral blood mononuclear cells of healthy donors. Target T cells were assessed for proliferation by incorporation of bromodeoxyuridine or carboxyfluorescein succinimidyl ester for production of cytokines such as IFN-gamma. Expression of TGFbeta and other candidate molecules by iPS-RPE cells was evaluated with flow cytometry, ELISA, multiplex cytokine array, immunohistochemistry, and qRT-PCR. RESULTS: The RPE cells we established from iPS cells had many characteristics of mature RPE cells but no characteristics of pluripotent stem cells. Cultured iPS-RPE cells inhibited cell proliferation and production of IFN-gamma by activated CD4(+) T cells. In some bystander T cells, iPS-derived RPE cells induced CD25(+)Foxp3(+) regulatory T cells in vitro. Induced pluripotent stem-RPE cells constitutively expressed TGFbeta and suppressed activation of T cells via soluble TGFbeta, because TGFbeta-downregulated iPS-RPE cells did not inhibit this T-cell activation. CONCLUSIONS: Cultured iPS-derived retinal cells fully suppress T-cell activation. Transplantation of iPS-RPE cells into the eye might be a therapy for retinal disorders.

Sugita, S., et al. (2018). "Natural Killer Cell Inhibition by HLA-E Molecules on Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelial Cells." Invest Ophthalmol Vis Sci **59**(5): 1719-1731.

Purpose: To determine whether human induced pluripotent stem (iPS) cell-derived retinal pigment epithelial (RPE) cells (iPS-RPE) can suppress natural killer (NK) cell activation. Methods: iPS-RPE cells were cocultured with peripheral blood mononuclear cells (PBMCs) or purified NK cells from healthy donors after stimulation with cytokines. To confirm expression of NK cell-specific markers, flow cytometry and quantitative RT-PCR (qRT-PCR) were performed. NK cells (or PBMCs) cocultured with iPS-RPE cells were assessed for proliferation by Ki-67 expression with flow cytometry, and NK suppression

by RPE cells was assessed for granzyme B production with ELISA. Human leukocyte antigen (HLA) expression including HLA-E on iPS-RPE cells was evaluated with flow cytometry and qRT-PCR. The effect of HLA-E downregulation was also investigated using small interfering RNA (siRNA) systems. Following iPS-RPE cell transplantation in vivo, we evaluated NK cell invasion in the retina with immunohistochemistry. Results: Activated NK cells expressed NK-related markers such as CD16, CD56, and CD11b, and NK cells produced cytotoxic agents such as granzyme B, perforin, and TNF-alpha. Human iPS-RPE cells inhibited cell proliferation and production of these cytotoxic agents by activated NK cells in vitro. iPS-RPE cells constitutively expressed HLA-E and suppressed NK cell activation through an interaction between HLA-E and CD94/NKG2A. Moreover, immunohistochemical evaluation of monkey RPE transplantation into in vivo immune rejection models showed no NK cell invasion in the retina in allografts or xenografts except for one xenografted eye. Conclusions: Cultured iPS cell-derived RPE cells greatly suppress NK cell activation. Thus, NK cells might be inactivated when exposed to this type of retinal cell.

Sugiura, M., et al. (2014). "Induced pluripotent stem cell generation-associated point mutations arise during the initial stages of the conversion of these cells." Stem Cell Reports **2**(1): 52-63.

A large number of point mutations have been identified in induced pluripotent stem cell (iPSC) genomes to date. Whether these mutations are associated with iPSC generation is an important and controversial issue. In this study, we approached this critical issue in different ways, including an assessment of iPSCs versus embryonic stem cells (ESCs), and an investigation of variant allele frequencies and the heterogeneity of point mutations within a single iPSC clone. Through these analyses, we obtained strong evidence that iPSC-generation-associated point mutations occur frequently in a transversion-predominant manner just after the onset of cell lineage conversion. The heterogeneity of the point mutation profiles within an iPSC clone was also revealed and reflects the history of the emergence of each mutation. Further, our results suggest a possible approach for establishing iPSCs with fewer point mutations.

Sui, L., et al. (2018). "Pancreatic Beta Cell Differentiation From Human Pluripotent Stem Cells." Curr Protoc Hum Genet **99**(1): e68.

Insulin-expressing beta cells are crucial for the maintenance of systemic glucose homeostasis. Elucidation of the molecular and cellular mechanisms of beta cell development, expansion, survival, and function are required for full understanding of the molecular pathogenesis of diabetes. However, access to human beta cells for such studies is limited by virtue of the logistics of acquisition, prior medical status of donor, and imperfect culture systems for maintaining beta cell identity and function after isolation from human pancreas. Here, a technique for generation of beta cells from human pluripotent stem cells (hPSCs) by modification of key signaling pathways during islet development is described. Up to 70% C-peptide-positive beta cells can be obtained from endodermal anlagen after 27 days of differentiation with specific growth factors and small molecules. Although 50% of them are monohormonal C-peptide-positive cells and have molecular and cellular characteristics consistent with human beta cells in the Islets of Langerhans, a sub-population co-expressing other endocrine markers are also generated, indicating the immaturity of these cells. (c) 2018 by John Wiley & Sons, Inc.

Sun, N. and H. Zhao (2014). "Seamless correction of the sickle cell disease mutation of the HBB gene in human induced pluripotent stem cells using TALENs." Biotechnol Bioeng **111**(5): 1048-1053.

Sickle cell disease (SCD) is the most common human genetic disease which is caused by a single mutation of human beta-globin (HBB) gene. The lack of long-term treatment makes the development of reliable cell and gene therapies highly desirable. Disease-specific patient-derived human induced pluripotent stem cells (hiPSCs) have great potential for developing novel cell and gene therapies. With the disease-causing mutations corrected in situ, patient-derived hiPSCs can restore normal cell functions and serve as a renewable autologous cell source for the treatment of genetic disorders. Here we successfully utilized transcription activator-like effector nucleases (TALENs), a recently emerged novel genome editing tool, to correct the SCD mutation in patient-derived hiPSCs. The TALENs we have engineered are highly specific and generate minimal off-target effects. In combination with piggyBac transposon, TALEN-mediated gene targeting leaves no residual ectopic sequences at the site of correction and the corrected hiPSCs retain full pluripotency and a normal karyotype. Our study demonstrates an important first step of using TALENs for the treatment of genetic diseases such as SCD, which represents a significant advance toward hiPSC-based cell and gene therapies.

Sun, Y. Q., et al. (2012). "Human pluripotent stem cell-derived mesenchymal stem cells prevent allergic airway inflammation in mice." Stem Cells **30**(12): 2692-2699.

We previously found that mesenchymal stem cells (MSCs) derived from human-induced pluripotent

stem cells (iPSCs) exerted immunomodulatory effects on Th2-mediated allergic rhinitis in vitro. However, their contribution to the asthma and allergic rhinitis in animal models remains unclear. In this study, we developed a mouse model of ovalbumin (OVA)-induced allergic inflammation in both the upper and lower airways and evaluated the effects of the systemic administration of human iPSC-MSCs and bone marrow-derived MSCs (BM-MSCs) on allergic inflammation. Our results showed that treatments with both the iPSC-MSCs and BM-MSCs before the challenge phase protected the animals from the majority of allergy-specific pathological changes. This protection included an inhibition of inflammatory cell infiltration and mucus production in the lung, a reduction in eosinophil infiltration in the nose, and a decrease in inflammatory cell infiltration in both the bronchoalveolar and nasal lavage fluids. In addition, treatment with iPSC-MSCs or BM-MSCs before the challenge phase resulted in reduced serum levels of Th2 immunoglobulins (e.g., IgE) and decreased levels of Th2 cytokines including interleukin (IL)-4, IL-5, or IL-13 in the bronchoalveolar and/or nasal lavage fluids. Similar therapeutic effects were observed when the animals were pretreated with human iPSC-MSCs before the sensitization phase. These data suggest that iPSC-MSCs may be used as an alternative strategy to adult MSCs in the treatment of asthma and allergic rhinitis.

Sung, L. Y., et al. (2014). "Telomere elongation and naive pluripotent stem cells achieved from telomerase haplo-insufficient cells by somatic cell nuclear transfer." Cell Rep **9**(5): 1603-1609.

Haplo-insufficiency of telomerase genes in humans leads to telomere syndromes such as dyskeratosis congenital and idiopathic pulmonary fibrosis. Generation of pluripotent stem cells from telomerase haplo-insufficient donor cells would provide unique opportunities toward the realization of patient-specific stem cell therapies. Recently, pluripotent human embryonic stem cells (ntESCs) have been efficiently achieved by somatic cell nuclear transfer (SCNT). We tested the hypothesis that SCNT could effectively elongate shortening telomeres of telomerase haplo-insufficient cells in the ntESCs with relevant mouse models. Indeed, telomeres of telomerase haplo-insufficient (Terc(+/-)) mouse cells are elongated in ntESCs. Moreover, ntESCs derived from Terc(+/-) cells exhibit naive pluripotency as evidenced by generation of Terc(+/-) ntESC clone pups by tetraploid embryo complementation, the most stringent test of naive pluripotency. These data suggest that SCNT could offer a powerful tool to reprogram telomeres and to discover the factors for robust restoration of telomeres and pluripotency of telomerase haplo-insufficient somatic cells.

Suzuki, H., et al. (2010). "Therapeutic angiogenesis by transplantation of induced pluripotent stem cell-derived Flk-1 positive cells." BMC Cell Biol **11**: 72.

BACKGROUND: Induced pluripotent stem (iPS) cells are the novel stem cell population induced from somatic cells. It is anticipated that iPS will be used in the expanding field of regenerative medicine. Here, we investigated whether implantation of fetal liver kinase-1 positive (Flk-1+) cells derived from iPS cells could improve angiogenesis in a mouse hind limb model of ischemia. RESULTS: Flk-1+ cells were induced from iPS cells after four to five days of culture. Hind limb ischemia was surgically induced and sorted Flk-1+ cells were directly injected into ischemic hind limbs of athymic nude mice. Revascularization of the ischemic hind limb was accelerated in mice that were transplanted with Flk-1+ cells compared with control mice, which were transplanted with vehicle, as evaluated by laser Doppler blood flowmetry. Transplantation of Flk-1+ cells also increased expression of VEGF mRNA in ischemic tissue compared to controls. CONCLUSIONS: Direct local implantation of iPS cell-derived Flk-1+ cells would salvage tissues from ischemia. These data indicate that iPS cells could be valuable in the therapeutic induction of angiogenesis.

Suzuki, N., et al. (2012). "Establishment of retinal progenitor cell clones by transfection with Pax6 gene of mouse induced pluripotent stem (iPS) cells." Neurosci Lett **509**(2): 116-120.

We previously reported that transfection of Pax6 gene which regulated early events in eye development into mouse ES cells brought about their differentiation into retinal progenitors. Here, we attempted to establish cloned retinal progenitors which had ability to further differentiate into photoreceptor like cells by transfecting mouse induced pluripotent stem (iPS) cells with Pax6 gene. Undifferentiated iPS cells were transfected with Pax6 cDNA, followed by selection with G418. After limiting dilution culture, we selected cloned Pax6-transfected cells, which simultaneously expressed mRNAs of Nestin, Musashi1, Six3 and Chx10 for further characterization. We obtained totally 8 clonally expanding Pax6-transfected cells. They started to express mRNAs of Brn3b, Cone-rod homeobox (Crx), pkc, CD73, rhodopsin and the gamma-subunit of rod cGMP phosphodiesterase (PDEgamma). Flow cytometric analysis revealed that almost half of the cells were CD73+, a marker of photoreceptor precursors. Western blotting confirmed cytoplasmic protein expression of rhodopsin. High KCl stimulation increased free Ca influx into the cells on

Ca(2+) imaging. iPS cells transfected with Pax6 gene, followed by subsequent limiting dilution culture became retinal progenitors including photoreceptor like cells. The cloned cell lines may be useful for analyzing differentiation requirement of retinal progenitors.

Takacs, E., et al. (2017). "Immunogenic Dendritic Cell Generation from Pluripotent Stem Cells by Ectopic Expression of Runx3." J Immunol **198**(1): 239-248.

Application of dendritic cells (DCs) to prime responses to tumor Ags provides a promising approach to immunotherapy. However, only a limited number of DCs can be manufactured from adult precursors. In contrast, pluripotent embryonic stem (ES) cells represent an inexhaustible source for DC production, although it remains a major challenge to steer directional differentiation because ES cell-derived cells are typically immature with impaired functional capacity. Consistent with this notion, we found that mouse ES cell-derived DCs (ES-DCs) represented less mature cells compared with bone marrow-derived DCs. This finding prompted us to compare the gene expression profile of the ES cell- and adult progenitor-derived, GM-CSF-instructed, nonconventional DC subsets. We quantified the mRNA level of 17 DC-specific transcription factors and observed that 3 transcriptional regulators (Irf4, Spi-B, and Runx3) showed lower expression in ES-DCs than in bone marrow-derived DCs. In light of this altered gene expression, we probed the effects of these transcription factors in developing mouse ES-DCs with an isogenic expression screen. Our analysis revealed that forced expression of Irf4 repressed ES-DC development, whereas, in contrast, Runx3 improved the ES-DC maturation capacity. Moreover, LPS-treated and Runx3-activated ES-DCs exhibited enhanced T cell activation and migratory potential. In summary, we found that ex vivo-generated ES-DCs had a compromised maturation ability and immunogenicity. However, ectopic expression of Runx3 enhances cytokine-driven ES-DC development and acts as an instructive tool for the generation of mature DCs with enhanced immunogenicity from pluripotent stem cells.

Takayama, K., et al. (2017). "Generation of safe and therapeutically effective human induced pluripotent stem cell-derived hepatocyte-like cells for regenerative medicine." Hepatol Commun **1**(10): 1058-1069.

Hepatocyte-like cells (HLCs) differentiated from human induced pluripotent stem (iPS) cells are expected to be applied for regenerative medicine. In this study, we attempted to generate safe and therapeutically effective human iPS-HLCs for hepatocyte transplantation. First, human iPS-HLCs were generated from a human leukocyte antigen-homozygous donor on the assumption that the allogenic transplantation might be carried out. Highly efficient hepatocyte differentiation was performed under a feeder-free condition using human recombinant laminin 111, laminin 511, and type IV collagen. The percentage of asialoglycoprotein receptor 1-positive cells was greater than 80%, while the percentage of residual undifferentiated cells was approximately 0.003%. In addition, no teratoma formation was observed even at 16 weeks after human iPS-HLC transplantation. Furthermore, harmful genetic somatic single-nucleotide substitutions were not observed during the hepatocyte differentiation process. We also developed a cryopreservation protocol for hepatoblast-like cells without negatively affecting their hepatocyte differentiation potential by programming the freezing temperature. To evaluate the therapeutic potential of human iPS-HLCs, these cells (1 x 10(6) cells/mouse) were intrasplenically transplanted into acute liver injury mice treated with 3 mL/kg CCl4 only once and chronic liver injury mice treated with 0.6 mL/kg CCl4 twice weekly for 8 weeks. By human iPS-HLC transplantation, the survival rate of the acute liver injury mice was significantly increased and the liver fibrosis level of chronic liver injury mice was significantly decreased. Conclusion: We were able to generate safe and therapeutically effective human iPS-HLCs for hepatocyte transplantation. (Hepatology Communications 2017;1:1058-1069).

Takayama, K. and H. Mizuguchi (2017). "Generation of human pluripotent stem cell-derived hepatocyte-like cells for drug toxicity screening." Drug Metab Pharmacokinet **32**(1): 12-20.

Because drug-induced liver injury is one of the main reasons for drug development failures, it is important to perform drug toxicity screening in the early phase of pharmaceutical development. Currently, primary human hepatocytes are most widely used for the prediction of drug-induced liver injury. However, the sources of primary human hepatocytes are limited, making it difficult to supply the abundant quantities required for large-scale drug toxicity screening. Therefore, there is an urgent need for a novel unlimited, efficient, inexpensive, and predictive model which can be applied for large-scale drug toxicity screening. Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are able to replicate indefinitely and differentiate into most of the body's cell types, including hepatocytes. It is expected that hepatocyte-like cells generated from human ES/iPS cells (human ES/iPS-HLCs) will be a useful tool for drug toxicity screening. To apply human ES/iPS-HLCs to various applications including drug toxicity screening, homogenous and functional HLCs must be differentiated from human ES/iPS cells. In this review, we will introduce the current status of hepatocyte

differentiation technology from human ES/iPS cells and a novel method to predict drug-induced liver injury using human ES/iPS-HLCs.

Takei, H., et al. (2018). "Skewed megakaryopoiesis in human induced pluripotent stem cell-derived haematopoietic progenitor cells harbouring calreticulin mutations." Br J Haematol **181**(6): 791-802.

Somatic mutations in the calreticulin (CALR) gene have been found in most patients with JAK2- and MPL-unmutated Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs). It has recently been shown that mutant CALR constitutively activates the thrombopoietin receptor MPL and, thus, plays a causal role in the development of MPNs. However, the roles of mutant CALR in human haematopoietic cell differentiation remain predominantly elusive. To examine the impact of the 5-base insertion mutant CALR gene (Ins5) on haematopoietic cell differentiation, we generated induced pluripotent stem cells from an essential thrombocythaemia (ET) patient harbouring a CALR-Ins5 mutation and from a healthy individual (WT). Megakaryopoiesis was more prominent in Ins5-haematopoietic progenitor cells (Ins5-HPCs) than in WT-HPCs, implying that the system recapitulates megakaryocytosis observed in the bone marrow of CALR-mutant ET patients. Ins5-HPCs exhibited elevated expression levels of GATA1 and GATA2, suggesting a premature commitment to megakaryocytic differentiation in progenitor cells. We also demonstrated that 3-hydroxy anagrelide markedly perturbed megakaryopoiesis, but not erythropoiesis. Collectively, we established an in vitro model system that recapitulates megakaryopoiesis caused by mutant CALR. This system can be used to validate therapeutic compounds for MPN patients harbouring CALR mutations and in detailed studies on mutant CALR in human haematological cell differentiation.

Talavera-Adame, D., et al. (2016). "Effective endothelial cell and human pluripotent stem cell interactions generate functional insulin-producing beta cells." Diabetologia **59**(11): 2378-2386.

AIMS/HYPOTHESIS: Endothelial cells (ECs) play an essential role in pancreatic organogenesis. We hypothesise that effective in vitro interactions between human microvascular endothelial cells (HMECs) and human pluripotent stem cells (hPSCs) results in the generation of functional pancreatic beta cells. METHODS: Embryoid bodies (EBs) derived from hPSCs were cultured alone (controls) or with ECs in collagen gels. Subsequently, cells were analysed for pancreatic beta cell markers, and then isolated and expanded. Insulin secretion in response to glucose was evaluated in vitro by static and dynamic (perifusion) assays, and in vivo by EB transplantation into immunodeficient mice. RESULTS: Co-cultured EBs had a higher expression of mature beta cells markers and enhanced insulin secretion in vitro, compared with controls. In mice, transplanted EBs had higher levels of human C-peptide secretion with a significant reduction in hyperglycaemia after the selective destruction of native pancreatic beta cells. In addition, there was significant in vitro upregulation of bone morphogenetic proteins 2 and 4 (BMP-2, 4) in co-cultured cells, compared with controls. CONCLUSIONS/INTERPRETATION: ECs provide essential signalling in vitro, such as activation of the BMP pathway, for derivation of functional insulin-producing beta cells from hPSCs.

Talavera-Adame, D., et al. (2016). "Erratum to: Effective endothelial cell and human pluripotent stem cell interactions generate functional insulin-producing beta cells." Diabetologia **59**(12): 2730.

Talbot, N. C., et al. (2017). "Bovine trophectoderm cells induced from bovine fibroblasts with induced pluripotent stem cell reprogramming factors." Mol Reprod Dev **84**(6): 468-485.

Thirteen independent induced bovine trophectroderm (iBT) cell lines were established by reprogramming bovine fetal liver-derived fibroblasts after viral-vector transduction with either six or eight factors, including POU5F1 (OCT4), KLF4, SOX2, MYC, NANOG, LIN28, SV40 large T antigen, and hTERT. Light- and electron-microscopy analysis showed that the iBT cells had epithelial cell morphology typical of bovine trophectoderm cells. Reverse-transcription-PCR assays indicated that all of the cell lines expressed interferon-tau (IFNT) at passages 1 or 2. At later passages (>/= passage 8), however, immunoblot and antiviral activity assays revealed that more than half of the iBT cell lines had stopped expressing IFNT. Messenger RNAs specific to trophectoderm differentiation and function were found in the iBT cell lines, and 2-dimensional-gel analysis for cellular proteins showed an expression pattern similar to that of trophectoderm cell lines derived from bovine blastocysts. Integration of some of the human reprogramming factors, including POU5F1, KLF4, SOX2, MYC, NANOG, and LIN28, were detected by PCR, but their transcription was mostly absent in the iBT cell lines. Gene expression assessment of endogenous bovine reprogramming factor orthologs revealed endogenous bLIN28 and bMYC transcripts in all; bSOX2 and bNANOG in none; and bKLF4 and bPOU5F1 in less than half of the iBT cell lines. These results demonstrate that bovine trophectoderm can be induced via reprogramming factor expression from bovine liver-derived fibroblasts, although other fibroblast populations-e.g., derived from fetal thigh

tissue-may produce similar results, albeit at lower frequencies.

Tamaoki, N., et al. (2010). "Dental pulp cells for induced pluripotent stem cell banking." J Dent Res **89**(8): 773-778.

Defined sets of transcriptional factors can reprogram human somatic cells to induced pluripotent stem (iPS) cells. However, many types of human cells are not easily accessible to minimally invasive procedures. Here we evaluated dental pulp cells (DPCs) as an optimal source of iPS cells, since they are easily obtained from extracted teeth and can be expanded under simple culture conditions. From all 6 DPC lines tested with the conventional 3 or 4 reprogramming factors, iPS cells were effectively established from 5 DPC lines. Furthermore, determination of the HLA types of 107 DPC lines revealed 2 lines homozygous for all 3 HLA loci and showed that if an iPS bank is established from these initial pools, the bank will cover approximately 20% of the Japanese population with a perfect match. Analysis of these data demonstrates the promising potential of DPC collections as a source of iPS cell banks for use in regenerative medicine.

Tamo, L., et al. (2018). "Generation of an alveolar epithelial type II cell line from induced pluripotent stem cells." Am J Physiol Lung Cell Mol Physiol.

Differentiation of primary alveolar type II epithelial cells (AEC type II) to AEC type I in culture is a major barrier in the study of the alveolar epithelium in vitro. The establishment of an AEC type II cell line derived from induced pluripotent stem cells (iPSC) represents a novel opportunity to study alveolar epithelial cell biology, for instance in the context of lung injury, fibrosis and repair. In the present study, we generated long lasting AECII from iPSC (iPSC-AEC). Long lasting iPSC-AECII (LL)-iPSC-AECII) displayed morphological characteristics of AECII including growth in a cobble stone monolayer, the presence of lamellar bodies and microvilli as shown by electron microscopy. Also, (LL)-iPSC-AECII expressed AEC type II proteins such as cytokeratin, surfactant protein C and lysotracker DND 26 (a marker for lamellar bodies). Furthermore, the (LL)-iPSC-AECII exhibited functional properties of AECII by an increase of transepithelial electrical resistance (TERR) over time, secretion of inflammatory mediators in biologically relevant quantities (interleukin-6, interleukin-8) and efficient in vitro alveolar epithelial wound repair. Consistent with the AECII phenotype, the cell line showed the ability to uptake and release surfactant protein B, to secrete phospholipids and to differentiate into AEC type I. In summary, we established a long lasting, but finite AEC type II cell line derived from iPSC as a novel cellular model to study alveolar epithelial cell biology in lung health and disease.

Tan, H. K., et al. (2014). "Human finger-prick induced pluripotent stem cells facilitate the development of stem cell banking." Stem Cells Transl Med **3**(5): 586-598.

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients can be a good model for studying human diseases and for future therapeutic regenerative medicine. Current initiatives to establish human iPSC (hiPSC) banking face challenges in recruiting large numbers of donors with diverse diseased, genetic, and phenotypic representations. In this study, we describe the efficient derivation of transgene-free hiPSCs from human finger-prick blood. Finger-prick sample collection can be performed on a "do-it-yourself" basis by donors and sent to the hiPSC facility for reprogramming. We show that single-drop volumes of finger-prick samples are sufficient for performing cellular reprogramming, DNA sequencing, and blood serotyping in parallel. Our novel strategy has the potential to facilitate the development of large-scale hiPSC banking worldwide.

Tan, R. P., et al. (2018). "Integration of induced pluripotent stem cell-derived endothelial cells with polycaprolactone/gelatin-based electrospun scaffolds for enhanced therapeutic angiogenesis." Stem Cell Res Ther **9**(1): 70.

BACKGROUND: Induced pluripotent stem-cell derived endothelial cells (iPSC-ECs) can be generated from any somatic cell and their iPSC sources possess unlimited self-renewal. Previous demonstration of their proangiogenic activity makes them a promising cell type for treatment of ischemic injury. As with many other stem cell approaches, the low rate of in-vivo survival has been a major limitation to the efficacy of iPSC-ECs to date. In this study, we aimed to increase the in-vivo lifetime of iPSC-ECs by culturing them on electrospun polycaprolactone (PCL)/gelatin scaffolds, before quantifying the subsequent impact on their proangiogenic function. METHODS: iPSC-ECs were isolated and stably transfected with a luciferase reporter to facilitate quantification of cell numbers and non-invasive imaging in-vivo PCL/gelatin scaffolds were engineered using electrospinning to obtain woven meshes of nanofibers. iPSC-ECs were cultured on scaffolds for 7 days. Subsequently, cell growth and function were assessed in vitro followed by implantation in a mouseback subcutaneous model for 7 days. RESULTS: Using a matrix of conditions, we found that scaffold blends with ratios of PCL:gelatin of 70:30 (PG73) spun at high flow rates supported the greatest levels of iPSC-EC growth, retention of phenotype, and function in

vitro. Implanting iPSC-ECs seeded on PG73 scaffolds in vivo improved their survival up to 3 days, compared to cells directly injected into control wounds, which were no longer observable within 1 h. Enhanced engraftment improved blood perfusion, observed through non-invasive laser Doppler imaging. Immunohistochemistry revealed a corresponding increase in host angiogenic mechanisms characterized by the enhanced recruitment of macrophages and the elevated expression of proangiogenic cytokines vascular endothelial growth factor and placental growth factor. CONCLUSIONS: Knowledge of these mechanisms combined with a deeper understanding of the scaffold parameters influencing this function provides the groundwork for optimizing future iPSC-EC therapies utilizing engraftment platforms. The development of combined scaffold and iPSC-EC therapies could ultimately improve therapeutic angiogenesis and the treatment of ischemic injury.

Tang, L., et al. (2017). "Modeling cadmium-induced endothelial toxicity using human pluripotent stem cell-derived endothelial cells." Sci Rep **7**(1): 14811.

Cadmium (Cd) is a harmful heavy metal that results in vascular diseases such as atherosclerosis. Prior evidence revealed that Cd induced endothelial cell (EC) death and dysfunction, supporting that ECs are a primary target of Cd-induced toxicity, and can cause severe pathologies of vascular diseases. However, the underlying mechanisms remain unclear. In this study, we investigated the mechanisms of Cd-induced endothelial toxicity in a human model system of H9 human pluripotent stem cell-derived endothelial cells (H9-ECs). We showed that H9-ECs were susceptible to CdCl2 induction, leading to detrimental changes of cell structure and significantly elevated level of apoptosis. We demonstrated that CdCl2-treated H9-ECs gave rise to a clear EC dysfunction phenotype and significantly differential transcriptomic profile. Signaling pathway analysis revealed that P38 or ERK signaling pathway is critical to cadmium-induced EC apoptosis and dysfunction, and inhibition of P38 or ERK effectively rescued CdCl2-induced endothelial toxicity in H9-ECs. Conclusively, hPSC-ECs can be a reliable model to recapitulate the EC pathological features and transcriptomic profile, which may provide a unique platform for understanding the cellular and molecular mechanisms of Cd-induced endothelial toxicity and for identifying therapeutic drugs for Cd-induced vascular diseases.

Tang, Z. H., et al. (2016). "Genetic Correction of Induced Pluripotent Stem Cells From a Deaf Patient With MYO7A Mutation Results in Morphologic and Functional Recovery of the Derived Hair Cell-Like Cells." Stem Cells Transl Med **5**(5): 561-571.

UNLABELLED: The genetic correction of induced pluripotent stem cells (iPSCs) induced from somatic cells of patients with sensorineural hearing loss (caused by hereditary factors) is a promising method for its treatment. The correction of gene mutations in iPSCs could restore the normal function of cells and provide a rich source of cells for transplantation. In the present study, iPSCs were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T; P-iPSCs), the asymptomatic father of the patient (MYO7A c.1184G>A mutation; CF-iPSCs), and a normal donor (MYO7A(WT/WT); C-iPSCs). One of MYO7A mutation sites (c.4118C>T) in the P-iPSCs was corrected using CRISPR/Cas9. The corrected iPSCs (CP-iPSCs) retained cell pluripotency and normal karyotypes. Hair cell-like cells induced from CP-iPSCs showed restored organization of stereocilia-like protrusions; moreover, the electrophysiological function of these cells was similar to that of cells induced from C-iPSCs and CF-iPSCs. These results might facilitate the development of iPSC-based gene therapy for genetic disorders. SIGNIFICANCE: Induced pluripotent stem cells (iPSCs) were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T). One of the MYO7A mutation sites (c.4118C>T) in the iPSCs was corrected using CRISPR/Cas9. The genetic correction of MYO7A mutation resulted in morphologic and functional recovery of hair cell-like cells derived from iPSCs. These findings confirm the hypothesis that MYO7A plays an important role in the assembly of stereocilia into stereociliary bundles. Thus, the present study might provide further insight into the pathogenesis of sensorineural hearing loss and facilitate the development of therapeutic strategies against monogenic disease through the genetic repair of patient-specific iPSCs.

Tani, K. (2015). "Towards the safer clinical translation of human induced pluripotent stem cell-derived cells to regenerative medicine." Mol Ther Methods Clin Dev **2**: 15032.

Tano, K., et al. (2014). "A novel in vitro method for detecting undifferentiated human pluripotent stem cells as impurities in cell therapy products using a highly efficient culture system." PLoS One **9**(10): e110496.

Innovative applications of cell therapy products (CTPs) derived from human pluripotent stem cells (hPSCs) in regenerative medicine are currently being developed. The presence of residual undifferentiated hPSCs in CTPs is a quality concern associated with tumorigencity. However, no simple in vitro method for direct detection of undifferentiated hPSCs that contaminate CTPs has been developed.

Here, we show a novel approach for direct and sensitive detection of a trace amount of undifferentiated human induced pluripotent stem cells (hiPSCs) using a highly efficient amplification method in combination with laminin-521 and Essential 8 medium. Essential 8 medium better facilitated the growth of hiPSCs dissociated into single cells on laminin-521 than in mTeSR1 medium. hiPSCs cultured on laminin-521 in Essential 8 medium were maintained in an undifferentiated state and they maintained the ability to differentiate into various cell types. Essential 8 medium allowed robust hiPSC proliferation plated on laminin-521 at low cell density, whereas mTeSR1 did not enhance the cell growth. The highly efficient culture system using laminin-521 and Essential 8 medium detected hiPSCs spiked into primary human mesenchymal stem cells (hMSCs) or human neurons at the ratio of 0.001%-0.01% as formed colonies. Moreover, this assay method was demonstrated to detect residual undifferentiated hiPSCs in cell preparations during the process of hMSC differentiation from hiPSCs. These results indicate that our highly efficient amplification system using a combination of laminin-521 and Essential 8 medium is able to detect a trace amount of undifferentiated hPSCs contained as impurities in CTPs and would contribute to quality assessment of hPSC-derived CTPs during the manufacturing process.

Tashiro, K., et al. (2012). "Inhibition of Lnk in mouse induced pluripotent stem cells promotes hematopoietic cell generation." Stem Cells Dev **21**(18): 3381-3390.

Embryonic stem (ES) cell- and induced pluripotent stem (iPS) cell-derived hematopoietic stem/progenitor cells (HSPCs) are considered as an unlimited source for HSPC transplantation. However, production of immature hematopoietic cells, especially HSPCs, from ES and iPS cells has been challenging. The adaptor protein Lnk has been shown to negatively regulate HSPC function via the inhibition of thrombopoietin (TPO) and stem cell factor signaling, and Lnk-deficient HSPCs show an enhanced self-renewal and repopulation capacity. In this study, we examined the role of Lnk on the hematopoietic differentiation from mouse ES and iPS cells by the inhibition of Lnk using a dominant-negative mutant of the Lnk (DN-Lnk) gene. We generated mouse ES and iPS cells stably expressing a DN-Lnk, and found that enforced expression of a DN-Lnk in ES and iPS cells led to an enhanced generation of Flk-1-positive mesodermal cells, thereby could increase in the expression of hematopoietic transcription factors, including Scl and Runx1. We also showed that the number of both total hematopoietic cells and immature hematopoietic cells with colony-forming potential in DN-Lnk-expressing cells was significantly increased in comparison with that in control cells. Furthermore, Lnk inhibition by the overexpression of the DN-Lnk gene augmented the TPO-induced phosphorylation of Erk1/2 and Akt, indicating the enhanced sensitivity to TPO. Adenovirus vector-mediated transient DN-Lnk gene expression in ES and iPS cells could also increase the hematopoietic cell production. Our data clearly showed that the inhibition of Lnk in ES and iPS cells could result in the efficient generation and expansion of hematopoietic cells.

Tashiro, S., et al. (2018). "High cell density suppresses BMP4-induced differentiation of human pluripotent stem cells to produce macroscopic spatial patterning in a unidirectional perfusion culture chamber." J Biosci Bioeng **126**(3): 379-388.

Spatial pattern formation is a critical step in embryogenesis. Bone morphogenetic protein 4 (BMP4) and its inhibitors are major factors for the formation of spatial patterns during embryogenesis. However, spatial patterning of the human embryo is unclear because of ethical issues and isotropic culture environments resulting from conventional culture dishes. Here, we utilized human pluripotent stem cells (hiPSCs) and a simple anisotropic (unidirectional perfusion) culture chamber, which creates unidirectional conditions, to measure the cell community effect. The influence of cell density on BMP4-induced differentiation was explored during static culture using a conventional culture dish. Immunostaining of the early differentiation marker SSEA-1 and the mesendoderm marker BRACHYURY revealed that high cell density suppressed differentiation, with small clusters of differentiated and undifferentiated cells formed. Addition of five-fold higher concentration of BMP4 showed similar results, suggesting that suppression was not caused by depletion of BMP4 but rather by high cell density. Quantitative RT-PCR array analysis showed that BMP4 induced multi-lineage differentiation, which was also suppressed under high-density conditions. We fabricated an elongated perfusion culture chamber, in which proteins were transported unidirectionally, and hiPSCs were cultured with BMP4. At low density, the expression was the same throughout the chamber. However, at high density, SSEA-1 and BRACHYURY were expressed only in upstream cells, suggesting that some autocrine/paracrine factors inhibited the action of BMP4 in downstream cells to form the spatial pattern. Human iPSCs cultured in a perfusion culture chamber might be useful for studying in vitro macroscopic pattern formation in human embryogenesis.

Tateno, H., et al. (2017). "Development of a practical sandwich assay to detect human pluripotent stem cells using cell culture media." Regen Ther **6**: 1-8.

Human pluripotent stem cells are considered to be ideal cell sources for regenerative medicine, but their clinical and industrial application is hindered by their tumorigenic potential. Previously we have identified a pluripotent stem cell-specific lectin rBC2LCN recognizing podocalyxin as a cell surface ligand. More recently, podocalyxin was found to be a soluble ligand of rBC2LCN that is secreted specifically from human pluripotent stem cells into cell culture media. Taking advantage of this phenomenon, we have previously developed a sandwich assay targeting the soluble podocalyxin using rBC2LCN as a capturing probe and another lectin rABA as an overlay probe to detect human pluripotent stem cells residing in cell therapy products derived from human pluripotent stem cells. A drawback to this, however, was that cell culture media containing fetal bovine serum was found to cause a substantial background signal to the sandwich assay. To reduce the background and increase the sensitivity, we screened different overlay probes to detect the soluble podocalyxin. Among them, an anti-keratan sulfate monoclonal antibody called R-10G showed the highest sensitivity and provided a low background signal to fetal bovine serum. The established sandwich assay using rBC2LCN and R-10G was proved to be powerful, which allowed the high-sensitive detection of human induced pluripotent stem cells residing among clinical-grade cardiomyocytes and neural stem cells, both derived from human induced pluripotent stem cells. The developed method has a possibility to be a standard technology to detect human induced pluripotent stem cells resided in various types of cell therapy products.

Tateno, H., et al. (2014). "Live-cell imaging of human pluripotent stem cells by a novel lectin probe rBC2LCN." Methods Mol Biol **1200**: 313-318.

We performed comprehensive glycome analysis of a large set of human pluripotent stem cells (hPSCs) using a high-density lectin microarray. We found that a recombinant lectin, rBC2LCN, binds exclusively to all of the undifferentiated hPSCs tested, but not to differentiated somatic cells. rBC2LCN can be used for both the staining and sorting of fixed and live hPSCs. rBC2LCN could serve as a novel detection reagent for hPSCs, particularly given that rBC2LCN is cost effective and, unlike conventional antibodies which require mammalian cells for their production, is easy to produce in a large amount (0.1 g/L) in an Escherichia coli expression system. Here we describe protocols for the fluorescence staining of fixed and live hPSCs and their detection by flow cytometry.

Taura, A., et al. (2014). "Effects of mouse utricle stromal tissues on hair cell induction from induced pluripotent stem cells." BMC Neurosci **15**: 121.

BACKGROUND: Hair cells are important for maintaining our sense of hearing and balance. However, they are difficult to regenerate in mammals once they are lost. Clarification of the molecular mechanisms underlying inner ear disorders is also impeded by the anatomical limitation of experimental access to the human inner ear. Therefore, the generation of hair cells, possibly from induced pluripotent stem (iPS) cells, is important for regenerative therapy and studies of inner ear diseases. RESULTS: We generated hair cells from mouse iPS cells using an established stepwise induction protocol. First, iPS cells were differentiated into the ectodermal lineage by floating culture. Next, they were treated with basic fibroblast growth factor to induce otic progenitor cells. Finally, the cells were co-cultured with three kinds of mouse utricle tissues: stromal tissue, stromal tissue + sensory epithelium, and the extracellular matrix of stromal tissue. Hair cell-like cells were successfully generated from iPS cells using mouse utricle stromal tissues. However, no hair cell-like cells with hair bundle-like structures were formed using other tissues. CONCLUSIONS: Hair cell-like cells were induced from mouse iPS cells using mouse utricle stromal tissues. Certain soluble factors from mouse utricle stromal cells might be important for induction of hair cells from iPS cells.

Tchao, J., et al. (2013). "Engineered Human Muscle Tissue from Skeletal Muscle Derived Stem Cells and Induced Pluripotent Stem Cell Derived Cardiac Cells." Int J Tissue Eng **2013**: 198762.

During development, cardiac and skeletal muscle share major transcription factors and sarcomere proteins which were generally regarded as specific to either cardiac or skeletal muscle but not both in terminally differentiated adult cardiac or skeletal muscle. Here, we investigated whether artificial muscle constructed from human skeletal muscle derived stem cells (MDSCs) recapitulates developmental similarities between cardiac and skeletal muscle. We constructed 3-dimensional collagen-based engineered muscle tissue (EMT) using MDSCs (MDSC-EMT) and compared the biochemical and contractile properties with EMT using induced pluripotent stem (iPS) cell-derived cardiac cells (iPS-EMT). Both MDSC-EMT and iPS-EMT expressed cardiac specific troponins, fast skeletal muscle myosin heavy chain, and connexin-43 mimicking developing cardiac or skeletal muscle. At the transcriptional level, MDSC-EMT and iPS-EMT upregulated both cardiac and skeletal muscle-specific genes and expressed Nkx2.5 and Myo-D proteins. MDSC-EMT displayed intracellular calcium ion transients and responses to isoproterenol. Contractile force measurements of MDSC-EMT demonstrated functional properties of immature cardiac and skeletal muscle in both tissues. Results suggest that the EMT

from MDSCs mimics developing cardiac and skeletal muscle and can serve as a useful in vitro functioning striated muscle model for investigation of stem cell differentiation and therapeutic options of MDSCs for cardiac repair.

Teichert, A. M., et al. (2014). "The neural stem cell lineage reveals novel relationships among spermatogonial germ stem cells and other pluripotent stem cells." Stem Cells Dev **23**(7): 767-778.

The embryonic stem cell (ESC) derived from the inner cell mass is viewed as the core pluripotent cell (PC) type from which all other cell types emanate. This familiar perspective derives from an embryological time line in which PCs are ordered according to their time of appearance. However, this schema does not take into account their potential for interconversion, thereby excluding this critical quality of PCs. The persistence of bona fide pluripotent adult stem cells has garnered increasing attention in recent years. Adult pluripotent spermatogonial germ stem cells (aSGSCs) arise from primordial germ cells (pGCs) that emerge from the epiblast during gastrulation. Adult definitive neural stem cells (dNSCs) arise clonally from pluripotent embryonic primitive neural stem cells (pNSCs), which can also be derived clonally from ESCs. To test for stem cell-type convertibility, we employed differentiation in the clonal lineage from ESCs to pNSCs to dNSCs, and revealed the relationships and lineage positioning among various PC populations, including spermatogonial germ cells (aSGSCs), epiblast-derived stem cells (Epi-SCs) and the bFGF, Activin, and BIO-derived stem cell (FAB-SC). Adult, murine aSGSCs assumed a 'pseudo-ESC' state in vitro, and then differentiated into dNSCs, but not pNSCs. Similarly, Epi-SCs and FAB-SCs only gave rise to dNSCs and not to pNSCs. The results of these experiments suggest a new pluripotency lineage model describing the relationship(s) among PCs that better reflects the transitions between these cell types in vitro.

Telugu, B. P., et al. (2011). "Leukemia inhibitory factor (LIF)-dependent, pluripotent stem cells established from inner cell mass of porcine embryos." J Biol Chem **286**(33): 28948-28953.

The pig is important for agriculture and as an animal model in human and veterinary medicine, yet despite over 20 years of effort, there has been a failure to generate pluripotent stem cells analogous to those derived from mouse embryos. Here we report the production of leukemia inhibitory factor-dependent, so-called naive type, pluripotent stem cells from the inner cell mass of porcine blastocysts by up-regulating expression of KLF4 and POU5F1. The alkaline phosphatase-positive colonies resulting from reprogramming resemble mouse embryonic stem cells in colony morphology, cell cycle interval, transcriptome profile, and expression of pluripotent markers, such as POU5F1, SOX2, and surface marker SSEA1. They are dependent on leukemia inhibitory factor signaling for maintenance of pluripotency, can be cultured over extended passage, and have the ability to form teratomas. These cells derived from the inner cell mass of pig blastocysts are clearly distinct from the FGF2-dependent "primed" induced pluripotent stem cells described recently from porcine mesenchymal cells. The data are consistent with the hypothesis that the up-regulation of KLF4, as well as POU5F1, is required to create and stabilize the naive pluripotent state and may explain why the derivation of embryonic stem cells from pigs and other ungulates has proved so difficult.

Templin, C., et al. (2012). "Transplantation and tracking of human-induced pluripotent stem cells in a pig model of myocardial infarction: assessment of cell survival, engraftment, and distribution by hybrid single photon emission computed tomography/computed tomography of sodium iodide symporter transgene expression." Circulation **126**(4): 430-439.

BACKGROUND: Evaluation of novel cellular therapies in large-animal models and patients is currently hampered by the lack of imaging approaches that allow for long-term monitoring of viable transplanted cells. In this study, sodium iodide symporter (NIS) transgene imaging was evaluated as an approach to follow in vivo survival, engraftment, and distribution of human-induced pluripotent stem cell (hiPSC) derivatives in a pig model of myocardial infarction. METHODS AND RESULTS: Transgenic hiPSC lines stably expressing a fluorescent reporter and NIS (NIS(pos)-hiPSCs) were established. Iodide uptake, efflux, and viability of NIS(pos)-hiPSCs were assessed in vitro. Ten (+/-2) days after induction of myocardial infarction by transient occlusion of the left anterior descending artery, catheter-based intramyocardial injection of NIS(pos)-hiPSCs guided by 3-dimensional NOGA mapping was performed. Dual-isotope single photon emission computed tomographic/computed tomographic imaging was applied with the use of (123)I to follow donor cell survival and distribution and with the use of (99m)TC-tetrofosmin for perfusion imaging. In vitro, iodide uptake in NIS(pos)-hiPSCs was increased 100-fold above that of nontransgenic controls. In vivo, viable NIS(pos)-hiPSCs could be visualized for up to 15 weeks. Immunohistochemistry demonstrated that hiPSC-derived endothelial cells contributed to vascularization. Up to 12 to 15 weeks after transplantation, no teratomas were detected. CONCLUSIONS: This study describes for the first

time the feasibility of repeated long-term in vivo imaging of viability and tissue distribution of cellular grafts in large animals. Moreover, this is the first report demonstrating vascular differentiation and long-term engraftment of hiPSCs in a large-animal model of myocardial infarction. NIS(pos)-hiPSCs represent a valuable tool to monitor and improve current cellular treatment strategies in clinically relevant animal models.

Thakar, N. Y., et al. (2015). "RELB Alters Proliferation of Human Pluripotent Stem Cells via IMP3- and LIN28-Mediated Modulation of the Expression of IGF2 and Other Cell-Cycle Regulators." Stem Cells Dev **24**(16): 1888-1900.

The molecular mechanisms that orchestrate the exit from pluripotency, cell cycle progression, and lineage-specific differentiation in human pluripotent stem cells (hPSCs) are poorly understood. RELB, a key protein in the noncanonical nuclear factor-kappaB (NFkappaB) signaling pathway, was previously implicated in controlling the switch between human embryonic stem cell (hESC) proliferation and differentiation. Here, we show that RELB enhances the proliferation of hESCs and human-induced pluripotent stem cells (hiPSCs) without affecting their pluripotency. We demonstrate that RELB does this by interacting with two RNA-binding proteins LIN28A and IMP3 (IGF2 mRNA-binding protein 3); further, these interactions control mRNA levels and protein expression of insulin-like growth factor 2 (IGF2) and key cell-cycle genes. Finally, after stress, these proteins co-localize in stress granules in hESCs and iPSCs. Our data identify RELB as a novel regulator of hPSC proliferation, and suggest a new function for RELB, in addition to its widely accepted role as a transcription factor, that involves recruitment of IMP3 and LIN28 to the cytosolic mRNA translation-control domains for post-transcriptional modulation of IGF2 and cell-cycle gene expression.

Theka, I., et al. (2013). "Rapid generation of functional dopaminergic neurons from human induced pluripotent stem cells through a single-step procedure using cell lineage transcription factors." Stem Cells Transl Med **2**(6): 473-479.

Current protocols for in vitro differentiation of human induced pluripotent stem cells (hiPSCs) to generate dopamine (DA) neurons are laborious and time-expensive. In order to accelerate the overall process, we have established a fast protocol by expressing the developmental transcription factors ASCL1, NURR1, and LMX1A. With this method, we were able to generate mature and functional dopaminergic neurons in as few as 21 days, skipping all the intermediate steps for inducting and selecting embryoid bodies and rosette-neural precursors. Strikingly, the resulting neuronal conversion process was very proficient, with an overall efficiency that was more than 93% of all the coinfected cells. hiPSC-derived DA neurons expressed all the critical molecular markers of the DA molecular machinery and exhibited sophisticated functional features including spontaneous electrical activity and dopamine release. This one-step protocol holds important implications for in vitro disease modeling and is particularly amenable for exploitation in high-throughput screening protocols.

Thoma, E. C., et al. (2016). "Establishment of a translational endothelial cell model using directed differentiation of induced pluripotent stem cells from Cynomolgus monkey." Sci Rep **6**: 35830.

Due to their broad differentiation potential, pluripotent stem cells (PSCs) offer a promising approach for generating relevant cellular models for various applications. While human PSC-based cellular models are already advanced, similar systems for non-human primates (NHPs) are still lacking. However, as NHPs are the most appropriate animals for evaluating the safety of many novel pharmaceuticals, the availability of in vitro systems would be extremely useful to bridge the gap between cellular and animal models. Here, we present a NHP in vitro endothelial cell system using induced pluripotent stem cells (IPSCs) from Cynomolgus monkey (Macaca fascicularis). Based on an adapted protocol for human IPSCs, we directly differentiated macaque IPSCs into endothelial cells under chemically defined conditions. The resulting endothelial cells can be enriched using immuno-magnetic cell sorting and display endothelial marker expression and function. RNA sequencing revealed that the differentiation process closely resembled vasculogenesis. Moreover, we showed that endothelial cells derived from macaque and human IPSCs are highly similar with respect to gene expression patterns and key endothelial functions, such as inflammatory responses. These data demonstrate the power of IPSC differentiation technology to generate defined cell types for use as translational in vitro models to compare cell type-specific responses across species.

Tolosa, L., et al. (2016). "Clinical Application of Pluripotent Stem Cells: An Alternative Cell-Based Therapy for Treating Liver Diseases?" Transplantation **100**(12): 2548-2557.

The worldwide shortage of donor livers for organ and hepatocyte transplantation has prompted the search for alternative therapies for intractable liver diseases. Cell-based therapy is envisaged as a useful therapeutic option to recover and stabilize the lost metabolic function for acute liver failure, end-stage and

congenital liver diseases, or for those patients who are not considered eligible for organ transplantation. In recent years, research to identify alternative and reliable cell sources for transplantation that can be derived by reproducible methods has been encouraged. Human pluripotent stem cells (PSCs), which comprise both embryonic and induced PSCs, may offer many advantages as an alternative to hepatocytes for liver cell therapy. Their capacity for expansion, hepatic differentiation and self-renewal make them a promising source of unlimited numbers of hepatocyte-like cells for treating and repairing damaged livers. Immunogenicity and tumorigenicity of human PSCs remain the bottleneck for successful clinical application. However, recent advances made to develop disease-corrected hepatocyte-like cells from patients' human-induced PSCs by gene editing have opened up many potential gateways for the autologous treatment of hereditary liver diseases, which may likely reduce the risk of rejection and the need for lifelong immunosuppression. Well-defined methods to reduce the expression of oncogenic genes in induced PSCs, including protocols for their complete and safe hepatic differentiation, should be established to minimize the tumorigenicity of transplanted cells. On top of this, such new strategies are currently being rigorously tested and validated in preclinical studies before they can be safely transferred to clinical practice with patients.

Tomokiyo, A., et al. (2017). "Generation of Neural Crest-Like Cells From Human Periodontal Ligament Cell-Derived Induced Pluripotent Stem Cells." J Cell Physiol **232**(2): 402-416.

Neural crest cells (NCC) hold great promise for tissue engineering, however the inability to easily obtain large numbers of NCC is a major factor limiting their use in studies of regenerative medicine. Induced pluripotent stem cells (iPSC) are emerging as a novel candidate that could provide an unlimited source of NCC. In the present study, we examined the potential of neural crest tissue-derived periodontal ligament (PDL) iPSC to differentiate into neural crest-like cells (NCLC) relative to iPSC generated from a non-neural crest derived tissue, foreskin fibroblasts (FF). We detected high HNK1 expression during the differentiation of PDL and FF iPSC into NCLC as a marker for enriching for a population of cells with NCC characteristics. We isolated PDL iPSC- and FF iPSC-derived NCLC, which highly expressed HNK1. A high proportion of the HNK1-positive cell populations generated, expressed the MSC markers, whilst very few cells expressed the pluripotency markers or the hematopoietic markers. The PDL and FF HNK1-positive populations gave rise to smooth muscle, neural, glial, osteoblastic and adipocytic like cells and exhibited higher expression of smooth muscle, neural, and glial cell-associated markers than the PDL and FF HNK1-negative populations. Interestingly, the HNK1-positive cells derived from the PDL-iPSC exhibited a greater ability to differentiate into smooth muscle, neural, glial cells and adipocytes, than the HNK1-positive cells derived from the FF-iPSC. Our work suggests that HNK1-enriched NCLC from neural crest tissue-derived iPSC more closely resemble the phenotypic and functional hallmarks of NCC compared to the HNK1-low population and non-neural crest iPSC-derived NCLC. J. Cell. Physiol. 232: 402-416, 2017. (c) 2016 Wiley Periodicals, Inc.

Tomotsune, D., et al. (2016). "Enrichment of Pluripotent Stem Cell-Derived Hepatocyte-Like Cells by Ammonia Treatment." PLoS One **11**(9): e0162693.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are potential resources for the regeneration of defective organs, including the liver. However, some obstacles must be overcome before this becomes reality. Undifferentiated cells that remain following differentiation have teratoma-forming potential. Additionally, practical applications require a large quantity of differentiated cells, so the differentiation process must be economical. Here we describe a DNA microarray-based global analysis of the gene expression profiles of differentiating human pluripotent stem cells. We identified differences and commonalities among six human pluripotent stem cell lines: the hESCs KhES1, KhES2, KhES3, and H1, and the iPSCs 201B7 and 243G1. Embryoid bodies (EBs) formed without requiring supplementation with inducing factors. EBs also expressed some liver-specific metabolic genes including the ammonia-metabolizing enzymes glutamine synthetase and carbamoyl-phosphate synthase 1. Real-time PCR analysis revealed hepatocyte-like differentiation of EBs treated with ammonia in Lanford medium. Analysis of DNA microarray data suggested that hepatocyte-like cells were the most abundant population in ammonia-treated cells. Furthermore, expression levels of undifferentiated pluripotent stem cell markers were drastically reduced, suggesting a reduced teratoma-forming capacity. These results indicate that treatment of EBs with ammonia in Lanford medium may be an effective inducer of hepatic differentiation in absence of expensive inducing factors.

Tong, C., et al. (2018). "Squaramide-Based Supramolecular Materials for Three-Dimensional Cell Culture of Human Induced Pluripotent Stem Cells and Their Derivatives." Biomacromolecules **19**(4): 1091-1099.

Synthetic hydrogel materials can recapitulate the natural cell microenvironment; however, it is

equally necessary that the gels maintain cell viability and phenotype while permitting reisolation without stress, especially for use in the stem cell field. Here, we describe a family of synthetically accessible, squaramide-based tripodal supramolecular monomers consisting of a flexible tris(2-aminoethyl)amine (TREN) core that self-assemble into supramolecular polymers and eventually into self-recovering hydrogels. Spectroscopic measurements revealed that monomer aggregation is mainly driven by a combination of hydrogen bonding and hydrophobicity. The self-recovering hydrogels were used to encapsulate NIH 3T3 fibroblasts as well as human-induced pluripotent stem cells (hiPSCs) and their derivatives in 3D. The materials reported here proved cytocompatible for these cell types with maintenance of hiPSCs in their undifferentiated state essential for their subsequent expansion or differentiation into a given cell type and potential for facile release by dilution due to their supramolecular nature.

Topol, A., et al. (2015). "Altered WNT Signaling in Human Induced Pluripotent Stem Cell Neural Progenitor Cells Derived from Four Schizophrenia Patients." Biol Psychiatry **78**(6): e29-34.

Trusler, O., et al. (2018). "Cell surface markers for the identification and study of human naive pluripotent stem cells." Stem Cell Res **26**: 36-43.

Characterisation of mouse pluripotent stem cells has revealed two distinct pluripotent states, naive and primed, that maintain characteristics of the pre and post implanted epiblast respectively. Recent studies have developed several culture systems that seek to recapitulate the naive phenomenon in human pluripotent stem cells. Therefore, robust methods to isolate these cells will be fundamental to assess their potential in modelling human development and disease. Here we review current methods for human naive pluripotent culture and collate a list of cell surface antigens that have been identified as markers to differentiate naive from primed human pluripotent stem cells. While these culture systems do display marker variability, and not all antigens mentioned were assessed in all methods, this review provides a resource for researchers of the human naive pluripotent stem cell state. SSEA-4, SSEA-3, CD24, CD75, CD7, CD77, CD130/GP130, CD57, CD90 and NLGN4X were all found to have a +/- expression profile in at least 2 methods, while +/- expression of Tra-1-81, CDH3, CD172a, CD107b, CD229 was reported in one method. Often it was reported that naive and primed cells could be defined using a low/medium/high expression of the following antigens TRA-1-60, PCDH1, GPR64, MHC Class I, however these markers were more likely to display expression pattern differences between methods. Studies using mouse naive cells indicate that they may have benefits over primed cells in modelling development and disease, and while it is yet to be determined if the same can be said about a human naive state, tools to identify this population should greatly further the field.

Tsai, Y., et al. (2015). "A chemically defined substrate for the expansion and neuronal differentiation of human pluripotent stem cell-derived neural progenitor cells." Stem Cell Res **15**(1): 75-87.

Due to the limitation of current pharmacological therapeutic strategies, stem cell therapies have emerged as a viable option for treating many incurable neurological disorders. Specifically, human pluripotent stem cell (hPSC)-derived neural progenitor cells (hNPCs), a multipotent cell population that is capable of near indefinite expansion and subsequent differentiation into the various cell types that comprise the central nervous system (CNS), could provide an unlimited source of cells for such cell-based therapies. However the clinical application of these cells will require (i) defined, xeno-free conditions for their expansion and neuronal differentiation and (ii) scalable culture systems that enable their expansion and neuronal differentiation in numbers sufficient for regenerative medicine and drug screening purposes. Current extracellular matrix protein (ECMP)-based substrates for the culture of hNPCs are expensive, difficult to isolate, subject to batch-to-batch variations, and, therefore, unsuitable for clinical application of hNPCs. Using a high-throughput array-based screening approach, we identified a synthetic polymer, poly(4-vinyl phenol) (P4VP), that supported the long-term proliferation and self-renewal of hNPCs. The hNPCs cultured on P4VP maintained their characteristic morphology, expressed high levels of markers of multipotency, and retained their ability to differentiate into neurons. Such chemically defined substrates will eliminate critical roadblocks for the utilization of hNPCs for human neural regenerative repair, disease modeling, and drug discovery.

Tseng, W. L., et al. (2017). "Imbalanced Production of Reactive Oxygen Species and Mitochondrial Antioxidant SOD2 in Fabry Disease-Specific Human Induced Pluripotent Stem Cell-Differentiated Vascular Endothelial Cells." Cell Transplant **26**(3): 513-527.

Fabry disease (FD) is an X-linked inherited lysosomal storage disease caused by alpha-galactosidase A (GLA) deficiency. Progressive intracellular accumulation of globotriaosylceramide (Gb3) is considered to be pathogenically responsible for the phenotype variability of FD that causes cardiovascular dysfunction; however, molecular mechanisms underlying the impairment of FD-

associated cardiovascular tissues remain unclear. In this study, we reprogrammed human induced pluripotent stem cells (hiPSCs) from peripheral blood cells of patients with FD (FD-iPSCs); subsequently differentiated them into vascular endothelial-like cells (FD-ECs) expressing CD31, VE-cadherin, and vWF; and investigated their ability to form vascular tube-like structures. FD-ECs recapitulated the FD pathophysiological phenotype exhibiting intracellular Gb3 accumulation under a transmission electron microscope. Moreover, compared with healthy control iPSC-derived endothelial cells (NC-ECs), reactive oxygen species (ROS) production considerably increased in FD-ECs. Microarray analysis was performed to explore the possible mechanism underlying Gb3 accumulation-induced ROS production in FD-ECs. Our results revealed that superoxide dismutase 2 (SOD2), a mitochondrial antioxidant, was significantly downregulated in FD-ECs. Compared with NC-ECs, AMPK activity was significantly enhanced in FD-ECs. Furthermore, to investigate the role of Gb3 in these effects, human umbilical vein endothelial cells (HUVECs) were treated with Gb3. After Gb3 treatment, we observed that SOD2 expression was suppressed and AMPK activity was enhanced in a dose-dependent manner. Collectively, our results indicate that excess accumulation of Gb3 suppressed SOD2 expression, increased ROS production, enhanced AMPK activation, and finally caused vascular endothelial dysfunction. Our findings suggest that dysregulated mitochondrial ROS may be a potential target for treating FD.

Tsuruya, K., et al. (2015). "A Paracrine Mechanism Accelerating Expansion of Human Induced Pluripotent Stem Cell-Derived Hepatic Progenitor-Like Cells." Stem Cells Dev **24**(14): 1691-1702.

Hepatic stem/progenitor cells in liver development have a high proliferative potential and the ability to differentiate into both hepatocytes and cholangiocytes. In this study, we focused on the cell surface molecules of human induced pluripotent stem (iPS) cell-derived hepatic progenitor-like cells (HPCs) and analyzed how these molecules modulate expansion of these cells. Human iPS cells were differentiated into immature hepatic lineage cells by cytokines. In addition to hepatic progenitor markers (CD13 and CD133), the cells were coimmunostained for various cell surface markers (116 types). The cells were analyzed by flow cytometry and in vitro colony formation culture with feeder cells. Twenty types of cell surface molecules were highly expressed in CD13(+)CD133(+) cells derived from human iPS cells. Of these molecules, CD221 (insulin-like growth factor receptor), which was expressed in CD13(+)CD133(+) cells, was quickly downregulated after in vitro expansion. The proliferative ability was suppressed by a neutralizing antibody and specific inhibitor of CD221. Overexpression of CD221 increased colony-forming ability. We also found that inhibition of CD340 (erbB2) and CD266 (fibroblast growth factor-inducible 14) signals suppressed proliferation. In addition, both insulin-like growth factor (a ligand of CD221) and tumor necrosis factor-like weak inducer of apoptosis (a ligand of CD266) were provided by feeder cells in our culture system. This study revealed the expression profiles of cell surface molecules in human iPS cell-derived HPCs and that the paracrine interactions between HPCs and other cells through specific receptors are important for proliferation.

Tucker, B. A., et al. (2011). "Exome sequencing and analysis of induced pluripotent stem cells identify the cilia-related gene male germ cell-associated kinase (MAK) as a cause of retinitis pigmentosa." Proc Natl Acad Sci U S A **108**(34): E569-576.

Retinitis pigmentosa (RP) is a genetically heterogeneous heritable disease characterized by apoptotic death of photoreceptor cells. We used exome sequencing to identify a homozygous Alu insertion in exon 9 of male germ cell-associated kinase (MAK) as the cause of disease in an isolated individual with RP. Screening of 1,798 unrelated RP patients identified 20 additional probands homozygous for this insertion (1.2%). All 21 affected probands are of Jewish ancestry. MAK encodes a kinase involved in the regulation of photoreceptor-connecting cilium length. Immunohistochemistry of human donor tissue revealed that MAK is expressed in the inner segments, cell bodies, and axons of rod and cone photoreceptors. Several isoforms of MAK that result from alternative splicing were identified. Induced pluripotent stem cells were derived from the skin of the proband and a patient with non-MAK-associated RP (RP control). In the RP control individual, we found that a transcript lacking exon 9 was predominant in undifferentiated cells, whereas a transcript bearing exon 9 and a previously unrecognized exon 12 predominated in cells that were differentiated into retinal precursors. However, in the proband with the Alu insertion, the developmental switch to the MAK transcript bearing exons 9 and 12 did not occur. In addition to showing the use of induced pluripotent stem cells to efficiently evaluate the pathogenicity of specific mutations in relatively inaccessible tissues like retina, this study reveals algorithmic and molecular obstacles to the discovery of pathogenic insertions and suggests specific changes in strategy that can be implemented to more fully harness the power of sequencing technologies.

Turunen, S., et al. (2017). "Direct Laser Writing of Tubular Microtowers for 3D Culture of Human

Pluripotent Stem Cell-Derived Neuronal Cells." ACS Appl Mater Interfaces **9**(31): 25717-25730.

As the complex structure of nervous tissue cannot be mimicked in two-dimensional (2D) cultures, the development of three-dimensional (3D) neuronal cell culture platforms is a topical issue in the field of neuroscience and neural tissue engineering. Computer-assisted laser-based fabrication techniques such as direct laser writing by two-photon polymerization (2PP-DLW) offer a versatile tool to fabricate 3D cell culture platforms with highly ordered geometries in the size scale of natural 3D cell environments. In this study, we present the design and 2PP-DLW fabrication process of a novel 3D neuronal cell culture platform based on tubular microtowers. The platform facilitates efficient long-term 3D culturing of human neuronal cells and supports neurite orientation and 3D network formation. Microtower designs both with or without intraluminal guidance cues and/or openings in the tower wall are designed and successfully fabricated from Ormocomp. Three of the microtower designs are chosen for the final culture platform: a design with openings in the wall and intralumial guidance cues (webs and pillars), a design with openings but without intraluminal structures, and a plain cylinder design. The proposed culture platform offers a promising concept for future 3D cultures in the field of neuroscience.

Uchida, N., et al. (2017). "Efficient Generation of beta-Globin-Expressing Erythroid Cells Using Stromal Cell-Derived Induced Pluripotent Stem Cells from Patients with Sickle Cell Disease." Stem Cells **35**(3): 586-596.

Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells represent an ideal source for in vitro modeling of erythropoiesis and a potential alternative source for red blood cell transfusions. However, iPS cell-derived erythroid cells predominantly produce epsilon- and gamma-globin without beta-globin production. We recently demonstrated that ES cell-derived sacs (ES sacs), known to express hemangioblast markers, allow for efficient erythroid cell generation with beta-globin production. In this study, we generated several iPS cell lines derived from bone marrow stromal cells (MSCs) and peripheral blood erythroid progenitors (EPs) from sickle cell disease patients, and evaluated hematopoietic stem/progenitor cell (HSPC) generation after iPS sac induction as well as subsequent erythroid differentiation. MSC-derived iPS sacs yielded greater amounts of immature hematopoietic progenitors (VEGFR2 + GPA-), definitive HSPCs (CD34 + CD45+), and megakaryoerythroid progenitors (GPA + CD41a+), as compared to EP-derived iPS sacs. Erythroid differentiation from MSC-derived iPS sacs resulted in greater amounts of erythroid cells (GPA+) and higher beta-globin (and betaS-globin) expression, comparable to ES sac-derived cells. These data demonstrate that human MSC-derived iPS sacs allow for more efficient erythroid cell generation with higher beta-globin production, likely due to heightened emergence of immature progenitors. Our findings should be important for iPS cell-derived erythroid cell generation. Stem Cells 2017;35:586-596.

Ueda, T. and S. Kaneko (2015). "[Pluripotent stem cells as a source for T cell research and clinical application]." Nihon Rinsho Meneki Gakkai Kaishi **38**(2): 101-108.

Recently, promising clinical outcomes of cancer immunotherapy including administration of an anti PD-1 antibody targeting for T cell reactivation has gained particular attention worldwide. Adoptive cell therapy with tumor infiltrating lymphocytes and TCR/CAR (Chimeric Antigen Receptor) transgenic T cells are also under development. Although it has become clearer that the efficacy of adoptive cell therapy correlate with the quality of infusing T cells, antigen specific T cells in patients with chronic infection and cancer have been exhausted. We have succeeded to generate rejuvenated antigen specific T cells by reprogramming to pluripotency and differentiation. In this article, we introduce fundamentals of this technology and describe its potential for adoptive cell therapy in the future.

Ueda, Y., et al. (2012). "Substrates for human pluripotent stem cell cultures in conditioned medium of mesenchymal stem cells." J Biomater Sci Polym Ed **23**(1-4): 153-165.

We aimed to establish a culture system of human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), free from xenogeneic proteins, Matrigel() and conditioned medium of mouse embryonic fibroblasts. The conditioned culture medium consisted of mesenchymal stem cells derived from human bone marrow. We examined surface properties suitable for hPSC culture by using self-assembled monolayers (SAMs) of alkanethiols with four different functional groups: CH(3), OH, COOH and NH(2). hPSCs neither adhered nor proliferated on surfaces with a water contact angle higher than 40 degrees . Based on this finding, the contact angle of a polystyrene (PSt) culture dish was reduced to less than 40 degrees , and COOH and OH groups were introduced to its surface by oxygen plasma treatment, making the PSt dish suitable for hPSC culture. This combination of a PSt dish treated with oxygen plasma treatment and conditioned medium of mesenchymal stem cells achieved a long-term maintenance of hPSCs without differentiation.

Ulrich, H. (2017). "Stem Cell Reviews and Reports: Induced Pluripotent Stem Cells, Embryonic Stem Cells and Development Section." Stem Cell Rev **13**(1): 3.

Unzu, C., et al. (2016). "Human Hepatocyte-Derived Induced Pluripotent Stem Cells: MYC Expression, Similarities to Human Germ Cell Tumors, and Safety Issues." Stem Cells Int **2016**: 4370142.

Induced pluripotent stem cells (iPSC) are a most promising approach to the development of a hepatocyte transplantable mass sufficient to induce long-term correction of inherited liver metabolic diseases, thus avoiding liver transplantation. Their intrinsic self-renewal ability and potential to differentiate into any of the three germ layers identify iPSC as the most promising cell-based therapeutics, but also as drivers of tumor development. Teratoma development currently represents the gold standard to assess iPSC pluripotency. We analyzed the tumorigenic potential of iPSC generated from human hepatocytes (HEP-iPSC) and compared their immunohistochemical profiles to that of tumors developed from fibroblast and hematopoietic stem cell-derived iPSC. HEP-iPSC generated tumors significantly presented more malignant morphological features than reprogrammed fibroblasts or CD34+ iPSC. Moreover, the protooncogene myc showed the strongest expression in HEP-iPSC, compared to only faint expression in the other cell subsets. Random integration of transgenes and the use of potent protooncogenes such as myc might be a risk factor for malignant tumor development if hepatocytes are used for reprogramming. Nonviral vector delivery systems or reprogramming of cells obtained from less invasive harvesting methods would represent interesting options for future developments in stem cell-based approaches for liver metabolic diseases.

Vaajasaari, H., et al. (2011). "Toward the defined and xeno-free differentiation of functional human pluripotent stem cell-derived retinal pigment epithelial cells." Mol Vis **17**: 558-575.

PURPOSE: The production of functional retinal pigment epithelium (RPE) cells from human embryonic (hESCs) and human induced pluripotent stem cells (hiPSCs) in defined and xeno-free conditions is highly desirable, especially for their use in cell therapy for retinal diseases. In addition, differentiated RPE cells provide an individualized disease model and drug discovery tool. In this study, we report the differentiation of functional RPE-like cells from several hESC lines and one hiPSC line in culture conditions, enabling easy translation to clinical quality cell production under Good Manufacturing Practice regulations. METHODS: Pluripotent stem cells were cultured on human fibroblast feeder cells in serum-free medium. The differentiation toward RPE was induced by removing basic fibroblast growth factor and feeder cells from the serum-free conditions. RPE differentiation was also achieved using xeno-free and defined culture conditions. The RPE cell morphology and pigmentation of the cells were analyzed and the expression of genes and proteins characteristic for RPE cells was evaluated. In vitro functionality of the cells was analyzed using ELISA measurements for pigment epithelium derived factor (PEDF) secretion and phagocytosis of photoreceptor outer segments (POS). The integrity of the generated RPE layers was analyzed using transepithelial electric resistance measurements. RESULTS: We generated putative RPE cells with typical pigmented cobblestone-like morphology. The expression of RPE-specific markers was confirmed at the gene and protein level. The differentiated cells were able to phagocytose POS and secrete PEDF characteristic of native RPE cells. In addition, cultured cells formed a polarized epithelium with high integrity and exhibited excellent transepithelial electric resistance values, indicating well established, tight junctions. Moreover, we introduced an improved method to generate functional putative RPE cells without xeno-components under defined conditions. CONCLUSIONS: We have developed a progressive differentiation protocol for the production of functional RPE-like cells from hESCs and hiPSCs. Our results demonstrate that putative hESC-RPE and hiPSC-RPE express genes and proteins characteristic for RPE cells, as well as being able to phagocytose POS and secrete PEDF. Furthermore, our results show that RPE-like cells can be differentiated in xeno-free and defined culture conditions, which is mandatory for Good Manufacturing Practice-production of these cells for clinical use.

Vale, K. L. D., et al. (2017). "The Effects of Photobiomodulation Delivered by Light-Emitting Diode on Stem Cells from Human Exfoliated Deciduous Teeth: A Study on the Relevance to Pluripotent Stem Cell Viability and Proliferation." Photomed Laser Surg **35**(12): 659-665.

OBJECTIVE: Photobiomodulation (PBM) can modulate the proliferation of some types of stem cells. However, few reports have addressed the effects of PBM delivered by light-emitting diode (LED) on stem cells obtained from the pulp tissue of deciduous teeth. The aim of the present study was to investigate the effect of PBM delivered by red LED (630 nm, 75 mW, 37 mW/cm(2)) with different radiant exposures on the cell cycle, mitochondrial membrane potential, and senescence of stem cells from human exfoliated deciduous teeth (SHED). MATERIALS AND METHODS: Cultures were irradiated with LED (2, 4, 8, 16, and 32 J/cm(2)). After 24 h, the cell cycle and

mitochondrial membrane potential of the cultures were evaluated using flow cytometry. Nonirradiated cultures served as control. RESULTS: Cultures irradiated with 16 J/cm(2) had higher percentages of cells in the synthesis phase than control cultures ($p < 0.05$), and no significant differences were found regarding the percentage of cells with viable mitochondria between irradiated and control cultures. No significant difference in cell senescence was found between control cultures and cultures irradiated with 2 or 16 J/cm(2). CONCLUSIONS: LED irradiation at 630 nm (37 mW/cm(2), 75 mW) with radiant exposure of 16 J/cm(2) was capable of inducing a proliferative response in stem cells from the pulp tissue of deciduous teeth without affecting mitochondrial function or inducing senescence.

Vallier, L., et al. (2009). "Signaling pathways controlling pluripotency and early cell fate decisions of human induced pluripotent stem cells." Stem Cells **27**(11): 2655-2666.

Human pluripotent stem cells from embryonic origins and those generated from reprogrammed somatic cells share many characteristics, including indefinite proliferation and a sustained capacity to differentiate into a wide variety of cell types. However, it remains to be demonstrated whether both cell types rely on similar mechanisms to maintain their pluripotent status and to control their differentiation. Any differences in such mechanisms would suggest that reprogramming of fibroblasts to generate induced pluripotent stem cells (iPSCs) results in novel states of pluripotency. In that event, current methods for expanding and differentiating human embryonic stem cells (ESCs) might not be directly applicable to human iPSCs. However, we show here that human iPSCs rely on activin/nodal signaling to control Nanog expression and thereby maintain pluripotency, thus revealing their mechanistic similarity to human ESCs. We also show that growth factors necessary and sufficient for achieving specification of human ESCs into extraembryonic tissues, neuroectoderm, and mesendoderm also drive differentiation of human iPSCs into the same tissues. Importantly, these experiments were performed in fully chemically defined medium devoid of factors that could obscure analysis of developmental mechanisms or render the resulting tissues incompatible with future clinical applications. Together these data reveal that human iPSCs rely on mechanisms similar to human ESCs to maintain their pluripotency and to control their differentiation, showing that these pluripotent cell types are functionally equivalent.

Vanova, T., et al. (2017). "Tyrosine Kinase Expressed in Hepatocellular Carcinoma, TEC, Controls Pluripotency and Early Cell Fate Decisions of Human Pluripotent Stem Cells via Regulation of Fibroblast Growth Factor-2 Secretion." Stem Cells **35**(9): 2050-2059.

Human pluripotent stem cells (hPSC) require signaling provided by fibroblast growth factor (FGF) receptors. This can be initiated by the recombinant FGF2 ligand supplied exogenously, but hPSC further support their niche by secretion of endogenous FGF2. In this study, we describe a role of tyrosine kinase expressed in hepatocellular carcinoma (TEC) kinase in this process. We show that TEC-mediated FGF2 secretion is essential for hPSC self-renewal, and its lack mediates specific differentiation. Following both short hairpin RNA- and small interfering RNA-mediated TEC knockdown, hPSC secretes less FGF2. This impairs hPSC proliferation that can be rescued by increasing amounts of recombinant FGF2. TEC downregulation further leads to a lower expression of the pluripotency markers, an improved priming towards neuroectodermal lineage, and a failure to develop cardiac mesoderm. Our data thus demonstrate that TEC is yet another regulator of FGF2-mediated hPSC pluripotency and differentiation. Stem Cells 2017;35:2050-2059.

Varga, N., et al. (2011). "Mesenchymal stem cell like (MSCl) cells generated from human embryonic stem cells support pluripotent cell growth." Biochem Biophys Res Commun **414**(3): 474-480.

Mesenchymal stem cell like (MSCl) cells were generated from human embryonic stem cells (hESC) through embryoid body formation, and isolated by adherence to plastic surface. MSCl cell lines could be propagated without changes in morphological or functional characteristics for more than 15 passages. These cells, as well as their fluorescent protein expressing stable derivatives, efficiently supported the growth of undifferentiated human embryonic stem cells as feeder cells. The MSCl cells did not express the embryonic (Oct4, Nanog, ABCG2, PODXL, or SSEA4), or hematopoietic (CD34, CD45, CD14, CD133, HLA-DR) stem cell markers, while were positive for the characteristic cell surface markers of MSCs (CD44, CD73, CD90, CD105). MSCl cells could be differentiated toward osteogenic, chondrogenic or adipogenic directions and exhibited significant inhibition of mitogen-activated lymphocyte proliferation, and thus presented immunosuppressive features. We suggest that cultured MSCl cells can properly model human MSCs and be applied as efficient feeders in hESC cultures.

Varun, D., et al. (2017). "A robust vitronectin-derived peptide for the scalable long-term expansion and neuronal differentiation of human pluripotent stem cell

(hPSC)-derived neural progenitor cells (hNPCs)." Acta Biomater **48**: 120-130.

Despite therapeutic advances, neurodegenerative diseases and disorders remain some of the leading causes of mortality and morbidity in the United States. Therefore, cell-based therapies to replace lost or damaged neurons and supporting cells of the central nervous system (CNS) are of great therapeutic interest. To that end, human pluripotent stem cell (hPSC) derived neural progenitor cells (hNPCs) and their neuronal derivatives could provide the cellular 'raw material' needed for regenerative medicine therapies for a variety of CNS disorders. In addition, hNPCs derived from patient-specific hPSCs could be used to elucidate the underlying mechanisms of neurodegenerative diseases and identify potential drug candidates. However, the scientific and clinical application of hNPCs requires the development of robust, defined, and scalable substrates for their long-term expansion and neuronal differentiation. In this study, we rationally designed a vitronectin-derived peptide (VDP) that served as an adhesive growth substrate for the long-term expansion of several hNPC lines. Moreover, VDP-coated surfaces allowed for the directed neuronal differentiation of hNPC at levels similar to cells differentiated on traditional extracellular matrix protein-based substrates. Overall, the ability of VDP to support the long-term expansion and directed neuronal differentiation of hNPCs will significantly advance the future translational application of these cells in treating injuries, disorders, and diseases of the CNS.

Veraitch, O., et al. (2013). "Human induced pluripotent stem cell-derived ectodermal precursor cells contribute to hair follicle morphogenesis in vivo." J Invest Dermatol **133**(6): 1479-1488.

Well-orchestrated epithelial-mesenchymal interactions are crucial for hair follicle (HF) morphogenesis. In this study, ectodermal precursor cells (EPCs) with the capacity to cross talk with hair-inductive dermal cells were generated from human induced pluripotent stem cells (hiPSCs) and assessed for HF-forming ability in vivo. EPCs derived from three hiPSC lines generated with 4 or 3 factors (POU5F1, SOX2, KLF4 +/- MYC) mostly expressed keratin 18, a marker of epithelial progenitors. When cocultured with human dermal papilla (DP) cells, a 4 factor 201B7 hiPSC-EPC line upregulated follicular keratinocyte (KC) markers more significantly than normal human adult KCs (NHKCs) and other hiPSC-EPC lines. DP cells preferentially increased DP biomarker expression in response to this line. Interestingly, 201B7 hiPSCs were shown to be ectodermal/epithelial prone, and the derived EPCs were putatively in a wingless-type MMTV integration site family (WNT)-activated state. Importantly, co-transplantation of 201B7 hiPSC-EPCs, but not NHKCs, with trichogenic mice dermal cells into immunodeficient mice resulted in HF formation. Human HF stem cell markers were detected in reconstituted HFs; however, a low frequency of human-derived cells implied that hiPSC-EPCs contributed to HF morphogenesis via direct repopulation and non-cell autonomous activities. The current study suggests a, to our knowledge, previously unrecognized advantage of using hiPSCs to enhance epithelial-mesenchymal interactions in HF bioengineering.

Veraitch, O., et al. (2017). "Induction of hair follicle dermal papilla cell properties in human induced pluripotent stem cell-derived multipotent LNGFR(+)THY-1(+) mesenchymal cells." Sci Rep **7**: 42777.

The dermal papilla (DP) is a specialised mesenchymal component of the hair follicle (HF) that plays key roles in HF morphogenesis and regeneration. Current technical difficulties in preparing trichogenic human DP cells could be overcome by the use of highly proliferative and plastic human induced pluripotent stem cells (hiPSCs). In this study, hiPSCs were differentiated into induced mesenchymal cells (iMCs) with a bone marrow stromal cell phenotype. A highly proliferative and plastic LNGFR(+)THY-1(+) subset of iMCs was subsequently programmed using retinoic acid and DP cell activating culture medium to acquire DP properties. The resultant cells (induced DP-substituting cells [iDPSCs]) exhibited up-regulated DP markers, interacted with human keratinocytes to up-regulate HF related genes, and when co-grafted with human keratinocytes in vivo gave rise to fibre structures with a hair cuticle-like coat resembling the hair shaft, as confirmed by scanning electron microscope analysis. Furthermore, iDPSCs responded to the clinically used hair growth reagent, minoxidil sulfate, to up-regulate DP genes, further supporting that they were capable of, at least in part, reproducing DP properties. Thus, LNGFR(+)THY-1(+) iMCs may provide material for HF bioengineering and drug screening for hair diseases.

Verpelli, C., et al. (2013). "Comparative neuronal differentiation of self-renewing neural progenitor cell lines obtained from human induced pluripotent stem cells." Front Cell Neurosci **7**: 175.

Most human neuronal disorders are associated with genetic alterations that cause defects in neuronal development and induce precocious neurodegeneration. In order to fully characterize the molecular mechanisms underlying the onset of these devastating diseases, it is important to establish in vitro models

able to recapitulate the human pathology as closely as possible. Here we compared three different differentiation protocols for obtaining functional neurons from human induced pluripotent stem cells (hiPSCs): human neural progenitors (hNPs) obtained from hiPSCs were differentiated by co-culturing them with rat primary neurons, glial cells or simply by culturing them on matrigel in neuronal differentiation medium, and the differentiation level was compared using immunofluorescence, biochemical and electrophysiological methods. We show that the differentiated neurons displayed distinct maturation properties depending on the protocol used and the faster morphological and functional maturation was obtained when hNPs were co-cultured with rat primary neurons.

Vignesh, S., et al. (2018). "Fabrication of micropatterned alginate-gelatin and k-carrageenan hydrogels of defined shapes using simple wax mould method as a platform for stem cell/induced Pluripotent Stem Cells (iPSC) culture." Int J Biol Macromol **112**: 737-744.

Micropatterning techniques involve soft lithography, which is laborious, expensive and restricted to a narrow spectrum of biomaterials. In this work we report, first time employment of patterned wax moulds for generation of micropatterned alginate-gelatin and kappa-carrageenan (kappa-CRG) hydrogel systems by a novel, simple and cost effective method. We generated and characterized uniform and reproducible micropatterned hydrogels of varying sizes and shapes such as square projections, square grooves, and circular grids and crisscrossed hillocks. The rheological analysis showed that kappa-carrageenan hydrogels had higher gel strength when compared to alginate-gelatin hydrogels. Human Mesenchymal stem cells (hMSCs) and Human Induced Pluripotent Stem Cells (hiPSCs) were found to be cytocompatible with these hydrogels. This micropatterned hydrogel system may have potential application in tissue engineering and also in understanding the basic biology behind the stem cell/iPSC fate.

Villa-Diaz, L. G., et al. (2013). "Concise review: The evolution of human pluripotent stem cell culture: from feeder cells to synthetic coatings." Stem Cells **31**(1): 1-7.

Current practices to maintain human pluripotent stem cells (hPSCs), which include induced pluripotent stem cells and embryonic stem cells, in an undifferentiated state typically depend on the support of feeder cells such as mouse embryonic fibroblasts (MEFs) or an extracellular matrix such as Matrigel. Culture conditions that depend on these undefined support systems limit our ability to interpret mechanistic studies aimed at resolving how hPSCs interact with their extracellular environment to remain in a unique undifferentiated state and to make fate-changing lineage decisions. Likewise, the xenogeneic components of MEFs and Matrigel ultimately hinder our ability to use pluripotent stem cells to treat debilitating human diseases. Many of these obstacles have been overcome by the development of synthetic coatings and bioreactors that support hPSC expansion and self-renewal within defined culture conditions that are free from xenogeneic contamination. The establishment of defined culture conditions and synthetic matrices will facilitate studies to more precisely probe the molecular basis of pluripotent stem cell self-renewal and differentiation. When combined with three-dimensional cultures in bioreactors, these systems will also enable large-scale expansion for future clinical applications.

Vlismas, A., et al. (2016). "Microarray Analyses Reveal Marked Differences in Growth Factor and Receptor Expression Between 8-Cell Human Embryos and Pluripotent Stem Cells." Stem Cells Dev **25**(2): 160-177.

Previous microarray analyses of RNAs from 8-cell (8C) human embryos revealed a lack of cell cycle checkpoints and overexpression of core circadian oscillators and cell cycle drivers relative to pluripotent human stem cells [human embryonic stem cells/induced pluripotent stem (hES/iPS)] and fibroblasts, suggesting growth factor independence during early cleavage stages. To explore this possibility, we queried our combined microarray database for expression of 487 growth factors and receptors. Fifty-one gene elements were overdetected on the 8C arrays relative to hES/iPS cells, including 14 detected at least 80-fold higher, which annotated to multiple pathways: six cytokine family (CSF1R, IL2RG, IL3RA, IL4, IL17B, IL23R), four transforming growth factor beta (TGFB) family (BMP6, BMP15, GDF9, ENG), one fibroblast growth factor (FGF) family [FGF14(FH4)], one epidermal growth factor member (GAB1), plus CD36, and CLEC10A. 8C-specific gene elements were enriched (73%) for reported circadian-controlled genes in mouse tissues. High-level detection of CSF1R, ENG, IL23R, and IL3RA specifically on the 8C arrays suggests the embryo plays an active role in blocking immune rejection and is poised for trophectoderm development; robust detection of NRG1, GAB1, -2, GRB7, and FGF14(FHF4) indicates novel roles in early development in addition to their known roles in later development. Forty-four gene elements were underdetected on the 8C arrays, including 11 at least 80-fold under the pluripotent cells: two cytokines (IFITM1, TNFRSF8), five TGFBs (BMP7, LEFTY1, LEFTY2, TDGF1, TDGF3), two FGFs (FGF2, FGF

receptor 1), plus ING5, and WNT6. The microarray detection patterns suggest that hES/iPS cells exhibit suppressed circadian competence, underexpression of early differentiation markers, and more robust expression of generic pluripotency genes, in keeping with an artificial state of continual uncommitted cell division. In contrast, gene expression patterns of the 8C embryo suggest that it is an independent circadian rhythm-competent equivalence group poised to signal its environment, defend against maternal immune rejection, and begin the rapid commitment events of early embryogenesis.

Wada, H., et al. (2011). "Successful differentiation to T cells, but unsuccessful B-cell generation, from B-cell-derived induced pluripotent stem cells." Int Immunol **23**(1): 65-74.

Forced expression of certain transcription factors in somatic cells results in generation of induced pluripotent stem (iPS) cells, which differentiate into various cell types. We investigated T-cell and B-cell lineage differentiation from iPS cells in vitro. To evaluate the impact of iPS cell source, murine splenic B-cell-derived iPS (B-iPS) cells were generated after retroviral transduction of four transcription factors (Oct4, Sox2, Klf4 and c-Myc). B-iPS cells were identical to embryonic stem (ES) cells and mouse embryonic fibroblast (MEF)-derived iPS cells in morphology, ES cell marker expression as well as teratoma and chimera mouse formation. Both B-iPS and MEF-derived iPS cells differentiated into lymphocytes in OP9 co-culture systems. Both efficiently differentiated into T-cell lineage that produced IFN-gamma on T-cell receptor stimulation. However, iPS cells including B-iPS cells were relatively resistant to B-cell lineage differentiation. One of the reasons of the failure of B-cell lineage differentiation seemed due to a defect of Pax5 expression in the differentiated cells. Therefore, current in vitro differentiation systems using iPS cells are sufficient for inducing T-cell but not B-cell lineage.

Wakao, S., et al. (2012). "Isolation of adult human pluripotent stem cells from mesenchymal cell populations and their application to liver damages." Methods Mol Biol **826**: 89-102.

We have found a novel type of pluripotent stem cells, Multilineage-differentiating stress enduring (Muse) cells that can be isolated from mesenchymal cell populations. Muse cells are characterized by stress tolerance, expression of pluripotency markers, self-renewal, and the ability to differentiate into endodermal-, mesodermal-, and ectodermal-lineage cells from a single cell, demonstrating that they are pluripotent stem cells. They can be isolated as cells positive for stage-specific embryonic antigen-3, a human pluripotent stem cell marker. Here, we introduce the isolation method for Muse cells and the effect of transplantation of these cells on chronic liver diseases.

Wakao, S., et al. (2012). "Regenerative Effects of Mesenchymal Stem Cells: Contribution of Muse Cells, a Novel Pluripotent Stem Cell Type that Resides in Mesenchymal Cells." Cells **1**(4): 1045-1060.

Mesenchymal stem cells (MSCs) are easily accessible and safe for regenerative medicine. MSCs exert trophic, immunomodulatory, anti-apoptotic, and tissue regeneration effects in a variety of tissues and organs, but their entity remains an enigma. Because MSCs are generally harvested from mesenchymal tissues, such as bone marrow, adipose tissue, or umbilical cord as adherent cells, MSCs comprise crude cell populations and are heterogeneous. The specific cells responsible for each effect have not been clarified. The most interesting property of MSCs is that, despite being adult stem cells that belong to the mesenchymal tissue lineage, they are able to differentiate into a broad spectrum of cells beyond the boundary of mesodermal lineage cells into ectodermal or endodermal lineages, and repair tissues. The broad spectrum of differentiation ability and tissue-repairing effects of MSCs might be mediated in part by the presence of a novel pluripotent stem cell type recently found in adult human mesenchymal tissues, termed multilineage-differentiating stress enduring (Muse) cells. Here we review recently updated studies of the regenerative effects of MSCs and discuss their potential in regenerative medicine.

Wakui, T., et al. (2017). "Method for evaluation of human induced pluripotent stem cell quality using image analysis based on the biological morphology of cells." J Med Imaging (Bellingham) **4**(4): 044003.

We propose an image analysis method for quality evaluation of human pluripotent stem cells based on biologically interpretable features. It is important to maintain the undifferentiated state of induced pluripotent stem cells (iPSCs) while culturing the cells during propagation. Cell culture experts visually select good quality cells exhibiting the morphological features characteristic of undifferentiated cells. Experts have empirically determined that these features comprise prominent and abundant nucleoli, less intercellular spacing, and fewer differentiating cellular nuclei. We quantified these features based on experts' visual inspection of phase contrast images of iPSCs and found that these features are effective for evaluating iPSC quality. We then developed an iPSC quality evaluation method using an image analysis technique. The method allowed

accurate classification, equivalent to visual inspection by experts, of three iPSC cell lines.

Wang, D., et al. (2018). "An in Vitro and in Vivo Study of the Effect of Dexamethasone on Immunoinhibitory Function of Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells." Cell Transplant **27**(9): 1340-1351.

Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) represent a promising cell source for patient-specific cell therapy. We previously demonstrated that they display an immunomodulatory effect on allergic airway inflammation. Glucocorticoids are powerful anti-inflammatory compounds and widely used in the therapy of allergic diseases. However, the effect of glucocorticoids on the immunomodulatory function of iPSC-MSCs remains unknown. This study aimed to determine the effect of dexamethasone (Dex) on the immunomodulatory function of iPSC-MSCs in vitro and in vivo. A total of three human iPSC-MSC clones were generated from amniocyte-derived iPSCs. Anti-CD3/CD28-induced peripheral blood mononuclear cell (PBMC) proliferation was used to assess the effect of Dex on the immunoinhibitory function of iPSC-MSCs in vitro. Mouse models of contact hypersensitivity (CHS) and allergic airway inflammation were induced, and the levels of inflammation in mice were analyzed with the treatments of iPSC-MSCs and Dex, alone and combined. The results showed that Dex did not interfere with the immunoinhibitory effect of iPSC-MSCs on PBMC proliferation. In CHS mice, simultaneous treatment with Dex did not affect the effect of iPSC-MSCs on the inflammation, both in regional draining lymph nodes and in inflamed ear tissue. In addition, co-administration of iPSC-MSCs with Dex decreased the local expression of interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha in the ears of CHS mice. In the mouse model of allergic airway inflammation, iPSC-MSC treatment combined with Dex resulted in a similar extent of reduction in pulmonary inflammation as iPSC-MSCs or Dex treatment alone. In conclusion, Dex does not significantly affect the immunomodulatory function of iPSC-MSCs both in vitro and in vivo. These findings may have implications when iPSC-MSCs and glucocorticoids are co-administered.

Wang, H., et al. (2016). "Induction of Germ Cell-like Cells from Porcine Induced Pluripotent Stem Cells." Sci Rep **6**: 27256.

The ability to generate germ cells from pluripotent stem cells (PSCs) is valuable for human regenerative medicine and animal breeding. Germ cell-like cells (GCLCs) have been differentiated from mouse and human PSCs, but not from porcine PSCs, which are considered an ideal model for stem cell applications. Here, we developed a defined culture system for the induction of primordial germ cell-like cells (PGCLCs) from porcine induced PSCs (piPSCs). The identity of the PGCLCs was characterized by observing cell morphology, detecting germ cell marker gene expression and evaluating epigenetic properties. PGCLCs could further differentiate into spermatogonial stem cell-like cells (SSCLCs) in vitro. Importantly, meiosis occurred during SSCLC induction. Xenotransplantation of GCLCs into seminiferous tubules of infertile immunodeficient mice resulted in immunohistochemically identifiable germ cells in vivo. Overall, our study provides a feasible strategy for directing piPSCs to the germ cell fate and lays a foundation for exploring germ cell development mechanisms.

Wang, J., et al. (2011). "Novel histone demethylase LSD1 inhibitors selectively target cancer cells with pluripotent stem cell properties." Cancer Res **71**(23): 7238-7249.

Histone modification determines epigenetic patterns of gene expression with methylation of histone H3 at lysine 4 (H3K4) often associated with active promoters. LSD1/KDM1 is a histone demethylase that suppresses gene expression by converting dimethylated H3K4 to mono- and unmethylated H3K4. LSD1 is essential for metazoan development, but its pathophysiologic functions in cancer remain mainly uncharacterized. In this study, we developed specific bioactive small inhibitors of LSD1 that enhance H3K4 methylation and derepress epigenetically suppressed genes in vivo. Strikingly, these compounds inhibited the proliferation of pluripotent cancer cells including teratocarcinoma, embryonic carcinoma, and seminoma or embryonic stem cells that express the stem cell markers Oct4 and Sox2 while displaying minimum growth-inhibitory effects on non-pluripotent cancer or normal somatic cells. RNA interference-mediated knockdown of LSD1 expression phenocopied these effects, confirming the specificity of small molecules and further establishing the high degree of sensitivity and selectivity of pluripotent cancer cells to LSD1 ablation. In support of these results, we found that LSD1 protein level is highly elevated in pluripotent cancer cells and in human testicular seminoma tissues that express Oct4. Using these novel chemical inhibitors as probes, our findings establish LSD1 and histone H3K4 methylation as essential cancer-selective epigenetic targets in cancer cells that have pluripotent stem cell properties.

Wang, L., et al. (2015). "Gene and MicroRNA Profiling of Human Induced Pluripotent Stem Cell-

Derived Endothelial Cells." Stem Cell Rev **11**(2): 219-227.

INTRODUCTION: The differentiated cell lineages from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) have shown their potential in regenerative medicine. However, the functional and transcriptional microRNA (miRNA) expression pattern during endothelial differentiation has yet to be characterized. METHODS: In this study, hESCs and hiPSCs were differentiated into endothelial cells (ECs). Then the endothelial-related gene profiling and miRNA profiling of hiPSCs, hESCs, hiPSCs derived endothelial cells (hiPSC-ECs), hESC derived endothelial cells (hESC-ECs) and human umbilical vein endothelial cells (HUVECs) were compared using RT-PCR Array. The data was analyzed using the data analysis system on QIAGEN's website. RESULTS: Our analysis demonstrated that the endothelial differentiation was triggered after EB formation and the EC-associated genes were up-regulated swiftly in both hESC-EBs and hiPSC-EBs; hiPSC-ECs and hESC-ECs had the similar EC-associated gene expression patterns. Moreover, we report here the first miRNA profiling study of hiPSC-ECs. Analyzing 376 unique miRNAs, we have identified several interesting miRNAs, including miR-20a, miR-20b, miR-222, miR-210, which have been previously reported to be involved in endothelial differentiation and show surprising expression patterns across our samples. We also identified novel miRNAs, such as miR-125a-5p, miR-149, miR-296-5p, miR-100, miR-27b, miR-181a and miR-137, which were up-regulated in both hiPSC-ECs and hESC-ECs during endothelial differentiation. CONCLUSION: hiPSC-ECs and hESC-ECs exhibited a high degree of similarity with HUVECs in EC-associated genes expression. And the miRNA profiling analysis revealed significant differences between hiPSCs and hESCs, but a high degree of similarity between hiPSC-ECs and hESC-ECs.

Wang, P., et al. (2018). "Metformin induces osteoblastic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells." J Tissue Eng Regen Med **12**(2): 437-446.

Metformin, a first-line antidiabetic drug used by millions of patients, has been shown to have potential osteogenic properties. The present study was performed to test the hypothesis that clinically relevant doses of metformin promote the osteogenic differentiation and mineralization of induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs). iPSC-MSCs were treated with metformin (10 mum) to assess cell viability, osteogenic differentiation, mineralization and activation of the LKB1/AMP-activated protein kinase (AMPK) signalling pathway, a surrogate marker of metformin action. To determine its potential application in MSC-based bone and periodontal tissue engineering, iPSC-MSCs were also treated with metformin when seeded on to calcium phosphate cement (CPC) scaffolds. Immunoblotting and cellular uptake assays showed that iPSC-MSCs express functional organic cation transporter-1 (OCT-1), a transmembrane protein that mediates the intracellular uptake of metformin. Although metformin treatment did not impair iPSC-MSC viability, it significantly stimulated alkaline phosphatase activity, enhanced mineralized nodule formation and increased expression of osteogenic markers, including Runt-related transcription factor 2 (RUNX2) and osterix. Inhibition of LKB1 activity, a common upstream AMPK kinase, markedly reversed metformin-induced AMPK activation, RUNX2 expression and nuclear localization. Moreover, metformin substantially increased mineralized nodule formation of iPSC-MSC seeded on CPC scaffolds. Collectively, functional OCT-expressing iPSC-MSCs responded to metformin by inducing an osteogenic effect in part mediated by the LKB1/AMPK pathway. Considering the widespread use of metformin in diabetics, this work may lead to novel tissue-engineering platforms where autogenous OCT-expressing iPSC-MSCs might be used to enhance bone and periodontal regeneration in diabetic patients prescribed with daily doses of metformin.

Wanjare, M., et al. (2015). "Biomechanical strain induces elastin and collagen production in human pluripotent stem cell-derived vascular smooth muscle cells." Am J Physiol Cell Physiol **309**(4): C271-281.

Blood vessels are subjected to numerous biomechanical forces that work harmoniously but, when unbalanced because of vascular smooth muscle cell (vSMC) dysfunction, can trigger a wide range of ailments such as cerebrovascular, peripheral artery, and coronary artery diseases. Human pluripotent stem cells (hPSCs) serve as useful therapeutic tools that may help provide insight on the effect that such biomechanical stimuli have on vSMC function and differentiation. In this study, we aimed to examine the effect of biomechanical strain on vSMCs derived from hPSCs. The effects of two types of tensile strain on hPSC-vSMC derivatives at different stages of differentiation were examined. The derivatives included smooth muscle-like cells (SMLCs), mature SMLCs, and contractile vSMCs. All vSMC derivatives aligned perpendicularly to the direction of cyclic uniaxial strain. Serum deprivation and short-term uniaxial strain had a synergistic effect in enhancing collagen type I, fibronectin, and elastin gene expression. Furthermore, long-term uniaxial strain deterred collagen type III gene expression, whereas long-term circumferential

strain upregulated both collagen type III and elastin gene expression. Finally, long-term uniaxial strain downregulated extracellular matrix (ECM) expression in more mature vSMC derivatives while upregulating elastin in less mature vSMC derivatives. Overall, our findings suggest that in vitro application of both cyclic uniaxial and circumferential tensile strain on hPSC-vSMC derivatives induces cell alignment and affects ECM gene expression. Therefore, mechanical stimulation of hPSC-vSMC derivatives using tensile strain may be important in modulating the phenotype and thus the function of vSMCs in tissue-engineered vessels.

Warlich, E., et al. (2014). "FAS-based cell depletion facilitates the selective isolation of mouse induced pluripotent stem cells." PLoS One **9**(7): e102171.

Cellular reprogramming of somatic cells into induced pluripotent stem cells (iPSC) opens up new avenues for basic research and regenerative medicine. However, the low efficiency of the procedure remains a major limitation. To identify iPSC, many studies to date relied on the activation of pluripotency-associated transcription factors. Such strategies are either retrospective or depend on genetically modified reporter cells. We aimed at identifying naturally occurring surface proteins in a systematic approach, focusing on antibody-targeted markers to enable live-cell identification and selective isolation. We tested 170 antibodies for differential expression between mouse embryonic fibroblasts (MEF) and mouse pluripotent stem cells (PSC). Differentially expressed markers were evaluated for their ability to identify and isolate iPSC in reprogramming cultures. Epithelial cell adhesion molecule (EPCAM) and stage-specific embryonic antigen 1 (SSEA1) were upregulated early during reprogramming and enabled enrichment of OCT4 expressing cells by magnetic cell sorting. Downregulation of somatic marker FAS was equally suitable to enrich OCT4 expressing cells, which has not been described so far. Furthermore, FAS downregulation correlated with viral transgene silencing. Finally, using the marker SSEA-1 we exemplified that magnetic separation enables the establishment of bona fide iPSC and propose strategies to enrich iPSC from a variety of human source tissues.

Watanabe, D., et al. (2018). "The Generation of Human gammadeltaT Cell-Derived Induced Pluripotent Stem Cells from Whole Peripheral Blood Mononuclear Cell Culture." Stem Cells Transl Med **7**(1): 34-44.

gammadeltaT cells constitute a small proportion of lymphocytes in peripheral blood. Unlike alphabetaT cells, the anti-tumor activities are exerted through several different pathways in a MHC-unrestricted manner. Thus, immunotherapy using gammadeltaT cells is considered to be effective for various types of cancer. Occasionally, however, ex vivo expanded cells are not as effective as expected due to cell exhaustion. To overcome the issue of T-cell exhaustion, researchers have generated induced pluripotent stem cells (iPSCs) that harbor the same T-cell receptor (TCR) genes as their original T-cells, which provide nearly limitless sources for antigen-specific cytotoxic T lymphocytes (CTLs). However, these technologies have focused on alphabetaT cells and require a population of antigen-specific CTLs, which are purified by cell sorting with HLA-peptide multimer, as the origin of iPS cells. In the present study, we aimed to develop an efficient and convenient system for generating iPSCs that harbor rearrangements of the TCRG and TCRD gene regions (gammadeltaT-iPSCs) without cell-sorting. We stimulated human whole peripheral blood mononuclear cell (PBMC) culture using Interleukin-2 and Zoledronate to activate gammadeltaT cells. Gene transfer into those cells with the Sendai virus vector resulted in gammadeltaT cell-dominant expression of exogenous genes. The introduction of reprogramming factors into the stimulated PBMC culture allowed us to establish iPSC lines. Around 70% of the established lines carried rearrangements at the TCRG and TCRD gene locus. The gammadeltaT-iPSCs could differentiate into hematopoietic progenitors. Our technology will pave the way for new avenues toward novel immunotherapy that can be applied for various types of cancer. Stem Cells Translational Medicine 2018;7:34-44.

Weed, L. S. and J. A. Mills (2017). "Strategies for retinal cell generation from human pluripotent stem cells." Stem Cell Investig **4**: 65.

Induced pluripotent stem cells (iPSCs) are specialized self-renewing cells that are generated by exogenously expressing pluripotency-associated transcription factors in somatic cells such as fibroblasts, peripheral blood mononuclear cells, or lymphoblastoid cell lines (LCLs). iPSCs are functionally similar to naturally pluripotent embryonic stem cells (ESCs) in their capacity to propagate indefinitely and potential to differentiate into all human cell types, and are devoid of the associated ethical complications of origin. iPSCs are useful for studying embryonic development, disease modeling, and drug screening. Additionally, iPSCs provide a personalized approach for pathological studies, particularly for diseases that lack appropriate animal models. Retinal cell differentiations using iPSCs have been successful in this regard. Several protocols to generate various retinal cells have been developed to maximize a specific cell type or, most recently, to mimic in vivo retinal structure and cellular environment. As differentiation protocols continue to

improve we are likely to see an increase in our basic understanding of various retinal degenerative diseases and the utilization of iPSCs in clinical trials.

Weist, R., et al. (2018). "Differential Expression of Cholinergic System Components in Human Induced Pluripotent Stem Cells, Bone Marrow-Derived Multipotent Stromal Cells, and Induced Pluripotent Stem Cell-Derived Multipotent Stromal Cells." Stem Cells Dev **27**(3): 166-183.

The components of the cholinergic system are evolutionary very old and conserved molecules that are expressed in typical spatiotemporal patterns. They are involved in signaling in the nervous system, whereas their functions in nonneuronal tissues are hardly understood. Stem cells present an attractive cellular system to address functional issues. This study therefore compared human induced pluripotent stem cells (iPSCs; from cord blood endothelial cells), mesenchymal stromal cells derived from iPSCs (iPSC-MSCs), and bone marrow-derived MSCs (BM-MSCs) from up to 33 different human donors with respect to gene expressions of components of the cholinergic system. The status of cells was identified and characterized by the detection of cell surface antigens using flow cytometry. Acetylcholinesterase expression in iPSCs declined during their differentiation into MSCs and was comparably low in BM-MSCs. Butyrylcholinesterase was present in iPSCs, increased upon transition from the three-dimensional embryoid body phase into monolayer culture, and declined upon further differentiation into iPSC-MSCs. In BM-MSCs a notable butyrylcholinesterase expression could be detected in only four donors, but was elusive in other patient-derived samples. Different nicotinic acetylcholine receptor subunits were preferentially expressed in iPSCs and during early differentiation into iPSC-MSCs, low expression was detected in iPS-MSCs and in BM-MSCs. The m2 and m3 variants of muscarinic acetylcholine receptors were detected in all stem cell populations. In BM-MSCs, these gene expressions varied between donors. Together, these data reveal the differential expression of cholinergic signaling system components in stem cells from specific sources and suggest the utility of our approach to establish informative biomarkers.

West, J. D. and E. J. Carrier (2018). "Precision Modeling of Pulmonary Hypertension Pathology with Induced Pluripotent Stem Cell-derived Cells." Am J Respir Crit Care Med **198**(2): 154-155.

West, M. D., et al. (2016). "Adult Versus Pluripotent Stem Cell-Derived Mesenchymal Stem Cells: The Need for More Precise Nomenclature." Curr Stem Cell Rep **2**: 299-303.

The complexity of human pluripotent stem cell (hPSC) fate represents both opportunity and challenge. In theory, all somatic cell types can be differentiated from hPSCs, opening the door to many opportunities in transplant medicine. However, such clinical applications require high standards of purity and identity, that challenge many existing protocols. This underscores the need for increasing precision in the description of cell identity during hPSC differentiation. We highlight one salient example, namely, the numerous published reports of hPSC-derived mesenchymal stem cells (MSCs). We suggest that many of these reports likely represent an improper use of certain cluster of differentiation (CD) antigens in defining bone marrow-derived MSCs. Instead, most such hPSC-derived mesenchymal cells are likely a complex mixture of embryonic anlagen, primarily of diverse mesodermal and neural crest origins, making precise identification, reproducible manufacture, and uniform differentiation difficult to achieve. We describe a potential path forward that may provide more precision in nomenclature, and cells with higher purity and identity for potential therapeutic use.

Wheeler, H. E., et al. (2015). "Modeling chemotherapeutic neurotoxicity with human induced pluripotent stem cell-derived neuronal cells." PLoS One **10**(2): e0118020.

There are no effective agents to prevent or treat chemotherapy-induced peripheral neuropathy (CIPN), the most common non-hematologic toxicity of chemotherapy. Therefore, we sought to evaluate the utility of human neuron-like cells derived from induced pluripotent stem cells (iPSCs) as a means to study CIPN. We used high content imaging measurements of neurite outgrowth phenotypes to compare the changes that occur to iPSC-derived neuronal cells among drugs and among individuals in response to several classes of chemotherapeutics. Upon treatment of these neuronal cells with the neurotoxic drug paclitaxel, vincristine or cisplatin, we identified significant differences in five morphological phenotypes among drugs, including total outgrowth, mean/median/maximum process length, and mean outgrowth intensity ($P < 0.05$). The differences in damage among drugs reflect differences in their mechanisms of action and clinical CIPN manifestations. We show the potential of the model for gene perturbation studies by demonstrating decreased expression of TUBB2A results in significantly increased sensitivity of neurons to paclitaxel (0.23 +/- 0.06 decrease in total neurite outgrowth, $P = 0.011$). The variance in several neurite outgrowth and apoptotic phenotypes upon treatment with one of the neurotoxic drugs is significantly greater between than within neurons derived from four different individuals ($P < 0.05$), demonstrating the potential of iPSC-derived

neurons as a genetically diverse model for CIPN. The human neuron model will allow both for mechanistic studies of specific genes and genetic variants discovered in clinical studies and for screening of new drugs to prevent or treat CIPN.

White, M. P., et al. (2013). "Limited gene expression variation in human embryonic stem cell and induced pluripotent stem cell-derived endothelial cells." Stem Cells **31**(1): 92-103.

Recent evidence suggests human embryonic stem cell (hESC) and induced pluripotent stem (iPS) cell lines have differences in their epigenetic marks and transcriptomes, yet the impact of these differences on subsequent terminally differentiated cells is less well understood. Comparison of purified, homogeneous populations of somatic cells derived from multiple independent human iPS and ES lines will be required to address this critical question. Here, we report a differentiation protocol based on embryonic development that consistently yields large numbers of endothelial cells (ECs) derived from multiple hESCs or iPS cells. Mesoderm differentiation of embryoid bodies was maximized, and defined growth factors were used to generate KDR(+) EC progenitors. Magnetic purification of a KDR(+) progenitor subpopulation resulted in an expanding, homogeneous pool of ECs that expressed EC markers and had functional properties of ECs. Comparison of the transcriptomes revealed limited gene expression variability between multiple lines of human iPS-derived ECs or between lines of ES- and iPS-derived ECs. These results demonstrate a method to generate large numbers of pure human EC progenitors and differentiated ECs from pluripotent stem cells and suggest individual lineages derived from human iPS cells may have significantly less variance than their pluripotent founders.

Whitt, J., et al. (2016). "Induced pluripotent stem cell-derived mesenchymal stem cells: A leap toward personalized therapies." Curr Stem Cell Res Ther **11**(2): 141-148.

Mesenchymal Stem/stromal cell (MSCs) transplantation procedures have been used since the 1960's to treat leukemia and other diseases, but due to the risks involved only patients with life threatening illnesses were typically subjected to the transplantation procedure until the last decade. Recent advancements in transplantation techniques have made it more feasible to use it for non-life-threatening diseases. However, the potential uses for stem cells are still limited by their rarity, and, in the case of allogeneic transplants, graft-vs.-host complications. An evolving alternative to conventional stem cell therapies is induced pluripotent stem-cell derived mesenchymal stem/stromal cells (iPSC- MSCs), which have a multi-lineage potential comparable to conventionally acquired MSCs with the added benefit of being less immunoreactive. However there are still many hurdles left to be overcome before they can be used regularly for personalized therapies. This review will focus on recent advancements that have been made regarding the role MSCs play in tumor development and the potential uses iPSC-MSCs may have in future cancer treatment.

Wilson, H. K., et al. (2015). "Exploring the effects of cell seeding density on the differentiation of human pluripotent stem cells to brain microvascular endothelial cells." Fluids Barriers CNS **12**: 13.

BACKGROUND: Brain microvascular-like endothelial cells (BMECs) derived from human pluripotent stem cells (hPSCs) have significant promise as tools for drug screening and studying the structure and function of the BBB in health and disease. The density of hPSCs is a key factor in regulating cell fate and yield during differentiation. Prior reports of hPSC differentiation to BMECs have seeded hPSCs in aggregates, leading to non-uniform cell densities that may result in differentiation heterogeneity. Here we report a singularized-cell seeding approach compatible with hPSC-derived BMEC differentiation protocols and evaluate the effects of initial hPSC seeding density on the subsequent differentiation, yield, and blood-brain barrier (BBB) phenotype. METHODS: A range of densities of hPSCs was seeded and differentiated, with the resultant endothelial cell yield quantified via VE-cadherin flow cytometry. Barrier phenotype of purified hPSC-derived BMECs was measured via transendothelial electrical resistance (TEER), and purification protocols were subsequently optimized to maximize TEER. Expression of characteristic vascular markers, tight junction proteins, and transporters was confirmed by immunocytochemistry and quantified by flow cytometry. P-glycoprotein and MRP-family transporter activity was assessed by intracellular accumulation assay. RESULTS: The initial hPSC seeding density of approximately 30,000 cells/cm(2) served to maximize the yield of VE-cadherin+ BMECs per input hPSC. BMECs displayed the highest TEER (>2,000 Omega x cm(2)) within this same range of initial seeding densities, although optimization of the BMEC purification method could minimize the seeding density dependence for some lines. Localization and expression levels of tight junction proteins as well as efflux transporter activity were largely independent of hPSC seeding density. Finally, the utility of the singularized-cell seeding approach was demonstrated by scaling the differentiation and purification process down from 6-well to 96-well culture without impacting BBB phenotype. CONCLUSIONS: Given the yield and

barrier dependence on initial seeding density, the singularized-cell seeding approach reported here should enhance the reproducibility and scalability of hPSC-derived BBB models, particularly for the application to new pluripotent stem cell lines.

Wong, J. C., et al. (2014). "The epigenetic bivalency of core pancreatic beta-cell transcription factor genes within mouse pluripotent embryonic stem cells is not affected by knockdown of the polycomb repressive complex 2, SUZ12." PLoS One **9**(5): e97820.

This study assesses changes in activator and repressor modifications to histones associated with the core transcription factor genes most highly upregulated or downregulated in pancreatic beta-cells relative to expression in an embryonic stem cell line. Epigenetic analysis of the Oct4, Utf1, Nanog and Sox2 (pluripotency) and Pdx1, Nkx6.1, Nkx2.2 and MafA (pancreatic beta-cells) transcription factor genes in embryonic stem cells and a beta-cell line (MIN6) showed the pluripotency genes were enriched for active (histone 3 trimethylated at lysine 4 and histone 3 acetylated at lysine 9) and depleted of repressor modifications (histone 3 trimethylated at lysine 27 and histone 3 trimethylated at lysine 9) around the transcription start site in mouse embryonic stem cells (D3), and this was reversed in MIN6 cells. The beta-cell transcription factors were bivalently enriched for activating (histone 3 trimethylated at lysine 4) and repressor (histone 3 trimethylated at lysine 27) modifications in embryonic stem cells but were monovalent for the activator modification (histone 3 trimethylated at lysine 4) in the beta-cells. The polycomb repressor complex 2 acts as a histone 3 lysine 27 methylase and an essential component of this complex, SUZ12, was enriched at the beta-cell transcription factors in embryonic stem cells and was reduced MIN6. Knock-down of SUZ12 in embryonic stem cells, however, did not reduce the level of histone 3 trimethylated at lysine 27 at beta-cell transcription factor loci or break the transcriptional repression of these genes in embryonic stem cells. This study shows the reduction in the total SUZ12 level was not a sufficient cause of the resolution of the epigenetic bivalency of beta-cell transcription factors in embryonic stem cells.

Wu, H. J., et al. (2017). "Human induced pluripotent stem cell-derived mesenchymal stem cells prevent adriamycin nephropathy in mice." Oncotarget **8**(61): 103640-103656.

Human induced pluripotent stem cell-derived mesenchymal stem cells (iPS-MSCs) are emerging as attractive options for use in cell replacement therapy, but their effect in kidney diseases remains unknown. Here, we showed that intravenous injection of iPS-MSCs protect against renal function loss in both short-term and long-term models of adriamycin nephropathy (AN). In the short-term AN model, iPS-MSCs conferred a substantial anti-apoptotic effect on tubular cells, associated with a downregulation of Bax and Bax/Bcl2 ratio and an upregulation of survivin expression. In vitro, conditioned medium from iPS-MSCs (iPSMSC-CM) significantly limited albumin-induced tubular apoptosis and enhanced tubular proliferation, accompanied by a reduced expression of tubular Bax and an elevated expression of Bcl2 and survivin. Oxidative stress was markedly attenuated by iPS-MSCs both in AN mice and in protein-overloaded tubular cells. In the long-term AN model, repeated injections of iPS-MSCs significantly inhibited tubulointerstitial fibrosis and reduced intrarenal deposition of collagen I, collagen IV and alphaSMA. Modulation of the hedgehog signaling pathway contributed to the anti-fibrotic effect of iPS-MSCs in chronic AN. Finally, we detected that most of the infused iPS-MSCs were entrapped in the lungs. In conclusion, our data support a beneficial role of iPS-MSCs in both acute and chronic AN.

Wu, S., et al. (2018). "Induced Pluripotent Stem Cells Promote Retinal Ganglion Cell Survival After Transplant." Invest Ophthalmol Vis Sci **59**(3): 1571-1576.

Purpose: The purpose of this study was to characterize whether induced pluripotent stem cells (iPSCs) affect survival of grafted retinal ganglion cells (RGCs) after transplantation. Methods: For in vitro studies, human iPSCs were either directly cocultured with mouse RGCs or plated in hanging inserts in RGC cultures for 1 week. For ex vivo studies, RGCs and iPSCs were seeded onto the inner surface of an adult rat retina explant and cultured for 1 week. For in vivo studies, RGCs and iPSCs were intravitreally coinjected into an adult rat eye 1 week before examining retinas by explant and immunostaining. Results: A dose-dependent increase in RGC survival was observed in RGC-iPSC direct cocultures, and RGC-iPSC indirect cocultures showed a similar RGC protective effect, but to a lesser extent than in direct coculture. Enhanced RGC survival was also identified in RGC-iPSC cotransplantations to adult retinas ex vivo and in vivo. In addition, RGCs with iPSC cotransplantation extended significantly longer neurites than RGC-only transplants. Conclusions: Human iPSCs promote transplanted RGC survival and neurite extension. This effect may be mediated at least partially through secretion of diffusible neuroprotective factors.

Xia, B., et al. (2017). "Gene expression profiling analysis of the effects of low-intensity pulsed ultrasound on induced pluripotent stem cell-derived

neural crest stem cells." Biotechnol Appl Biochem **64**(6): 927-937.

Low-intensity pulsed ultrasound (LIPUS) is a noninvasive technique that has been shown to affect cell proliferation, migration, and differentiation and promote the regeneration of damaged peripheral nerve. Our previous studies had proved that LIPUS can significantly promote the neural differentiation of induced pluripotent stem cell-derived neural crest stem cells (iPSCs-NCSCs) and enhance the repair of rat-transected sciatic nerve. To further explore the underlying mechanisms of LIPUS treatment of iPSCs-NCSCs, this study reported the gene expression profiling analysis of iPSCs-NCSCs before and after LIPUS treatment using the RNA-sequencing (RNA-Seq) method. It was found that expression of 76 genes of iPSCs-NCSCs cultured in a serum-free neural induction medium and expression of 21 genes of iPSCs-NCSCs cultured in a neuronal differentiation medium were significantly changed by LIPUS treatment. The differentially expressed genes are related to angiogenesis, nervous system activity and functions, cell activities, and so on. The RNA-seq results were further verified by a quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR). High correlation was observed between the results obtained from qRT-PCR and RNA-Seq. This study presented new information on the global gene expression patterns of iPSCs-NCSCs after LIPUS treatment and may expand the understanding of the complex molecular mechanism of LIPUS treatment of iPSCs-NCSCs.

Xia, G., et al. (2015). "Genome modification leads to phenotype reversal in human myotonic dystrophy type 1 induced pluripotent stem cell-derived neural stem cells." Stem Cells **33**(6): 1829-1838.

Myotonic dystrophy type 1 (DM1) is caused by expanded CTG repeats in the 3'-untranslated region (3' UTR) of the DMPK gene. Correcting the mutation in DM1 stem cells would be an important step toward autologous stem cell therapy. The objective of this study is to demonstrate in vitro genome editing to prevent production of toxic mutant transcripts and reverse phenotypes in DM1 stem cells. Genome editing was performed in DM1 neural stem cells (NSCs) derived from human DM1 induced pluripotent stem (iPS) cells. An editing cassette containing SV40/bGH polyA signals was integrated upstream of the CTG repeats by TALEN-mediated homologous recombination (HR). The expression of mutant CUG repeats transcript was monitored by nuclear RNA foci, the molecular hallmarks of DM1, using RNA fluorescence in situ hybridization. Alternative splicing of microtubule-associated protein tau (MAPT) and muscleblind-like (MBNL) proteins were analyzed to further monitor the phenotype reversal after genome modification. The cassette was successfully inserted into DMPK intron 9 and this genomic modification led to complete disappearance of nuclear RNA foci. MAPT and MBNL 1, 2 aberrant splicing in DM1 NSCs were reversed to normal pattern in genome-modified NSCs. Genome modification by integration of exogenous polyA signals upstream of the DMPK CTG repeat expansion prevents the production of toxic RNA and leads to phenotype reversal in human DM1 iPS-cells derived stem cells. Our data provide proof-of-principle evidence that genome modification may be used to generate genetically modified progenitor cells as a first step toward autologous cell transfer therapy for DM1.

Xie, J., et al. (2015). "Electrospun nanofibers of hydroxyapatite/collagen/chitosan promote osteogenic differentiation of the induced pluripotent stem cell-derived mesenchymal stem cells." J Control Release **213**: e53.

Xing, K., et al. (2018). "Establishment of a human trisomy 18 induced pluripotent stem cell line from amniotic fluid cells." Intractable Rare Dis Res **7**(2): 94-99.

Trisomy 18 (18T) is the second most common autosomal trisomy syndrome in humans, but the detailed mechanism of its pathology remains unclear due to the lack of appropriate models of this disease. To resolve this problem, the current study reprogrammed human 18T amniotic fluid cells (AFCs) into an induced pluripotent stem cell (iPSC) line by introducing integration-free episomal vectors carrying pCXLE-hOCT3/4-shp53-F, pCXLE-hSK, pCXLE-hUL, and pCXWB-EBNA1. The pluripotency of 18T-iPSCs was subsequently validated by alkaline phosphatase staining, detection of iPSC biomarkers using real-time PCR and flow cytometry, detection of embryoid body (EB) formation, and detection of in vivo teratoma formation. Moreover, this study also investigated the transcriptomic profiles of 18T-iPSCs using RNA sequencing, and several gene clusters associated with the clinical manifestations of 18T were identified. In summary, the generated induced pluripotent stem cells line has typical pluripotency characteristics and can provide a useful tool with which to understand the development of 18T.

Xu, L., et al. (2018). "Effect of leukocyte inhibitory factor on neuron differentiation from human induced pluripotent stem cell-derived neural precursor cells." Int J Mol Med **41**(4): 2037-2049.

Direct derivation of human induced pluripotent stem cells into neural precursor cells and differentiation of these into neurons holds great promise in the cell therapy of neurodegenerative

diseases. However, the availability and survival rate of neurons requires improvement. In the present study, it was found that the addition of 5 ng/ml leukocyte inhibitory factor (LIF) during the process of differentiation significantly improved the expression of neuronspecific class III betatubulin (TUJ1) and microtubuleassociated protein 2 (MAP2), as detected by immunofluorescence and western blotting. In addition, LIF improved the cell viability, increased the expression of phosphorylatedprotein kinase B (AKT), downregulated the expression of proinflammatory cytokines, including interleukin1alpha (IL1alpha) and tumor necrosis factoralpha (TNF-alpha), and upregulated the expression of antiinflammatory cytokines, including interleukin10 (IL10) and transforming growth factorbeta (TGF-beta). After adding the phosphatidylinositol 3-kinase (PI3K)/AKT signaling inhibitor LY294002 or wortmannin to the LIF differentiation group, LIF-induced changes in the protein expression of TUJ1 and MAP2 were reversed, but this effect could not be prevented by rapamycin, a mechanistic target of rapamycin signaling inhibitor. The expression of cytokines associated with inflammation and cell viability was reversed by LY294002 and wortmannin, but not by rapamycin. In conclusion, LIF could improve neuronal differentiation and survival through the activation of PI3K/AKT signaling and the antiinflammatory effect. The antiinflammatory effect may be mediated by the activation of PI3K/AKT.

Xu, Y., et al. (2012). "Efficient commitment to functional CD34+ progenitor cells from human bone marrow mesenchymal stem-cell-derived induced pluripotent stem cells." PLoS One **7**(4): e34321.

The efficient commitment of a specialized cell type from induced pluripotent stem cells (iPSCs) without contamination from unknown substances is crucial to their use in clinical applications. Here, we propose that CD34+ progenitor cells, which retain hematopoietic and endothelial cell potential, could be efficiently obtained from iPSCs derived from human bone marrow mesenchymal stem cells (hBMMSC-iPSCs) with defined factors. By treatment with a cocktail containing mesodermal, hematopoietic, and endothelial inducers (BMP4, SCF, and VEGF, respectively) for 5 days, hBMMSC-iPSCs expressed the mesodermal transcription factors Brachyury and GATA-2 at higher levels than untreated groups ($P<0.05$). After culturing with another hematopoietic and endothelial inducer cocktail, including SCF, Flt3L, VEGF and IL-3, for an additional 7-9 days, CD34+ progenitor cells, which were undetectable in the initial iPSC cultures, reached nearly 20% of the total culture. This was greater than the relative number of progenitor cells produced from human-skin-fibroblast-derived iPSCs (hFib-iPSCs) or from the spontaneous differentiation groups ($P<0.05$), as assessed by flow cytometry analysis. These induced cells expressed hematopoietic transcription factors TAL-1 and GATA-2 [corrected]. They developed into various hematopoietic colonies when exposed to semisolid media with hematopoietic cytokines such as EPO and G-CSF. Hematopoietic cell lineages were identified by phenotype analysis with Wright-Giemsa staining. The endothelial potential of the cells was also verified by the confirmation of the formation of vascular tube-like structures and the expression of endothelial-specific markers CD31 and VE-CADHERIN. Efficient induction of CD34+ progenitor cells, which retain hematopoietic and endothelial cell potential with defined factors, provides an opportunity to obtain patient-specific cells for iPSC therapy and a useful model for the study of the mechanisms of hematopoiesis and drug screening.

Xue, Y., et al. (2013). "Generating a non-integrating human induced pluripotent stem cell bank from urine-derived cells." PLoS One **8**(8): e70573.

Induced pluripotent stem cell (iPS cell) holds great potential for applications in regenerative medicine, drug discovery, and disease modeling. We describe here a practical method to generate human iPS cells from urine-derived cells (UCs) under feeder-free, virus-free, serum-free condition and without oncogene c-MYC. We showed that this approach could be applied in a large population with different genetic backgrounds. UCs are easily accessible and exhibit high reprogramming efficiency, offering advantages over other cell types used for the purpose of iPS generation. Using the approach described in this study, we have generated 93 iPS cell lines from 20 donors with diverse genetic backgrounds. The non-viral iPS cell bank with these cell lines provides a valuable resource for iPS cells research, facilitating future applications of human iPS cells.

Yamagata, K. and J. Ueda (2013). "Long-term live-cell imaging of mammalian preimplantation development and derivation process of pluripotent stem cells from the embryos." Dev Growth Differ **55**(4): 378-389.

Mammalian fertilization is a process in which two highly specialized haploid gametes unite and endow totipotency to the resulting diploid zygote. This is followed by cell proliferation and the onset of differentiation during the brief period leading up to implantation. In these processes, a number of cellular components and structures are regulated spatially and temporally, as seen in repeated cell division, cell cycle progression, and epigenetic reprogramming. In mammals, the numbers of oocytes and embryos that can be collected are very limited. Therefore, analyses

of molecular mechanisms are hampered because of difficulties in conducting biochemical analyses on such limited material. Furthermore, immunostaining methods require cell fixation and are insufficient for understanding ontogeny, because the processes observed in fertilization and early embryonic development progress in time-dependent manners and each phenomenon is connected with others by cause-and-effect relationships. Consequently, it is important to develop an experimental system that enables molecular imaging without affecting embryonic development. To achieve the above advantages, especially retrospective and prospective analyses, we have established a live-cell imaging system that enables observations under minimally invasive conditions. Using this approach, we have succeeded in visualizing and predicting the developmental potential of embryos after various perturbations. We also succeeded in imaging embryonic stem (ES) cell derivation in natural conditions. In this review, we describe a brief history of embryonic imaging and detailed protocols. We also discuss promising aspects of imaging in the fields of developmental and stem cell biology.

Yamazaki, K., et al. (2016). "Functional Comparison of Neuronal Cells Differentiated from Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells under Different Oxygen and Medium Conditions." J Biomol Screen **21**(10): 1054-1064.

Because neurons are difficult to obtain from humans, generating functional neurons from human induced pluripotent stem cells (hiPSCs) is important for establishing physiological or disease-relevant screening systems for drug discovery. To examine the culture conditions leading to efficient differentiation of functional neural cells, we investigated the effects of oxygen stress (2% or 20% O2) and differentiation medium (DMEM/F12:Neurobasal-based [DN] or commercial [PhoenixSongs Biologicals; PS]) on the expression of genes related to neural differentiation, glutamate receptor function, and the formation of networks of neurons differentiated from hiPSCs (201B7) via long-term self-renewing neuroepithelial-like stem (lt-NES) cells. Expression of genes related to neural differentiation occurred more quickly in PS and/or 2% O2 than in DN and/or 20% O2, resulting in high responsiveness of neural cells to glutamate, N-methyl-d-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and (S)-3,5-dihydroxyphenylglycine (an agonist for mGluR1/5), as revealed by calcium imaging assays. NMDA receptors, AMPA receptors, mGluR1, and mGluR5 were functionally validated by using the specific antagonists MK-801, NBQX, JNJ16259685, and 2-methyl-6-(phenylethynyl)-pyridine, respectively. Multielectrode array analysis showed that spontaneous firing occurred earlier in cells cultured in 2% O2 than in 20% O2. Optimization of O2 tension and culture medium for neural differentiation of hiPSCs can efficiently generate physiologically relevant cells for screening systems.

Yamazoe, T., et al. (2015). "Potent tumor tropism of induced pluripotent stem cells and induced pluripotent stem cell-derived neural stem cells in the mouse intracerebral glioma model." Int J Oncol **46**(1): 147-152.

Although neural and mesenchymal stem cells have been well-known to have a strong glioma tropism, this activity in induced pluripotent stem cells (iPSCs) has not yet been fully studied. In the present study, we tested tumor tropic activity of mouse iPSCs and neural stem cells derived from the iPSC (iPS-NSCs) using in vitro Matrigel invasion chamber assay and in vivo mouse intracranial tumor model. Both iPSC and iPS-NSC had a similar potent in vitro tropism for glioma conditioned media. The migrated iPSCs to the gliomas kept expressing Nanog-GFP gene, suggesting no neuronal or glial differentiation. iPSCs or iPS-NSCs labeled with 5-bromo-2-deoxyuridine were intracranially implanted in the contralateral hemisphere to the GL261 glioma cell implantation in the allogeneic C57BL/6 mouse. Active migration of both stem cells was observed 7 days after implantation. Again, the iPSCs located in the tumor area expressed Nanog-GFP gene, suggesting that the migrated cells were still iPSCs. These findings demonstrated that both iPSCs and iPS-NSCs had potent glioma tropism and could be candidates as vehicles in stem cell-based glioma therapy.

Yan, B. and D. K. Singla (2013). "Transplanted induced pluripotent stem cells mitigate oxidative stress and improve cardiac function through the Akt cell survival pathway in diabetic cardiomyopathy." Mol Pharm **10**(9): 3425-3432.

Recent evidence suggests transplanted stem cells improve left ventricular function in diabetic induced cardiomyopathy (DICM). However, little is known about the mechanisms by which induced pluripotent stem (iPS) cells or factors released from these cells inhibit adverse cardiac remodeling in DICM. The present study was designed to determine molecular mediators and pathways regulated by transplanted iPS cells and their conditioned media (CM) in DICM. Animals were divided into four experimental groups such as control, streptozotocin (STZ), STZ+iPS-CM, and STZ+iPS cells. Experimental diabetes was induced in C57BL/6 mice by intraperitoneal STZ injections (100 mg/kg body weight for 2 consecutive days). Following STZ injections, iPS cells or CM was given

intravenously for 3 consecutive days. Animals were humanely killed, and hearts were harvested at D14. Animals transplanted with iPS cells or CM demonstrated a significant reduction in apoptosis, mediated by Akt upregulation and ERK1/2 downregulation, and inhibition of interstitial fibrosis via MMP-9 suppression compared with the STZ group. Oxidative stress was significantly hindered in iPS cell and CM groups as evidenced by diminished pro-oxidant expression and enhanced antioxidant (catalase and MnSOD) concentration. Echocardiography data suggest a significant improvement in cardiac function in cells and CM groups in comparison to STZ. In conclusion, our data strongly suggest that iPS cells and CM attenuate oxidative stress and associated apoptosis and fibrosis. Moreover, we also suggest that increased antioxidant levels, decreased adverse cardiac remodeling, and improved cardiac function is mediated by iPS CM and cells in DICM through multiple autocrine and paracrine mechanisms.

Yanagimachi, M. D., et al. (2013). "Robust and highly-efficient differentiation of functional monocytic cells from human pluripotent stem cells under serum- and feeder cell-free conditions." PLoS One **8**(4): e59243.

Monocytic lineage cells (monocytes, macrophages and dendritic cells) play important roles in immune responses and are involved in various pathological conditions. The development of monocytic cells from human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) is of particular interest because it provides an unlimited cell source for clinical application and basic research on disease pathology. Although the methods for monocytic cell differentiation from ESCs/iPSCs using embryonic body or feeder co-culture systems have already been established, these methods depend on the use of xenogeneic materials and, therefore, have a relatively poor-reproducibility. Here, we established a robust and highly-efficient method to differentiate functional monocytic cells from ESCs/iPSCs under serum- and feeder cell-free conditions. This method produced 1.3 x 10(6) +/- 0.3 x 10(6) floating monocytes from approximately 30 clusters of ESCs/iPSCs 5-6 times per course of differentiation. Such monocytes could be differentiated into functional macrophages and dendritic cells. This method should be useful for regenerative medicine, disease-specific iPSC studies and drug discovery.

Yang, D., et al. (2016). "Enrichment of G2/M cell cycle phase in human pluripotent stem cells enhances HDR-mediated gene repair with customizable endonucleases." Sci Rep **6**: 21264.

Efficient gene editing is essential to fully utilize human pluripotent stem cells (hPSCs) in regenerative medicine. Custom endonuclease-based gene targeting involves two mechanisms of DNA repair: homology directed repair (HDR) and non-homologous end joining (NHEJ). HDR is the preferred mechanism for common applications such knock-in, knock-out or precise mutagenesis, but remains inefficient in hPSCs. Here, we demonstrate that synchronizing synchronizing hPSCs in G2/M with ABT phase increases on-target gene editing, defined as correct targeting cassette integration, 3 to 6 fold. We observed improved efficiency using ZFNs, TALENs, two CRISPR/Cas9, and CRISPR/Cas9 nickase to target five genes in three hPSC lines: three human embryonic stem cell lines, neural progenitors and diabetic iPSCs. neural progenitors and diabetic iPSCs. Reversible synchronization has no effect on pluripotency or differentiation. The increase in on-target gene editing is locus-independent and specific to the cell cycle phase as G2/M phase enriched cells show a 6-fold increase in targeting efficiency compared to cells in G1 phase. Concurrently inhibiting NHEJ with SCR7 does not increase HDR or improve gene targeting efficiency further, indicating that HR is the major DNA repair mechanism after G2/M phase arrest. The approach outlined here makes gene editing in hPSCs a more viable tool for disease modeling, regenerative medicine and cell-based therapies.

Yang, J., et al. (2012). "Tumor tropism of intravenously injected human-induced pluripotent stem cell-derived neural stem cells and their gene therapy application in a metastatic breast cancer model." Stem Cells **30**(5): 1021-1029.

Human pluripotent stem cells can serve as an accessible and reliable source for the generation of functional human cells for medical therapies. In this study, we used a conventional lentiviral transduction method to derive human-induced pluripotent stem (iPS) cells from primary human fibroblasts and then generated neural stem cells (NSCs) from the iPS cells. Using a dual-color whole-body imaging technology, we demonstrated that after tail vein injection, these human NSCs displayed a robust migratory capacity outside the central nervous system in both immunodeficient and immunocompetent mice and homed in on established orthotopic 4T1 mouse mammary tumors. To investigate whether the iPS cell-derived NSCs can be used as a cellular delivery vehicle for cancer gene therapy, the cells were transduced with a baculoviral vector containing the herpes simplex virus thymidine kinase suicide gene and injected through tail vein into 4T1 tumor-bearing mice. The transduced NSCs were effective in inhibiting the growth of the orthotopic 4T1 breast tumor and the metastatic spread of the cancer cells in the presence of ganciclovir, leading to prolonged survival of the tumor-

bearing mice. The use of iPS cell-derived NSCs for cancer gene therapy bypasses the sensitive ethical issue surrounding the use of cells derived from human fetal tissues or human embryonic stem cells. This approach may also help to overcome problems associated with allogeneic transplantation of other types of human NSCs.

Yang, J., et al. (2016). "Induced pluripotent stem cells in Alzheimer's disease: applications for disease modeling and cell-replacement therapy." Mol Neurodegener **11**(1): 39.

Alzheimer's disease (AD) is the most common cause of dementia in those over the age of 65. While a numerous of disease-causing genes and risk factors have been identified, the exact etiological mechanisms of AD are not yet completely understood, due to the inability to test theoretical hypotheses on non-postmortem and patient-specific research systems. The use of recently developed and optimized induced pluripotent stem cells (iPSCs) technology may provide a promising platform to create reliable models, not only for better understanding the etiopathological process of AD, but also for efficient anti-AD drugs screening. More importantly, human-sourced iPSCs may also provide a beneficial tool for cell-replacement therapy against AD. Although considerable progress has been achieved, a number of key challenges still require to be addressed in iPSCs research, including the identification of robust disease phenotypes in AD modeling and the clinical availabilities of iPSCs-based cell-replacement therapy in human. In this review, we highlight recent progresses of iPSCs research and discuss the translational challenges of AD patients-derived iPSCs in disease modeling and cell-replacement therapy.

Ye, L., et al. (2009). "Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases." Proc Natl Acad Sci U S A **106**(24): 9826-9830.

The innovation of reprogramming somatic cells to induced pluripotent stem cells provides a possible new approach to treat beta-thalassemia and other genetic diseases such as sickle cell anemia. Induced pluripotent stem (iPS) cells can be made from these patients' somatic cells and the mutation in the beta-globin gene corrected by gene targeting, and the cells differentiated into hematopoietic cells to be returned to the patient. In this study, we reprogrammed the skin fibroblasts of a patient with homozygous beta(0) thalassemia into iPS cells, and showed that the iPS cells could be differentiated into hematopoietic cells that synthesized hemoglobin. Prenatal diagnosis and selective abortion have been effective in decreasing the number of beta-thalassemia births in some countries that have instituted carrier screening and genetic counseling. To make use of the cells from the amniotic fluid or chorionic villus sampling that are used for prenatal diagnosis, we also showed that these cells could be reprogrammed into iPS cells. This raises the possibility of providing a new option following prenatal diagnosis of a fetus affected by a severe illness. Currently, the parents would choose either to terminate the pregnancy or continue it and take care of the sick child after birth. The cells for prenatal diagnosis can be converted into iPS cells for treatment in the perinatal periods. Early treatment has the advantage of requiring much fewer cells than adult treatment, and can also prevent organ damage in those diseases in which damage can begin in utero or at an early age.

Ye, L., et al. (2014). "Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells." Cell Stem Cell **15**(6): 750-761.

Human induced pluripotent stem cells (hiPSCs) hold promise for myocardial repair following injury, but preclinical studies in large animal models are required to determine optimal cell preparation and delivery strategies to maximize functional benefits and to evaluate safety. Here, we utilized a porcine model of acute myocardial infarction (MI) to investigate the functional impact of intramyocardial transplantation of hiPSC-derived cardiomyocytes, endothelial cells, and smooth muscle cells, in combination with a 3D fibrin patch loaded with insulin growth factor (IGF)-encapsulated microspheres. hiPSC-derived cardiomyocytes integrated into host myocardium and generated organized sarcomeric structures, and endothelial and smooth muscle cells contributed to host vasculature. Trilineage cell transplantation significantly improved left ventricular function, myocardial metabolism, and arteriole density, while reducing infarct size, ventricular wall stress, and apoptosis without inducing ventricular arrhythmias. These findings in a large animal MI model highlight the potential of utilizing hiPSC-derived cells for cardiac repair.

Ye, L., et al. (2013). "Blood cell-derived induced pluripotent stem cells free of reprogramming factors generated by Sendai viral vectors." Stem Cells Transl Med **2**(8): 558-566.

The discovery of induced pluripotent stem cells (iPSCs) holds great promise for regenerative medicine since it is possible to produce patient-specific pluripotent stem cells from affected individuals for potential autologous treatment. Using nonintegrating cytoplasmic Sendai viral vectors, we generated iPSCs efficiently from adult mobilized CD34(+) and

peripheral blood mononuclear cells. After 5-8 passages, the Sendai viral genome could not be detected by real-time quantitative reverse transcription-polymerase chain reaction. Using the spin embryoid body method, we showed that these blood cell-derived iPSCs could efficiently be differentiated into hematopoietic stem and progenitor cells without the need of coculture with either mouse or human stromal cells. We obtained up to 40% CD34(+) of which ~25% were CD34(+)/CD43(+) hematopoietic precursors that could readily be differentiated into mature blood cells. Our study demonstrated a reproducible protocol for reprogramming blood cells into transgene-free iPSCs by the Sendai viral vector method. Maintenance of the genomic integrity of iPSCs without integration of exogenous DNA should allow the development of therapeutic-grade stem cells for regenerative medicine.

Ye, Z. and L. Cheng (2010). "Potential of human induced pluripotent stem cells derived from blood and other postnatal cell types." Regen Med **5**(4): 521-530.

Human induced pluripotent stem (iPS) cells have been generated from various cell types including blood cells, and offer certain advantages as a starting population for reprogramming postnatal somatic cells. Unlike adult stem cells, iPS cells can proliferate limitlessly in culture while retaining their potential to differentiate into any cell type, including hematopoietic lineages. Derivation of patient-specific iPS cells, in combination with improved hematopoietic differentiation protocols, provides an alternative to generate histocompatible stem cells for bone marrow transplantation. In addition, the ability to reprogram blood cells and redifferentiate iPS cells back to hematopoietic lineages provides opportunities to establish novel models for acquired and inherited blood diseases. This article will summarize recent progress in human iPS cells derived from blood cells and hematopoietic differentiation from iPS cells. Advantages of blood as a source for reprogramming and applications in regenerative medicine will be discussed.

Yi, F., et al. (2012). "Human induced pluripotent stem cells derived hepatocytes: rising promise for disease modeling, drug development and cell therapy." Protein Cell **3**(4): 246-250.

Recent advances in the study of human hepatocytes derived from induced pluripotent stem cells (iPSC) represent new promises for liver disease study and drug discovery. Human hepatocytes or hepatocyte-like cells differentiated from iPSC recapitulate many functional properties of primary human hepatocytes and have been demonstrated as a powerful and efficient tool to model human liver metabolic diseases and facilitate drug development process. In this review, we summarize the recent progress in this field and discuss the future perspective of the application of human iPSC derived hepatocytes.

Yin, X., et al. (2017). "Growth/differentiation factor-5 promotes in vitro/vivo periodontal specific differentiation of induced pluripotent stem cell-derived mesenchymal stem cells." Exp Ther Med **14**(5): 4111-4117.

Mesenchymal stem cells (MSCs) derived from induced pluripotent stem cells (iPSCs) represent a promising alternative source of MSCs for effective periodontal regeneration. Scientific evidence has demonstrated that growth/differentiation factor-5 (GDF-5) supports regeneration of periodontal tissues and has a key role in MSC differentiation. The present study investigated the effects of recombinant human GDF-5 (rhGDF-5) on periodontal specific differentiation of iPSC-derived MSCs (iPSC-MSCs) and bone marrow mesenchymal stem cells (BMSCs). rhGDF-5 treatment in vitro significantly enhanced the expression levels of marker genes associated with osteogenesis (OCN), fibrogenesis (periostin) and cementogenesis (CAP) in the iPSC-MSCs compared with untreated controls (all $P<0.05$). Interestingly, the rhGDF-5-treated BMSCs failed to exhibit overexpression of periostin and CAP despite highly upregulated expression of OCN. In the presence of rhGDF-5, both the iPSC-MSCs and BMSCs demonstrated marked formation of mineralized nodules. Notably, rhGDF-5 greatly promoted periodontal specific differentiation of the iPSC-MSCs encapsulated in hyaluronic acid (HA) hydrogels in vivo as determined by immunohistochemical and immunofluorescence staining. The majority of the PKH67-labeled iPSC-MSCs implanted with rhGDF-5 exhibited strong expression of OCN, periostin and CAP. In conclusion, iPSC-MSCs demonstrate high periodontal specific differentiation potential in response to rhGDF-5 both in vitro and in vivo. The delivery of iPSC-MSCs and rhGDF-5 with HA hydrogel may have beneficial effects in regenerative periodontal therapy.

Yokobayashi, S., et al. (2017). "Clonal variation of human induced pluripotent stem cells for induction into the germ cell fate." Biol Reprod **96**(6): 1154-1166.

The mechanisms for human germ cell development have remained largely unknown, due to the difficulty in obtaining suitable experimental materials. The establishment of an in vitro system to reconstitute human germ cell development will thus provide a critical opportunity to understand its mechanisms at a molecular level. It has previously been shown that human induced pluripotent stem cells (hiPSCs) are first induced into incipient mesoderm-like

cells (iMeLCs), which are in turn induced into primordial germ-cell like cells (PGCLCs) with gene expression properties similar to early migratory PGCs. Here, we report that the efficiency of PGCLC induction varies among hiPSC clones, and, interestingly, the clonal variations in PGCLC induction efficiency are reflected in the gene expression states of the iMeLCs. Remarkably, the expression levels of EOMES, MIXL1, or T in the iMeLCs are positively correlated with the efficiency of subsequent PGCLC generation, while the expressions of CDH1, SOX3, or FGF2 are negatively correlated. These results indicate that the expression changes of these genes occurring during iMeLC induction are key markers indicative of successful induction of PGCLCs, and furthermore, that hiPSC clones have different properties that influence their responsivity to the iMeLC induction. Our study thus provides important insights into the mechanism of hPGC specification as well as the development of a better strategy for inducing human germ cell fate from PSCs in vitro.

Yoshida, S., et al. (2018). "Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes by Soluble Factors from Human Mesenchymal Stem Cells." Mol Ther.

In this study, we proposed that the functionality or phenotype of differentiated cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) might be modified by co-culture with mesenchymal stem cells (MSCs), resulting in an improved therapeutic potential for failing myocardial tissues. Structural, motility, electrophysiological, and metabolic analyses revealed that iPSC-CMs co-cultured with MSCs displayed aligned myofibrils with A-, H-, and I-bands that could contract and relax quickly, indicating the promotion of differentiation and the establishment of the iPSC-CM structural framework, and showed clear gap junctions and an electric pacing of >2 Hz, indicating enhanced cell-cell interactions. In addition, soluble factors excreted by MSCs, including several cytokines and exosomes, enhanced cardiomyocyte-specific marker production, produced more energy under normal and stressed conditions, and reduced reactive oxygen species production by iPSC-CMs under stressed condition. Notably, gene ontology and pathway analysis revealed that microRNAs and proteins in the exosomes impacted the functionality and maturation of iPSC-CMs. Furthermore, cell sheets consisting of a mixture of iPSC-CMs and MSCs showed longer survival and enhanced therapeutic effects compared with those consisting of iPSC-CMs alone. This may lead to a new type of iPSC-based cardiomyogenesis therapy for patients with heart failure.

Yoshida, Y. and S. Yamanaka (2010). "Recent stem cell advances: induced pluripotent stem cells for disease modeling and stem cell-based regeneration." Circulation **122**(1): 80-87.

Yu, D., et al. (2014). "Niemann-Pick Disease Type C: Induced Pluripotent Stem Cell-Derived Neuronal Cells for Modeling Neural Disease and Evaluating Drug Efficacy." J Biomol Screen **19**(8): 1164-1173.

Niemann-Pick disease type C (NPC) is a rare neurodegenerative disorder caused by recessive mutations in the NPC1 or NPC2 gene that result in lysosomal accumulation of unesterified cholesterol in patient cells. Patient fibroblasts have been used for evaluation of compound efficacy, although neuronal degeneration is the hallmark of NPC disease. Here, we report the application of human NPC1 neural stem cells as a cell-based disease model to evaluate nine compounds that have been reported to be efficacious in the NPC1 fibroblasts and mouse models. These cells are differentiated from NPC1 induced pluripotent stem cells and exhibit a phenotype of lysosomal cholesterol accumulation. Treatment of these cells with hydroxypropyl-beta-cyclodextrin, methyl-beta-cyclodextrin, and delta-tocopherol significantly ameliorated the lysosomal cholesterol accumulation. Combined treatment with cyclodextrin and delta-tocopherol shows an additive or synergistic effect that otherwise requires 10-fold higher concentration of cyclodextrin alone. In addition, we found that hydroxypropyl-beta-cyclodextrin is much more potent and efficacious in the NPC1 neural stem cells compared to the NPC1 fibroblasts. Miglustat, suberoylanilide hydroxamic acid, curcumin, lovastatin, pravastatin, and rapamycin did not, however, have significant effects in these cells. The results demonstrate that patient-derived NPC1 neural stem cells can be used as a model system for evaluation of drug efficacy and study of disease pathogenesis.

Yu, J., et al. (2007). "Induced pluripotent stem cell lines derived from human somatic cells." Science **318**(5858): 1917-1920.

Somatic cell nuclear transfer allows trans-acting factors present in the mammalian oocyte to reprogram somatic cell nuclei to an undifferentiated state. We show that four factors (OCT4, SOX2, NANOG, and LIN28) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem (ES) cells. These induced pluripotent human stem cells have normal karyotypes, express telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Such

induced pluripotent human cell lines should be useful in the production of new disease models and in drug development, as well as for applications in transplantation medicine, once technical limitations (for example, mutation through viral integration) are eliminated.

Yu, L., et al. (2018). "Low Cell-Matrix Adhesion Reveals Two Subtypes of Human Pluripotent Stem Cells." Stem Cell Reports **11**(1): 142-156.

We show that a human pluripotent stem cell (hPSC) population cultured on a low-adhesion substrate developed two hPSC subtypes with different colony morphologies: flat and domed. Notably, the dome-like cells showed higher active proliferation capacity and increased several pluripotent genes' expression compared with the flat monolayer cells. We further demonstrated that cell-matrix adhesion mediates the interaction between cell morphology and expression of KLF4 and KLF5 through a serum response factor (SRF)-based regulatory double loop. Our results provide a mechanistic view on the coupling among adhesion, stem cell morphology, and pluripotency, shedding light on the critical role of cell-matrix adhesion in the induction and maintenance of hPSC.

Yu, X., et al. (2015). "Upregulated sirtuin 1 by miRNA-34a is required for smooth muscle cell differentiation from pluripotent stem cells." Cell Death Differ **22**(7): 1170-1180.

microRNA-34a (miR-34a) and sirtuin 1 (SirT1) have been extensively studied in tumour biology and longevity/aging, but little is known about their functional roles in smooth muscle cell (SMC) differentiation from pluripotent stem cells. Using well-established SMC differentiation models, we have demonstrated that miR-34a has an important role in SMC differentiation from murine and human embryonic stem cells. Surprisingly, deacetylase sirtuin 1 (SirT1), one of the top predicted targets, was positively regulated by miR-34a during SMC differentiation. Mechanistically, we demonstrated that miR-34a promoted differentiating stem cells' arrest at G0/G1 phase and observed a significantly decreased incorporation of miR-34a and SirT1 RNA into Ago2-RISC complex upon SMC differentiation. Importantly, we have identified SirT1 as a transcriptional activator in the regulation of SMC gene programme. Finally, our data showed that SirT1 modulated the enrichment of H3K9 tri-methylation around the SMC gene-promoter regions. Taken together, our data reveal a specific regulatory pathway that miR-34a positively regulates its target gene SirT1 in a cellular context-dependent and sequence-specific manner and suggest a functional role for this pathway in SMC differentiation from stem cells in vitro and in vivo.

Yuan, S. H., et al. (2011). "Cell-surface marker signatures for the isolation of neural stem cells, glia and neurons derived from human pluripotent stem cells." PLoS One **6**(3): e17540.

BACKGROUND: Neural induction of human pluripotent stem cells often yields heterogeneous cell populations that can hamper quantitative and comparative analyses. There is a need for improved differentiation and enrichment procedures that generate highly pure populations of neural stem cells (NSC), glia and neurons. One way to address this problem is to identify cell-surface signatures that enable the isolation of these cell types from heterogeneous cell populations by fluorescence activated cell sorting (FACS). METHODOLOGY/PRINCIPAL FINDINGS: We performed an unbiased FACS- and image-based immunophenotyping analysis using 190 antibodies to cell surface markers on naive human embryonic stem cells (hESC) and cell derivatives from neural differentiation cultures. From this analysis we identified prospective cell surface signatures for the isolation of NSC, glia and neurons. We isolated a population of NSC that was CD184(+)/CD271(-)/CD44(-)/CD24(+) from neural induction cultures of hESC and human induced pluripotent stem cells (hiPSC). Sorted NSC could be propagated for many passages and could differentiate to mixed cultures of neurons and glia in vitro and in vivo. A population of neurons that was CD184(-)/CD44(-)/CD15(LOW)/CD24(+) and a population of glia that was CD184(+)/CD44(+) were subsequently purified from cultures of differentiating NSC. Purified neurons were viable, expressed mature and subtype-specific neuronal markers, and could fire action potentials. Purified glia were mitotic and could mature to GFAP-expressing astrocytes in vitro and in vivo. CONCLUSIONS/SIGNIFICANCE: These findings illustrate the utility of immunophenotyping screens for the identification of cell surface signatures of neural cells derived from human pluripotent stem cells. These signatures can be used for isolating highly pure populations of viable NSC, glia and neurons by FACS. The methods described here will enable downstream studies that require consistent and defined neural cell populations.

Yuan, T., et al. (2013). "Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion." Stem Cell Res Ther **4**(3): 73.

INTRODUCTION: Stroke is a major cause of permanent neurologic damage, with few effective

treatments available to restore lost function. Induced pluripotent stem cells (iPSCs) have the potential to generate all cell types in vitro and can be generated from a stroke patient. Therefore, iPSCs are attractive donor sources of genetically identical "patient-specific" cells to hold promise in therapy for stroke. In the present study, we established a four-stage culture system by using serum-free medium and retinoic acid (RA) to differentiate iPSCs into neural stem cells (NSCs) effectively and stably. Our hypothesis was that iPSC-derived NSCs would survive, migrate, and differentiate in vivo, and improve neurologic function after transplantation into the brains of rats with ischemic stroke. METHODS: Human iPSCs (iPS-S-01) and human ESCs (HuES17) were used to differentiate into NSCs by using our four-stage culture system. iPSCs and differentiated NSCs were characterized by immunocytochemistry staining and reverse transcription-polymerase chain reaction (RT-PCR) analysis. After establishment of focal cerebral ischemia with occlusion of the middle cerebral artery (MCA) and cell transplantation, animals were killed at 1 week and 2 weeks to analyze survival, migration, and differentiation of implanted cells in brain tissue. Animal behavior was evaluated via rope grabbing, beam walking, and Morris water maze tests. RESULTS: iPSCs were efficiently induced into NSCs by using a newly established four-stage induction system in vitro. iPSCs expressed pluripotency-associated genes Oct4, Sox2, and Nanog before NSC differentiation. The iPSC-derived NSCs spontaneously differentiated into neurons and astrocytes, which highly express beta-tubulin and glial fibrillary acidic protein (GFAP), respectively. On transplantation into the striatum, CM-DiI labeled iPSC-derived NSCs were found to migrate into the ischemia area at 1 week and 2 weeks, and animal-function recovery was significantly improved in comparison with control groups at 3 weeks. CONCLUSIONS: The four-stage induction system is stable and effective to culture, differentiate, and induce iPSCs to NSCs by using serum-free medium combined with retinoic acid (RA). Implanted iPSC-derived NSCs were able to survive, migrate into the ischemic brain area to differentiate into mature neural cells, and seem to have potential to restore lost neurologic function from damage due to stroke in a rat model.

Yuan, T. F. and O. Arias-Carrion (2008). "Locally induced neural stem cells/pluripotent stem cells for in vivo cell replacement therapy." Int Arch Med **1**(1): 17.

Neural stem cells hold the key to innovative new treatments for age-associated degeneration and traumatic injury to the brain and spinal cord. We hypothesized that the in vivo induced pluripotent stem cells or neural stem cells through "forced gene expression" can be used to repair damaged brain areas or treat degenerative diseases. Hopefully, these in vivo patient-specific stem cells can bring a new avenue for cell replacement therapies.

Yuan, X., et al. (2017). "Extracellular vesicles from human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs) protect against renal ischemia/reperfusion injury via delivering specificity protein (SP1) and transcriptional activating of sphingosine kinase 1 and inhibiting necroptosis." Cell Death Dis **8**(12): 3200.

Renal ischemia-reperfusion is a main cause of acute kidney injury (AKI), which is associated with high mortality. Here we show that extracellular vesicles (EVs) secreted from hiPSC-MSCs play a critical role in protection against renal I/R injury. hiPSC-MSCs-EVs can fuse with renal cells and deliver SP1 into target cells, subsequently active SK1 expression and increase S1P formation. Chromatin immunoprecipitation (ChIP) analyses and luciferase assay were used to confirm SP1 binds directly to the SK1 promoter region and promote promoter activity. Moreover, SP1 inhibition (MIT) or SK1 inhibition (SKI-II) completely abolished the renal protective effect of hiPSC-MSCs-EVs in rat I/R injury mode. However, pre-treatment of necroptosis inhibitor Nec-1 showed no difference with the administration of hiPSC-MSCs-EVs only. We then generated an SP1 knockout hiPSC-MSC cell line by CRISPR/Cas9 system and found that SP1 knockout failed to show the protective effect of hiPSC-MSCs-EVs unless restoring the level of SP1 by Ad-SP1 in vitro and in vivo. In conclusion, this study describes an anti-necroptosis effect of hiPSC-MSCs-EVs against renal I/R injury via delivering SP1 into target renal cells and intracellular activating the expression of SK1 and the generation of S1P. These findings suggest a novel mechanism for renal protection against I/R injury, and indicate a potential therapeutic approach for a variety of renal diseases and renal transplantation.

Yuan, Y., et al. (2014). "Cell cycle synchronization of leukemia inhibitory factor (LIF)-dependent porcine-induced pluripotent stem cells and the generation of cloned embryos." Cell Cycle **13**(8): 1265-1276.

Nuclear transfer (NT) from porcine iPSC to create cloned piglets is unusually inefficient. Here we examined whether such failure might be related to the cell cycle stage of donor nuclei. Porcine iPSC, derived here from the inner cell mass of blastocysts, have a prolonged S phase and are highly sensitive to drugs normally used for synchronization. However, a double-blocking procedure with 0.3 muM aphidicolin for 10 h followed by 20 ng/ml nocodazole for 4 h arrested 94.3% of the cells at G2/M and, after release from the block, provided 70.1% cells in the subsequent G1 phase without causing any significant loss of cell viability or

pluripotent phenotype. Nuclei from different cell cycle stages were used as donors for NT to in vitro-matured metaphase II oocytes. G2/M nuclei were more efficient than either G1 and S stage nuclei in undergoing first cleavage and in producing blastocysts, but all groups had a high incidence of chromosomal/nuclear abnormalities at 2 h and 6 h compared with non-synchronized NT controls from fetal fibroblasts. Many G2 embryos extruded a pseudo-second polar body soon after NT and, at blastocyst, tended to be either polyploid or diploid. By contrast, the few G1 blastocysts that developed were usually mosaic or aneuploid. The poor developmental potential of G1 nuclei may relate to lack of a G1/S check point, as the cells become active in DNA synthesis shortly after exit from mitosis. Together, these data provide at least a partial explanation for the almost complete failure to produce cloned piglets from piPSC.

Yuhuan, X., et al. (2018). "Generation of GZKHQi001-A and GZWWTi001-A, two induced pluripotent stem cell lines derived from peripheral blood mononuclear cells of Duchenne muscular dystrophy patients." Stem Cell Res **28**: 25-28.

Duchenne muscular dystrophy (DMD) is an X-linked disease caused by mutations in the DMD gene, which spans ~2.4Mb of genomic sequence at locus Xp21. This mutation results in the loss of the protein dystrophin. DMD patients die in their second or third decade due to either respiratory failure or cardiomyopathy, as the absence of dystrophin leads to myofiber membrane fragility and necrosis, eventually resulting in muscle atrophy and contractures. Currently, there is no effective treatment for DMD, therefore induced pluripotent stem cells from DMD patients would be a powerful tool for studying disease mechanisms.

Yukawa, H., et al. (2010). "Transduction of cell-penetrating peptides into induced pluripotent stem cells." Cell Transplant **19**(6): 901-909.

Induced pluripotent stem (iPS) cells have recently been generated by Yamanaka's group, and then followed by others. iPS cells are expected to have clinical applications including an important role in regenerative medicine. This study focused on the cell-penetrating peptides (CPPs) for differentiation or functional application of iPS cells, because several transduction domains can deliver a large size-independent variety of molecules into cells. Two CPPs, Texas Red-R8 and Rhodamine-TAT, were generated as representative CPPs and these CPPs were tested to determine their ability to penetrate the membrane of iPS cells. Both CPPs were transduced in iPS cells through macropinocytosis classified in endocytosis within 2 h in a manner consistent with many other cells, and no cytotoxicity and influence on their undifferentiated state was observed. In conclusion, CPPs can be utilized for their differentiation or functional application in iPS cells.

Yun, Y. I., et al. (2017). "Comparison of the anti-inflammatory effects of induced pluripotent stem cell-derived and bone marrow-derived mesenchymal stromal cells in a murine model of corneal injury." Cytotherapy **19**(1): 28-35.

BACKGROUND AIMS: Mesenchymal stromal cells (MSCs) offer tremendous potential for therapeutic applications for inflammatory diseases. However, tissue-derived MSCs, such as bone marrow-derived MSCs (BM-MSCs), have considerable donor variations and limited expandability. It was recently demonstrated that MSCs derived from induced pluripotent stem cells (iPSC-MSCs) have less pro-tumor potential and greater expandability of homogenous cell population. In this study, we investigated the anti-inflammatory effects and mechanism of iPSC-MSCs in a murine model of chemical and mechanical injury to the cornea and compared the effects with those of BM-MSCs. METHODS: To create an injury, ethanol was applied to the corneal surface in mice, and the corneal epithelium was removed with a blade. Immediately after injury, mice received an intravenous injection of (i) iPSC-MSCs, (ii) BM-MSCs or (iii) vehicle. Clinical, histological and molecular assays were performed in the cornea to evaluate inflammation. RESULTS: We found that corneal opacity was significantly reduced by iPSC-MSCs or BM-MSCs. Histological examination revealed that the swelling and inflammatory infiltration in the cornea were markedly decreased in mice treated with iPSC-MSCs or BM-MSCs. Corneal levels of tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-6 were lower in iPSC-MSC- and BM-MSC-treated mice, compared with vehicle-treated controls. In contrast, iPSC-MSCs with a knockdown of the TNF-alpha stimulating gene (TSG)-6 did not suppress the levels of inflammatory cytokines and failed to reduce corneal opacity. CONCLUSIONS: Together these data demonstrate that iPSC-MSCs exert therapeutic effects in the cornea by reducing inflammation in part through the expression of TSG-6, and the effects are similar to those seen with BM-MSCs.

Zahedi, A., et al. (2016). "Evaluating Cell Processes, Quality, and Biomarkers in Pluripotent Stem Cells Using Video Bioinformatics." PLoS One **11**(2): e0148642.

There is a foundational need for quality control tools in stem cell laboratories engaged in basic research, regenerative therapies, and toxicological

studies. These tools require automated methods for evaluating cell processes and quality during in vitro passaging, expansion, maintenance, and differentiation. In this paper, an unbiased, automated high-content profiling toolkit, StemCellQC, is presented that non-invasively extracts information on cell quality and cellular processes from time-lapse phase-contrast videos. Twenty four (24) morphological and dynamic features were analyzed in healthy, unhealthy, and dying human embryonic stem cell (hESC) colonies to identify those features that were affected in each group. Multiple features differed in the healthy versus unhealthy/dying groups, and these features were linked to growth, motility, and death. Biomarkers were discovered that predicted cell processes before they were detectable by manual observation. StemCellQC distinguished healthy and unhealthy/dying hESC colonies with 96% accuracy by non-invasively measuring and tracking dynamic and morphological features over 48 hours. Changes in cellular processes can be monitored by StemCellQC and predictions can be made about the quality of pluripotent stem cell colonies. This toolkit reduced the time and resources required to track multiple pluripotent stem cell colonies and eliminated handling errors and false classifications due to human bias. StemCellQC provided both user-specified and classifier-determined analysis in cases where the affected features are not intuitive or anticipated. Video analysis algorithms allowed assessment of biological phenomena using automatic detection analysis, which can aid facilities where maintaining stem cell quality and/or monitoring changes in cellular processes are essential. In the future StemCellQC can be expanded to include other features, cell types, treatments, and differentiating cells.

Zanella, F. and F. Sheikh (2016). "Patient-Specific Induced Pluripotent Stem Cell Models: Generation and Characterization of Cardiac Cells." Methods Mol Biol **1353**: 147-162.

The generation of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes has been of utmost interest for the study of cardiac development, cardiac disease modeling, and evaluation of cardiotoxic effects of novel candidate drugs. Several protocols have been developed to guide human stem cells toward the cardiogenic path. Pioneering work used serum to promote cardiogenesis; however, low cardiogenic throughputs, lack of chemical definition, and batch-to-batch variability of serum lots constituted a considerable impediment to the implementation of those protocols to large-scale cell biology. Further work focused on the manipulation of pathways that mouse genetics indicated to be fundamental in cardiac development to promote cardiac differentiation in stem cells. Although extremely elegant, those serum-free protocols involved the use of human recombinant cytokines that tend to be quite costly and which can also be variable between lots. The latest generation of cardiogenic protocols aimed for a more cost-effective and reproducible definition of the conditions driving cardiac differentiation, using small molecules to manipulate cardiogenic pathways overriding the need for cytokines. This chapter details methods based on currently available cardiac differentiation protocols for the generation and characterization of robust numbers of hiPSC-derived cardiomyocytes under chemically defined conditions.

Zanotelli, M. R., et al. (2016). "Stable engineered vascular networks from human induced pluripotent stem cell-derived endothelial cells cultured in synthetic hydrogels." Acta Biomater **35**: 32-41.

UNLABELLED: Here, we describe an in vitro strategy to model vascular morphogenesis where human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) are encapsulated in peptide-functionalized poly(ethylene glycol) (PEG) hydrogels, either on standard well plates or within a passive pumping polydimethylsiloxane (PDMS) tri-channel microfluidic device. PEG hydrogels permissive towards cellular remodeling were fabricated using thiol-ene photopolymerization to incorporate matrix metalloproteinase (MMP)-degradable crosslinks and CRGDS cell adhesion peptide. Time lapse microscopy, immunofluorescence imaging, and RNA sequencing (RNA-Seq) demonstrated that iPSC-ECs formed vascular networks through mechanisms that were consistent with in vivo vasculogenesis and angiogenesis when cultured in PEG hydrogels. Migrating iPSC-ECs condensed into clusters, elongated into tubules, and formed polygonal networks through sprouting. Genes upregulated for iPSC-ECs cultured in PEG hydrogels relative to control cells on tissue culture polystyrene (TCP) surfaces included adhesion, matrix remodeling, and Notch signaling pathway genes relevant to in vivo vascular development. Vascular networks with lumens were stable for at least 14days when iPSC-ECs were encapsulated in PEG hydrogels that were polymerized within the central channel of the microfluidic device. Therefore, iPSC-ECs cultured in peptide-functionalized PEG hydrogels offer a defined platform for investigating vascular morphogenesis in vitro using both standard and microfluidic formats. STATEMENT OF SIGNIFICANCE: Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) cultured in synthetic hydrogels self-assemble into capillary networks through mechanisms consistent with in vivo vascular morphogenesis.

Zeng, J., et al. (2017). "Generation of "Off-the-Shelf" Natural Killer Cells from Peripheral Blood Cell-

Derived Induced Pluripotent Stem Cells." Stem Cell Reports **9**(6): 1796-1812.

Current donor cell-dependent strategies can only produce limited "made-to-order" therapeutic natural killer (NK) cells for limited patients. To provide unlimited "off-the-shelf" NK cells that serve many recipients, we designed and demonstrated a holistic manufacturing scheme to mass-produce NK cells from induced pluripotent stem cells (iPSCs). Starting with a highly accessible human cell source, peripheral blood cells (PBCs), we derived a good manufacturing practice-compatible iPSC source, PBC-derived iPSCs (PBC-iPSCs) for this purpose. Through our original protocol that excludes CD34+ cell enrichment and spin embryoid body formation, high-purity functional and expandable NK cells were generated from PBC-iPSCs. Above all, most of these NK cells expressed no killer cell immunoglobulin-like receptors (KIRs), which renders them unrestricted by recipients' human leukocyte antigen genotypes. Hence, we have established a practical "from blood cell to stem cells and back with less (less KIRs)" strategy to generate abundant "universal" NK cells from PBC-iPSCs for a wide range of patients.

Zhan, M., et al. (2012). "The B-MYB transcriptional network guides cell cycle progression and fate decisions to sustain self-renewal and the identity of pluripotent stem cells." PLoS One **7**(8): e42350.

Embryonic stem cells (ESCs) are pluripotent and have unlimited self-renewal capacity. Although pluripotency and differentiation have been examined extensively, the mechanisms responsible for self-renewal are poorly understood and are believed to involve an unusual cell cycle, epigenetic regulators and pluripotency-promoting transcription factors. Here we show that B-MYB, a cell cycle regulated phosphoprotein and transcription factor critical to the formation of inner cell mass, is central to the transcriptional and co-regulatory networks that sustain normal cell cycle progression and self-renewal properties of ESCs. Phenotypically, B-MYB is robustly expressed in ESCs and induced pluripotent stem cells (iPSCs), and it is present predominantly in a hypo-phosphorylated state. Knockdown of B-MYB results in functional cell cycle abnormalities that involve S, G2 and M phases, and reduced expression of critical cell cycle regulators like ccnb1 and plk1. By conducting gene expression profiling on control and B-MYB deficient cells, ChIP-chip experiments, and integrative computational analyses, we unraveled a highly complex B-MYB-mediated transcriptional network that guides ESC self-renewal. The network encompasses critical regulators of all cell cycle phases and epigenetic regulators, pluripotency transcription factors, and differentiation determinants. B-MYB along with E2F1 and c-MYC preferentially co-regulate cell cycle target genes. B-MYB also co-targets genes regulated by OCT4, SOX2 and NANOG that are significantly associated with stem cell differentiation, embryonic development, and epigenetic control. Moreover, loss of B-MYB leads to a breakdown of the transcriptional hierarchy present in ESCs. These results coupled with functional studies demonstrate that B-MYB not only controls and accelerates cell cycle progression in ESCs it contributes to fate decisions and maintenance of pluripotent stem cell identity.

Zhang, J., et al. (2012). "Regulation of cell proliferation of human induced pluripotent stem cell-derived mesenchymal stem cells via ether-a-go-go 1 (hEAG1) potassium channel." Am J Physiol Cell Physiol **303**(2): C115-125.

The successful generation of a high yield of mesenchymal stem cells (MSCs) from human induced pluripotent stem cells (iPSCs) may represent an unlimited cell source with superior therapeutic benefits for tissue regeneration to bone marrow (BM)-derived MSCs. We investigated whether the differential expression of ion channels in iPSC-MSCs was responsible for their higher proliferation capacity than BM-MSCs. The expression of ion channels for K(+), Na(+), Ca(2+), and Cl(-) was examined by RT-PCR. The electrophysiological properties of iPSC-MSCs and BM-MSCs were then compared by patch-clamp experiments to verify their functional roles. Significant mRNA expression of ion channel genes including KCa1.1, KCa3.1, KCNH1, Kir2.1, SCN9A, CACNA1C, and Clcn3 was observed in both human iPSC-MSCs and BM-MSCs, whereas Kir2.2 and Kir2.3 were only detected in human iPSC-MSCs. Five types of currents [big-conductance Ca(2+)-activated K(+) current (BK(Ca)), delayed rectifier K(+) current (IK(DR)), inwardly rectifying K(+) current (I(Kir)), Ca(2+)-activated K(+) current (IK(Ca)), and chloride current (I(Cl))] were found in iPSC-MSCs (83%, 47%, 11%, 5%, and 4%, respectively) but only four of them (BK(Ca), IK(DR), I(Kir), and IK(Ca)) were identified in BM-MSCs (76%, 25%, 22%, and 11%, respectively). Cell proliferation was examined with MTT or bromodeoxyuridine assay, and doubling times were 2.66 and 3.72 days for iPSC-MSCs and BM-MSCs, respectively, showing a 1.4-fold discrepancy. Blockade of IK(DR) with short hairpin RNA or human ether-a-go-go 1 (hEAG1) channel blockers, 4-AP and astemizole, significantly reduced the rate of proliferation of human iPSC-MSCs. These treatments also decreased the rate of proliferation of human BM-MSCs albeit to a lesser extent. These findings demonstrate that the hEAG1 channel plays a crucial role in controlling the proliferation rate of human iPSC-MSCs and to a lesser extent in BM-MSCs.

Zhang, J., et al. (2017). "Functional characterization of human pluripotent stem cell-derived arterial endothelial cells." Proc Natl Acad Sci U S A **114**(30): E6072-E6078.

Here, we report the derivation of arterial endothelial cells from human pluripotent stem cells that exhibit arterial-specific functions in vitro and in vivo. We combine single-cell RNA sequencing of embryonic mouse endothelial cells with an EFNB2-tdTomato/EPHB4-EGFP dual reporter human embryonic stem cell line to identify factors that regulate arterial endothelial cell specification. The resulting xeno-free protocol produces cells with gene expression profiles, oxygen consumption rates, nitric oxide production levels, shear stress responses, and TNFalpha-induced leukocyte adhesion rates characteristic of arterial endothelial cells. Arterial endothelial cells were robustly generated from multiple human embryonic and induced pluripotent stem cell lines and have potential applications for both disease modeling and regenerative medicine.

Zhang, J., et al. (2014). "Overexpression of myocardin induces partial transdifferentiation of human-induced pluripotent stem cell-derived mesenchymal stem cells into cardiomyocytes." Physiol Rep **2**(2): e00237.

Mesenchymal stem cells (MSCs) derived from human-induced pluripotent stem cells (iPSCs) show superior proliferative capacity and therapeutic potential than those derived from bone marrow (BM). Ectopic expression of myocardin further improved the therapeutic potential of BM-MSCs in a mouse model of myocardial infarction. The aim was of this study was to assess whether forced myocardin expression in iPSC-MSCs could further enhance their transdifferentiation to cardiomyocytes and improve their electrophysiological properties for cardiac regeneration. Myocardin was overexpressed in iPSC-MSCs using viral vectors (adenovirus or lentivirus). The expression of smooth muscle cell and cardiomyocyte markers, and ion channel genes was examined by reverse transcription-polymerase chain reaction (RT-PCR), immunofluorescence staining and patch clamp. The conduction velocity of the neonatal rat ventricular cardiomyocytes cocultured with iPSC-MSC monolayer was measured by multielectrode arrays recording plate. Myocardin induced the expression of alpha-MHC, GATA4, alpha-actinin, cardiac MHC, MYH11, calponin, and SM alpha-actin, but not cTnT, beta-MHC, and MLC2v in iPSC-MSCs. Overexpression of myocardin in iPSC-MSC enhanced the expression of SCN9A and CACNA1C, but reduced that of KCa3.1 and Kir2.2 in iPSC-MSCs. Moreover, BKCa, IKir, ICl, Ito and INa.TTX were detected in iPSC-MSC with myocardin overexpression; while only BKCa, IKir, ICl, IKDR, and IKCa were noted in iPSC-MSC transfected with green florescence protein. Furthermore, the conduction velocity of iPSC-MSC was significantly increased after myocardin overexpression. Overexpression of myocardin in iPSC-MSCs resulted in partial transdifferentiation into cardiomyocytes phenotype and improved the electrical conduction during integration with mature cardiomyocytes.

Zhang, J., et al. (2017). "A Genome-wide Analysis of Human Pluripotent Stem Cell-Derived Endothelial Cells in 2D or 3D Culture." Stem Cell Reports **8**(4): 907-918.

A defined protocol for efficiently deriving endothelial cells from human pluripotent stem cells was established and vascular morphogenesis was used as a model system to understand how synthetic hydrogels influence global biological function compared with common 2D and 3D culture platforms. RNA sequencing demonstrated that gene expression profiles were similar for endothelial cells and pericytes cocultured in polyethylene glycol (PEG) hydrogels or Matrigel, while monoculture comparisons identified distinct vascular signatures for each cell type. Endothelial cells cultured on tissue-culture polystyrene adopted a proliferative phenotype compared with cells cultured on or encapsulated in PEG hydrogels. The proliferative phenotype correlated to increased FAK-ERK activity, and knockdown or inhibition of ERK signaling reduced proliferation and expression for cell-cycle genes while increasing expression for "3D-like" vasculature development genes. Our results provide insight into the influence of 2D and 3D culture formats on global biological processes that regulate cell function.

Zhang, J., et al. (2018). "Generation of a human induced pluripotent stem cell line from urinary cells of a patient with primary congenital glaucoma using integration free Sendai technology." Stem Cell Res **29**: 162-165.

We have generated a human induced pluripotent stem cell (iPSC) line derived from urinary cells of a 10years old patient with primary congenital glaucoma (PCG). The cells were reprogrammed with the human OSKM transcription factors using the Sendai-virus delivery system and shown to have full differentiation potential. The line is available and registered in the human pluripotent stem cell registry as BIOi001-A.

Zhang, N., et al. (2010). "Characterization of Human Huntington's Disease Cell Model from Induced Pluripotent Stem Cells." PLoS Curr **2**: RRN1193.

Huntington's disease (HD) is a dominantly inherited neurodegenerative disease caused by a CAG

repeat expansion in the first exon of the gene Huntingtin (Htt). A dramatic pathological change in HD is the massive loss of striatal neurons as the disease progresses. A useful advance in HD would be the generation of a human-derived HD model to use for drug screening and understanding mechanisms of HD. We utilized the recently established human iPS cell line derived from HD patient fibroblasts to derive neuronal precursors and human striatal neurons. To achieve this goal, the differentiation of the HD-iPS cells into striatal fate required several steps. First, we generated nestin+/PAX6+/SOX1+/OCT4- neural stem cells (NSCs) from HD-iPS cells using the method of embryoid body formation. HD-NSCs were then subjected to a differentiation condition combining morphogens and neurotrophins to induce striatal lineage commitment. Striatal neuronal precursors/immature neurons stained with beta-III tubulin, calbindin and GABA but not DARPP-32 (dopamine- and cyclic AMP-regulated phosphoprotein, Mr = 32,000) were produced in this step. Finally, maturation and terminal differentiation of the striatal neuronal precursors/immature neurons resulted in striatal neurons expressing markers like DARPP-32. The HD-iPS cells derived striatal neurons and neuronal precursors contain the same CAG expansion as the mutation in the HD patient from whom the iPS cell line was established. Moreover, the HD-NSCs showed enhanced caspase activity upon growth factor deprivation compared to normal NSCs (from iPS or H9 NSCs). Therefore, these differentiated cells may produce a human HD cell model useful in the study of HD mechanisms and drug screening.

Zhang, R., et al. (2015). "Generation of mouse pluripotent stem cell-derived proliferating myeloid cells as an unlimited source of functional antigen-presenting cells." Cancer Immunol Res **3**(6): 668-677.

The use of dendritic cells (DC) to prime tumor-associated antigen-specific T-cell responses provides a promising approach to cancer immunotherapy. Embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) can differentiate into functional DCs, thus providing an unlimited source of DCs. However, the previously established methods of generating practical volumes of DCs from pluripotent stem cells (PSC) require a large number of PSCs at the start of the differentiation culture. In this study, we generated mouse proliferating myeloid cells (pMC) as a source of antigen-presenting cells (APC) using lentivirus-mediated transduction of the c-Myc gene into mouse PSC-derived myeloid cells. The pMCs could propagate almost indefinitely in a cytokine-dependent manner, while retaining their potential to differentiate into functional APCs. After treatment with IL4 plus GM-CSF, the pMCs showed impaired proliferation and differentiated into immature DC-like cells (pMC-DC) expressing low levels of major histocompatibility complex (MHC)-I, MHC-II, CD40, CD80, and CD86. In addition, exposure to maturation stimuli induced the production of TNFalpha and IL12p70, and enhanced the expression of MHC-II, CD40, and CD86, which is thus suggestive of typical DC maturation. Similar to bone marrow-derived DCs, they stimulated a primary mixed lymphocyte reaction. Furthermore, the in vivo transfer of pMC-DCs pulsed with H-2K(b)-restricted OVA257-264 peptide primed OVA-specific cytotoxic T cells and elicited protection in mice against challenge with OVA-expressing melanoma. Overall, myeloid cells exhibiting cytokine-dependent proliferation and DC-like differentiation may be used to address issues associated with the preparation of DCs.

Zhang, S., et al. (2014). "The influence of a spatiotemporal 3D environment on endothelial cell differentiation of human induced pluripotent stem cells." Biomaterials **35**(12): 3786-3793.

Current EC differentiation protocols are inefficient, and the phenotypes of the differentiated ECs are only briefly stable, which significantly inhibits their utility for basic science research. Here, a remarkably more efficient hiPSC-EC differentiation protocol that incorporates a three-dimensional (3D) fibrin scaffold is presented. With this protocol, up to 45% of the differentiated hiPSCs assumed an EC phenotype, and after purification, greater than 95% of the cells displayed the EC phenotype (based on CD31 expression). The hiPSC-ECs continued to display EC characteristics for 4 weeks in vitro. Gene and protein expression levels of CD31, CD144 and von Willebrand factor-8 (vWF-8) were significantly up-regulated in differentiated hiPSC-ECs. hiPSC-ECs also have biological function to up-take Dil-conjugated acetylated LDL (Dil-ac-LDL) and form tubular structures on Matrigel. Collectively, these data demonstrate that a 3D differentiation protocol can efficiently generate ECs from hiPSCs and, furthermore, the differentiated hiPSC-ECs are functional and can maintain EC fate up to 4 weeks in vitro.

Zhang, X., et al. (2013). "Pluripotent stem cell protein Sox2 confers sensitivity to LSD1 inhibition in cancer cells." Cell Rep **5**(2): 445-457.

Gene amplification of Sox2 at 3q26.33 is a common event in squamous cell carcinomas (SCCs) of the lung and esophagus, as well as several other cancers. Here, we show that the expression of LSD1/KDM1 histone demethylase is significantly elevated in Sox2-expressing lung SCCs. LSD1-specific inhibitors selectively impair the growth of Sox2-expressing lung SCCs, but not that of Sox2-negative

cells. Sox2 expression is associated with sensitivity to LSD1 inhibition in lung, breast, ovarian, and other carcinoma cells. Inactivation of LSD1 reduces Sox2 expression, promotes G1 cell-cycle arrest, and induces genes for differentiation by selectively modulating the methylation states of histone H3 at lysines 4 (H3K4) and 9 (H3K9). Reduction of Sox2 further suppresses Sox2-dependent lineage-survival oncogenic potential, elevates trimethylation of histone H3 at lysine 27 (H3K27) and enhances growth arrest and cellular differentiation. Our studies suggest that LSD1 serves as a selective epigenetic target for therapy in Sox2-expressing cancers.

Zhang, Y., et al. (2015). "Potent Paracrine Effects of human induced Pluripotent Stem Cell-derived Mesenchymal Stem Cells Attenuate Doxorubicin-induced Cardiomyopathy." Sci Rep **5**: 11235.

Transplantation of bone marrow mesenchymal stem cells (BM-MSCs) can protect cardiomyocytes against anthracycline-induced cardiomyopathy (AIC) through paracrine effects. Nonetheless the paracrine effects of human induced pluripotent stem cell-derived MSCs (iPSC-MSCs) on AIC are poorly understood. In vitro studies reveal that doxorubicin (Dox)-induced reactive oxidative stress (ROS) generation and cell apoptosis in neonatal rat cardiomyocytes (NRCMs) are significantly reduced when treated with conditioned medium harvested from BM-MSCs (BM-MSCs-CdM) or iPSC-MSCs (iPSC-MSCs-CdM). Compared with BM-MSCs-CdM, NRCMs treated with iPSC-MSCs-CdM exhibit significantly less ROS and cell apoptosis in a dose-dependent manner. Transplantation of BM-MSCs-CdM or iPSC-MSCs-CdM into mice with AIC remarkably attenuated left ventricular (LV) dysfunction and dilatation. Compared with BM-MSCs-CdM, iPSC-MSCs-CdM treatment showed better alleviation of heart failure, less cardiomyocyte apoptosis and fibrosis. Analysis of common and distinct cytokines revealed that macrophage migration inhibitory factor (MIF) and growth differentiation factor-15 (GDF-15) were uniquely overpresented in iPSC-MSC-CdM. Immunodepletion of MIF and GDF-15 in iPSC-MSCs-CdM dramatically decreased cardioprotection. Injection of GDF-15/MIF cytokines could partially reverse Dox-induced heart dysfunction. We suggest that the potent paracrine effects of iPSC-MSCs provide novel "cell-free" therapeutic cardioprotection against AIC, and that MIF and GDF-15 in iPSC-MSCs-CdM are critical for these enhanced cardioprotective effects.

Zhao, C. and M. Ikeya (2018). "Generation and Applications of Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells." Stem Cells Int **2018**: 9601623.

Mesenchymal stem cells (MSCs) are adult stem cells with fibroblast-like morphology and isolated from the bone marrow via plastic adhesion. Their multipotency and immunoregulatory properties make MSCs possible therapeutic agents, and an increasing number of publications and clinical trials have highlighted their potential in regenerative medicine. However, the finite proliferative capacity of MSCs limits their scalability and global dissemination as a standardized therapeutic product. Furthermore, adult tissue provenance could constrain accessibility, impinge on cellular potency, and incur greater exposure to disease-causing pathogens based on the donor. These issues could be circumvented by the derivation of MSCs from pluripotent stem cells. In this paper, we review methods that induce and characterize MSCs derived from induced pluripotent stem cells (iPSCs) and introduce MSC applications to disease modeling, pathogenic mechanisms, and drug discovery. We also discuss the potential applications of MSCs in regenerative medicine including cell-based therapies and issues that should be overcome before iPSC-derived MSC therapy will be applied in the clinic.

Zhao, L., et al. (2017). "Characterization of the single-cell derived bovine induced pluripotent stem cells." Tissue Cell **49**(5): 521-527.

Single-cell derived bovine induced pluripotent stem cells (iPSCs) were generated by the introduction of piggyBac transposons with CAG promoting transcription factors (Oct3/4, Sox2, Klf4 and cMyc). In the study, the bovine iPSCs colony from single cell could passage more than 50 passages after enzymatic dissociation into single cells. These bovine iPSCs cells kept the normal karyotype and displayed dome shaped clones similar to mouse embryonic stem cells. They showed pluripotency in many ways, including their expression of pluripotency markers, such as OCT3/4, NANOG, SOX2, SSEA1, SSEA4, and AP in immunofluorescence assay, Oct4, Nanog, Sox2, Klf4 and cMyc in RT-PCR. Additionally, single-cell derived bovine iPSCs formed embryoid bodies and teratomas that all subsequently gave rise to differentiated cells from all three embryonic germ layers. The results showed that our reprogramming method could obtain high efficiency single-cell cloning bovine iPSCs, and the efficiency of single cell cloning is 40%.

Zhao, M. T., et al. (2017). "Cell Type-Specific Chromatin Signatures Underline Regulatory DNA Elements in Human Induced Pluripotent Stem Cells and Somatic Cells." Circ Res **121**(11): 1237-1250.

RATIONALE: Regulatory DNA elements in the human genome play important roles in determining the transcriptional abundance and spatiotemporal gene expression during embryonic heart development and

somatic cell reprogramming. It is not well known how chromatin marks in regulatory DNA elements are modulated to establish cell type-specific gene expression in the human heart. OBJECTIVE: We aimed to decipher the cell type-specific epigenetic signatures in regulatory DNA elements and how they modulate heart-specific gene expression. METHODS AND RESULTS: We profiled genome-wide transcriptional activity and a variety of epigenetic marks in the regulatory DNA elements using massive RNA-seq (n=12) and ChIP-seq (chromatin immunoprecipitation combined with high-throughput sequencing; n=84) in human endothelial cells (CD31(+)CD144(+)), cardiac progenitor cells (Sca-1(+)), fibroblasts (DDR2(+)), and their respective induced pluripotent stem cells. We uncovered 2 classes of regulatory DNA elements: class I was identified with ubiquitous enhancer (H3K4me1) and promoter (H3K4me3) marks in all cell types, whereas class II was enriched with H3K4me1 and H3K4me3 in a cell type-specific manner. Both class I and class II regulatory elements exhibited stimulatory roles in nearby gene expression in a given cell type. However, class I promoters displayed more dominant regulatory effects on transcriptional abundance regardless of distal enhancers. Transcription factor network analysis indicated that human induced pluripotent stem cells and somatic cells from the heart selected their preferential regulatory elements to maintain cell type-specific gene expression. In addition, we validated the function of these enhancer elements in transgenic mouse embryos and human cells and identified a few enhancers that could possibly regulate the cardiac-specific gene expression. CONCLUSIONS: Given that a large number of genetic variants associated with human diseases are located in regulatory DNA elements, our study provides valuable resources for deciphering the epigenetic modulation of regulatory DNA elements that fine-tune spatiotemporal gene expression in human cardiac development and diseases.

Zhao, Q., et al. (2012). "Safeguarding pluripotent stem cells for cell therapy with a non-viral, non-integrating episomal suicide construct." Biomaterials **33**(29): 7261-7271.

Pluripotent stem cells provide an unlimited cell source for cell therapy. However, residual pluripotent stem cells after differentiation can form tumors. Modifying stem cells with suicide constructs through integrating plasmid DNA and viral vectors has been attempted to specifically eliminate residual pluripotent stem cells after differentiation. However, integration of foreign DNA has the potential of insertional mutagenesis, position effects and silencing. Scaffold/matrix attachment region (S/MAR)-based plasmid DNA can be maintained extra-chromosomally, offering a safer alternative to integrating vectors for this purpose. Here, we report the design of an S/MAR-based suicide construct capable of episomal maintenance and specifically killing pluripotent stem cells but not differentiated cells in the presence of ganciclovir. Treating cells differentiated from episomal suicide construct-modified stem cells with ganciclovir reduces the tumor formation risk after cell transplantation. Tumors formed by such modified pluripotent stem cells could be inhibited by ganciclovir administration. This episomal suicide construct enables negative selection of residual pluripotent stem cells in vitro and control of tumors formed from residual pluripotent stem cells in vivo.

Zhao, Q., et al. (2017). "Cardiotoxicity evaluation using human embryonic stem cells and induced pluripotent stem cell-derived cardiomyocytes." Stem Cell Res Ther **8**(1): 54.

BACKGROUND: Cardiotoxicity remains an important concern in drug discovery. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have become an attractive platform to evaluate cardiotoxicity. However, the consistency between human embryonic stem cell-derived cardiomyocytes (hESC-CMs) and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in prediction of cardiotoxicity has yet to be elucidated. METHODS: Here we screened the toxicities of four representative drugs (E-4031, isoprenaline, quinidine, and haloperidol) using both hESC-CMs and hiPSC-CMs, combined with an impedance-based bioanalytical method. RESULTS: It showed that both hESC-CMs and hiPSC-CMs can recapitulate cardiotoxicity and identify the effects of well-characterized compounds. CONCLUSIONS: The combined platform of hPSC-CMs and an impedance-based bioanalytical method could improve preclinical cardiotoxicity screening, holding great potential for increasing drug development accuracy.

Zhao, Y., et al. (2013). "Hydrogen sulfide augments the proliferation and survival of human induced pluripotent stem cell-derived mesenchymal stromal cells through inhibition of BKCa." Cytotherapy **15**(11): 1395-1405.

BACKGROUND: Hydrogen sulfide (H2S) is an endogenously generated gaseous transmitter known for its cytoprotective effect mediated by the PI3K-Akt signaling pathway. Human induced pluripotent stem cell (hiPSC)-derived mesenchymal stromal cells (MSCs), or hiPSC-MSCs, represent an alternative source of MSCs for autologous cell therapy. The big-conductance Ca(2+)-activated outward K(+) currents (BKCa), known to mediate cell proliferation, have been detected in >80% of hiPSC-MSCs. The present study aimed to explore the effect of H2S on survival and proliferation of hiPSC-MSCs and investigate the

mediatory role of BKCa. METHODS: Effects of H2S on proliferation and survival of hiPSC-MSCs were measured by 5-bromo-2-deoxyuridine incorporation, population doubling and cell cycle assays, and by 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide assay and 4'-6-diamidino-2-phenylindole staining, respectively. BKCa was recorded by means of the whole-cell patch-clamp technique. The expressions of KCa 1.1 (encoding BKCa) and apoptosis-related genes were measured by reverse transcriptase-polymerase chain reaction. The phosphorylation of Akt was assessed by Western blot analysis. RESULTS: Exogenously administered NaHS (an H2S donor, 50-300 mumol/L) significantly promoted proliferation of hiPSC-MSCs. NaHS prevented the hypoxia-induced apoptosis and suppressed BKCa currents without altering the expression levels of alpha- and beta-KCa 1.1. In addition, NaHS increased the phosphorylation of Akt and decreased the expression of Caspase 8 and Bax in hiPSC-MSCs. Paxilline (1 mumol/L), a BKCa blocker, showed similar effects on promoting cell proliferation and phosphorylation of Akt and suppression of apoptotic genes in hiPSC-MSCs. CONCLUSIONS: Our data confirmed that H2S arguments the proliferation and survival of hiPSC-MSCs through activation of the PI3K-Akt pathway and that such effects could be mediated through inhibition of BKCa.

Zhao, Y., et al. (2018). "In Vitro Modeling of Human Germ Cell Development Using Pluripotent Stem Cells." Stem Cell Reports **10**(2): 509-523.

Due to differences across species, the mechanisms of cell fate decisions determined in mice cannot be readily extrapolated to humans. In this study, we developed a feeder- and xeno-free culture protocol that efficiently induced human pluripotent stem cells (iPSCs) into PLZF+/GPR125+/CD90+ spermatogonium-like cells (SLCs). These SLCs were enriched with key genes in germ cell development such as MVH, DAZL, GFRalpha1, NANOS3, and DMRT1. In addition, a small fraction of SLCs went through meiosis in vitro to develop into haploid cells. We further demonstrated that this chemically defined induction protocol faithfully recapitulated the features of compromised germ cell development of PSCs with NANOS3 deficiency or iPSC lines established from patients with non-obstructive azoospermia. Taken together, we established a powerful experimental platform to investigate human germ cell development and pathology related to male infertility.

Zheng, Y. L., et al. (2015). "Mesenchymal Stem Cells Obtained from Synovial Fluid Mesenchymal Stem Cell-Derived Induced Pluripotent Stem Cells on a Matrigel Coating Exhibited Enhanced Proliferation and Differentiation Potential." PLoS One **10**(12): e0144226.

Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) serve as a promising source for cell-based therapies in regenerative medicine. However, optimal methods for transforming iPSCs into MSCs and the characteristics of iPSC-MSCs obtained from different methods remain poorly understood. In this study, we developed a one-step method for obtaining iPSC-MSCs (CD146+STRO-1+ MSCs) from human synovial fluid MSC-derived induced iPSCs (SFMSC-iPSCs). CD146-STRO-1-SFMSCs were reprogrammed into iPSCs by transduction with lentivirus-mediated Sox2, Oct-3/4, klf4, and c-Myc. SFMSC-iPSCs were maintained with mTeSR1 medium in Matrigel-coated culture plates. Single dissociated cells were obtained by digesting the SFMSC-iPSCs with trypsin. The dissociated cells were then plated into Matrigel-coated culture plate with alpha minimum essential medium supplemented with 10% fetal bovine serum, 1x Glutamax, and the ROCK inhibitor Y-27632. Cells were then passaged in standard cell culture plates with alpha minimum essential medium supplemented with 10% fetal bovine serum and 1x Glutamax. After passaging in vitro, the cells showed a homogenous spindle-shape similar to their ancestor cells (SFMSCs), but with more robust proliferative activity. Flow cytometric analysis revealed typical MSC surface markers, including expression of CD73, CD90, CD105, and CD44 and lack of CD45, CD34, CD11b, CD19, and HLA-DR. However, these cells were positive for CD146 and stro-1, which the ancestor cells were not. Moreover, the cells could also be induced to differentiate in osteogenic, chondrogenic, and adipogenic lineages in vitro. The differentiation potential was improved compared with the ancestor cells in vitro. The cells were not found to exhibit oncogenicity in vivo. Therefore, the method presented herein facilitated the generation of STRO-1+CD146+ MSCs from SFMSC-iPSCs exhibiting enhanced proliferation and differentiation potential.

Zhu, D., et al. (2014). "Induced pluripotent stem cell-derived neural stem cells transduced with baculovirus encoding CD40 ligand for immunogene therapy in mouse models of breast cancer." Hum Gene Ther **25**(8): 747-758.

The interaction between CD40 ligand (CD40L) and CD40 can directly inhibit growth of CD40-positive carcinoma cells and may indirectly inhibit tumor growth through coordination of immune responses. Many efforts in CD40L cancer gene therapy have been focused on direct CD40L gene transfer into malignant target cells. This in vivo gene therapy approach relies on high-efficiency gene transfer and could be

technically challenging for the treatment of certain cancers, especially multisite metastases. We report herein an alternative means of using the tumor-homing property of neural stem cells (NSCs) to deliver CD40L molecules into tumor tissues. NSCs were derived from human induced pluripotent stem cells, transduced in vitro with a baculoviral vector encoding CD40L, and intravenously injected into immunocompetent mice with orthotopic and metastatic breast cancers. Through a bystander mechanism of intercellular transfer of CD40L from the donor NSCs to tumor target cells, the treatment impeded tumor growth, leading to prolonged survival of the tumor-bearing mice. We further showed that compared with the stem cell-based gene therapy that employed a suicide gene, the CD40L immunogene therapy did not cause liver and kidney injury in the treated mice. This new approach may be particularly valuable for metastatic cancer treatments after systemic stem cell administration.

Zhu, H., et al. (2018). "Concise Review: Human Pluripotent Stem Cells to Produce Cell-Based Cancer Immunotherapy." Stem Cells **36**(2): 134-145.

Human pluripotent stem cells (PSCs) provide a promising resource to produce immune cells for adoptive cellular immunotherapy to better treat and potentially cure otherwise lethal cancers. Cytotoxic T cells and natural killer (NK) cells can now be routinely produced from human PSCs. These PSC-derived lymphocytes have phenotype and function similar to primary lymphocytes isolated from peripheral blood. PSC-derived T and NK cells have advantages compared with primary immune cells, as they can be precisely engineered to introduce improved anti-tumor activity and produced in essentially unlimited numbers. Stem Cells 2018;36:134-145.

Zhu, Q., et al. (2016). "Prospect of Human Pluripotent Stem Cell-Derived Neural Crest Stem Cells in Clinical Application." Stem Cells Int **2016**: 7695836.

Neural crest stem cells (NCSCs) represent a transient and multipotent cell population that contributes to numerous anatomical structures such as peripheral nervous system, teeth, and cornea. NCSC maldevelopment is related to various human diseases including pigmentation abnormalities, disorders affecting autonomic nervous system, and malformations of teeth, eyes, and hearts. As human pluripotent stem cells including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) can serve as an unlimited cell source to generate NCSCs, hESC/hiPSC-derived NCSCs can be a valuable tool to study the underlying mechanisms of NCSC-associated diseases, which paves the way for future therapies for these abnormalities. In addition, hESC/hiPSC-derived NCSCs with the capability of differentiating to various cell types are highly promising for clinical organ repair and regeneration. In this review, we first discuss NCSC generation methods from human pluripotent stem cells and differentiation mechanism of NCSCs. Then we focus on the clinical application potential of hESC/hiPSC-derived NCSCs on peripheral nerve injuries, corneal blindness, tooth regeneration, pathological melanogenesis, Hirschsprung disease, and cardiac repair and regeneration.

Zhu, W., et al. (2017). "Pluripotent Stem Cell Derived Cardiac Cells for Myocardial Repair." J Vis Exp(120).

Human induced pluripotent stem cells (hiPSCs) must be fully differentiated into specific cell types before administration, but conventional protocols for differentiating hiPSCs into cardiomyocytes (hiPSC-CMs), endothelial cells (hiPSC-ECs), and smooth muscle cells (SMCs) are often limited by low yield, purity, and/or poor phenotypic stability. Here, we present novel protocols for generating hiPSC-CMs, -ECs, and -SMCs that are substantially more efficient than conventional methods, as well as a method for combining cell injection with a cytokine-containing patch created over the site of administration. The patch improves both the retention of the injected cells, by sealing the needle track to prevent the cells from being squeezed out of the myocardium, and cell survival, by releasing insulin-like growth factor (IGF) over an extended period. In a swine model of myocardial ischemia-reperfusion injury, the rate of engraftment was more than two-fold greater when the cells were administered with the cytokine-containing patch comparing to the cells without patch, and treatment with both the cells and the patch, but not with the cells alone, was associated with significant improvements in cardiac function and infarct size.

Zhu, X., et al. (2018). "Generation of ZZUi008-A, a transgene-free, induced pluripotent stem cell line derived from chorionic villi cells of a fetus with Duchenne muscular dystrophy." Stem Cell Res **32**: 47-50.

Duchenne muscular dystrophy (DMD) is a common X-linked recessive disorder for which there is no present cure. In this paper, we reported the generation of ZZUi008-A, an induced pluripotent stem cell(iPSC) line derived from chorionic villus(CV) cells of a fetus with a deletion mutation in exon 33 of the dystrophin gene (DMD). The cell line was generated using feeder-free and virus-free conditions, and the established cell line retains the original DMD mutation, a normal karyotype, expresses pluripotency markers, able to differentiate into three lineages. This ZZUi008-A cell line could provide a promising tool to study this complex disease.

Zhu, Y., et al. (2017). "Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis." Stem Cell Res Ther **8**(1): 64.

BACKGROUND: Osteoarthritis (OA) is the most common joint disease worldwide. In the past decade, mesenchymal stem cells (MSCs) have been used widely for the treatment of OA. A potential mechanism of MSC-based therapies has been attributed to the paracrine secretion of trophic factors, in which exosomes may play a major role. In this study, we aimed to compare the effectiveness of exosomes secreted by synovial membrane MSCs (SMMSC-Exos) and exosomes secreted by induced pluripotent stem cell-derived MSCs (iMSC-Exos) on the treatment of OA. METHODS: Induced pluripotent stem cell-derived MSCs and synovial membrane MSCs were characterized by flow cytometry. iMSC-Exos and SMMSC-Exos were isolated using an ultrafiltration method. Tunable resistive pulse-sensing analysis, transmission electron microscopy, and western blots were used to identify exosomes. iMSC-Exos and SMMSC-Exos were injected intra-articularly in a mouse model of collagenase-induced OA and the efficacy of exosome injections was assessed by macroscopic, histological, and immunohistochemistry analysis. We also evaluated the effects of iMSC-Exos and SMMSC-Exos on proliferation and migration of human chondrocytes by cell-counting and scratch assays, respectively. RESULTS: The majority of iMSC-Exos and SMMSC-Exos were approximately 50-150 nm in diameter and expressed CD9, CD63, and TSG101. The injection of iMSC-Exos and SMMSC-Exos both attenuated OA in the mouse OA model, but iMSC-Exos had a superior therapeutic effect compared with SMMSC-Exos. Similarly, chondrocyte migration and proliferation were stimulated by both iMSC-Exos and SMMSC-Exos, with iMSC-Exos exerting a stronger effect. CONCLUSIONS: The present study demonstrated that iMSC-Exos have a greater therapeutic effect on OA than SMMSC-Exos. Because autologous iMSCs are theoretically inexhaustible, iMSC-Exos may represent a novel therapeutic approach for the treatment of OA.

Zou, C., et al. (2012). "Efficient derivation and genetic modifications of human pluripotent stem cells on engineered human feeder cell lines." Stem Cells Dev **21**(12): 2298-2311.

Derivation of pluripotent stem cells (iPSCs) induced from somatic cell types and the subsequent genetic modifications of disease-specific or patient-specific iPSCs are crucial steps in their applications for disease modeling as well as future cell and gene therapies. Conventional procedures of these processes require co-culture with primary mouse embryonic fibroblasts (MEFs) to support self-renewal and clonal growth of human iPSCs as well as embryonic stem cells (ESCs). However, the variability of MEF quality affects the efficiencies of all these steps. Furthermore, animal sourced feeders may hinder the clinical applications of human stem cells. In order to overcome these hurdles, we established immortalized human feeder cell lines by stably expressing human telomerase reverse transcriptase, Wnt3a, and drug resistance genes in adult mesenchymal stem cells. Here, we show that these immortalized human feeders support efficient derivation of virus-free, integration-free human iPSCs and long-term expansion of human iPSCs and ESCs. Moreover, the drug-resistance feature of these feeders also supports nonviral gene transfer and expression at a high efficiency, mediated by piggyBac DNA transposition. Importantly, these human feeders exhibit superior ability over MEFs in supporting homologous recombination-mediated gene targeting in human iPSCs, allowing us to efficiently target a transgene into the AAVS1 safe harbor locus in recently derived integration-free iPSCs. Our results have great implications in disease modeling and translational applications of human iPSCs, as these engineered human cell lines provide a more efficient tool for genetic modifications and a safer alternative for supporting self-renewal of human iPSCs and ESCs.

Zou, L., et al. (2016). "Angiogenic activity mediates bone repair from human pluripotent stem cell-derived osteogenic cells." Sci Rep **6**: 22868.

Human pluripotent stem cells provide a standardized resource for bone repair. However, criteria to determine which exogenous cells best heal orthopedic injuries remain poorly defined. We evaluated osteogenic progenitor cells derived from both human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs). Phenotypic and genotypic analyses demonstrated that these hESCs/hiPSCs are similar in their osteogenic differentiation efficiency and they generate osteogenic cells comparable to osteogenic cells derived from mesenchymal stromal cells (BM-MSCs). However, expression of angiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor in these osteogenic progenitor cells are markedly different, suggesting distinct pro-angiogenic potential of these stem cell derivatives. Studies to repair a femur non-union fracture demonstrate only osteogenic progenitor cells with higher pro-angiogenic potential significantly enhance bone repair in vivo. Together, these studies highlight a key role of pro-angiogenic potential of transplanted osteogenic cells for effective cell-mediated bone repair.

Zovoilis, A., et al. (2010). "Embryonic stem cell-related miRNAs are involved in differentiation of pluripotent cells originating from the germ line." Mol Hum Reprod **16**(11): 793-803.

Cells originating from the germ cell lineage retain the remarkable property under special culture conditions to give rise to cells with embryonic stem cell (ESC) properties, such as the multipotent adult germline stem cells (maGSCs) derived from adult mouse testis. To get an insight into the mechanisms that control pluripotency and differentiation in these cells, we studied how differences observed during in vitro differentiation between ESCs and maGSCs are associated with differences at the level of microRNAs (miRNAs). In this work, we provide for a first time a connection between germ cell origin of maGSCs and their specific miRNA expression profile. We found that maGSCs express higher levels of germ cell markers characteristic for primordial germ cells (PGCs) and spermatogonia compared with ESCs. Retained expression of miR-290 cluster has been previously reported in maGSCs during differentiation and it was associated with higher Oct-4 levels. Here, we show that this property is also shared by another pluripotent cell line originating from the germ line, the embryonic germ cells. In addition, we provide proof that the specific miRNA expression profile of maGSCs has an impact on their differentiation potential. Low levels of miR-302 in maGSCs during the first 10 days of leukaemia inhibitory factor deprivation are shown to be necessary for the maintenance of high levels of early germ cell markers.

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