Pluripotent Stem Cell Research Literatures

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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; pluripotent; 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, 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., et al. (2010). "Sustained factor VIII production in hemophiliac mice 1 year after engraftment with induced pluripotent stem cell-derived factor VIII producing endothelial cells." Blood Coagul Fibrinolysis **21**(5): 502-504.

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.

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.

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.

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.

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.

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. Copyright (c) 2017 John Wiley & Sons, Ltd.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Sato, M., et al. (2016). "Efficient CRISPR/Cas9-based gene correction in induced pluripotent stem cells established from fibroblasts of patients with sickle cell disease." Stem Cell Investig **3**: 78.

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. (2010). "[Establishment of pluripotent stem cell-derived dendritic cells for clinical application]." Rinsho Ketsueki **51**(11): 1668-1673.

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.

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.

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.

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.

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.

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.

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.

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.

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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