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.

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. 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.

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

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

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

Blimp1 (Prdm1), the key determinant of primordial germ cells (PGCs), plays a combinatorial role with Prdm14 during PGC specification from postimplantation epiblast cells. They together initiate epigenetic reprogramming in early germ cells toward an underlying pluripotent state, which is equivalent to embryonic stem cells (ESCs). Whereas Prdm14 alone can promote reprogramming and is important for the propagation of the pluripotent state, it is not known whether Blimp1 is similarly involved. By using a genetic approach, we demonstrate that Blimp1 is dispensable for the derivation and maintenance of ESCs and postimplantation epiblast stem cells

(epiSCs). Notably, Blimp1 is also dispensable for reprogramming epiSCs to ESCs. Thus, although Blimp1 is obligatory for PGC specification, it is not required for the reversion of epiSCs to ESCs and for their maintenance thereafter. This study suggests that reprogramming, including that of somatic cells to ESCs, may not entail an obligatory route through a Blimp1-positive PGC-like state.

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

During heart development, epicardial progenitors contribute various cardiac lineages including smooth muscle cells, cardiac fibroblasts, and endothelial cells. However, their specific contribution to the human endothelium has not yet been resolved, at least in part due to the inability to expand and maintain human primary or pluripotent stem cell (hPSC)-derived epicardial cells. Here we first generated CDH5-2A-eGFP knock-in hPSC lines and differentiated them into self-renewing WT1+ epicardial cells, which gave rise to endothelial cells upon VEGF treatment in vitro. In addition, we found that the percentage of endothelial cells correlated with WT1 expression in a WT1-2A-eGFP reporter line. The resulting endothelial cells displayed many endocardium-like endothelial cell properties, including high expression levels of endocardial-specific markers, nutrient transporters and well-organized tight junctions. These findings suggest that human epicardial progenitors may have the capacity to form endocardial endothelium during development and have implications for heart regeneration and cardiac tissue engineering.

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

The 49-member human ATP binding cassette (ABC) gene family encodes 44 membrane transporters for lipids, ions, peptides or xenobiotics, four translation factors without transport activity, as they lack transmembrane domains, and one pseudogene. To understand the roles of ABC genes in pluripotency and multipotency, we performed a sensitive qRT-PCR analysis of their expression in embryonic stem cells (hESCs), bone marrow-derived mesenchymal stem cells (hMSCs) and hESC-derived hMSCs (hES-MSCs). We confirm that hES-MSCs represent an intermediate developmental stage between hESCs and hMSCs. We observed that 44 ABCs were significantly expressed in

hESCs, 37 in hES-MSCs and 35 in hMSCs. These variations are mainly due to plasma membrane transporters with low but significant gene expression: 18 are expressed in hESCs compared with 16 in hES-MSCs and 8 in hMSCs, suggesting important roles in pluripotency. Several of these ABCs shared similar substrates but differ regarding gene regulation. ABCA13 and ABCB4, similarly to ABCB1, could be new markers to select primitive hMSCs with specific plasma membrane transporter (low) phenotypes. ABC proteins performing basal intracellular functions, including translation factors and mitochondrial heme transporters, showed the highest constant gene expression among the three populations. Peptide transporters in the endoplasmic reticulum, Golgi and lysosome were well expressed in hESCs and slightly upregulated in hMSCs, which play important roles during the development of stem cell niches in bone marrow or meningeal tissue. These results will be useful to study specific cell cycle regulation of pluripotent stem cells or ABC dysregulation in complex pathologies, such as cancers or neurological disorders.

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

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

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

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

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

Hepatotoxicity is an enormous and increasing problem for the pharmaceutical industry. Early detection of problems during the drug discovery pathway is advantageous to minimize costs and improve patient safety. However, current cellular models are sub-optimal. This review addresses the potential use of pluripotent stem cells in the generation of hepatic cell lineages. It begins by highlighting the scale of the problem faced by the pharmaceutical industry, the precise nature of drug-induced liver injury and where in the drug discovery pathway the need for additional cell models arises. Current research

is discussed, mainly for generating hepatocyte-like cells rather than other liver cell-types. In addition, an effort is made to identify where some of the major barriers remain in translating what is currently hypothesis-driven laboratory research into meaningful platform technologies for the pharmaceutical industry.

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

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

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

BACKGROUND: We previously reported that induced pluripotent stem cell-derived cardiomyocytes manifest beat rate variability (BRV) resembling heart rate variability (HRV) in the human sinoatrial node. We now hypothesized the BRV-HRV continuum originates in pacemaker cells. OBJECTIVE: To investigate whether cellular BRV is a source of HRV dynamics, we hypothesized 3 levels of interaction among different cardiomyocyte entities: (1) single pacemaker cells, (2) networks of electrically coupled pacemaker cells, and (3) the in situ sinoatrial node. METHODS: We measured BRV/HRV properties in single pacemaker cells, induced pluripotent stem cell-derived contracting embryoid bodies (EBs), and

electrocardiograms from the same individual. RESULTS: Pronounced BRV/HRV was present at all 3 levels. The coefficient of variance of interbeat intervals and Poincare plot indices SD1 and SD2 for single cells were 20 times greater than those for EBs (P <.05) and the in situ heart (the latter two were similar; P >.05). We also compared BRV magnitude among single cells, small EBs (~5-10 cells), and larger EBs (>10 cells): BRV indices progressively increased with the decrease in the cell number (P <.05). Disrupting intracellular Ca (2+) handling markedly augmented BRV magnitude, revealing a unique bimodal firing pattern, suggesting that intracellular mechanisms contribute to BRV/HRV and the fractal behavior of heart rhythm. CONCLUSION: The decreased BRV magnitude in transitioning from the single cell to the EB suggests that the HRV of in situ hearts originates from the summation and integration of multiple cell-based oscillators. Hence, complex interactions among multiple pacemaker cells and intracellular Ca (2+) handling determine HRV in humans and cardiomyocyte networks.

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

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

experimentally tractable human cellular system for studying mechanisms of chromosomal number control, which is of critical relevance to human development and disease.

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

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

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

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

enriches the EBCs for cells that undergo neural differentiation after transplantation.

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

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

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

We have examined the effects of administration of stem cell-factor (SCF) on the number and distribution of pluripotent hematopoietic stem cells (PHSC) in normal mice. Using the competitive repopulation assay we found that in vivo administration of SCF increases the absolute number of PHSC per mouse threefold. The increased numbers of PHSC are found in the peripheral blood and spleen of the SCF-treated animals. The spleen and peripheral blood stem cells completely repopulated the erythroid, myeloid, and lymphoid lineages of irradiated or W/Wv hosts, similar to bone marrow PHSC. PHSC from the peripheral blood of SCF-treated mice have a lineage marker-negative, c-kit-positive phenotype that is

indistinguishable from that of bone marrow PHSC. The increase in the absolute number of spleen PHSC is associated with efficient gene transfer to these cells without prior treatment with 5-fluorouracil. This is a US government work. There are no restrictions on its use.

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

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

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

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

karyotyping were used to verify the genomic integrity and stability of the established iPSC lines. Our results suggest that RNA-based technology eliminates the risk of genomic integrations or aberrations, an important step toward a clinical-grade cell source for regenerative medicine such as treatment of cartilage defects and osteoarthritis.

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

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

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

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

analysis to identify cell surface markers with elevated expression in this population, thereby facilitating isolation and purification of this hPSC-derived cell population.

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

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

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

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

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

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

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

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

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

The process of X chromosome inactivation (XCI) during reprogramming to produce human induced pluripotent stem cells (iPSCs), as well as during the extensive programming that occurs in human preimplantation development, is not well-understood. Indeed, studies of XCI during reprogramming to

iPSCs report cells with two active X chromosomes and/or cells with one inactive X chromosome. Here, we examine expression of the long noncoding RNA, XIST, in single cells of human embryos through the oocyte-to-embryo transition and in new mRNA reprogrammed iPSCs. We show that XIST is first expressed beginning at the 4-cell stage, coincident with the onset of embryonic genome activation in an asynchronous manner. Additionally, we report that mRNA reprogramming produces iPSCs that initially express XIST transcript; however, expression is rapidly lost with culture. Loss of XIST and H3K27me3 enrichment at the inactive X chromosome at late passage results in X chromosome expression changes. Our data may contribute to applications in disease modeling and potential translational applications of female stem cells.

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

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

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

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

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

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

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

The ataxia telangiectasia mutated (ATM) kinase is a key transducer of the cellular response to DNA double strand breaks and its deficiency causes ataxia-telangiectasia (A-T), a pleiotropic genetic disorder primarily characterized by cerebellar neuropathy, immunodeficiency and cancer predisposition. While enormous progress has been achieved in elucidating the biochemical and functional regulation of ATM in DNA damage response, and more recently in redox signalling and antioxidant defence, the factors that

make neurons in A-T extremely vulnerable remain unclear. Given also that ATM knockout mice do not recapitulate the central nervous system phenotype, a number of human neural stem cell (hNSC) model systems have been developed to provide insights into the mechanisms of neurodegeneration associated with ATM dysfunction. Here we review the hNSC systems developed by us an others to model A-T.

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.

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.

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

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease characterized by neuromuscular degeneration and the progressive loss of upper and lower motor neurons (MNs), causing weakness and paralysis. However, the underlying mechanisms of this disease are still unknown and there is no cure, or even treatment to stop or reverse its pathology. Consequently, most ALS patients die within 3 - 5 years after disease onset. While considerable progress has been made in studying animal models of ALS, they lack clinical suitability due to genetic differences. However, the recent development of induced pluripotent stem cells (iPSCs) has made it possible to

study human disease-specific neuronal and glial cells to identify disease mechanisms and develop phenotypic screens for drug discovery. iPSCs provide researchers with a model of naturally occurring pathology under the human genetic background and MNs differentiated from human iPSCs bearing ALS-associated mutations offer a powerful model to study disease pathology. This paper reviews recent methods of differentiating iPSCs into neuronal cells and suggests further applications of these iPSCs-derived cells for ALS disease modeling, drug screening, and possible cell-based therapy.

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

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

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

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

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

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

Dannenmann, B., et al. (2015). "High glutathione and glutathione peroxidase-2 levels mediate cell-type-specific DNA damage protection in human induced

pluripotent stem cells." Stem Cell Reports **4**(5): 886-898.

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

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

Pluripotent stem cells possess the unique property of differentiating into all other cell types of the human body. Further, the discovery of induced pluripotent stem cells (iPSCs) in 2006 has opened up new avenues in clinical medicine. In simple language, iPSCs are nothing but somatic cells reprogrammed genetically to exhibit pluripotent characteristics. This process utilizes retroviruses/lentiviruses/adenovirus/plasmids to incorporate candidate genes into somatic cells isolated from any part of the human body. It is also possible to develop disease-specific iPSCs which are most likely to revolutionize research in respect to the pathophysiology of most debilitating diseases, as these can be mimicked ex vivo in the laboratory. These models can also be used to study the safety and efficacy of known drugs or potential drug candidates for a particular diseased condition, limiting the need for animal studies and considerably reducing the time and money required to develop new drugs. Recently, functional neurons, cardiomyocytes, pancreatic islet cells, hepatocytes and retinal cells have been derived from human iPSCs, thus re-confirming the pluripotency and differentiation capacity of these cells. These findings further open up the possibility of using iPSCs in cell replacement therapy for various degenerative disorders. In this review we highlight the development of iPSCs by different methods, their

biological characteristics and their prospective applications in regenerative medicine and drug screening. We further discuss some practical limitations pertaining to this technology and how they can be averted for the betterment of human life.

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

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

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.

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

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

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

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

detachment from the inductive stromal feeder layer. These findings suggest that hESC may be useful in deciphering the yet enigmatic mechanisms of specificity of VZV infection and replication.

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

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

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

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

vitro into primordial germ cells, which are then cultured after xenografting reconstructed artificial ovaries. Progress in making human oocytes artificially is proving challenging. The usefulness of these artificial gametes, from assessing environmental exposure toxicity to optimising medical treatments to prevent negative off-target effects on fertility, may prove invaluable, as may basic discoveries on the fundamental mechanisms of gametogenesis.

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

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

hiPSC-NSC transplantation with tPA to attenuate stroke's adverse effects.

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

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

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

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

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

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

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

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

Fauser, A. A., et al. (1982). "Cytotoxic T-cell clones derived from pluripotent stem cells (CFU-

GEMM) of patients with Hodgkin's lymphoma." Blood **60**(6): 1317-1320.

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

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

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

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

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

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

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

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.

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

The discovery of microRNAs (miRNAs - small non-coding RNAs of approximately 22 nt) heralded a new and exciting era in biology. During this period miRNAs have gone from ignominy due to their origin mainly in 'junk DNA' to notoriety where they can be at once characterized as being all powerful (a single miRNA can target and potentially silence several hundred genes) and yet marginal (a given gene can be targeted by several miRNAs such that a given miRNA typically exerts a modest repression) [1-4]. The emerging paradox is exemplified by miRNAs that are prominently expressed in embryonic stem (ES) cells. The collective importance of miRNAs is firmly established by the fact that Dicer-/- mouse embryos die on day 7.5 due to defects in differentiation [5]. However, oppositely correlated expression that is expected of conventional repressors is increasingly being defied in multiple systems in relation to miRNA-mRNA target pairs. This is most evident in ES cells where miR-290-295 and 302 clusters the most abundant ES cell miRNAs are found to be driven by pluripotency genes Oct4, Nanog and Sox2 and also target these genes in 'incoherent feed-forward loops' [7]. Here the miRNAs are co-expressed and positively correlated with these targets that they repress suggesting that one of their primary roles is to fine tune gene expression rather than act as ON/OFF switches. On the other hand, let-7 family members that are notably low in ES cells and rapidly induced upon differentiation exhibit more conventional anti-correlated expression patterns with their targets [7-8]. In an intricately designed auto-regulatory loop, LIN28, a key 'keeper' of the pluripotent state binds and represses the processing of let-7 (a key 'keeper' of the differentiated state) [9-11]. One of the let-7 family members, let-7g targets and represses LIN28 through four 3'-UTR binding sites [12]. We propose that LIN28/let-7 pair has the potential to act as a 'toggle switch' that balances the decision to maintain pluripotency vs. differentiation. We also propose that the c-Myc/E2F driven miR17-92 cluster that together controls the G1 to S transition is fundamental for ES self-renewal and cell proliferation [13-18]. In that context it is no surprise that LIN28 and c-Myc (and therefore let-7 and miR-17-92 by association) and more recently Oct4/Sox2 regulated miR-302 has been shown to be among a handful of factors shown to be necessary and sufficient to convert differentiated cells to induced pluripotent stem (iPS) cells [19-29]. It is also no surprise that activation of miR-17-92 (OncomiRs) and down-regulation of let-7 (tumor suppressors) is a recurring theme in relation to cancers from multiple systems [30-48]. We speculate that the LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 networks are fundamental to properties of pluripotency and self-renewal associated with embryonic stem cells. We also speculate that ES cell miRNA-mRNA associations may also regulate tissue homeostasis and regeneration in the fully developed adult. Consequently, the appropriate regulation of LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 gene networks will be critical for the success of regenerative strategies that involve iPS cells. Any perturbation in key ES cell miRNA-mRNA networks during any of the above processes maybe a hallmark of (CSCs).

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

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

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

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

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

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

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

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

Cellular pluripotency is associated with expression of the homeobox transcription factor genes NANOG, SOX2, and POU5F1 (OCT3/4 protein). Some reports suggest that mesenchymal progenitor cells (MPCs) may express increased quantities of these genes, creating the possibility that MPCs are more "pluripotent" than other adult cell types. The objective of this study was to determine whether equine bone marrow-derived MPCs had gene expression or DNA methylation patterns that differed from either early fetal-derived or terminally differentiated adult cells. Specifically, this study compared DNA methylation of the NANOG and SOX2 promoter regions and concurrent gene expression of NANOG, SOX2, and POU5F1 in equine induced pluripotent stem (iPS) cells, fetal fibroblasts, fetal brain cells, adult chondrocytes, and MPCs. Results indicate that NANOG and POU5F1 were not detectable in appreciable quantities

in tissues other than the equine iPS cell lines. Equine iPS cells expressed large quantities of all three genes examined. Significantly increased quantities of SOX2 were noted in iPS cells and both fetal-derived cell types compared with adult cells. MPCs and adult chondrocytes expressed equivalent, low quantities of SOX2. Further, NANOG and SOX2 expression inversely correlated with the DNA methylation pattern in the promoter region, such that as gene expression increased, DNA methylation decreased. The equine iPS cell lines examined demonstrated DNA methylation and gene expression patterns that were consistent with pluripotency features described in other species. Results do not support previous reports that NANOG, SOX2, and POU5F1 are poised for increased activity in MPCs compared with other adult cells.

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

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

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

The periodontal ligament (PDL) plays an important role in anchoring teeth in the bone socket. Damage to the PDL, such as after severe inflammation, can be treated with a therapeutic strategy that uses stem cells derived from PDL tissue (PDLSCs), a strategy that has received intense scrutiny over the past decade. However, there is an insufficient number of PDLSCs within the PDL for treating such damage. Therefore, we sought to induce the differentiation of induced pluripotent stem (iPS) cells into PDLSCs as an initial step toward PDL therapy. To this end, we first induced iPS cells into neural crest (NC)-like cells. We then captured the p75 neurotrophic receptor-positive cells (iPS-NC cells) and cultured them on an extracellular matrix (ECM) produced by human PDL cells (iPS-NC-PDL cells). These iPS-NC-PDL cells showed reduced expression of embryonic stem cell and NC cell markers as compared with iPS and iPS-NC cells, and enrichment of mesenchymal stem cell markers. The cells also had a higher proliferative capacity, multipotency, and elevated expression of PDL-related markers than iPS-NC cells cultured on fibronectin and laminin (iPS-NC-FL cells) or ECM produced by human skin fibroblast cells (iPS-NC-SF cells). Overall, we present a culture method to produce high number of PDLSC-like cells from iPS cells as a first step toward a strategy for PDL regeneration.

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

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

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

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

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

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

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

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

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

Renal podocyte survival depends upon the dynamic regulation of a complex cell architecture that links the glomerular basement membrane to integrins, ion channels, and receptors. Alport syndrome is a heritable chronic kidney disease where mutations in alpha3, alpha4, or alpha5 collagen genes promote podocyte death. In rodent models of renal failure, activation of the calcium-sensing receptor (CaSR) can protect podocytes from stress-related death. In this study, we assessed CaSR function in podocyte-like cells derived from induced-pluripotent stem cells from two patients with Alport Syndrome (AS1 & AS2) and a renal disease free individual [normal human mesangial cell (NHMC)], as well as a human immortalized podocyte-like (HIP) cell line. Extracellular calcium elicited concentration-dependent elevations of intracellular calcium in all podocyte-like

cells. NHMC and HIP, but not AS1 or AS2 podocyte-like cells, also showed acute reductions in intracellular calcium prior to elevation. In NHMC podocyte-like cells this acute reduction was blocked by the large-conductance potassium channel (KCNMA1) inhibitors iberiotoxin (10 nM) and tetraethylammonium (5 mM), as well as the focal adhesion kinase inhibitor PF562271 (N-methyl-N-(3-((2-(2-oxo-2,3-dihydro-1H-indol-5-ylamino)-5-trifluoromethyl-pyrim idin-4-ylamino)-methyl)-pyridin-2-yl)-methanesulfonamide, 10 nM). Quantitative polymerase chain reaction (qPCR) and immunolabeling showed the presence of KCNMA1 transcript and protein in all podocyte-like cells tested. Cultivation of AS1 podocytes on decellularized plates of NHMC podocyte-like cells partially restored acute reductions in intracellular calcium in response to extracellular calcium. We conclude that the AS patient-derived podocyte-like cells used in this study showed dysfunctional integrin signaling and potassium channel function, which may contribute to podocyte death seen in Alport syndrome.

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." <u>Biochem Biophys Res Commun</u> **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.

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

UNLABELLED: Recent developments in cell therapy using human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) hold great promise for treating ischemic cardiovascular tissues. However, poor post-transplantation viability largely limits the potential of stem cell therapy. Although the extracellular matrix (ECM) has become increasingly recognized as an important cell survival factor, conventional approaches primarily rely on single ECMs for in vivo co-delivery with cells, even though the endothelial basement membrane is comprised of a milieu of different ECMs. To address this limitation, we developed a combinatorial ECM microarray platform to simultaneously interrogate hundreds of micro-scale multi-component chemical compositions of ECMs on iPSC-EC response. After seeding iPSC-ECs onto ECM microarrays, we performed high-throughput analysis of the effects of combinatorial ECMs on iPSC-EC survival, endothelial phenotype, and nitric oxide production under conditions of

hypoxia (1% O2) and reduced nutrients (1% fetal bovine serum), as is present in ischemic injury sites. Using automated image acquisition and analysis, we identified combinatorial ECMs such as collagen IV+gelatin+heparan sulfate+laminin and collagen IV+fibronectin+gelatin+heparan sulfate+laminin that significantly improved cell survival, nitric oxide production, and CD31 phenotypic expression, in comparison to single-component ECMs. These results were further validated in conventional cell culture platforms and within three-dimensional scaffolds. Furthermore, this approach revealed complex ECM interactions and non-intuitive cell behavior that otherwise could not be easily determined using conventional cell culture platforms. Together these data suggested that iPSC-EC delivery within optimal combinatorial ECMs may improve their survival and function under the condition of hypoxia with reduced nutrients. STATEMENT OF SIGNIFICANCE: Human endothelial cells (ECs) derived from induced pluripotent stem cells (iPSC-ECs) are promising for treating diseases associated with reduced nutrient and oxygen supply like heart failure. However, diminished iPSC-EC survival after implantation into diseased environments limits their therapeutic potential. Since native ECs interact with numerous extracellular matrix (ECM) proteins for functional maintenance, we hypothesized that combinatorial ECMs may improve cell survival and function under conditions of reduced oxygen and nutrients. We developed a high-throughput system for simultaneous screening of iPSC-ECs cultured on multi-component ECM combinations under the condition of hypoxia and reduced serum. Using automated image acquisition and analytical algorithms, we identified combinatorial ECMs that significantly improved cell survival and function, in comparison to single ECMs. Furthermore, this approach revealed complex ECM interactions and non-intuitive cell behavior that otherwise could not be easily determined.

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

Pluripotent stem cells display significant heterogeneity in gene expression, but whether this diversity is an inherent feature of the pluripotent state remains unknown. Single-cell gene expression analysis in cell subsets defined by surface antigen expression revealed that human embryonic stem cell cultures exist as a continuum of cell states, even under defined conditions that drive self-renewal. The majority of the population expressed canonical pluripotency transcription factors and could differentiate into derivatives of all three germ layers. A

minority subpopulation of cells displayed high self-renewal capacity, consistently high transcripts for all pluripotency-related genes studied, and no lineage priming. This subpopulation was characterized by its expression of a particular set of intercellular signaling molecules whose genes shared common regulatory features. Our data support a model of an inherently metastable self-renewing population that gives rise to a continuum of intermediate pluripotent states, which ultimately become primed for lineage specification.

Hoveizi, E., et al. (2015). "In vitro comparative survey of cell adhesion and proliferation of human induced pluripotent stem cells on surfaces of polymeric electrospun nanofibrous and solution-cast film scaffolds." J Biomed Mater Res A **103**(9): 2952-2958.

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

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

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

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

Patient-specific induced pluripotent stem cells (iPSCs) have the potential to be useful in the treatment of human diseases. While prior studies have reported multiple methods to generate iPSCs, DNA methylation continues to limit the totipotency and reprogramming efficiency of iPSCs. Here, we first show the competency of embryonic germ cells (EGCs) as a reprogramming catalyst capable of effectively promoting reprogramming induced by four defined factors, including Oct4, Sox2, Klf4 and c-Myc. Combining EGC extracts with these four factors resulted in formation of more embryonic stem cell-like colonies than did factors alone. Notably, expression of imprinted genes was higher with combined induction than with factors alone. Moreover, iPSCs derived from

the combined inductors tended to have more global hypomethylation. Our research not only provides evidence that EGC extracts could activate DNA demethylation and reprogram imprinted genes, but also establishes a new way to enhance reprogramming of iPSCs, which remains a critical safety concern for potential use of iPSCs in regenerative medicine.

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

Human induced pluripotent stem cells (iPSCs) can be derived from various types of somatic cells by transient overexpression of 4 Yamanaka factors (OCT4, SOX2, C-MYC, and KLF4). Patient-specific iPSC derivatives (e.g., neuronal, cardiac, hepatic, muscular, and endothelial cells [ECs]) hold great promise in drug discovery and regenerative medicine. In this study, we aimed to evaluate whether the cellular origin can affect the differentiation, in vivo behavior, and single-cell gene expression signatures of human iPSC-derived ECs. We derived human iPSCs from 3 types of somatic cells of the same individuals: fibroblasts (FB-iPSCs), ECs (EC-iPSCs), and cardiac progenitor cells (CPC-iPSCs). We then differentiated them into ECs by sequential administration of Activin, BMP4, bFGF, and VEGF. EC-iPSCs at early passage (10 < P < 20) showed higher EC differentiation propensity and gene expression of EC-specific markers (PECAM1 and NOS3) than FB-iPSCs and CPC-iPSCs. In vivo transplanted EC-iPSC-ECs were recovered with a higher percentage of CD31(+) population and expressed higher EC-specific gene expression markers (PECAM1, KDR, and ICAM) as revealed by microfluidic single-cell quantitative PCR (qPCR). In vitro EC-iPSC-ECs maintained a higher CD31(+) population than FB-iPSC-ECs and CPC-iPSC-ECs with long-term culturing and passaging. These results indicate that cellular origin may influence lineage differentiation propensity of human iPSCs; hence, the somatic memory carried by early passage iPSCs should be carefully considered before clinical translation.

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

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

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

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

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

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

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

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

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

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

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

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

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

The use of transgenic markers in pluripotent stem cells allows the facile isolation of transient cell populations that appear at certain phases of embryonic development. Here, we describe a procedure for

deriving cardiac progenitors from mouse pluripotent stem cells carrying a GFP reporter under the control of an Nkx2.5 enhancer sequence. The cells are propagated under standard conditions and are differentiated using the hanging-droplet method with medium optimized for commitment to the cardiac lineage. Cardiac progenitors are isolated from the differentiation culture using fluorescence-activated cell sorting (FACS) and can be cultured further for functional characterization and experimentation. The protocols described here can be applied to both embryonic and induced pluripotent stem cells and can easily be adapted to transgenic lines carrying other cardiac cell lineage reporters.

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

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

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

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

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

Human pluripotent stem cells are a potentially powerful cellular resource for application in regenerative medicine. Because such applications require large numbers of human pluripotent stem cell-derived cells, a scalable culture system of human pluripotent stem cell needs to be developed. Several suspension culture systems for human pluripotent stem cell expansion exist; however, it is difficult to control the thickness of cell aggregations in these systems, leading to increased cell death likely caused by limited diffusion of gases and nutrients into the aggregations. Here, we describe a scalable culture system using the cell fiber technology for the expansion of human induced pluripotent stem (iPS) cells. The cells were encapsulated and cultured within the core region of core-shell hydrogel microfibers, resulting in the formation of rod-shaped or fiber-shaped cell aggregations with sustained thickness and high

viability. By encapsulating the cells with type I collagen, we demonstrated a long-term culture of the cells by serial passaging at a high expansion rate (14-fold in four days) while retaining its pluripotency. Therefore, our culture system could be used for large-scale expansion of human pluripotent stem cells for use in regenerative medicine.

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

OBJECTIVES: Compared with using autologous tissue, the use of artificial materials in the regeneration of tracheal defects is minimally invasive. However, this technique requires early epithelialization on the inner side of the artificial trachea. After differentiation from induced pluripotent stem cells (iPSCs), tracheal epithelial tissues may be used to produce artificial tracheas. Herein, we aimed to demonstrate that after differentiation from fluorescent protein-labeled iPSCs, tracheal epithelial tissues survived in nude rats with tracheal defects. METHODS: Red fluorescent tdTomato protein was electroporated into mouse iPSCs to produce tdTomato-labeled iPSCs. Embryoid bodies derived from these iPSCs were then cultured in differentiation medium supplemented with growth factors, followed by culture on air-liquid interfaces for further differentiation into tracheal epithelium. The cells were implanted with artificial tracheas into nude rats with tracheal defects on day 26 of cultivation. On day 7 after implantation, the tracheas were exposed and examined histologically. RESULTS: Tracheal epithelial tissue derived from tdTomato-labeled iPSCs survived in the tracheal defects. Moreover, immunochemical analyses showed that differentiated tissues had epithelial structures similar to those of proximal tracheal tissues. CONCLUSIONS: After differentiation from iPSCs, tracheal epithelial tissues survived in rat bodies, warranting the use of iPSCs for epithelial regeneration in tracheal defects.

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

BACKGROUND: Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterised by developmental defects and tumorigenesis, such as medulloblastomas and basal cell carcinomas, caused by mutations of the patched-1 (PTCH1) gene. In this article, we seek to demonstrate a mosaicism containing double mutations in PTCH1 in an individual with NBCCS. METHODS AND RESULTS: A de novo germline mutation of PTCH1 (c.272delG) was detected in a 31-year-old woman with

NBCCS. Gene analysis of two out of four induced pluripotent stem cell (iPSC) clones established from the patient unexpectedly revealed an additional mutation, c.274delT. Deep sequencing confirmed a low-prevalence somatic mutation (5.5%-15.6% depending on the tissue) identical to the one found in iPSC clones. CONCLUSIONS: This is the first case of mosaicism unequivocally demonstrated in NBCCS. Furthermore, the mosaicism is unique in that the patient carries one normal and two mutant alleles. Because these mutations are located in close proximity, reversion error is likely to be involved in this event rather than a spontaneous mutation. In addition, this study indicates that gene analysis of iPSC clones can contribute to the detection of mosaicism containing a minor population carrying a second mutation.

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

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

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

We recently reported a robust and defined culture system for the specification of human primordial germ cell-like cells (hPGCLCs) from human pluripotent

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

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

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

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

Expanded human T cells from a Japanese healthy male were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, WT-iPSC1, was confirmed by the expressions of stem cell markers and the differentiation capability into three germ layers. WT-iPSC1 may be a useful cell resource as a normal control for the comparative study using disease-specific iPSCs.

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

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

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

Expanded human T cells from a Japanese healthy male were used to generate integration-free induced pluripotent stem cells (iPSCs) by exogenous expression of four reprogramming factors, OCT3/4, SOX2, cMYC, KLF4, using Sendai virus vector (SeVdp). The authenticity of established iPSC line, WT-iPSC4, was confirmed by the expressions of stem

cell markers and the differentiation capability into three germ layer. WT-iPSC4 may be a useful cell resource as a normal control for the comparative study using disease-specific iPSCs.

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

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

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

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

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

It is generally accepted that the difficulty in obtaining a sufficient number of functional dendritic cells (DCs) poses a serious problem in DC-based immunotherapy. Therefore, we used induced pluripotent stem (iPS) cell-derived DCs (iPSDCs) instead. If the therapeutic efficacy of iPSDCs was equivalent to that of bone marrow-derived DCs (BMDCs), then the above-mentioned problems may

be solved. In this study, we generated iPSDCs from iPS cells and compared their capacity to mature and migrate to the regional lymph nodes with that of BMDCs. We adenovirally transduced the hgp100 gene, which codes for a natural tumor antigen, into the DCs and immunized the mice with these genetically modified DCs. The cytotoxic activity of CD8(+) cytotoxic T lymphocytes (CTLs) was assayed using a 51Cr-release assay. The therapeutic efficacy of the vaccination was examined in a subcutaneous tumor model. Our results demonstrated that iPSDCs equaled BMDCs in terms of their maturation and migration capacity. Furthermore, hgp100-specific CTLs were generated in mice that were immunized with the genetically modified iPSDCs. These CTLs exhibited a high level of cytotoxicity against B16 cells, which is similar to that exhibited by CTLs generated in BMDCs immunized mice. Moreover, vaccination with genetically modified iPSDCs elicited a high level of therapeutic efficacy equaling that of vaccination with BMDCs. This study clarified experimentally that genetically modified iPSDCs are equivalent to BMDCs in terms of tumor-associated antigen-specific therapeutic antitumor immunity. This vaccination strategy may therefore be useful for future clinical application as a cancer vaccine.

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

Tissues of the small intestine are crucial to understanding drug disposition because these tissues regulate the bioavailability of drugs. However, no evaluation system is currently available for precise and comprehensive analysis of intestinal pharmacokinetics. To address this, functional intestinal epithelial cells were generated from human induced pluripotent stem (iPS) cells for use in pharmacokinetic studies. An improved intestinal differentiation method was established by screening a variety of small molecule compounds against cells during differentiation. The mRNA expression levels of intestinal markers, drug transporters, and CYP3A4 were found to increase following treatment with compounds that act as inhibitors of mitogen-activated protein kinase, DNA methyltransferase, and transforming growth factor-beta. Therefore, we inferred that these compounds enhanced differentiation into intestinal epithelial cells. The differentiated intestinal epithelial cells in the presence of these compounds possessed drug-metabolizing enzyme activities, such as those of CYPs, UDP-glucuronosyltransferase, and sulfotransferase. In addition, these cells had the ability to induce CYP3A4 in the presence of 1alpha,25-dihydroxyvitamin D3.

The differentiated intestinal epithelial cells seeded on cell culture inserts formed loose-tight junctions, similar to those in the human small intestine, rather than Caco-2 cells. The cells exhibited polarity, such as apical and basal sides. We also demonstrated that the uptake and efflux transport activities in the cells occurred via peptide transporter and breast cancer resistance protein, respectively. Taken together, it was suggested that human iPS cell-derived intestinal epithelial cells are pharmacokinetically functional, and represent a promising model system for pharmacokinetic studies of drug candidates.

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

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

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, 1×10^{-4} M 2-mercaptoethanol, 1 mmol/L NEAA, 2mM L-glutamine and 1% penicillin-streptomycin. iPS cells were differentiated to HLCs by sequential culture using a

four step differentiation protocol: (I) Generation of embryoid bodies (EBs) in suspension culture; (II) Induction of definitive endoderm (DE) from 2 days old EBs by growth in human activin-A (100 ng/ml) and basic fibroblasts growth factor (bFGF2) (100 ng/ml) on matrigel coated plates; (III) Induction of hepatic progenitors by co-culture with non-parenchymal human hepatic stellate cell line (TWNT-1); and (IV) Maturation by culture in dexamethasone. Characterization was performed by RT-PCR and functional assays. RESULTS: The generated HLCs showed microscopically morphological phenotype of human hepatocytes, expressed liver-specific genes (ASGPR, Albumin, AFP, Sox17, Fox A2), secreted human liver-specific proteins such as albumin, synthesized urea and metabolized ammonia. CONCLUSION: Functional HLCs were generated from human iPS cells, which could be used for autologus hepatocyte transplantation for liver failure and as in vitro model for determining the metabolic and toxicological properties of drug compounds.

Jeong, H. C., et al. (2017). "Technical approaches to induce selective cell death of pluripotent stem cells." Cell Mol Life Sci **74**(14): 2601-2611.
Despite the recent promising results of clinical trials using human pluripotent stem cell (hPSC)-based cell therapies for age-related macular degeneration (AMD), the risk of teratoma formation resulting from residual undifferentiated hPSCs remains a serious and critical hurdle for broader clinical implementation. To mitigate the tumorigenic risk of hPSC-based cell therapy, a variety of approaches have been examined to ablate the undifferentiated hPSCs based on the unique molecular properties of hPSCs. In the present review, we offer a brief overview of recent attempts at selective elimination of undifferentiated hPSCs to decrease the risk of teratoma formation in hPSC-based cell therapy.

Jerebtsova, M., et al. (2012). "HIV-1 Resistant CDK2-Knockdown Macrophage-Like Cells Generated from 293T Cell-Derived Human Induced Pluripotent Stem Cells." Biology (Basel) **1**(2): 175-195.
A major challenge in studies of human diseases involving macrophages is low yield and heterogeneity of the primary cells and limited ability of these cells for transfections and genetic manipulations. To address this issue, we developed a simple and efficient three steps method for somatic 293T cells reprogramming into monocytes and macrophage-like cells. First, 293T cells were reprogrammed into induced pluripotent stem cells (iPSCs) through a transfection-mediated expression of two factors, Oct-4 and Sox2, resulting in a high yield of iPSC. Second, the obtained iPSC were differentiated into monocytes

using IL-3 and M-CSF treatment. And third, monocytes were differentiated into macrophage-like cells in the presence of M-CSF. As an example, we developed HIV-1-resistant macrophage-like cells from 293T cells with knockdown of CDK2, a factor critical for HIV-1 transcription. Our study provides a proof-of-principle approach that can be used to study the role of host cell factors in HIV-1 infection of human macrophages.

Ji, H., et al. (2017). "Application of induced pluripotent stem cells to model smooth muscle cell function in vascular diseases." Curr Opin Biomed Eng **1**: 38-44.
Vascular smooth muscle cells (SMC) play an essential role in remodeling the vasculature during disease progression. Induced pluripotent stem cells (iPSC) provide an attractive approach to obtain autologous SMC source for patient-specific disease modeling. Here we discuss the current methods to 1) derive functional SMC from iPSC, 2) model vascular diseases using SMC generated from patient-derived iPSC, and 3) modulate microenvironmental cues to enhance cellular differentiation and functionality and better mimic the physiological environment. We emphasize that continuous exploration of biomaterial technologies to engineer a more SMC-specific microenvironment will provide further insight on complex vascular diseases.

Ji, Z. and B. Tian (2009). "Reprogramming of 3' untranslated regions of mRNAs by alternative polyadenylation in generation of pluripotent stem cells from different cell types." PLoS One **4**(12): e8419.
BACKGROUND: The 3' untranslated regions (3'UTRs) of mRNAs contain cis elements involved in post-transcriptional regulation of gene expression. Over half of all mammalian genes contain multiple polyadenylation sites that lead to different 3'UTRs for a gene. Studies have shown that the alternative polyadenylation (APA) pattern varies across tissues, and is dynamically regulated in proliferating or differentiating cells. Generation of induced pluripotent stem (iPS) cells, in which differentiated cells are reprogrammed to an embryonic stem (ES) cell-like state, has been intensively studied in recent years. However, it is not known how 3'UTRs are regulated during cell reprogramming. METHODS/MAIN FINDINGS: Using a computational method that robustly examines APA across DNA microarray data sets, we analyzed 3'UTR dynamics in generation of iPS cells from different cell types. We found that 3'UTRs shorten during reprogramming of somatic cells, the extent of which depends on the type of source cell. By contrast, reprogramming of spermatogonial cells involves 3'UTR lengthening. The

alternative polyadenylation sites that are highly responsive to change of cell state in generation of iPS cells are also highly regulated during embryonic development in opposite directions. Compared with other sites, they are more conserved, can lead to longer alternative 3'UTRs, and are associated with more cis elements for polyadenylation. Consistently, reprogramming of somatic cells and germ cells involves significant upregulation and downregulation, respectively, of mRNAs encoding polyadenylation factors, and RNA processing is one of the most significantly regulated biological processes during cell reprogramming. Furthermore, genes containing target sites of ES cell-specific microRNAs (miRNAs) in different portions of 3'UTR are distinctively regulated during cell reprogramming, suggesting impact of APA on miRNA targeting. CONCLUSIONS/SIGNIFICANCE: Taken together, these findings indicate that reprogramming of 3'UTRs by APA, which result from regulation of both general polyadenylation activity and cell type-specific factors and can reset post-transcriptional gene regulatory programs in the cell, is an integral part of iPS cell generation, and the APA pattern can be a good biomarker for cell type and state, useful for sample classification. The results also suggest that perturbation of the mRNA polyadenylation machinery or RNA processing activity may facilitate generation of iPS cells.

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

Endothelial cells (ECs) that are differentiated from induced pluripotent stem cells (iPSCs) can be used in establishing disease models for personalized drug discovery or developing patient-specific vascularized tissues or organoids. However, a number of technical challenges are often associated with iPSC-ECs in culture, including instability of the endothelial phenotype and limited cell proliferative capacity over time. Early senescence is believed to be the primary mechanism underlying these limitations. Sirtuin1 (SIRT1) is an NAD (+)-dependent deacetylase involved in the regulation of cell senescence, redox state, and inflammatory status. We hypothesize that overexpression of the SIRT1 gene in iPSC-ECs will maintain EC phenotype, function, and proliferative capacity by overcoming early cell senescence. SIRT1 gene was packaged into a lentiviral vector (LV-SIRT1) and transduced into iPSC-ECs at passage 4. Beginning with passage 5, iPSC-ECs exhibited a fibroblast-like morphology, whereas iPSC-ECs overexpressing SIRT1 maintained EC cobblestone morphology. SIRT1 overexpressing iPSC-ECs also exhibited a

higher percentage of canonical markers of endothelia (LV-SIRT1 61.8% CD31(+) vs. LV-empty 31.7% CD31(+), P < 0.001; LV-SIRT1 46.3% CD144(+) vs. LV-empty 20.5% CD144(+), P < 0.02), with a higher nitric oxide synthesis, lower beta-galactosidase production indicating decreased senescence (3.4% for LV-SIRT1 vs. 38.6% for LV-empty, P < 0.001), enhanced angiogenesis, increased deacetylation activity, and higher proliferation rate. SIRT1 overexpressing iPSC-ECs continued to proliferate through passage 9 with high purity of EC-like characteristics, while iPSC-ECs without SIRT1 overexpression became senescent after passage 5. Taken together, SIRT1 overexpression in iPSC-ECs maintains EC phenotype, improves EC function, and extends cell lifespan, overcoming critical hurdles associated with the use of iPSC-ECs in translational research.

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

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

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

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are defined as pluripotent in view of their self-renewal ability and potential to differentiate to cells of all three germ layers. Recent studies have indicated that microRNAs (miRNAs) play an important role in the maintenance of pluripotency and cell cycle regulation. We used a microarray based approach to identify miRNAs that were enriched in hESCs when compared to differentiated cells and at the same time showed significant expression changes between different phases of cell cycle. We identified 34 candidate miRNAs and performed functional studies on one of these, miR-1305, which showed the highest expression change during cell cycle transition. Overexpression of miR-1305 induced differentiation of pluripotent stem cells, increased cell apoptosis and sped up G1/S transition, while its downregulation facilitated the maintenance of pluripotency and increased cell survival. Using target prediction software and luciferase based reporter assays we identified POLR3G as a downstream target by which miR-1305 regulates the fine balance between maintenance of pluripotency and onset of differentiation. Overexpression of POLR3G rescued pluripotent stem cell differentiation induced by miR-1305 overexpression. In contrast, knock-down of POLR3G expression abolished the miR-1305-knockdown mediated enhancement of pluripotency, thus validating its role as miR-1305 target in human pluripotent stem cells. Together our data point to an important role for miR-1305 as a novel regulator of pluripotency, cell survival and cell cycle and uncovers new mechanisms and networks by which these processes are intertwined in human pluripotent stem cells. Stem Cells 2016;34:2306-2317.

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

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

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

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

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

Hepatocytes derived from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) could provide a defined and renewable source of human cells relevant for cell replacement therapies and toxicology studies. However, before patient-specific iPSCs can be routinely used for these purposes, there is a dire need to critically compare these cells to the golden standard--hESCs. In this study, we aimed at investigating the differences and similarities at the transcriptional level between hepatocyte-like cells (HLCs) derived from both hESCs and iPSCs. Two independent protocols for deriving HLCs from hESCs and iPSCs were adopted and further characterization included immunocytochemistry, real-time (RT)-polymerase chain reaction, and in vitro functional assays. Comparative microarray-based gene expression profiling was conducted on these cells and compared to the transcriptomes of human fetal liver and adult liver progenitors. HLCs derived from hESCs and human iPSCs showed significant functional similarities, similar expression of genes important for liver physiology and common pathways. However, specific differences between the 2 cell types could be

observed. For example, among the cytochrome P450 gene family, CYP19A1, CYP1A1, and CYP11A1 were enriched in hESC-derived HLCs, and CYP46A1 and CYP26A1 in iPSC-derived HLCs. HLCs derived from hESCs and human iPSCs exhibited broad similarities but as well meaningful differences. We identified common upregulated transcription factors, which might serve as a source for generating a cocktail of factors able to directly transdifferentiate somatic cells into HLCs. The findings may be vital to the refinement of protocols for the efficient derivation of functional patient-specific HLCs for regenerative and toxicology studies.

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

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

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

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

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

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

overcome before iPSC-derived MSC therapy can be used widely in the clinic.

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

PURPOSE: Aquaporins (AQPs), a family of transmembrane water channel proteins, are essential for allowing passive water transport through retinal pigmented epithelial (RPE) cells. Even though human native RPE cells and immortalized human RPEs have been shown to express AQPs, the expression of AQPs during the differentiation in stem cell-derived RPE remains to be elucidated. METHODS: In human embryonic (hESCs) and induced pluripotent stem cells (hiPSCs)-derived RPE cells, the expression of several AQPs was determined by quantitative real-time PCR and the localization of AQP1 was assessed with confocal microscopy. The functionality of AQP water channels was determined by cell volume assay in hESC-derived RPE cells. RESULTS: AQP1, AQP3, AQP4, AQP5, AQP6, AQP7, AQP10, AQP11, and AQP12 were expressed in hESC- and hiPSC-derived RPE cells. Furthermore, the expression of AQP1 and AQP11 genes were significantly upregulated during the maturation of both hESC and iPSC into RPE. Confocal microscopy shows the expression of AQP1 at the apical plasma membrane of polarized cobblestone hESC- and hiPSC-derived RPE cells. Lastly, aquaporin inhibitors significantly reduced AQP functionality in hESC-RPE cells. CONCLUSIONS: hESC-RPE and hiPSC-RPE cells express several AQP genes, which are functional in mature hESC-derived RPE cells. The localization of AQP1 on the apical plasma membrane in mature RPE cells derived from both hESC and hiPSC suggests its functionality. These data propose that hESC- and hiPSC-derived RPE cells, grown and differentiated under serum-free conditions, resemble their native counterpart in the human eye.

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

AIMS: Mouse and human fibroblasts can be directly reprogrammed to pluripotency by the ectopic expression of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) to yield induced pluripotent stem (iPS) cells. iPS cells can be generated even without the expression of c-Myc. The present study examined patterns of differentiation of mouse iPS cells into cardiomyocytes in three different cell lines reprogrammed by three or four factors. METHODS AND RESULTS: During the induction of differentiation on feeder-free gelatinized dishes, genes

involved in cardiogenesis were expressed as in embryonic stem cells and myogenic contraction occurred in two iPS cell lines. However, in one iPS cell line (20D17) generated by four factors, the expression of cardiac-specific genes and the beating activity were extremely low. Treating iPS cells with trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, increased Nkx2.5 expression in all iPS cell lines. While the basal Nkx2.5 expression was very low in 20D17, the TSA-induced increase was the greatest. TSA also induced the expression of contractile proteins in 20D17. Furthermore, we demonstrated the increased mRNA level of Oct3/4 and nuclear protein level of HDAC4 in 20D17 compared with the other two iPS cell lines. DNA microarray analysis identified genes whose expression is up- or down-regulated in 20D17. CONCLUSIONS: Mouse iPS cells differentiate into cardiomyocytes in a cell line-dependent manner. TSA induces myocardial differentiation in mouse iPS cells and might be useful to overcome cell line variation in the differentiation efficiency.

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

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

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

Myelomeningocele (MMC) is a congenital disease without genetic abnormalities. Neurological symptoms are irreversibly impaired after birth, and no effective treatment has been reported to date. Only surgical repairs have been reported so far. In this study, we performed antenatal treatment of MMC with an

artificial skin using induced pluripotent stem cells (iPSCs) generated from a patient with Down syndrome (AF-T21-iPSCs) and twin-twin transfusion syndrome (AF-TTTS-iPSCs) to a rat model. We manufactured three-dimensional skin with epidermis generated from keratinocytes derived from AF-T21-iPSCs and AF-TTTS-iPSCs and dermis of human fibroblasts and collagen type I. For generation of epidermis, we developed a protocol using Y-27632 and epidermal growth factor. The artificial skin was successfully covered over MMC defect sites during pregnancy, implying a possible antenatal surgical treatment with iPSC technology.

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

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

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

The potential for tumor formation from transplanted human induced pluripotent stem cell (hiPSC) derivatives represents a high risk in their application to regenerative medicine. We examined the genetic origin and characteristics of tumors, that were formed when 13 hiPSC lines, established by ourselves,

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

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

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

transformation and de-differentiation suggest that tumorigenic risks of differentiated cells arise when they are exposed to suitable niches in vivo. Thus, removal of only the undifferentiated cells from iPSC-derivatives before transplantation does not solve the problem. Elucidation of mechanisms of reversibility and control of epigenetic changes is discussed as a safety bottleneck for hiPSC therapy.

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

Induced pluripotent stem cells (iPSCs) have radically advanced the field of regenerative medicine by making possible the production of patient-specific pluripotent stem cells from adult individuals. By developing iPSCs to treat HIV, there is the potential for generating a continuous supply of therapeutic cells for transplantation into HIV-infected patients. In this study, we have used human hematopoietic stem cells (HSCs) to generate anti-HIV gene expressing iPSCs for HIV gene therapy. HSCs were dedifferentiated into continuously growing iPSC lines with four reprogramming factors and a combination anti-HIV lentiviral vector containing a CCR5 short hairpin RNA (shRNA) and a human/rhesus chimeric TRIM5alpha gene. Upon directed differentiation of the anti-HIV iPSCs toward the hematopoietic lineage, a robust quantity of colony-forming CD133(+) HSCs were obtained. These cells were further differentiated into functional end-stage macrophages which displayed a normal phenotypic profile. Upon viral challenge, the anti-HIV iPSC-derived macrophages exhibited strong protection from HIV-1 infection. Here, we demonstrate the ability of iPSCs to develop into HIV-1 resistant immune cells and highlight the potential use of iPSCs for HIV gene and cellular therapies.

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

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

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

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

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

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

Kaneko, S. (2016). "In Vitro Generation of Antigen-Specific T Cells from Induced Pluripotent

Stem Cells of Antigen-Specific T Cell Origin." Methods Mol Biol **1393**: 67-73.

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

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

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

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

Understanding the steps and cues that allow hematopoietic cells to be generated during development holds great clinical as well as biological interest. Analysis of these events in mice has provided many important insights into the processes involved,

but features that might be unique to humans remain challenging to elucidate because they cannot be studied directly in vivo. Human embryonic stem or induced pluripotent stem cells offer attractive in vitro alternatives to analyze the process. Here we review recent efforts to develop defined and quantitative systems to address outstanding developmental questions against a background of what we know about the development of hematopoietic cells in the fetus and derived from mouse embryonic stem cells.

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

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

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

Given the difficulties inherent in maintaining human pluripotent stem cells (hPSCs) in a healthy state, hPSCs should be routinely characterized using several established standard criteria during expansion for research or therapeutic purposes. hPSC colony

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

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

Developmental exposure to endocrine disruptors has resulted in the increased incidence of infertility and testicular germ cell tumors (T2GCT) in young men residing in developed countries. Unlike T1GCT (infants and young children) and T3GCT (aged men), T2GCT arise from CIS/GCNIS that develops from pre-CIS. Pre-CIS represents undifferentiated, growth-arrested gonocytes that persist in fetal testes due to endocrine disruption. However, whether pre-CIS truly exist, do CIS develop into T2GCT, why no CIS in T1GCT/T3GCT, why germ cell tumors (GCT) also occur along midline at extra-gonadal sites, why T1GCT show partial erasure and T2GCT show complete erasure of genomic imprints are open questions that are awaiting answers. We propose that rather than pre-CIS, pluripotent, very small embryonic-like stem cells (VSELs) get affected by exposure to endocrine disruption. Since VSELs are developmentally equivalent to primordial germ cells (PGCs), T2GCT cells show complete erasure of genomic imprints and CIS represents growth-arrested clonally expanding stem/progenitor cells. PGCs/VSELs migrate along the midline to various organs and this explains why GCT occur along the midline, T1GCT show partial erasure of imprints as they develop from migrating PGCs. T3GCT possibly

reflects effects of aging due to compromised differentiation and expansion of pre-meiotic spermatocytes. Absent spermatogenesis in pre-pubertal and aged testes explains absence of CIS in T1GCT and T3GCT. Endocrine disruptors possibly alter epigenetic state of VSELs and thus rather than maintaining normal tissue homeostasis, VSELs undergo increased proliferation and compromised differentiation resulting in reduced sperm count, infertility and TGCT. This newly emerging understanding offers alternate premise to explain TGCT and warrants further exploration.

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

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

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

Cell surface glycans vary widely, depending on cell properties. We hypothesized that glycan expression on induced pluripotent stem cells (iPSCs) might change during cardiomyogenic differentiation toward the myocardial phenotype. N-glycans were isolated from iPSCs, iPSC-derived cardiomyocytes (iPSC-CM), and original C57BL/6 mouse myocardium (Heart). Their structures were analyzed by a mapping technique based on HPLC elution times and MALDI-TOF/MS spectra. Sixty-eight different N-glycans were isolated; the structures of 60 of these N-glycans were identified. The quantity of high-mannose type (immature) N-glycans on the iPSCs decreased with

cardiomyogenic differentiation, but did not reach the low levels observed in the heart. We observed a similar reduction in neutral N-glycans and an increase in fucosylated or sialyl N-glycans. Some structural differences were detected between iPSC-CM and Heart. No N-glycolyl neuraminic acid (NeuGc) structures were detected in iPSC-CM, whereas the heart contained numerous NeuGc structures, corresponding to the expression of cytidine monophosphate-N-acetylneuraminic acid hydroxylase. Furthermore, several glycans containing Galalpha1-6 Gal, rarely identified in the other cells, were detected in the iPSC-CM. The expression of N-glycan on murine iPSCs changed toward the myocardial phenotype during cardiomyogenic differentiation, leaving the structural differences of NeuGc content or Galalpha1-6 Gal structures. Further studies will be warranted to reveal the meaning of the difference of N-glycans between the iPSC-CM and the myocardium.

Keller, K. C., et al. (2014). "Suspension culture of pluripotent stem cells: effect of shear on stem cell fate." Crit Rev Eukaryot Gene Expr **24**(1): 1-13.
Despite significant promise, the routine usage of suspension cell culture to manufacture stem cell-derived differentiated cells has progressed slowly. Suspension culture is an innovative way of either expanding or differentiating cells and sometimes both are combined into a single bioprocess. Its advantages over static 2D culturing include a homogeneous and controllable culture environment and producing a large quantity of cells in a fraction of time. This feature makes suspension cell culture ideal for use in stem cell research and eventually ideal in the large-scale production of differentiated cells for regenerative medicine. Because of their tremendous differentiation capacities and unlimited growth properties, pluripotent stem cells (PSCs) in particular are considered potential sources for future cell-replacement therapies. Currently, expansion of PSCs is accomplished in 2D, which only permits a limited amount of cell growth per culture flask before cells need to be passaged. However, before stem cells can be applied clinically, several aspects of their expansion, such as directed growth, but also differentiation, need to be better controlled. This review will summarize recent advantages in suspension culture of PSCs, while at the same time highlighting current challenges.

Kempf, H., et al. (2016). "Bulk cell density and Wnt/TGFbeta signalling regulate mesendodermal patterning of human pluripotent stem cells." Nat Commun **7**: 13602.
In vitro differentiation of human pluripotent stem cells (hPSCs) recapitulates early aspects of human embryogenesis, but the underlying processes are poorly understood and controlled. Here we show that modulating the bulk cell density (BCD: cell number per culture volume) deterministically alters anteroposterior patterning of primitive streak (PS)-like priming. The BCD in conjunction with the chemical WNT pathway activator CHIR99021 results in distinct paracrine microenvironments codifying hPSCs towards definitive endoderm, precardiac or presomitic mesoderm within the first 24 h of differentiation, respectively. Global gene expression and secretome analysis reveals that TGFss superfamily members, antagonist of Nodal signalling LEFTY1 and CER1, are paracrine determinants restricting PS progression. These data result in a tangible model disclosing how hPSC-released factors deflect CHIR99021-induced lineage commitment over time. By demonstrating a decisive, functional role of the BCD, we show its utility as a method to control lineage-specific differentiation. Furthermore, these findings have profound consequences for inter-experimental comparability, reproducibility, bioprocess optimization and scale-up.

Kim, B. J., et al. (2017). "Modeling Group B Streptococcus and Blood-Brain Barrier Interaction by Using Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells." mSphere **2**(6).
Bacterial meningitis is a serious infection of the central nervous system (CNS) that occurs after bacteria interact with and penetrate the blood-brain barrier (BBB). The BBB is comprised of highly specialized brain microvascular endothelial cells (BMECs) that function to separate the circulation from the CNS and act as a formidable barrier for toxins and pathogens. Certain bacteria, such as Streptococcus agalactiae (group B Streptococcus [GBS]), possess the ability to interact with and penetrate the BBB to cause meningitis. Modeling bacterial interaction with the BBB in vitro has been limited to primary and immortalized BMEC culture. While useful, these cells often do not retain BBB-like properties, and human primary cells have limited availability. Recently, a human induced pluripotent stem cell (iPSC)-derived BMEC model has been established that is readily renewable and retains key BBB phenotypes. Here, we sought to evaluate whether the iPSC-derived BMECs were appropriate for modeling bacterial interaction with the BBB. Using GBS as a model meningeal pathogen, we demonstrate that wild-type GBS adhered to, invaded, and activated the iPSC-derived BMECs, while GBS mutants known to have diminished BBB interaction were attenuated in the iPSC-derived model. Furthermore, bacterial infection resulted in the disruption of tight junction components ZO-1, occludin, and claudin-5. Thus, we show for the first time that the iPSC-derived BBB model can be utilized

to study BBB interaction with a bacterial CNS pathogen. IMPORTANCE Here for the first time, human iPSC-derived BMECs were used to model bacterial interaction with the BBB. Unlike models previously used to study these interactions, iPSC-derived BMECs possess robust BBB properties, such as the expression of complex tight junctions that are key components for the investigation of bacterial effects on the BBB. Here, we demonstrated that GBS interacts with the iPSC-derived BMECs and specifically disrupts these tight junctions. Thus, using this BBB model may allow researchers to uncover novel mechanisms of BBB disruption during meningitis that are inaccessible to immortalized or primary cell models that lack substantial tight junctions.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Understanding mechanisms that govern cell fate determination of human induced pluripotent stem cells (hiPSCs) could assist in maintenance of the undifferentiated state during cell expansion. We used

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

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

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

expanding cultures of undifferentiated and pluripotent hiPSCs. We focus on the regulation of migration-dependent balance between cell-cell and cell-substrate interactions. This review offers novel insights into the regulation and processing of stem cells for research in regenerative medicine.

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

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

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

Aim: One of the parameters that greatly affects homeostasis in the body is the pH. Regarding reproductive biology, germ cells, such as oocytes or sperm, are exposed to severe changes in pH, resulting in dramatic changes in their characteristics. To date, the effect of the pH has not been investigated

regarding the reprogramming of somatic cells and the maintenance and differentiation of pluripotent stem cells. Methods: In order to investigate the effects of the pH on cell culture, the methods to produce induced pluripotent stem cells (iPSCs) and to differentiate embryonic stem cells (ESCs) into mesendoderm and neuroectoderm were performed at each medium pH from 6.6 to 7.8. Using the cells of the Oct4-GFP (green fluorescent protein) carrying mouse, the effects of pH changes were examined on the timing and colony formation at cell reprogramming and on the cell morphology and direction of the differentiation of the ESCs. Results: The colony formation rate and timing of the reprogramming of the somatic cells varied depending on the pH of the culture medium. In addition, mesendodermal differentiation of the mouse ESCs was enhanced at the high pH level of 7.8. Conclusion: These results suggest that the pH in the culture medium is one of the key factors in the induction of the reprogramming of somatic cells and in the differentiation of pluripotent stem cells.

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

Induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (iMSCs) serve as a unique source for cell therapy. We investigated whether exosomes from iMSCs promote the proliferation of human keratinocytes (HaCaT) and human dermal fibroblasts (HDFs). iPSCs were established from human Wharton's jelly MSCs and were allowed to differentiate into iMSCs. Exosomes were collected from the culture supernatant of MSCs (MSC-exo) and iMSCs (iMSC-exo), and their characteristics were investigated. Both exosome types possessed basic characteristics of exosomes and were taken up by skin cells in vitro and in vivo. A significant increase in HaCaT proliferation was observed with iMSC-exo, although both exosomes increased the viability and cell cycle progression in HaCaT and HDFs. No significant difference was observed in the closure of wound scratch and the expression of reparative genes between cells treated with the two exosome types. Both exosomes enhanced the secretion of collagen in HaCaT and HDFs; however, an increase in fibronectin level was observed only in HaCaT, and this effect was better with iMSC-exo treatment. Only iMSC-exo increased the phosphorylation of extracellular signal-regulated kinase (ERK)-1/2. Our results indicate that iMSC-exo promote the proliferation of skin cells by stimulating ERK1/2 and highlight the application of iMSCs for producing exosomes.

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

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

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

Mesenchymal stem cells (MSCs) are being tested in a wide range of human diseases; however, loss of potency and inconsistent quality severely limit their use. To overcome these issues, we have utilized a developmental precursor called the hemangioblast as an intermediate cell type in the derivation of a highly potent and replenishable population of MSCs from human embryonic stem cells (hESCs). This method circumvents the need for labor-intensive hand-picking, scraping, and sorting that other hESC-MSC derivation methods require. Moreover, unlike previous reports on hESC-MSCs, we have systematically evaluated their immunomodulatory properties and in vivo potency. As expected, they dynamically secrete a range of bioactive factors, display enzymatic activity, and suppress T-cell proliferation that is induced by either allogeneic cells or mitogenic stimuli. However, they also display unique immunophenotypic properties, as

well as a smaller size and >30,000-fold proliferative capacity than bone marrow-derived MSCs. In addition, this is the first report which demonstrates that hESC-MSCs can inhibit CD83 up-regulation and IL-12p70 secretion from dendritic cells and enhance regulatory T-cell populations induced by interleukin 2 (IL-2). This is also the first report which shows that hESC-MSCs have therapeutic efficacy in two different autoimmune disorder models, including a marked increase in survival of lupus-prone mice and a reduction of symptoms in an autoimmune model of uveitis. Our data suggest that this novel and therapeutically active population of MSCs could overcome many of the obstacles that plague the use of MSCs in regenerative medicine and serve as a scalable alternative to current MSC sources.

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

Strategies for therapeutic cell transplantation have been assessed for use in the treatment of massive peripheral nerve defects. To support safe and efficient cell transplantation, we have focused on the purification of cells using cell surface markers. Our group previously reported low-affinity nerve growth factor receptor (LNGFR)- and thymocyte antigen-1 (THY-1)-positive neural crest-like cells (LT-NCLCs), generated from human induced pluripotent stem cells (hiPSCs). In the present study, we investigated the efficacy of transplantation of hiPSC-derived LT-NCLCs in a murine massive peripheral nerve defect model. Animals with a sciatic nerve defect were treated with a bridging silicone tube prefilled with LT-NCLCs or medium in the transplantation (TP) and negative control (NC) groups, respectively. The grafted LT-NCLCs survived and enhanced myelination and angiogenesis, as compared to the NC group. Behavioral analysis indicated that motor functional recovery in the TP group was superior to that in the NC group, and similar to that in the autograft (Auto) group. LT-NCLCs promoted axonal regrowth and remyelination by Schwann cells. Transplantation of LT-NCLCs is a promising approach for nerve regeneration treatment of massive peripheral nerve defects.

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

Hemopoietic stem cell factor (SCF), which is the ligand for the proto-oncogene c-kit receptor (allelic with W locus) and the product of Sl locus of the mouse, has recently been cloned. The human homologue has

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

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

Induced pluripotent stem (iPS) cells have attracted a great deal attention as a new pluripotent stem cell type that can be generated from somatic cells, such as fibroblasts, by introducing the transcription factors Oct3/4, Sox2, Klf4, and c-Myc. The mechanism of generation, however, is not fully understood. Two mechanistic theories have been proposed; the stochastic model purports that every cell type has the potential to be reprogrammed to become an iPS cell and the elite model proposes that iPS cell generation occurs only from a subset of cells. Some reports have provided theoretical support for the stochastic model, but a recent publication demonstrated findings that support the elite model, and

thus the mechanism of iPS cell generation remains under debate. To enhance our understanding of iPS cells, it is necessary to clarify the properties of the original cell source, i.e., the components of the original populations and the potential of each population to become iPS cells. In this review, we discuss the two theories and their implications in iPS cell research.

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

The difficulty in obtaining a sufficient number of functional dendritic cells (DCs)is a well-known serious problem in DCbased immunotherapy. Therefore, we used induced pluripotent stem cell-derived DCs (iPSDCs). We have reported that mouse iPSDCs are equivalent to BMDCs, in terms of maturation and antigen presentation. In this study, the antitumor immune response of human iPSDCs expressing the carcinoembryonic antigen was examined, to determine its clinical application in gastrointestinal cancer. Human iPS cells were established from healthy human fibroblasts using a Sendai virus vector, and human iPSDCs were differentiated under a feeder-free culture. Additionally, the surface marker expression, cytokine production, and migratory capacity of human iPSDCs were equivalent to those of monocyte-derived DCs (MoDCs). After 3 cycles of stimulation of autologous PBMCs by genetically modified DCs, the 51Cr-release assay was performed. The lymphocytes stimulated by iPSDCs-CEA showed cytotoxic activity against LCL-CEA and CEA652-pulsed LCL, but showed no cytotoxicity against LCL-LacZ. In addition, they showed cytotoxic activity against CEA-positive human cancer cell lines, MKN45 and HT29, but showed no cytotoxicity against CEA-negative human cancer cell line MKN1. In conclusion, CEA-specific CTLs responses could be induced by iPSDCs-CEA. This vaccination strategy may be useful in future clinical applications of cancer vaccines.

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

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

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

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

Kobayashi, T. and H. Nakauchi (2011). "[From cell therapy to organ regeneration therapy: generation

of functional organs from pluripotent stem cells]." Nihon Rinsho **69**(12): 2148-2155.

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

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

Purpose: We aimed to establish purification and culture systems for retinal ganglion cells (RGCs) differentiated from mouse and human pluripotent stem cells (PSC) for in vitro and regenerative medicine studies. Methods: We used a two-step immunopanning method to purify RGCs from mouse and human PSC-derived three-dimensional (3D) retinal organoids. To assess the method, we purified RGCs from 3D retinal organoids derived from embryonic stem cells (ESCs) generated from Thy1-EGFP transgenic (TG) mice. In addition, 3D retinal organoids differentiated from human induced PSCs (iPSCs) were cultured for up to differentiation day (DD) 120, and RGCs were purified by immunopanning. RGC marker expressions were confirmed by immunostaining and reverse transcription-quantitative PCR. The purified RGCs were cultured, and neurite outgrowth was measured and analyzed using an IncuCyte Zoom system. Results: Mouse RGCs purified from Thy1-EGFP TG mouse retinas and the ESC-derived 3D retinas could be maintained for approximately 2 to 3 weeks, expressing the markers BRN3B and SMI-312. Purified RGCs from human iPSC-derived retinal organoids expressed RGC markers and could be maintained for up to 4 weeks. The RGCs collected at DD 90 to 110 extended longer neurites than those collected at younger stages. Conclusions: We successfully purified RGCs from mouse and human PSC-derived 3D retinal organoids cultured for approximately 120 days. RGCs from older retinal organoids would be useful for neurite tracking. This method would be effective not only for studying the pathology of human RGC diseases but also for therapeutic drug studies and RGC transplantation.

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

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

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

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have the potential to differentiate into various types of cells including skeletal muscle cells. The approach of converting ESCs/iPSCs into skeletal muscle cells offers hope for patients afflicted with the skeletal muscle diseases such as the Duchenne muscular dystrophy (DMD). Patient-derived iPSCs are an especially ideal cell source to obtain an unlimited number of myogenic cells that escape immune rejection after engraftment. Currently, there are several approaches to induce differentiation of ESCs and iPSCs to skeletal muscle. A key to the generation of skeletal muscle cells from ESCs/iPSCs is the mimicking of embryonic mesodermal induction followed by myogenic

induction. Thus, current approaches of skeletal muscle cell induction of ESCs/iPSCs utilize techniques including overexpression of myogenic transcription factors such as MyoD or Pax3, using small molecules to induce mesodermal cells followed by myogenic progenitor cells, and utilizing epigenetic myogenic memory existing in muscle cell-derived iPSCs. This review summarizes the current methods used in myogenic differentiation and highlights areas of recent improvement.

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

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

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

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

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

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

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

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

post-transplantation but limited teratoma formation. Thus, these data suggest the potential to derive pluripotent cells from human testis biopsies but indicate a need for novel strategies to optimize hMGSC culture conditions and reprogramming.

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

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

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

BACKGROUND: For cell therapies to treat diabetes, it is important to produce a sufficient number of pancreatic endocrine cells that function similarly to primary islets. Induced pluripotent stem (iPS) cells represent a potentially unlimited source of functional pancreatic endocrine cells. However, the use of iPS cells for laboratory studies and cell-based therapies is hampered by their high tumorigenic potential and limited ability to generate pure populations of differentiated cell types in vitro. The purpose of this study was to establish a pancreatic stem cell line from iPS cells derived from mouse fibroblasts. METHODS: Mouse iPS cells were induced to differentiate into insulin-producing cells by a multi-step differentiation

protocol, which was conducted as described previously with minor modifications. Selection of the pancreatic stem cell was based on morphology and Pdx1 expression. The pancreatic potential of the pancreatic stem cells was evaluated using a reverse transcription PCR, real-time PCR, immunofluorescence, and a glucose challenge test. To assess potential tumorigenicity of the pancreatic stem cells, the cells were injected into the quadriceps femoris muscle of the left hindlimb of nude mice. RESULTS: The iPS-derived pancreatic stem cells expressed the transcription factor--Pdx1--a marker of pancreatic development, and continued to divide actively beyond passage 80. Endocrine cells derived from these pancreatic stem cells expressed insulin and pancreatic genes, and they released insulin in response to glucose stimulation. Mice injected with the pancreatic stem cells did not develop tumors, in contrast to mice injected with an equal number of iPS cells. CONCLUSION: This strategy provides a new approach for generation of insulin-producing cells that is more efficient and safer than using iPS cells. We believe that this approach will help to develop a patient-specific cell transplantation therapy for diabetes in the near future.

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

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

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

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

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

Germ cells undergo epigenome reprogramming for proper development of the next generation. The realization of germ cell derivation from human and mouse pluripotent stem cells offers unprecedented opportunity for investigation of germline development. Primordial germ cells reconstituted in vitro (PGC-like cells [PGCLCs]) show progressive dilution of genomic DNA methylation, tightly linked with chromatin remodeling, during their specification. PGCLCs can be further expanded by plane culture, allowing maintenance of the gene-expression profiles of early PGCs and continuance of the DNA methylation erasure, thereby establishing an epigenetic `blank slate'. PGCLCs undergo further epigenome regulation to acquire the male or female fates. These findings will provide a foundation for basic germ cell biology and for in-depth evaluations of in vitro gametogenesis.

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

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

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

Tissue-engineered constructs are rendered useless without a functional vasculature owing to a lack of nutrients and oxygen. Cell-based approaches to reconstruct blood vessels can yield structures that mimic native vasculature and aid transplantation. Vascular derivatives of human induced pluripotent stem cells (hiPSCs) offer opportunities to generate patient-specific therapies and potentially provide unlimited amounts of vascular cells. To be used in engineered vascular constructs and confer therapeutic benefit, vascular derivatives must exhibit additional key properties, including extracellular matrix (ECM) production to confer structural integrity and growth factor production to facilitate integration. In this study, we examine the hypothesis that vascular cells derived from hiPSCs exhibit these critical properties to facilitate their use in engineered tissues. hiPSCs were codifferentiated toward early vascular cells (EVCs), a bicellular population of endothelial cells (ECs) and pericytes, under varying low-oxygen differentiation

conditions; subsequently, ECs were isolated and passaged. We found that EVCs differentiated under low-oxygen conditions produced copious amounts of collagen IV and fibronectin as well as vascular endothelial growth factor and angiopoietin 2. EVCs differentiated under atmospheric conditions did not demonstrate such abundant ECM expression, but exhibited greater expression of angiopoietin 1. Isolated ECs could proliferate up to three passages while maintaining the EC marker vascular endothelial cadherin. Isolated ECs demonstrated an increased propensity to produce ECM compared with their EVC correlates and took on an arterial-like fate. These findings illustrate that hiPSC vascular derivates hold great potential for therapeutic use and should continue to be a preferred cell source for vascular construction.

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

Unlike mouse results, cloning efficiency of nuclear transfer from porcine induced pluripotent stem cells (piPSCs) is very low. The present study was performed to investigate the effect of cell cycle inhibitors on the cell cycle synchronization of piPSCs. piPSCs were generated using combination of six human transcriptional factors under stem cell culture condition. To examine the efficiency of cell cycle synchronization, piPSCs were cultured on a matrigel coated plate with stem cell media and they were treated with staurosporine (STA, 20 nM), daidzein (DAI, 100 muM), roscovitine (ROSC, 10 muM), or olomoucine (OLO, 200 muM) for 12 h. Flow Cytometry (FACs) data showed that piPSCs in control were in G1 (37.5+/-0.2%), S (34.0+/-0.6%) and G2/M (28.5+/-0.4%). The proportion of cells at G1 in DAI group was significantly higher than that in control, while STA, ROSC and OLO treatments could not block the cell cycle of piPSCs. Both of viability and apoptosis were affected by STA and ROSC treatment, but there were no significantly differences between control and DAI groups. Real-Time qPCR and FACs results revealed that DAI treatment did not affect the expression of pluripotent gene, Oct4. In case of OLO, it did not affect both of viability and apoptosis, but Oct4 expression was significantly decreased. Our results suggest that DAI could be used for synchronizing piPSCs at G1 stage and has any deleterious effect on survival and pluripotency sustaining of piPSCs.

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

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

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

Interleukin-3 (IL-3) is capable of supporting the proliferation of a broad range of hematopoietic cell types, whereas granulocyte colony-stimulating factor (G-CSF) and macrophage CSF (M-CSF) represent critical cytokines in myeloid differentiation. When this was investigated in a pluripotent-stem-cell-based hematopoietic differentiation model, IL-3/G-CSF or IL-3/M-CSF exposure resulted in the continuous generation of myeloid cells from an intermediate myeloid-cell-forming complex containing CD34(+) clonogenic progenitor cells for more than 2 months. Whereas IL-3/G-CSF directed differentiation toward CD45(+)CD11b (+)CD15(+)CD16(+)CD66b (+) granulocytic cells of various differentiation stages up to a segmented morphology displaying the capacity of cytokine-directed migration, respiratory burst response,

and neutrophil-extracellular-trap formation, exposure to IL-3/M-CSF resulted in CD45(+)CD11b (+)CD14(+)CD163(+)CD68(+) monocyte/macrophage-type cells capable of phagocytosis and cytokine secretion. Hence, we show here that myeloid specification of human pluripotent stem cells by IL-3/G-CSF or IL-3/M-CSF allows for prolonged and large-scale production of myeloid cells, and thus is suited for cell-fate and disease-modeling studies as well as gene- and cell-therapy applications.

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

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

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

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

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

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

as compared to RA/EGF-treated cultures. Our results show that modulating Notch pathway during in vitro differentiation of hiPSC-derived otic/placodal progenitors is a valuable strategy to promote the expression of human otic sensory lineage genes.

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

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

how they affect cell functions and contribute to pathogenesis.

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

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

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

The development of dengue antivirals and vaccine has been hampered by the incomplete understanding of molecular mechanisms of dengue virus (DENV) infection and pathology, partly due to the limited suitable cell culture or animal models that can capture the comprehensive cellular changes induced by DENV. In this study, we differentiated human pluripotent stem cells (hPSCs) into hepatocytes, one of the target cells of DENV, to investigate various aspects of DENV-hepatocyte interaction. hPSC-

derived hepatocyte-like cells (HLCs) supported persistent and productive DENV infection. The activation of interferon pathways by DENV protected bystander cells from infection and protected the infected cells from massive apoptosis. Furthermore, DENV infection activated the NF-kappaB pathway, which led to production of proinflammatory cytokines and downregulated many liver-specific genes such as albumin and coagulation factor V. Our study demonstrates the utility of hPSC-derived hepatocytes as an in vitro model for DENV infection and reveals important aspects of DENV-host interactions.

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

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

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

BACKGROUND: Ex vivo manufacture of red blood cells from stem cells is a potential means to ensure an adequate and safe supply of blood cell products. Advances in somatic cell reprogramming of human induced pluripotent stem cells have opened the door to generating specific cells for cell therapy. Human induced pluripotent stem cells represent a potentially unlimited source of stem cells for erythroid generation for transfusion medicine. DESIGN AND METHODS: We characterized the erythroid differentiation and maturation of human induced pluripotent stem cell lines obtained from human fetal (IMR90) and adult fibroblasts (FD-136) compared to those of a human embryonic stem cell line (H1). Our

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

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

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

Lewandowski, J., et al. (2018). "The impact of in vitro cell culture duration on the maturation of human cardiomyocytes derived from induced pluripotent stem

cells of myogenic origin." Cell Transplant **27**(7): 1047-1067.

Ischemic heart disease, also known as coronary artery disease (CAD), poses a challenge for regenerative medicine. iPSC technology might lead to a breakthrough due to the possibility of directed cell differentiation delivering a new powerful source of human autologous cardiomyocytes. One of the factors supporting proper cell maturation is in vitro culture duration. In this study, primary human skeletal muscle myoblasts were selected as a myogenic cell type reservoir for genetic iPSC reprogramming. Skeletal muscle myoblasts have similar ontogeny embryogenetic pathways (myoblasts vs. cardiomyocytes), and thus, a greater chance of myocardial development might be expected, with maintenance of acquired myogenic cardiac cell characteristics, from the differentiation process when iPSCs of myoblastoid origin are obtained. Analyses of cell morphological and structural changes, gene expression (cardiac markers), and functional tests (intracellular calcium transients) performed at two in vitro culture time points spanning the early stages of cardiac development (day 20 versus 40 of cell in vitro culture) confirmed the ability of the obtained myogenic cells to acquire adult features of differentiated cardiomyocytes. Prolonged 40-day iPSC-derived cardiomyocytes (iPSC-CMs) revealed progressive cellular hypertrophy; a better-developed contractile apparatus; expression of marker genes similar to human myocardial ventricular cells, including a statistically significant CX43 increase, an MHC isoform switch, and a troponin I isoform transition; more efficient intercellular calcium handling; and a stronger response to beta-adrenergic stimulation.

Li, C., et al. (2013). "Arf tumor suppressor and miR-205 regulate cell adhesion and formation of extraembryonic endoderm from pluripotent stem cells." Proc Natl Acad Sci U S A **110**(12): E1112-1121.

Induction of the Arf tumor suppressor (encoded by the alternate reading frame of the Cdkn2a locus) following oncogene activation engages a p53-dependent transcriptional program that limits the expansion of incipient cancer cells. Although the p19(Arf) protein is not detected in most tissues of fetal or young adult mice, it is physiologically expressed in the fetal yolk sac, a tissue derived from the extraembryonic endoderm (ExEn). Expression of the mouse p19(Arf) protein marks late stages of ExEn differentiation in cultured embryoid bodies (EBs) derived from either embryonic stem cells or induced pluripotent stem cells. Arf inactivation delays differentiation of the ExEn lineage within EBs, but not the formation of other germ cell lineages from

pluripotent progenitors. Arf is required for the timely induction of ExEn cells in response to Ras/Erk signaling and, in turn, acts through p53 to ensure the development, but not maintenance, of the ExEn lineage. Remarkably, a significant temporal delay in ExEn differentiation detected during the maturation of Arf-null EBs is rescued by enforced expression of mouse microRNA-205 (miR-205), a microRNA up-regulated by p19(Arf) and p53 that controls ExEn cell migration and adhesion. The noncanonical and canonical roles of Arf in ExEn development and tumor suppression, respectively, may be conceptually linked through mechanisms that govern cell attachment and migration.

Lv, Y., et al. (2013). "Effects of low-intensity pulsed ultrasound on cell viability, proliferation and neural differentiation of induced pluripotent stem cells-derived neural crest stem cells." Biotechnol Lett **35**(12): 2201-2212.

Low-intensity pulsed ultrasound (LIPUS) acting on induced pluripotent stem cells-derived neural crest stem cells (iPSCs-NCSCs) is considered a promising therapy to improve the efficacy of injured peripheral nerve regeneration. Effects of LIPUS on cell viability, proliferation and neural differentiation of iPSCs-NCSCs were examined respectively in this study. LIPUS at 500 mW cm (-2) enhanced the viability and proliferation of iPSCs-NCSCs after 2 days and, after 4 days, up-regulated gene and protein expressions of NF-M, Tuj1, S100beta and GFAP in iPSCs-NCSCs whereas after 7 days expression of only NF-M, S100beta and GFAP were up-regulated. LIPUS treatment at an appropriate intensity can, therefore, be an efficient and cost-effective method to enhance cell viability, proliferation and neural differentiation of iPSCs-NCSCs in vitro for peripheral nerve tissue engineering.

Ma, H., et al. (2018). "Establishment of human pluripotent stem cell-derived pancreatic beta-like cells in the mouse pancreas." Proc Natl Acad Sci U S A **115**(15): 3924-3929.

Type 1 diabetes is characterized by autoimmune destruction of beta cells located in pancreatic islets. However, tractable in vivo models of human pancreatic beta cells have been limited. Here, we generated xenogeneic human pancreatic beta-like cells in the mouse pancreas by orthotopic transplantation of stem cell-derived beta (SC-beta) cells into the pancreas of neonatal mice. The engrafted beta-like cells expressed beta cell transcription factors and markers associated with functional maturity. Engrafted human cells recruited mouse endothelial cells, suggesting functional integration. Human insulin was detected in the blood circulation of transplanted mice for months

after transplantation and increased upon glucose stimulation. In addition to beta-like cells, human cells expressing markers for other endocrine pancreas cell types, acinar cells, and pancreatic ductal cells were identified in the pancreata of transplanted mice, indicating that this approach allows studying other human pancreatic cell types in the mouse pancreas. Our results demonstrate that orthotopic transplantation of human SC-beta cells into neonatal mice is an experimental platform that allows the generation of mice with human pancreatic beta-like cells in the endogenous niche.

Ma, M. S., et al. (2015). "Pluripotent stem cells for Schwann cell engineering." Stem Cell Rev **11**(2): 205-218.

Tissue engineering of Schwann cells (SCs) can serve a number of purposes, such as in vitro SC-related disease modeling, treatment of peripheral nerve diseases or peripheral nerve injury, and, potentially, treatment of CNS diseases. SCs can be generated from autologous stem cells in vitro by recapitulating the various stages of in vivo neural crest formation and SC differentiation. In this review, we survey the cellular and molecular mechanisms underlying these in vivo processes. We then focus on the current in vitro strategies for generating SCs from two sources of pluripotent stem cells, namely embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Different methods for SC engineering from ESCs and iPSCs are reviewed and suggestions are proposed for optimizing the existing protocols. Potential safety issues regarding the clinical application of iPSC-derived SCs are discussed as well. Lastly, we will address future aspects of SC engineering.

Ma, R., et al. (2015). "Thyroid cell differentiation from murine induced pluripotent stem cells." Front Endocrinol (Lausanne) **6**: 56.

BACKGROUND: Here, we demonstrate the successful differentiation of induced pluripotent stem (iPS) cells into functional thyroid cells indicating the therapeutic potential of this approach when applied to individuals with thyroid deficiency. RESEARCH DESIGN AND METHODS: Using embryonic murine fibroblasts, we generated iPS cells with a single lentiviral "stem cell cassette" vector and then differentiated these iPS cells into thyroid cells after transfection with PAX8 and NKX2-1 by Activin A and TSH stimulation. RESULTS: The generated iPS cells expressed pluripotent stem cell markers as assessed using both reverse transcription quantitative PCRs and immunofluorescence staining with ~0.5% reprograming efficiency. Compared to control cells, the expression of thyroid-specific genes NIS, TSHR, Tg, and TPO were greatly enhanced in PAX8(+)NKX2-1(+) iPS cells after differentiation. On stimulation with TSH, these differentiated iPS cells were also capable of dose-dependent cAMP generation and radioiodine uptake indicative of functional thyroid epithelial cells. Furthermore, the cells formed three-dimensional follicles in culture, and "thyroid organoids" formed after PAX8(+)NKX2-1(+) iPS cells transplanted into nude mice, and all expressed Tg protein as judged immunohistochemically. Taken together, thyroid epithelial cells differentiated from iPS cells, which were themselves derived from murine fibroblasts, exhibited very similar properties to thyroid cells previously developed from traditional murine embryonic stem cells. CONCLUSION: Thyroid cells differentiated from iPS cells offer the opportunity to examine the detailed transcriptional regulation of thyroid cell differentiation and may provide a useful future source for individualized regenerative cell therapy.

MacLean, G. A., et al. (2018). "Downregulation of Endothelin Receptor B Contributes to Defective B Cell Lymphopoiesis in Trisomy 21 Pluripotent Stem Cells." Sci Rep **8**(1): 8001.

Individuals with Trisomy 21 (T21) exhibit numerous hematological abnormalities, including reductions in numbers of circulating B and T lymphocytes. To elucidate molecular mechanisms underlying these phenotypes, we differentiated human isogenic disomic and trisomic pluripotent cells, and observed that trisomic cells showed defects in B cell, but not T cell differentiation. Global gene expression of differentiated, trisomic B cells revealed reduced expression of genes encoding endothelin signaling components, namely the Endothelin Receptor B (EDNRB), and its ligand Endothelin1 (EDN1). Depletion of EDNRB mRNA in cord blood-derived CD34(+) cells led to defective B cell differentiation, supporting a hypothesis that low EDNRB expression in T21 contributes to intrinsic lymphoid defects. Further evidence for the role of the EDNRB pathway in B cell differentiation was obtained through CRISPR/Cas9 gene targeting in disomic and trisomic iPS cells. Knockout of EDNRB in both cell backgrounds reduced the capacity for B cell differentiation. Collectively, this work identifies downregulation of EDNRB as a causative factor for impaired B lymphocyte generation in trisomic cells, which may contribute to defects in immune function associated with T21. Furthermore, a novel role for endothelin signaling in regulation of B cell development has been identified.

Magdy, T., et al. (2018). "Human Induced Pluripotent Stem Cell (hiPSC)-Derived Cells to Assess

Drug Cardiotoxicity: Opportunities and Problems." Annu Rev Pharmacol Toxicol **58**: 83-103.

Billions of US dollars are invested every year by the pharmaceutical industry in drug development, with the aim of introducing new drugs that are effective and have minimal side effects. Thirty percent of in-pipeline drugs are excluded in an early phase of preclinical and clinical screening owing to cardiovascular safety concerns, and several lead molecules that pass the early safety screening make it to market but are later withdrawn owing to severe cardiac side effects. Although the current drug safety screening methodologies can identify some cardiotoxic drug candidates, they cannot accurately represent the human heart in many aspects, including genomics, transcriptomics, and patient- or population-specific cardiotoxicity. Despite some limitations, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a powerful and evolving technology that has been shown to recapitulate many attributes of human cardiomyocytes and their drug responses. In this review, we discuss the potential impact of the inclusion of the hiPSC-CM platform in premarket candidate drug screening.

Maguire, E. M., et al. (2017). "Differentiation and Application of Induced Pluripotent Stem Cell-Derived Vascular Smooth Muscle Cells." Arterioscler Thromb Vasc Biol **37**(11): 2026-2037.

Vascular smooth muscle cells (VSMCs) play a role in the development of vascular disease, for example, neointimal formation, arterial aneurysm, and Marfan syndrome caused by genetic mutations in VSMCs, but little is known about the mechanisms of the disease process. Advances in induced pluripotent stem cell technology have now made it possible to derive VSMCs from several different somatic cells using a selection of protocols. As such, researchers have set out to delineate key signaling processes involved in triggering VSMC gene expression to grasp the extent of gene regulatory networks involved in phenotype commitment. This technology has also paved the way for investigations into diseases affecting VSMC behavior and function, which may be treatable once an identifiable culprit molecule or gene has been repaired. Moreover, induced pluripotent stem cell-derived VSMCs are also being considered for their use in tissue-engineered blood vessels as they may prove more beneficial than using autologous vessels. Finally, while several issues remains to be clarified before induced pluripotent stem cell-derived VSMCs can become used in regenerative medicine, they do offer both clinicians and researchers hope for both treating and understanding vascular disease. In this review, we aim to update the recent progress on VSMC generation from stem cells and the underlying molecular mechanisms of VSMC differentiation. We will also explore how the use of induced pluripotent stem cell-derived VSMCs has changed the game for regenerative medicine by offering new therapeutic avenues to clinicians, as well as providing researchers with a new platform for modeling of vascular disease.

Makinen, M., et al. (2013). "Fluorescent probes as a tool for cell population tracking in spontaneously active neural networks derived from human pluripotent stem cells." J Neurosci Methods **215**(1): 88-96.

Applications such as 3D cultures and tissue modelling require cell tracking with non-invasive methods. In this work, the suitability of two fluorescent probes, CellTracker, CT, and long chain carbocyanine dye, DiD, was investigated for long-term culturing of labeled human pluripotent stem cell-derived neural cells. We found that these dyes did not affect the cell viability. However, proliferation was decreased in DiD labeled cell population. With both dyes the labeling was stable up to 4 weeks. CT and DiD labeled cells could be co-cultured and, importantly, these mixed populations had their normal ability to form spontaneous electrical network activity. In conclusion, human neural cells can be successfully labeled with these two fluorescent probes without significantly affecting the cell characteristics. These labeled cells could be utilized further in e.g. building controlled neuronal networks for neurotoxicity screening platforms, combining cells with biomaterials for 3D studies, and graft development.

Maldonado, M., et al. (2015). "The effects of electrospun substrate-mediated cell colony morphology on the self-renewal of human induced pluripotent stem cells." Biomaterials **50**: 10-19.

The development of xeno-free, chemically defined stem cell culture systems has been a primary focus in the field of regenerative medicine to enhance the clinical application of pluripotent stem cells (PSCs). In this regard, various electrospun substrates with diverse physiochemical properties were synthesized utilizing various polymer precursors and surface treatments. Human induced pluripotent stem cells (IPSCs) cultured on these substrates were characterized by their gene and protein expression to determine the effects of the substrate physiochemical properties on the cells' self-renewal, i.e., proliferation and the maintenance of pluripotency. The results showed that surface chemistry significantly affected cell colony formation via governing the colony edge propagation. More importantly, when surface chemistry of the substrates was uniformly controlled by collagen conjugation, the stiffness of substrate was inversely related to the sphericity, a degree of three

dimensionality in colony morphology. The differences in sphericity subsequently affected spontaneous differentiation of IPSCs during a long-term culture, implicating that the colony morphology is a deciding factor in the lineage commitment of PSCs. Overall, we show that the capability of controlling IPSC colony morphology by electrospun substrates provides a means to modulate IPSC self-renewal.

Malecki, M., et al. (2013). "Safeguarding Stem Cell-Based Regenerative Therapy against Iatrogenic Cancerogenesis: Transgenic Expression of DNASE1, DNASE1L3, DNASE2, DFFB Controlled By POLA1 Promoter in Proliferating and Directed Differentiation Resisting Human Autologous Pluripotent Induced Stem Cells Leads to their Death." J Stem Cell Res Ther **Suppl 9**(5).

INTRODUCTION: The worst possible complication of using stem cells for regenerative therapy is iatrogenic cancerogenesis. The ultimate goal of our work is to develop a self-triggering feedback mechanism aimed at causing death of all stem cells, which resist directed differentiation, keep proliferating, and can grow into tumors. SPECIFIC AIM: The specific aim was threefold: (1) to genetically engineer the DNA constructs for the human, recombinant DNASE1, DNASE1L3, DNASE2, DFFB controlled by POLA promoter; (2) to bioengineer anti-SSEA-4 antibody guided vectors delivering transgenes to human undifferentiated and proliferating pluripotent stem cells; (3) to cause death of proliferating and directed differentiation resisting stem cells by transgenic expression of the human recombinant the DNases (hrDNases). METHODS: The DNA constructs for the human, recombinant DNASE1, DNASE1L3, DNASE2, DFFB controlled by POLA promoter were genetically engineered. The vectors targeting specifically SSEA-4 expressing stem cells were bioengineered. The healthy volunteers' bone marrow mononuclear cells (BMMCs) were induced into human, autologous, pluripotent stem cells with non-integrating plasmids. Directed differentiation of the induced stem cells into endothelial cells was accomplished with EGF and BMP. The anti-SSEA 4 antibodies' guided DNA vectors delivered the transgenes for the human recombinant DNases' into proliferating stem cells. RESULTS: Differentiation of the pluripotent induced stem cells into the endothelial cells was verified by highlighting formation of tight and adherens junctions through transgenic expression of recombinant fluorescent fusion proteins: VE cadherin, claudin, zona occludens 1, and catenin. Proliferation of the stem cells was determined through highlighting transgenic expression of recombinant fluorescent proteins controlled by POLA promoter, while also reporting expression of the transgenes for

the hrDNases. Expression of the transgenes for the DNases resulted in complete collapse of the chromatin architecture and degradation of the proliferating cells' genomic DNA. The proliferating stem cells, but not the differentiating ones, were effectively induced to die. CONCLUSION: Herein, we describe attaining the proof-of-concept for the strategy, whereby transgenic expression of the genetically engineered human recombinant DNases in proliferating and directed differentiation resisting stem cells leads to their death. This novel strategy reduces the risk of iatrogenic neoplasms in stem cell therapy.

Malik, N., et al. (2014). "Comparison of the gene expression profiles of human fetal cortical astrocytes with pluripotent stem cell derived neural stem cells identifies human astrocyte markers and signaling pathways and transcription factors active in human astrocytes." PLoS One **9**(5): e96139.

Astrocytes are the most abundant cell type in the central nervous system (CNS) and have a multitude of functions that include maintenance of CNS homeostasis, trophic support of neurons, detoxification, and immune surveillance. It has only recently been appreciated that astrocyte dysfunction is a primary cause of many neurological disorders. Despite their importance in disease very little is known about global gene expression for human astrocytes. We have performed a microarray expression analysis of human fetal astrocytes to identify genes and signaling pathways that are important for astrocyte development and maintenance. Our analysis confirmed that the fetal astrocytes express high levels of the core astrocyte marker GFAP and the transcription factors from the NFI family which have been shown to play important roles in astrocyte development. A group of novel markers were identified that distinguish fetal astrocytes from pluripotent stem cell-derived neural stem cells (NSCs) and NSC-derived neurons. As in murine astrocytes, the Notch signaling pathway appears to be particularly important for cell fate decisions between the astrocyte and neuronal lineages in human astrocytes. These findings unveil the repertoire of genes expressed in human astrocytes and serve as a basis for further studies to better understand astrocyte biology, especially as it relates to disease.

Manos, P. D., et al. (2011). "Live-cell immunofluorescence staining of human pluripotent stem cells." Curr Protoc Stem Cell Biol **Chapter 1**: Unit 1C 12.

Antibodies are instrumental tools in stem cell identification, purification, and analysis. Most commonly, cell samples are either dissociated to obtain a single-cell suspension suitable for FACS analysis or cell sorting, or fixed in situ for

immunostaining and fluorescence microscopy imaging. This unit describes an alternative method in which live adherent cells are stained and imaged in situ without the need for cell dissociation, fixation, or fluorescent reporter genes. This minimally invasive method is particularly useful for identification and distinction of fully and partially reprogrammed induced pluripotent stem cells (iPSCs). The unit also describes the use of mCD49e and hCD29 antibodies in live-cell (vital) imaging. mCD49e strongly stains mouse embryonic fibroblast (MEF) feeder cells in human pluripotent stem cell cultures, whereas hCD29 recognizes an antigen expressed on undifferentiated and many differentiated cells. A distinguishing feature of hCD29 in live-cell staining is that its antigen is precluded from detection wherever cells have formed tight epithelial junctions (e.g., in the center but not the periphery of pluripotent stem cell colonies) due to basolateral location. A non-fluorescent fixed-cell staining protocol is also provided for medium- to high-throughput quantification of stem cell experiments without an automated microscope. The discussion addresses technical limitations, pitfalls, troubleshooting, and potential applications, such as identification of emerging bona fide human iPSC colonies in reprogramming experiments.

Mao, D., et al. (2017). "A Synthetic Hybrid Molecule for the Selective Removal of Human Pluripotent Stem Cells from Cell Mixtures." Angew Chem Int Ed Engl **56**(7): 1765-1770.
A major hurdle in stem cell therapy is the tumorigenic risk of residual undifferentiated stem cells. This report describes the design and evaluation of synthetic hybrid molecules that efficiently reduce the number of human induced pluripotent stem cells (hiPSCs) in cell mixtures. The design takes advantage of Kyoto probe 1 (KP-1), a fluorescent chemical probe for hiPSCs, and clinically used anticancer drugs. Among the KP-1-drug conjugates we synthesized, we found an exceptionally selective, chemically tractable molecule that induced the death of hiPSCs. Mechanistic analysis suggested that the high selectivity originates from the synergistic combination of transporter-mediated efflux and the cytotoxicity mode of action. The present study offers a chemical and mechanistic rationale for designing selective, safe, and simple reagents for the preparation of non-tumorigenic clinical samples.

Marion, R. M., et al. (2009). "Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells." Cell Stem Cell **4**(2): 141-154.
Telomere shortening is associated with organismal aging. iPS cells have been recently derived from old patients; however, it is not known whether telomere chromatin acquires the same characteristics as in ES cells. We show here that telomeres are elongated in iPS cells compared to the parental differentiated cells both when using four (Oct3/4, Sox2, Klf4, cMyc) or three (Oct3/4, Sox2, Klf4) reprogramming factors and both from young and aged individuals. We demonstrate genetically that, during reprogramming, telomere elongation is usually mediated by telomerase and that iPS telomeres acquire the epigenetic marks of ES cells, including a low density of trimethylated histones H3K9 and H4K20 and increased abundance of telomere transcripts. Finally, reprogramming efficiency of cells derived from increasing generations of telomerase-deficient mice shows a dramatic decrease in iPS cell efficiency, a defect that is restored by telomerase reintroduction. Together, these results highlight the importance of telomere biology for iPS cell generation and functionality.

Martin, G. R. (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells." Proc Natl Acad Sci U S A **78**(12): 7634-7638.
This report describes the establishment directly from normal preimplantation mouse embryos of a cell line that forms teratocarcinomas when injected into mice. The pluripotency of these embryonic stem cells was demonstrated conclusively by the observation that subclonal cultures, derived from isolated single cells, can differentiate into a wide variety of cell types. Such embryonic stem cells were isolated from inner cell masses of late blastocysts cultured in medium conditioned by an established teratocarcinoma stem cell line. This suggests that such conditioned medium might contain a growth factor that stimulates the proliferation or inhibits the differentiation of normal pluripotent embryonic cells, or both. This method of obtaining embryonic stem cells makes feasible the isolation of pluripotent cells lines from various types of noninbred embryo, including those carrying mutant genes. The availability of such cell lines should made possible new approaches to the study of early mammalian development.

Mashimo, Y. and K. Kamei (2015). "Microfluidic Image Cytometry for Single-Cell Phenotyping of Human Pluripotent Stem Cells." Methods Mol Biol **1346**: 85-98.
A microfluidic human pluripotent stem cell (hPSC) array has been developed for robust and reproducible hPSC culture methods to assess chemically defined serum- and feeder-free culture conditions. This microfluidic platform, combined with image cytometry, enables the systematic analysis of multiple simultaneously detected marker expression in

individual cells, for screening of various chemically defined media across hPSC lines, and the study of phenotypic responses.

Mathapati, S., et al. (2016). "Small-Molecule-Directed Hepatocyte-Like Cell Differentiation of Human Pluripotent Stem Cells." Curr Protoc Stem Cell Biol **38**: 1G 6 1-1G 6 18.

Hepatocyte-like cells (HLCs) generated in vitro from human pluripotent stem cells (hPSCs) provide an invaluable resource for basic research, regenerative medicine, drug screening, toxicology, and modeling of liver disease and development. This unit describes a small-molecule-driven protocol for in vitro differentiation of hPSCs into HLCs without the use of growth factors. hPSCs are coaxed through a developmentally relevant route via the primitive streak to definitive endoderm (DE) using the small molecule CHIR99021 (a Wnt agonist), replacing the conventional growth factors Wnt3A and activin A. The small-molecule-derived DE is then differentiated to hepatoblast-like cells in the presence of dimethyl sulfoxide. The resulting hepatoblasts are then differentiated to HLCs with N-hexanoic-Tyr, Ile-6 aminohexanoic amide (Dihexa, a hepatocyte growth factor agonist) and dexamethasone. The protocol provides an efficient and reproducible procedure for differentiation of hPSCs into HLCs utilizing small molecules. (c) 2016 by John Wiley & Sons, Inc.

Matsumoto, T., et al. (2016). "Functional Neurons Generated from T Cell-Derived Induced Pluripotent Stem Cells for Neurological Disease Modeling." Stem Cell Reports **6**(3): 422-435.

Modeling of neurological diseases using induced pluripotent stem cells (iPSCs) derived from the somatic cells of patients has provided a means of elucidating pathogenic mechanisms and performing drug screening. T cells are an ideal source of patient-specific iPSCs because they can be easily obtained from samples. Recent studies indicated that iPSCs retain an epigenetic memory relating to their cell of origin that restricts their differentiation potential. The classical method of differentiation via embryoid body formation was not suitable for T cell-derived iPSCs (TiPSCs). We developed a neurosphere-based robust differentiation protocol, which enabled TiPSCs to differentiate into functional neurons, despite differences in global gene expression between TiPSCs and adult human dermal fibroblast-derived iPSCs. Furthermore, neurons derived from TiPSCs generated from a juvenile patient with Parkinson's disease exhibited several Parkinson's disease phenotypes. Therefore, we conclude that TiPSCs are a useful tool for modeling neurological diseases.

Matsumura, T., et al. (2014). "Single-cell cloning and expansion of human induced pluripotent stem cells by a microfluidic culture device." Biochem Biophys Res Commun **453**(1): 131-137.

The microenvironment of cells, which includes basement proteins, shear stress, and extracellular stimuli, should be taken into consideration when examining physiological cell behavior. Although microfluidic devices allow cellular responses to be analyzed with ease at the single-cell level, few have been designed to recover cells. We herein demonstrated that a newly developed microfluidic device helped to improve culture conditions and establish a clonality-validated human pluripotent stem cell line after tracing its growth at the single-cell level. The device will be a helpful tool for capturing various cell types in the human body that have not yet been established in vitro.

Matsuo, T., et al. (2015). "Efficient long-term survival of cell grafts after myocardial infarction with thick viable cardiac tissue entirely from pluripotent stem cells." Sci Rep **5**: 16842.

Poor engraftment of cells after transplantation to the heart is a common and unresolved problem in the cardiac cell therapies. We previously generated cardiovascular cell sheets entirely from pluripotent stem cells with cardiomyocytes, endothelial cells and vascular mural cells. Though sheet transplantation showed a better engraftment and improved cardiac function after myocardial infarction, stacking limitation (up to 3 sheets) by hypoxia hampered larger structure formation and long-term survival of the grafts. Here we report an efficient method to overcome the stacking limitation. Insertion of gelatin hydrogel microspheres (GHMs) between each cardiovascular cell sheet broke the viable limitation via appropriate spacing and fluid impregnation with GHMs. Fifteen sheets with GHMs (15-GHM construct; >1 mm thickness) were stacked within several hours and viable after 1 week in vitro. Transplantation of 5-GHM constructs (approximately $2 \times 10(6)$ 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.

Muffat, J., et al. (2018). "Human induced pluripotent stem cell-derived glial cells and neural progenitors display divergent responses to Zika and dengue infections." Proc Natl Acad Sci U S A **115**(27): 7117-7122.

Maternal Zika virus (ZIKV) infection during pregnancy is recognized as the cause of an epidemic of microcephaly and other neurological anomalies in human fetuses. It remains unclear how ZIKV accesses the highly vulnerable population of neural progenitors of the fetal central nervous system (CNS), and which cell types of the CNS may be viral reservoirs. In contrast, the related dengue virus (DENV) does not elicit teratogenicity. To model viral interaction with cells of the fetal CNS in vitro, we investigated the tropism of ZIKV and DENV for different induced pluripotent stem cell-derived human cells, with a particular focus on microglia-like cells. We show that ZIKV infected isogenic neural progenitors, astrocytes, and microglia-like cells (pMGLs), but was only cytotoxic to neural progenitors. Infected glial cells propagated ZIKV and maintained ZIKV load over time, leading to viral spread to susceptible cells. DENV triggered stronger immune responses and could

be cleared by neural and glial cells more efficiently. pMGLs, when cocultured with neural spheroids, invaded the tissue and, when infected with ZIKV, initiated neural infection. Since microglia derive from primitive macrophages originating in proximity to the maternal vasculature, they may act as a viral reservoir for ZIKV and establish infection of the fetal brain. Infection of immature neural stem cells by invading microglia may occur in the early stages of pregnancy, before angiogenesis in the brain rudiments. Our data are also consistent with ZIKV and DENV affecting the integrity of the blood-brain barrier, thus allowing infection of the brain later in life.

Mulyasasmita, W., et al. (2014). "Avidity-controlled hydrogels for injectable co-delivery of induced pluripotent stem cell-derived endothelial cells and growth factors." J Control Release **191**: 71-81.

To translate recent advances in induced pluripotent stem cell biology to clinical regenerative medicine therapies, new strategies to control the co-delivery of cells and growth factors are needed. Building on our previous work designing Mixing-Induced Two-Component Hydrogels (MITCHs) from engineered proteins, here we develop protein-polyethylene glycol (PEG) hybrid hydrogels, MITCH-PEG, which form physical gels upon mixing for cell and growth factor co-delivery. MITCH-PEG is a mixture of C7, which is a linear, engineered protein containing seven repeats of the CC43 WW peptide domain (C), and 8-arm star-shaped PEG conjugated with either one or two repeats of a proline-rich peptide to each arm (P1 or P2, respectively). Both 20kDa and 40kDa star-shaped PEG variants were investigated, and all four PEG-peptide variants were able to undergo a sol-gel phase transition when mixed with the linear C7 protein at constant physiological conditions due to noncovalent hetero-dimerization between the C and P domains. Due to the dynamic nature of the C-P physical crosslinks, all four gels were observed to be reversibly shear-thinning and self-healing. The P2 variants exhibited higher storage moduli than the P1 variants, demonstrating the ability to tune the hydrogel bulk properties through a biomimetic peptide-avidity strategy. The 20kDa PEG variants exhibited slower release of encapsulated vascular endothelial growth factor (VEGF), due to a decrease in hydrogel mesh size relative to the 40kDa variants. Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) adopted a well-spread morphology within three-dimensional MITCH-PEG cultures, and MITCH-PEG provided significant protection from cell damage during ejection through a fine-gauge syringe needle. In a mouse hindlimb ischemia model of peripheral arterial disease, MITCH-PEG co-delivery of hiPSC-ECs and VEGF was found

to reduce inflammation and promote muscle tissue regeneration compared to a saline control.

Munsie, M. J., et al. (2000). "Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei." Curr Biol **10**(16): 989-992.

Pluripotent human stem cells isolated from early embryos represent a potentially unlimited source of many different cell types for cell-based gene and tissue therapies [1-3]. Nevertheless, if the full potential of cell lines derived from donor embryos is to be realised, the problem of donor-recipient tissue matching needs to be overcome. One approach, which avoids the problem of transplant rejection, would be to establish stem cell lines from the patient's own cells through therapeutic cloning [3,4]. Recent studies have shown that it is possible to transfer the nucleus from an adult somatic cell to an unfertilised oocyte that is devoid of maternal chromosomes, and achieve embryonic development under the control of the transferred nucleus [5-7]. Stem cells isolated from such a cloned embryo would be genetically identical to the patient and pose no risk of immune rejection. Here, we report the isolation of pluripotent murine stem cells from reprogrammed adult somatic cell nuclei. Embryos were generated by direct injection of mechanically isolated cumulus cell nuclei into mature oocytes. Embryonic stem (ES) cells isolated from cumulus-cell-derived blastocysts displayed the characteristic morphology and marker expression of conventional ES cells and underwent extensive differentiation into all three embryonic germ layers (endoderm, mesoderm and ectoderm) in tumours and in chimaeric foetuses and pups. The ES cells were also shown to differentiate readily into neurons and muscle in culture. This study shows that pluripotent stem cells can be derived from nuclei of terminally differentiated adult somatic cells and offers a model system for the development of therapies that rely on autologous, human pluripotent stem cells.

Munst, S., et al. (2018). "In vitro segregation and isolation of human pluripotent stem cell-derived neural crest cells." Methods **133**: 65-80.

The neural crest (NC) is a transient embryonic cell population with remarkable characteristics. After delaminating from the neural tube, NC cells (NCCs) migrate extensively, populate nearly every tissue of the body and differentiate into highly diverse cell types such as peripheral neurons and glia, but also mesenchymal cells including chondrocytes, osteocytes, and adipocytes. While the NC has been extensively studied in several animal models, little is known about human NC development. A number of methods have been established to derive NCCs in vitro from human

pluripotent stem cells (hPSC). Typically, these protocols comprise several cell culture steps to enrich for NCCs in the neural derivatives of the differentiating hPSCs. Here we report on a remarkable and hitherto unnoticed in vitro segregation phenomenon that enables direct extraction of virtually pure NCCs during the earliest stages of hPSC differentiation. Upon aggregation to embryoid bodies (EB) and replating, differentiating hPSCs give rise to a population of NCCs, which spontaneously segregate from the EB outgrowth to form conspicuous, macroscopically visible atoll-shaped clusters in the periphery of the EB outgrowth. Isolation of these NC clusters yields p75NTR (+)/SOXE (+) NCCs, which differentiate to peripheral neurons and glia as well as mesenchymal derivatives. Our data indicate that differentiating hPSC cultures recapitulate, in a simplified manner, the physical segregation of central nervous system (CNS) tissue and NCCs. This phenomenon may be exploited for NCC purification and for studying segregation and differentiation processes observed during early human NC development in vitro.

Nagamatsu, G. and T. Suda (2013). "Conversion of primordial germ cells to pluripotent stem cells: methods for cell tracking and culture conditions." Methods Mol Biol **1052**: 49-56.

Primordial germ cells (PGCs) are unipotent cells committed to germ lineage: PGCs can only differentiate into gametes in vivo. However, upon fertilization, germ cells acquire the capacity to differentiate into all cell types in the body, including germ cells. Therefore, germ cells are thought to have the potential for pluripotency. PGCs can convert to pluripotent stem cells in vitro when cultured under specific conditions that include bFGF, LIF, and the membrane-bound form of SCF (mSCF). Here, the culture conditions which efficiently convert PGCs to pluripotent embryonic germ (EG) cells are described, as well as methods used for identifying pluripotent candidate cells during culture.

Nagamoto, Y., et al. (2015). "Efficient Engraftment of Human Induced Pluripotent Stem Cell-Derived Hepatocyte-Like Cells in uPA/SCID Mice by Overexpression of FNK, a Bcl-xL Mutant Gene." Cell Transplant **24**(6): 1127-1138.

Human liver chimeric mice are expected to be applied for drug toxicity tests and human hepatitis virus research. Human induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs) are a highly attractive donor source for the generation of human liver chimeric mice because they can be produced on a large scale and established from an individual. Although these cells have been

successfully used to generate human liver chimeric mice, there is still room for improvement in the repopulation efficiency. To enhance the repopulation efficacy, the human iPSC-HLCs were transduced with an adenovirus vector (Ad-FNK) expressing FNK, a hyperactive mutant gene from Bcl-xL, which was expected to inhibit apoptosis in the process of integration into liver parenchyma. We then transplanted Ad-FNK-transduced human iPSC-HLCs into urokinase-type plasminogen activator-transgenic severe combined immunodeficiency (uPA/SCID) mice (FNK mice) and evaluated the repopulation efficacy. The antiapoptotic effects of the human iPSC-HLCs were enhanced by FNK overexpression in vitro. Human albumin levels in the transplanted mice were significantly increased by transplantation of Ad-FNK-transduced human iPSC-HLCs (about 24,000 ng/ml). Immunohistochemical analysis with an anti-human alphaAT antibody revealed greater repopulation efficacy in the livers of FNK mice than control mice. Interestingly, the expression levels of human hepatocyte-related genes in the human iPSC-HLCs of FNK mice were much higher than those in the human iPSC-HLCs before transplantation. We succeeded in improving the repopulation efficacy of human liver chimeric mice generated by transplanting the Ad-FNK-transduced human iPSC-HLCs into uPA/SCID mice. Our method using ectopic expression of FNK was useful for generating human chimeric mice with high chimerism.

Nagamoto, Y., et al. (2012). "The promotion of hepatic maturation of human pluripotent stem cells in 3D co-culture using type I collagen and Swiss 3T3 cell sheets." Biomaterials **33**(18): 4526-4534.

Hepatocyte-like cells differentiated from human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) are known to be a useful cell source for drug screening. We recently developed an efficient hepatic differentiation method from hESCs and hiPSCs by sequential transduction of FOXA2 and HNF1alpha. It is known that the combination of three-dimensional (3D) culture and co-culture, namely 3D co-culture, can maintain the functions of primary hepatocytes. However, hepatic maturation of hESC- or hiPSC-derived hepatocyte-like cells (hEHs or hiPHs, respectively) by 3D co-culture systems has not been examined. Therefore, we utilized a cell sheet engineering technology to promote hepatic maturation. The gene expression levels of hepatocyte-related markers (such as cytochrome P450 enzymes and conjugating enzymes) and the amount of albumin secretion in the hEHs or hiPHs, which were 3D co-cultured with the Swiss 3T3 cell sheet, were significantly up-regulated in comparison with those in the hEHs or hiPHs cultured in a monolayer.

Furthermore, we found that type I collagen synthesized in Swiss 3T3 cells plays an important role in hepatic maturation. The hEHs or hiPHs that were 3D co-cultured with the Swiss 3T3 cell sheet would be powerful tools for medical applications, such as drug screening.

Nagasaka, R., et al. (2017). "Image-based cell quality evaluation to detect irregularities under same culture process of human induced pluripotent stem cells." J Biosci Bioeng **123**(5): 642-650.

To meet the growing demand for human induced pluripotent stem cells (iPSCs) for various applications, technologies that enable the manufacturing of iPSCs on a large scale should be developed. There are several technological challenges in iPSC manufacturing technology. Image-based cell quality evaluation technology for monitoring iPSC quality in culture enables the manufacture of intact cells for further applications. Although several studies have reported the effectiveness of image-based evaluation of iPSCs, it remains challenging to detect irregularities that may arise using the same processing operations during quality evaluation of automated processing. In this study, we investigated the evaluation performance of image-based cell quality analysis in detecting small differences that can result from human measurement, even when the same protocol is followed. To imitate such culture conditions, by image-analysis guided colony pickup, we changed the proportions of morphologically different subpopulations: "good morphology, regular morphology correlated with undifferentiation marker expression" and "bad morphology, irregular morphology correlated with loss of undifferentiation marker expression". In addition, comprehensive gene-expression and metabolomics analyses were carried out for the same samples to investigate performance differences. Our data shows an example of investigating the usefulness and sensitivity of quality evaluation methods for iPSC quality monitoring.

Nakamura, M. and H. Okano (2013). "Cell transplantation therapies for spinal cord injury focusing on induced pluripotent stem cells." Cell Res **23**(1): 70-80.

Stimulated by the 2012 Nobel Prize in Physiology or Medicine awarded for Shinya Yamanaka and Sir John Gurdon, there is an increasing interest in the induced pluripotent stem (iPS) cells and reprograming technologies in medical science. While iPS cells are expected to open a new era providing enormous opportunities in biomedical sciences in terms of cell therapies and regenerative medicine, safety-related concerns for iPS cell-based cell therapy should be resolved prior to the clinical application of

iPS cells. In this review, the pre-clinical investigations of cell therapy for spinal cord injury (SCI) using neural stem/progenitor cells derived from iPS cells, and their safety issues in vivo, are outlined. We also wish to discuss the strategy for the first human trails of iPS cell-based cell therapy for SCI patients.

Nakamura, S., et al. (2014). "Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells." Cell Stem Cell **14**(4): 535-548.

The donor-dependent supply of platelets is frequently insufficient to meet transfusion needs. To address this issue, we developed a clinically applicable strategy for the derivation of functional platelets from human pluripotent stem cells (PSCs). This approach involves the establishment of stable immortalized megakaryocyte progenitor cell lines (imMKCLs) from PSC-derived hematopoietic progenitors through the overexpression of BMI1 and BCL-XL to respectively suppress senescence and apoptosis and the constrained overexpression of c-MYC to promote proliferation. The resulting imMKCLs can be expanded in culture over extended periods (4-5 months), even after cryopreservation. Halting the overexpression of c-MYC, BMI1, and BCL-XL in growing imMKCLs led to the production of CD42b (+) platelets with functionality comparable to that of native platelets on the basis of a range of assays in vitro and in vivo. The combination of robust expansion capacity and efficient platelet production means that appropriately selected imMKCL clones represent a potentially inexhaustible source of hPSC-derived platelets for clinical application.

Nakane, T., et al. (2017). "Impact of Cell Composition and Geometry on Human Induced Pluripotent Stem Cells-Derived Engineered Cardiac Tissue." Sci Rep **7**: 45641.

The current study describes a scalable, porous large-format engineered cardiac tissue (LF-ECT) composed of human induced pluripotent stem cells (hiPSCs) derived multiple lineage cardiac cells with varied 3D geometries and cell densities developed towards the goal of scale-up for large animal pre-clinical studies. We explored multiple 15 x 15 mm ECT geometries using molds with rectangular internal staggered posts (mesh, ME), without posts (plain sheet, PS), or long parallel posts (multiple linear bundles, ML) and a gel matrix containing hiPSC-derived cardiomyocytes, endothelial, and vascular mural cells matured in vitro for 14 days. ME-ECTs displayed the lowest dead cell ratio (p < 0.001) and matured into 0.5 mm diameter myofiber bundles with greater 3D cell alignment and higher active stress than PS-ECTs. Increased initial ECT cell number beyond 6 M per

construct resulted in reduced cell survival and lower active stress. The 6M-ME-ECTs implanted onto 1 week post-infarct immune tolerant rat hearts engrafted, displayed evidence for host vascular coupling, and recovered myocardial structure and function with reduced scar area. We generated a larger (30 x 30 mm) ME-ECT to confirm scalability. Thus, large-format ECTs generated from hiPSC-derived cardiac cells may be feasible for large animal preclinical cardiac regeneration paradigms.

Nakayama, C., et al. (2018). "The development of induced pluripotent stem cell-derived mesenchymal stem/stromal cells from normal human and RDEB epidermal keratinocytes." J Dermatol Sci **91**(3): 301-310.

BACKGROUND: Epidermolysis bullosa (EB) is a group of hereditary disorders caused by mutations in the genes encoding structural molecules of the dermal-epidermal junction (DEJ). Cell-based therapies such as allogeneic mesenchymal stem/stromal cell (MSC) transplantation have recently been explored for severe EB types, such as recessive dystrophic EB (RDEB). However, hurdles exist in current MSC-based therapies, such as limited proliferation from a single cell source and limited cell survival due to potential allogenic rejection. OBJECTIVES: We aimed to develop MSCs from keratinocyte-derived induced pluripotent stem cells (iPSCs). METHODS: Keratinocyte-derived iPSCs (KC-iPSCs) of a healthy human and an RDEB patient were cultured with activin A, 6-bromoindirubin-3'-oxime and bone morphogenetic protein 4 to induce mesodermal lineage formation. These induced cells were subjected to immunohistochemical analysis, flow cytometric analysis and RNA microarray analysis in vitro, and were injected subcutaneously and intravenously to wounded immunodeficient mice to assess their wound-healing efficacy. RESULTS: After their induction, KC-iPSC-induced cells were found to be compatible with MSCs. Furthermore, with the subcutaneous and intravenous injection of the KC-iPSC-induced cells into wounded immunodeficient mice, human type VII collagen was detected at the DEJ of epithelized areas. CONCLUSIONS: We successfully established iPSC-derived MSCs from keratinocytes (KC-iPSC-MSCs) of a normal human and an RDEB patient. KC-iPSC-MSCs may have potential in therapies for RDEB.

Nakayama, M. (2009). "Cell Therapy Using Induced Pluripotent Stem (iPS) Cells Meets Next-Next Generation DNA Sequencing Technology." Curr Genomics **10**(5): 303-305.

The recent development of induced pluripotent stem (iPS) cell technology brings cell and gene therapies to patients one large step closer to reality.

Technical improvements in various research fields sometimes come together fortuitously, leading to approaches to treating disease. If iPS cell technology continues to progress smoothly as expected and is actually applied to patients, the next logical step to ensuring the success of iPS cell therapy is to make use of next-next generation DNA sequencing technology and bioinformatics of recipient genomes. Before a patient-derived iPS cell colony is used for clinical therapy in a patient, the colony should undergo whole-genome DNA sequencing, thus avoiding risks associated with spontaneously mutagenized iPS cells. Researchers participating in the Human Genome Project need to take full advantage of both technologies-iPS cell technology and DNA sequencing-as doing so will help us achieve the original long-term goal of the project: developing therapies that will benefit human health.

Narsinh, K. H., et al. (2011). "Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells." J Clin Invest **121**(3): 1217-1221.

Human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) are promising candidate cell sources for regenerative medicine. However, despite the common ability of hiPSCs and hESCs to differentiate into all 3 germ layers, their functional equivalence at the single cell level remains to be demonstrated. Moreover, single cell heterogeneity amongst stem cell populations may underlie important cell fate decisions. Here, we used single cell analysis to resolve the gene expression profiles of 362 hiPSCs and hESCs for an array of 42 genes that characterize the pluripotent and differentiated states. Comparison between single hESCs and single hiPSCs revealed markedly more heterogeneity in gene expression levels in the hiPSCs, suggesting that hiPSCs occupy an alternate, less stable pluripotent state. hiPSCs also displayed slower growth kinetics and impaired directed differentiation as compared with hESCs. Our results suggest that caution should be exercised before assuming that hiPSCs occupy a pluripotent state equivalent to that of hESCs, particularly when producing differentiated cells for regenerative medicine aims.

Natarajan, K. N., et al. (2017). "Single cell transcriptomics of pluripotent stem cells: reprogramming and differentiation." Curr Opin Genet Dev **46**: 66-76.

Single-cell transcriptomics serves as a powerful tool to identify cell states within populations of cells, and to dissect underlying heterogeneity at high resolution. Single-cell transcriptomics on pluripotent stem cells has provided new insights into cellular variation, subpopulation structures and the interplay of cell cycle with pluripotency. The single-cell perspective has helped to better understand gene regulation and regulatory networks during exit from pluripotency, cell-fate determination as well as molecular mechanisms driving cellular reprogramming of somatic cells to induced pluripotent stage. Here we review the recent progress and significant findings from application of single-cell technologies on pluripotent stem cells along with a brief outlook on new combinatorial single-cell approaches that further unravel pluripotent stem cell states.

Naujok, O. and S. Lenzen (2012). "[Pluripotent stem cells for cell replacement therapy of diabetes]." Dtsch Med Wochenschr **137**(20): 1062-1066.

The use of pluripotent stem cells (PSCs) harbours great potential for a future use in the cell replacement therapy of diabetes mellitus. The in vitro differentiation of human or mouse embryonic stem cells has yielded pancreatic progenitor cells, but not authentic insulin-producing beta cells. Induced pluripotent cells are a class of a pluripotent stem cells potentially suited as a cell source for cell replacement therapy. These patient specific pluripotent cells are generated by reprogramming but unfortunately accumulate genetic and epigenetic errors during reprogramming, precluding their use for therapeutic purposes in humans.

Navara, C. S., et al. (2013). "Derivation of induced pluripotent stem cells from the baboon: a nonhuman primate model for preclinical testing of stem cell therapies." Cell Reprogram **15**(6): 495-502.

Development of effective pluripotent stem cell-based therapies will require safety and efficacy testing in a clinically relevant preclinical model such as nonhuman primates (NHPs). Baboons and macaques are equally similar to humans genetically and both have been extensively used for biomedical research. Macaques are preferred for human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) research whereas baboons are preferred for transplantation studies because of the greater similarity of their anatomy and immunogenetic system to those of humans. We generated four induced pluripotent stem cell (iPSC) lines from skin cells of the olive baboon (Papio anubis). Each line shows the distinct morphology of primate pluripotent stem cells, including flat colonies with well-defined borders and a high nuclear/cytoplasm ratio. Each is positive for the pluripotency markers OCT4, SOX2, NANOG, and SSEA4. Pluripotency was confirmed in two lines by teratoma formation with representative tissues from each germ layer, whereas a third produced cells from all three germ layers following embryoid body

differentiation. Three lines have a normal male karyotype and the fourth is missing the short arm of one copy of chromosome 18. This may serve as an in vitro model for the human developmental disorder 18p-, which impacts 1 in 50,000 births/year. These iPSC lines represent the first step toward establishing the baboon as a NHP model for developing stem cell-based therapies.

Nemade, H., et al. (2018). "Cell death mechanisms of the anti-cancer drug etoposide on human cardiomyocytes isolated from pluripotent stem cells." Arch Toxicol **92**(4): 1507-1524.

Etoposide (ETP) and anthracyclines are applied for wide anti-cancer treatments. However, the ETP-induced cardiotoxicity remains to be a major safety issue and the underlying cardiotoxic mechanisms are not well understood. This study is aiming to unravel the cardiotoxicity profile of ETP in comparison to anthracyclines using physiologically relevant human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). Using xCELLigence real-time cell analyser (RTCA), we found that single high dose of ETP induces irreversible increase in hPSC-CMs beating rate and decrease in beating amplitude. We also identified 58 deregulated genes consisting of 33 upregulated and 25 downregulated genes in hPSC-CMs after ETP treatment. Gene ontology (GO) and pathway analysis showed that most upregulated genes are enriched in GO categories like positive regulation of apoptotic process, regulation of cell death, and mitochondria organization, whereas most downregulated genes were enriched in GO categories like cytoskeletal organization, muscle contraction, and Ca (2+) ion homeostasis. Moreover, we also found upregulation in 5 miRNAs (has-miR-486-3p, has-miR-34c-5p, has-miR-4423-3p, has-miR-182-5p, and has-miR-139-5p) which play role in muscle contraction, arginine and proline metabolism, and hypertrophic cardiomyopathy (HCM). Immunostaining and transmission electron microscopy also confirmed the cytoskeletal and mitochondrial damage in hPSC-CMs treated with ETP, as well as noticeable alterations in intracellular calcium handling and mitochondrial membrane potential were also observed. The apoptosis inhibitor, Pifithrin-alpha, found to protect hPSC-CMs from ETP-induced cardiotoxicity, whereas hPSC-CMs treated with ferroptosis inhibitor, Liproxstatin-1, showed significant recovery in hPSC-CMs functional properties like beating rate and amplitude after ETP treatment. We suggest that the damage to mitochondria is a major contributing factor involved in ETP-induced cardiotoxicity and the activation of the p53-mediated ferroptosis pathway by ETP is likely the critical pathway in ETP-induced cardiotoxicity. We also conclude that the genomic biomarkers identified

in this study will significantly contribute to develop and predict potential cardiotoxic effects of novel anti-cancer drugs in vitro.

Ng, J., et al. (2016). "Immunomodulatory Properties of Induced Pluripotent Stem Cell-Derived Mesenchymal Cells." J Cell Biochem **117**(12): 2844-2853.

MSC-like populations derived from induced pluripotent stem cells (iPSC-MSC) serve as an alternative stem cell source due to their high proliferative capacity. In this study, we assessed the immunomodulatory potential of iPSC-MSC generated from periodontal ligament (PDL) and gingival (GF) tissue. The iPSC-MSC lines exhibited a similar level of suppression of mitogen-stimulated peripheral blood mononuclear cells (PBMNC) proliferation compared to their respective parental fibroblast populations in vitro. Moreover, iPSC-MSC demonstrated the ability to suppress T-cells effector cells, Th1/Th2/Th17 populations, and increase levels of Treg cells. In order to investigate the mechanisms involved, expression of common MSC-derived soluble factors known to supress lymphocyte proliferation were assessed in iPSC-MSC cultured with PBMNC with direct cell-cell contact or separated in transwells. Real-time PCR analysis of factors known to be involved in MSC mediated immune regulation, found a general trend of elevated IDO1 and IL6 transcript levels in iPSC-MSC lines and their respective primary cells co-cultured with activated PBMNC, with a wide range of gene expression levels between the different mesenchymal cell types. The results suggest that different iPSC-MSC may be useful as a potential alternative source of cells for future clinical use in therapeutic applications because of their potent immunosuppressive properties. J. Cell. Biochem. 117: 2844-2853, 2016. (c) 2016 Wiley Periodicals, Inc.

Nguyen, Q. H., et al. (2018). "Single-cell RNA-seq of human induced pluripotent stem cells reveals cellular heterogeneity and cell state transitions between subpopulations." Genome Res **28**(7): 1053-1066.

Heterogeneity of cell states represented in pluripotent cultures has not been described at the transcriptional level. Since gene expression is highly heterogeneous between cells, single-cell RNA sequencing can be used to identify how individual pluripotent cells function. Here, we present results from the analysis of single-cell RNA sequencing data from 18,787 individual WTC-CRISPRi human induced pluripotent stem cells. We developed an unsupervised clustering method and, through this, identified four subpopulations distinguishable on the basis of their pluripotent state, including a core

pluripotent population (48.3%), proliferative (47.8%), early primed for differentiation (2.8%), and late primed for differentiation (1.1%). For each subpopulation, we were able to identify the genes and pathways that define differences in pluripotent cell states. Our method identified four transcriptionally distinct predictor gene sets composed of 165 unique genes that denote the specific pluripotency states; using these sets, we developed a multigenic machine learning prediction method to accurately classify single cells into each of the subpopulations. Compared against a set of established pluripotency markers, our method increases prediction accuracy by 10%, specificity by 20%, and explains a substantially larger proportion of deviance (up to threefold) from the prediction model. Finally, we developed an innovative method to predict cells transitioning between subpopulations and support our conclusions with results from two orthogonal pseudotime trajectory methods.

Nissenbaum, J., et al. (2013). "Global indiscriminate methylation in cell-specific gene promoters following reprogramming into human induced pluripotent stem cells." Stem Cell Reports **1**(6): 509-517.

Molecular reprogramming of somatic cells into human induced pluripotent stem cells (iPSCs) is accompanied by extensive changes in gene expression patterns and epigenetic marks. To better understand the link between gene expression and DNA methylation, we have profiled human somatic cells from different embryonic cell types (endoderm, mesoderm, and parthenogenetic germ cells) and the iPSCs generated from them. We show that reprogramming is accompanied by extensive DNA methylation in CpG-poor promoters, sparing CpG-rich promoters. Intriguingly, methylation in CpG-poor promoters occurred not only in downregulated genes, but also in genes that are not expressed in the parental somatic cells or their respective iPSCs. These genes are predominantly tissue-specific genes of other cell types from different lineages. Our results suggest a role of DNA methylation in the silencing of the somatic cell identity by global nonspecific methylation of tissue-specific genes from all lineages, regardless of their expression in the parental somatic cells.

Niu, Z., et al. (2013). "Germ-like cell differentiation from induced pluripotent stem cells (iPSCs)." Cell Biochem Funct **31**(1): 12-19.

Historically, our understanding of molecular genetic aspects of germ cell development has been limited. Recently, results demonstrated that the derivation of pluripotent stem cells may provide the necessary genetic system to study germ cell development. Here, we characterized an induced pluripotent stem cell (iPSC) line, which can spontaneously differentiate into embryonic bodies (EBs) after 3 days of suspension culture, expressing specific markers of three germ layers. Then, we induced the iPSCs to differentiate into germ cells by culturing adherent EBs in retinoic acid (RA) and porcine follicular fluid (PFF) differentiation medium or seminiferous tubule transplantation. Our results indicated that RA and PFF were beneficial for the derivation of germ cells and oocyte-like cells from iPSCs, and iPSCs transplantation could make a contribution to repairing the testis of infertile mice. Our study offers an approach for further study on the development and the differentiation of germ cells derived from iPSCs.

No, J. G., et al. (2015). "Cell-free extract from porcine induced pluripotent stem cells can affect porcine somatic cell nuclear reprogramming." J Reprod Dev **61**(2): 90-98.

Pretreatment of somatic cells with undifferentiated cell extracts, such as embryonic stem cells and mammalian oocytes, is an attractive alternative method for reprogramming control. The properties of induced pluripotent stem cells (iPSCs) are similar to those of embryonic stem cells; however, no studies have reported somatic cell nuclear reprogramming using iPSC extracts. Therefore, this study aimed to evaluate the effects of porcine iPSC extracts treatment on porcine ear fibroblasts and early development of porcine cloned embryos produced from porcine ear skin fibroblasts pretreated with the porcine iPSC extracts. The Chariot (TM) reagent system was used to deliver the iPSC extracts into cultured porcine ear skin fibroblasts. The iPSC extracts-treated cells (iPSC-treated cells) were cultured for 3 days and used for analyzing histone modification and somatic cell nuclear transfer. Compared to the results for nontreated cells, the trimethylation status of histone H3 lysine residue 9 (H3K9) in the iPSC-treated cells significantly decreased. The expression of Jmjd2b, the H3K9 trimethylation-specific demethylase gene, significantly increased in the iPSC-treated cells; conversely, the expression of the proapoptotic genes, Bax and p53, significantly decreased. When the iPSC-treated cells were transferred into enucleated porcine oocytes, no differences were observed in blastocyst development and total cell number in blastocysts compared with the results for control cells. However, H3K9 trimethylation of pronuclear-stage-cloned embryos significantly decreased in the iPSC-treated cells. Additionally, Bax and p53 gene expression in the blastocysts was significantly lower in iPSC-treated cells than in control cells. To our knowledge, this study is the first to show that an extracts of porcine

iPSCs can affect histone modification and gene expression in porcine ear skin fibroblasts and cloned embryos.

Nomura, Y., et al. (2012). "Human periodontal ligament fibroblasts are the optimal cell source for induced pluripotent stem cells." Histochem Cell Biol **137**(6): 719-732.

Among the various kinds of fibroblasts existing in the human body, the periodontal ligament (PDL) fibroblasts have been suggested as multipotent cells. Periodontal ligament fibroblasts are characterized by rapid turnover, a high remodeling capacity and remarkable capacity for renewal and repair. They also differentiate into osteoblasts and cementoblasts. We established iPS cells from human PDL fibroblasts by introducing the ES cell markers Oct3/4, Sox2, Nanog, Klf4 and Lin28 by retrovirus transduction, even without the oncogene c-Myc. The iPS cells established in this study expressed the ES cell markers and formed teratomas in SCID mice. The c-Myc expression level in the PDL fibroblasts was higher than that in the iPS cells by quantitative RT-PCR. Therefore, we have concluded that PDL fibroblasts could be an optimal cell source for iPS cells.

Nong, K., et al. (2016). "Hepatoprotective effect of exosomes from human-induced pluripotent stem cell-derived mesenchymal stromal cells against hepatic ischemia-reperfusion injury in rats." Cytotherapy **18**(12): 1548-1559.

BACKGROUND: This study aimed to evaluate the effect of exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) on hepatic ischemia-reperfusion (I/R) injury. METHODS: Exosomes were isolated and concentrated from conditioned medium using ultracentrifugation and ultrafiltration. hiPSC-MSCs-Exo were injected systemically via the inferior vena cava in a rat model of 70% warm hepatic I/R injury, and the therapeutic effect was evaluated. The serum levels of transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were measured using an automatic analyzer. The expression of inflammatory factors was measured using enzyme-linked immunosorbent assay (ELISA). Histological changes indicated changes in pathology and inflammatory infiltration in liver tissue. Apoptosis of hepatic cells in liver tissue was measured using terminal-deoxynucleoitidyl transferase mediated nick end labeling (TUNEL) staining along with apoptotic markers. RESULTS: hiPSCs were efficiently induced into hiPSC-MSCs with typical MSC characteristics. hiPSC-MSCs-Exo had diameters ranging from 50 to 60 nm and expressed exosomal markers (CD9, CD63 and CD81). Hepatocyte necrosis and sinusoidal congestion were markedly suppressed with a lower Suzuki score after hiPSC-MSCs-Exo administration. The levels of the hepatocyte injury markers AST and ALT were significantly lower in the treated group than in the control group. Inflammatory markers, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and high mobility group box 1 (HMGB1), were significantly reduced after administration of hiPSC-MSCs-Exo, which suggests that the exosomes have a role in suppressing the inflammatory response. Additionally, in liver tissues from the experimental group, the levels of apoptotic markers, such as caspase-3 and bax, were significantly lower and the levels of oxidative markers, such as glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), were significantly higher than in the control group. These data point to an anti-apoptotic, anti-oxidative stress response role for hiPSC-MSCs-Exo. CONCLUSIONS: Our results demonstrated that hiPSC-MSCs-Exo alleviate hepatic I/R injury, possibly via suppression of inflammatory responses, attenuation of the oxidative stress response and inhibition of apoptosis.

Nurnberg, E., et al. (2018). "Basal glucocorticoid receptor activation induces proliferation and inhibits neuronal differentiation of human induced pluripotent stem cell-derived neuronal precursor cells." J Steroid Biochem Mol Biol **182**: 119-126.

Glucocorticoids (GC) have first been shown to originate from the adrenal glands where synthesis and release is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. Recently, it was shown that GC and other steroid hormones are also synthesized in the central nervous system, so-called neurosteroids. GC bind to specific GC receptors (GR) which function as ligand-activated transcription factors. GR are expressed in nearly all cell types in the brain, and therefore GC have a strong impact on neuronal development. Most knowledge of the influence of GC on neurodevelopment has been obtained from animal research. Recent advances in stem cell technology made it possible to generate neuronal precursor cells (NPCs) and neurons from human induced pluripotent stem cells (hiPSCs). To explore the cellular mechanism of GC affecting human neuronal development, we quantified the proliferation and differentiation of hiPSCs-derived NPCs in the absence and presence of the selective high-affinity GR agonist dexamethasone and the selective GR antagonist mifepristone, respectively. Our results show that inhibition of GR significantly reduced proliferation of NPCs and promoted differentiation whereas GR activation suppressed neuronal differentiation. This implies that neuronal GC must be present in NPCs for proliferation. Consequently we identified the presence

of 11-beta-hydroxylase CYP11B1, which hydroxylates the respective steroid precursors to bioactive GC, in NPCs. We propose that hiPSC technology offers an ideal system to get more insight into the synthesising and regulatory pathways in steroidogenesis in human neurons and to differentiate between the mechanism by which adrenal GC and neuronal GC impact on neurodevelopment.

O'Brien, C. M., et al. (2017). "New Monoclonal Antibodies to Defined Cell Surface Proteins on Human Pluripotent Stem Cells." Stem Cells **35**(3): 626-640.

The study and application of human pluripotent stem cells (hPSCs) will be enhanced by the availability of well-characterized monoclonal antibodies (mAbs) detecting cell-surface epitopes. Here, we report generation of seven new mAbs that detect cell surface proteins present on live and fixed human ES cells (hESCs) and human iPS cells (hiPSCs), confirming our previous prediction that these proteins were present on the cell surface of hPSCs. The mAbs all show a high correlation with POU5F1 (OCT4) expression and other hPSC surface markers (TRA-160 and SSEA-4) in hPSC cultures and detect rare OCT4 positive cells in differentiated cell cultures. These mAbs are immunoreactive to cell surface protein epitopes on both primed and naive state hPSCs, providing useful research tools to investigate the cellular mechanisms underlying human pluripotency and states of cellular reprogramming. In addition, we report that subsets of the seven new mAbs are also immunoreactive to human bone marrow-derived mesenchymal stem cells (MSCs), normal human breast subsets and both normal and tumorigenic colorectal cell populations. The mAbs reported here should accelerate the investigation of the nature of pluripotency, and enable development of robust cell separation and tracing technologies to enrich or deplete for hPSCs and other human stem and somatic cell types. Stem Cells 2017;35:626-640.

Ohgushi, M., et al. (2010). "Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells." Cell Stem Cell **7**(2): 225-239.

Human embryonic stem cells (hESCs), unlike mouse ones (mESCs), are vulnerable to apoptosis upon dissociation. Here, we show that the apoptosis, which is of a nonanoikis type, is caused by ROCK-dependent hyperactivation of actomyosin and efficiently suppressed by the myosin inhibitor Blebbistatin. The actomyosin hyperactivation is triggered by the loss of E-cadherin-dependent intercellular contact and also observed in dissociated mouse epiblast-derived pluripotent cells but not in mESCs. We reveal that Abr, a unique Rho-GEF family

factor containing a functional Rac-GAP domain, is an indispensable upstream regulator of the apoptosis and ROCK/myosin hyperactivation. Rho activation coupled with Rac inhibition is induced in hESCs upon dissociation, but not in Abr-depleted hESCs or mESCs. Furthermore, artificial Rho or ROCK activation with Rac inhibition restores the vulnerability of Abr-depleted hESCs to dissociation-induced apoptosis. Thus, the Abr-dependent "Rho-high/Rac-low" state plays a decisive role in initiating the dissociation-induced actomyosin hyperactivation and apoptosis in hESCs.

Ohgushi, M. and Y. Sasai (2011). "Lonely death dance of human pluripotent stem cells: ROCKing between metastable cell states." Trends Cell Biol **21**(5): 274-282.

Two kinds of human pluripotent cells, human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), promise new avenues for medical innovation. These human cells share many similarities with mouse counterparts, including pluripotency, and they exhibit several unique properties. This review examines the diversity of mammalian pluripotent cells from a perspective of metastable pluripotency states. An intriguing phenomenon unique to human pluripotent stem cells is dissociation-induced apoptosis, which has been a technical problem for various cellular manipulations. The discovery that this apoptosis is suppressed by ROCK inhibitors brought revolutionary change to this troublesome situation. We discuss possible links of the metastable pluripotent state to ROCK-dependent human embryonic stem cell apoptosis and summarize recent progress in molecular understandings of this phenomenon.

Ohnishi, H., et al. (2015). "Limited hair cell induction from human induced pluripotent stem cells using a simple stepwise method." Neurosci Lett **599**: 49-54.

Disease-specific induced pluripotent stem cells (iPS) cells are expected to contribute to exploring useful tools for studying the pathophysiology of inner ear diseases and to drug discovery for treating inner ear diseases. For this purpose, stable induction methods for the differentiation of human iPS cells into inner ear hair cells are required. In the present study, we examined the efficacy of a simple induction method for inducing the differentiation of human iPS cells into hair cells. The induction of inner ear hair cell-like cells was performed using a stepwise method mimicking inner ear development. Human iPS cells were sequentially transformed into the preplacodal ectoderm, otic placode, and hair cell-like cells. As a first step, preplacodal ectoderm induction, human iPS

cells were seeded on a Matrigel-coated plate and cultured in a serum free N2/B27 medium for 8 days according to a previous study that demonstrated spontaneous differentiation of human ES cells into the preplacodal ectoderm. As the second step, the cells after preplacodal ectoderm induction were treated with basic fibroblast growth factor (bFGF) for induction of differentiation into otic-placode-like cells for 15 days. As the final step, cultured cells were incubated in a serum free medium containing Matrigel for 48 days. After preplacodal ectoderm induction, over 90% of cultured cells expressed the genes that express in preplacodal ectoderm. By culture with bFGF, otic placode marker-positive cells were obtained, although their number was limited. Further 48-day culture in serum free media resulted in the induction of hair cell-like cells, which expressed a hair cell marker and had stereocilia bundle-like constructions on their apical surface. Our results indicate that hair cell-like cells are induced from human iPS cells using a simple stepwise method with only bFGF, without the use of xenogeneic cells.

Ohtani-Kaneko, R., et al. (2017). "Characterisation of human induced pluripotent stem cell-derived endothelial cells under shear stress using an easy-to-use microfluidic cell culture system." Biomed Microdevices **19**(4): 91.

Induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) can contribute to elucidating the pathogenesis of heart and vascular diseases and developing their treatments. Their precise characteristics in fluid flow however remain unclear. Therefore, the aim of the present study is to characterise these features. We cultured three types of ECs in a microfluidic culture system: commercially available human iPS-ECs, human umbilical vein endothelial cells (HUVECs) and human umbilical artery endothelial cells (HUAECs). We then examined the mRNA expression levels of endothelial marker gene cluster of differentiation 31 (CD31), fit-related receptor tyrosine kinase (Flk-1), and the smooth muscle marker gene smooth muscle alpha-actin, and investigated changes in plasminogen activator inhibitor-1 (PAI-1) secretion and intracellular F-actin arrangement following heat stress. We also compared expressions of the arterial and venous marker genes ephrinB2 and EphB4, and the endothelial gap junction genes connexin (Cx) 37, 40, and 43 under fluidic shear stress to determine their arterial or venous characteristics. We found that iPS-ECs had similar endothelial marker gene expressions and exhibited similar increases in PAI-1 secretion under heat stress as HUVECs and HUAECs. In addition, F-actin arrangement in iPSC-ECs also responded to heat stress, as previously reported. However, they had different

expression patterns of arterial and venous marker genes and Cx genes under different fluidic shear stress levels, showing that iPSC-ECs exhibit different characteristics from arterial and venous ECs. This microfluidic culture system equipped with variable shear stress control will provide an easy-to-use assay tool to examine characteristics of iPS-ECs generated by different protocols in various laboratories and contribute to basic and applied biomedical researches on iPS-ECs.

Okada, A., et al. (2016). "Selective Differentiation into Hematopoietic and Cardiac Cells from Pluripotent Stem Cells Based on the Expression of Cell Surface Markers." Methods Mol Biol **1341**: 181-195.

Flk1-expressing (+) mesodermal cells are useful source for the generation of hematopoietic cells and cardiomyocytes from pluripotent stem cells (PSCs). However, they have been reported as a heterogenous population that includes hematopoietic and cardiac progenitors. Therefore, to provide a method for a highly efficient production of hematopoietic cells and cardiomyocytes, cell surface markers are often used for separating these progenitors in Flk1(+) cells. Our recent study has shown that the expression of coxsackievirus and adenovirus receptor (CAR), a tight junction component molecule, could divide mouse and human PSC- and mouse embryo-derived Flk1(+) cells into Flk1(+)CAR (-) and Flk1(+)CAR (+) cells. Flk1(+)CAR (-) and Flk1(+)CAR (+) cells efficiently differentiated into hematopoietic cells and cardiomyocytes, respectively. These results indicate that CAR is a novel cell surface marker for separating PSC-derived Flk1(+) mesodermal cells into hematopoietic and cardiac progenitors. We herein describe a differentiation method from PSCs into hematopoietic cells and cardiomyocytes based on CAR expression.

Okano, H., et al. (2013). "Steps toward safe cell therapy using induced pluripotent stem cells." Circ Res **112**(3): 523-533.

The enthusiasm for producing patient-specific human embryonic stem cells using somatic nuclear transfer has somewhat abated in recent years because of ethical, technical, and political concerns. However, the interest in generating induced pluripotent stem cells (iPSCs), in which pluripotency can be obtained by transcription factor transduction of various somatic cells, has rapidly increased. Human iPSCs are anticipated to open enormous opportunities in the biomedical sciences in terms of cell therapies for regenerative medicine and stem cell modeling of human disease. On the other hand, recent reports have emphasized the pitfalls of iPSC technology, including

the potential for genetic and epigenetic abnormalities, tumorigenicity, and immunogenicity of transplanted cells. These constitute serious safety-related concerns for iPSC-based cell therapy. However, preclinical data supporting the safety and efficacy of iPSCs are also accumulating. In this Review, recent achievements and future tasks for safe iPSC-based cell therapy are summarized, using regenerative medicine for repair strategies in the damaged central nervous system (CNS) as a model. Insights on safety and preclinical use of iPSCs in cardiovascular repair model are also discussed.

Okeyo, K. O., et al. (2015). "Cell Adhesion Minimization by a Novel Mesh Culture Method Mechanically Directs Trophoblast Differentiation and Self-Assembly Organization of Human Pluripotent Stem Cells." Tissue Eng Part C Methods **21**(10): 1105-1115.

Mechanical methods for inducing differentiation and directing lineage specification will be instrumental in the application of pluripotent stem cells. Here, we demonstrate that minimization of cell-substrate adhesion can initiate and direct the differentiation of human pluripotent stem cells (hiPSCs) into cyst-forming trophoblast lineage cells (TLCs) without stimulation with cytokines or small molecules. To precisely control cell-substrate adhesion area, we developed a novel culture method where cells are cultured on microstructured mesh sheets suspended in a culture medium such that cells on mesh are completely out of contact with the culture dish. We used microfabricated mesh sheets that consisted of open meshes (100 approximately 200 mum in pitch) with narrow mesh strands (3-5 mum in width) to provide support for initial cell attachment and growth. We demonstrate that minimization of cell adhesion area achieved by this culture method can trigger a sequence of morphogenetic transformations that begin with individual hiPSCs attached on the mesh strands proliferating to form cell sheets by self-assembly organization and ultimately differentiating after 10-15 days of mesh culture to generate spherical cysts that secreted human chorionic gonadotropin (hCG) hormone and expressed caudal-related homeobox 2 factor (CDX2), a specific marker of trophoblast lineage. Thus, this study demonstrates a simple and direct mechanical approach to induce trophoblast differentiation and generate cysts for application in the study of early human embryogenesis and drug development and screening.

Onuma, Y., et al. (2013). "rBC2LCN, a new probe for live cell imaging of human pluripotent stem cells." Biochem Biophys Res Commun **431**(3): 524-529.

Cell surface biomarkers have been applied to discriminate pluripotent human embryonic stem cells and induced pluripotent stem cells from differentiated cells. Here, we demonstrate that a recombinant lectin probe, rBC2LCN, a new tool for fluorescence-based imaging and flow cytometry analysis of pluripotent stem cells, is an alternative to conventional pluripotent maker antibodies. Live or fixed colonies of both human embryonic stem cells and induced pluripotent stem cells were visualized in culture medium containing fluorescent dye-labeled rBC2LCN. Fluorescent dye-labeled rBC2LCN was also successfully used to separate live pluripotent stem cells from a mixed cell population by flow cytometry.

Oshima, K., et al. (2010). "Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells." Cell **141**(4): 704-716.

Mechanosensitive sensory hair cells are the linchpin of our senses of hearing and balance. The inability of the mammalian inner ear to regenerate lost hair cells is the major reason for the permanence of hearing loss and certain balance disorders. Here, we present a stepwise guidance protocol starting with mouse embryonic stem and induced pluripotent stem cells, which were directed toward becoming ectoderm capable of responding to otic-inducing growth factors. The resulting otic progenitor cells were subjected to varying differentiation conditions, one of which promoted the organization of the cells into epithelial clusters displaying hair cell-like cells with stereociliary bundles. Bundle-bearing cells in these clusters responded to mechanical stimulation with currents that were reminiscent of immature hair cell transduction currents.

Otsu, M., et al. (2014). "Pluripotent stem cell-derived neural stem cells: From basic research to applications." World J Stem Cells **6**(5): 651-657.

Basic research on pluripotent stem cells is designed to enhance understanding of embryogenesis, whereas applied research is designed to develop novel therapies and prevent diseases. Attainment of these goals has been enhanced by the establishment of embryonic stem cell lines, the technological development of genomic reprogramming to generate induced-pluripotent stem cells, and improvements in vitro techniques to manipulate stem cells. This review summarizes the techniques required to generate neural cells from pluripotent stem cells. In particular, this review describes current research applications of a simple neural differentiation method, the neural stem sphere method, which we developed.

Ouchi, T., et al. (2016). "LNGFR (+)THY-1(+) human pluripotent stem cell-derived neural crest-like

cells have the potential to develop into mesenchymal stem cells." Differentiation **92**(5): 270-280.

Mesenchymal stem cells (MSCs) are defined as non-hematopoietic, plastic-adherent, self-renewing cells that are capable of tri-lineage differentiation into bone, cartilage or fat in vitro. Thus, MSCs are promising candidates for cell-based medicine. However, classifications of MSCs have been defined retrospectively; moreover, this conventional criterion may be inaccurate due to contamination with other hematopoietic lineage cells. Human MSCs can be enriched by selection for LNGFR and THY-1, and this population may be analogous to murine PDGFRalpha (+)Sca-1(+) cells, which are developmentally derived from neural crest cells (NCCs). Murine NCCs were labeled by fluorescence, which provided definitive proof of neural crest lineage, however, technical considerations prevent the use of a similar approach to determine the origin of human LNGFR (+)THY-1(+) MSCs. To further clarify the origin of human MSCs, human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) were used in this study. Under culture conditions required for the induction of neural crest cells, human ESCs and iPSCs-derived cells highly expressed LNGFR and THY-1. These LNGFR (+)THY-1(+) neural crest-like cells, designated as LT-NCLCs, showed a strong potential to differentiate into both mesenchymal and neural crest lineages. LT-NCLCs proliferated to form colonies and actively migrated in response to serum concentration. Furthermore, we transplanted LT-NCLCs into chick embryos, and traced their potential for survival, migration and differentiation in the host environment. These results suggest that LNGFR (+)THY-1(+) cells identified following NCLC induction from ESCs/iPSCs shared similar potentials with multipotent MSCs.

Ould-Brahim, F., et al. (2018). "Metformin Preconditioning of Human Induced Pluripotent Stem Cell-Derived Neural Stem Cells Promotes Their Engraftment and Improves Post-Stroke Regeneration and Recovery." Stem Cells Dev **27**(16): 1085-1096.

While transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) shows therapeutic potential in animal stroke models, major concerns for translating hiPSC therapy to the clinic are efficacy and safety. Therefore, there is a demand to develop an optimal strategy to enhance the engraftment and regenerative capacity of transplanted hiPSC-NSCs to produce fully differentiated neural cells to replace lost brain tissues. Metformin, an FDA-approved drug, is an optimal neuroregenerative agent that not only promotes NSC proliferation but also drives NSCs toward differentiation. In this regard, we hypothesize that

preconditioning of hiPSC-NSCs with metformin before transplantation into the stroke-damaged brain will improve engraftment and regenerative capabilities of hiPSC-NSCs, ultimately enhancing functional recovery. In this study, we show that pretreatment of hiPSC-NSCs with metformin enhances the proliferation and differentiation of hiPSC-NSCs in culture. Furthermore, metformin-preconditioned hiPSC-NSCs show increased engraftment 1 week post-transplantation in a rat endothelin-1 focal ischemic stroke model. In addition, metformin-preconditioned cell grafts exhibit increased survival compared to naive cell grafts at 7 weeks post-transplantation. Analysis of the grafts demonstrates that metformin preconditioning enhances the differentiation of hiPSC-NSCs at the expense of their proliferation. As an outcome, rats receiving metformin-preconditioned cells display accelerated gross motor recovery and reduced infarct volume. These studies represent a vital step forward in the optimization of hiPSC-NSC-based transplantation to promote post-stroke recovery.

Ovchinnikov, D. A., et al. (2014). "Transgenic human ES and iPS reporter cell lines for identification and selection of pluripotent stem cells in vitro." Stem Cell Res **13**(2): 251-261.

Optimization of pluripotent stem cell expansion and differentiation is facilitated by biological tools that permit non-invasive and dynamic monitoring of pluripotency, and the ability to select for an undifferentiated input cell population. Here we report on the generation and characterisation of clonal human embryonic stem (HES3, H9) and human induced pluripotent stem cell lines (UQEW01i-epifibC11) that have been stably modified with an artificial EOS (C3+) promoter driving expression of EGFP and puromycin resistance-conferring proteins. We show that EGFP expression faithfully reports on the pluripotency status of the cells in these lines and that antibiotic selection allows for an efficient elimination of differentiated cells from the cultures. We demonstrate that the extinction of the expression of the pluripotency reporter during differentiation closely correlates with the decrease in expression of conventional pluripotency markers, such as OCT4 (POU5F1), TRA-1-60 and SSEA4 when screening across conditions with various levels of pluripotency-maintaining or differentiation-inducing signals. We further illustrate the utility of these lines for real-time monitoring of pluripotency in embryoid bodies and microfluidic bioreactors.

Ozawa, M., et al. (2014). "Development of FGF2-dependent pluripotent stem cells showing naive state characteristics from murine preimplantation inner cell mass." Stem Cell Res **13**(1): 75-87.

Two distinct types of embryonic pluripotent stem cells can be established from either the inner cell mass (ICM) of preimplantation blastocyst (leukemia inhibitory factor (LIF)-dependent embryonic stem cell, ESC, called naive state) or the epiblast of postimplantation fetuses (fibroblast growth factor 2 (FGF2)-dependent epiblast stem cells, EpiSC, called primed state). Here, we report that naive pluripotent stem cell was established from the ICM, but maintained its self-renewal by treatment with FGF2 and mouse embryonic fibroblasts (MEFs) when they were exposed FGF2 during establishment. This cell line is competent to contribute to chimeric animals, including germ cells, at high efficiency. The ERK1/2, SMAD2/3, and JAK/STAT3 pathways are essential to maintain self-renewal. Inhibition of ERK1/2 or SMAD2/3 initiates transition to a naive state ESC-like state, whereas inhibition of JAK/STAT3 promotes a primed EpiSC-like character. Our present results could provide novel insights into understanding the growth factor environment and ICM plasticity, and mechanisms which orchestrate the pluripotency of embryonic stem cells and the capacity for chimeric contributions.

Ozeki, N., et al. (2017). "Gelatin scaffold combined with bone morphogenetic protein-4 induces odontoblast-like cell differentiation involving integrin profile changes, autophagy-related gene 10, and Wnt5 sequentially in human induced pluripotent stem cells." Differentiation **93**: 1-14.

While human induced pluripotent stem (hiPS) cells have potential use in regenerative medicine, there are no reports on odontoblastic differentiation of hiPS cells. In the current study, to examine integrin profiles and explore the early signaling cascade of odontoblastic differentiation in hiPS cells, we investigated the regulation of autophagy-related gene (Atg) and wingless/int1 (Wnt) signaling in gelatin scaffold (GS) combined with bone morphogenetic protein (BMP)-4 (GS/BMP-4)-mediated odontoblastic differentiation. Following GS/BMP-4 treatment, there was a dramatic loss of alpha3 and alpha6 integrins, and reciprocal strong induction of alpha1 integrin expression in the differentiated cells. GS/BMP-4 increased the mRNA and protein levels of Atg10, Lrp5/Fzd9 (an Atg10 receptor), and Wnt5 together with the amount of autophagosomes and autophagic fluxes. Treatment with siRNAs against Atg10 and Wnt5a individually suppressed the GS/BMP-4-induced increase in odontoblastic differentiation. The odontoblastic phenotype was inhibited by chloroquine, but increased after treatment with rapamycin (an autophagy enhancer). Taken together with our previous findings, we have replicated our results from the rodent system in a novel human system. We have revealed a unique sequential cascade involving Atg10, Wnt5a, alpha1 integrin, and matrix metalloproteinase-3 in GS/BMP-4-induced differentiation of hiPS cells into odontoblast-like cells at a relatively early stage.

Ozeki, N., et al. (2017). "MicroRNA-211 and autophagy-related gene 14 signaling regulate osteoblast-like cell differentiation of human induced pluripotent stem cells." Exp Cell Res **352**(1): 63-74.

MicroRNAs (miRNAs) have been the subject of recent attention as key regulatory factors in cell differentiation. In the current study, to explore the early signaling cascade of osteogenic differentiation of human induced pluripotent stem (hiPS) cells, we investigated miR-211 regulation and autophagy-related gene (Atg) signaling in osteogenic differentiation. In addition to reciprocal strong induction of miR-211 expression in differentiated cells following osteogenic differentiation, we found abundant Argonaute 3 bound to miR-211. There were also dramatic increases in the mRNA and protein levels of Atg14 together with increases in the amount of autophagosomes as well as autophagic fluxes. While transfection of a miR-211 inhibitor abrogated the induction of Atg14, autophagy events, osteoblast differentiation markers, and induction of calcification were suppressed markedly. Treatment with small interfering RNAs against Atg14 also suppressed the osteogenic differentiation medium (ODM)-induced increase in osteogenic differentiation. The osteogenic phenotype was inhibited by chloroquine (an autophagy inhibitor), but increased after treatment with rapamycin (an autophagy inducer). Taken together with our previous findings, we have revealed a unique sequential cascade involving miR-211 and Atg14 in ODM-induced differentiation of hiPS cells into osteoblast-like cells at a relatively early stage.

Paik, D. T., et al. (2018). "Large-Scale Single-Cell RNA-Seq Reveals Molecular Signatures of Heterogeneous Populations of Human Induced Pluripotent Stem Cell-Derived Endothelial Cells." Circ Res.

Rationale: Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) have risen as a useful tool in cardiovascular research, offering a wide gamut of translational and clinical applications. However, inefficiency of the currently available iPSC-EC differentiation protocol and underlying heterogeneity of derived iPSC-ECs remain as major limitations of iPSC-EC technology. Objective: Here we performed droplet-based single-cell RNA-sequencing (scRNA-seq) of the human iPSCs following iPSC-EC differentiation. Droplet-based scRNA-seq enables analysis of thousands of cells in parallel, allowing comprehensive analysis of

transcriptional heterogeneity. Methods and Results: Bona fide iPSC-EC cluster was identified by scRNA-seq, which expressed high levels of endothelial-specific genes. iPSC-ECs, sorted by CD144 antibody-conjugated magnetic sorting, exhibited standard endothelial morphology and function including tube formation, response to inflammatory signals, and production of nitric oxide. Non-endothelial cell populations resulting from the differentiation protocol were identified, which included immature and atrial-like cardiomyocytes, hepatic-like cells, and vascular smooth muscle cells. Furthermore, scRNA-seq analysis of purified iPSC-ECs revealed transcriptional heterogeneity with four major subpopulations, marked by robust enrichment of CLDN5, APLNR, GJA5, and ESM1 genes respectively. Conclusions: Massively parallel, droplet-based scRNA-seq allowed meticulous analysis of thousands of human iPSCs subjected to iPSC-EC differentiation. Results showed inefficiency of the differentiation technique, which can be improved with further studies based on identification of molecular signatures that inhibit expansion of non-endothelial cell types. Subtypes of bona fide human iPSC-ECs were also identified, allowing us to sort for iPSC-ECs with specific biological function and identity.

Paik, I., et al. (2012). "Rapid micropatterning of cell lines and human pluripotent stem cells on elastomeric membranes." Biotechnol Bioeng **109**(10): 2630-2641.

Tissue function during development and in regenerative medicine completely relies on correct cell organization and patterning at micro and macro scales. We describe a rapid method for patterning mammalian cells including human embryonic stem cells (HESCs) and induced pluripotent stem cells (iPSCs) on elastomeric membranes such that micron-scale control of cell position can be achieved over centimeter-length scales. Our method employs surface engineering of hydrophobic polydimethylsiloxane (PDMS) membranes by plasma polymerization of allylamine. Deposition of plasma polymerized allylamine (ppAAm) using our methods may be spatially restricted using a micro-stencil leaving faithful hydrophilic ppAAm patterns. We employed airbrushing to create aerosols which deposit extracellular matrix (ECM) proteins (such as fibronectin and Matrigel) onto the same patterned ppAAm rich regions. Cell patterns were created with a variety of well characterized cell lines (e.g., NIH-3T3, C2C12, HL1, BJ6, HESC line HUES7, and HiPSC line IPS2). Individual and multiple cell line patterning were also achieved. Patterning remains faithful for several days and cells are viable and proliferate. To demonstrate the utility of our technique we have patterned cells in a variety of configurations.

The ability to rapidly pattern cells at high resolution over macro scales should aid future tissue engineering efforts for regenerative medicine applications and in creating in vitro stem cell niches.

Parameswaran, V., et al. (2007). "Development of a pluripotent ES-like cell line from Asian sea bass (Lates calcarifer)--an oviparous stem cell line mimicking viviparous ES cells." Mar Biotechnol (NY) **9**(6): 766-775.

We report a pluripotent embryonic stem cell-like cell line designated as SBES from blastula stage embryos of Asian sea bass (Lates calcarifer), which is an economically important cultivable and edible marine fish species in India. The SBES cells were cultured at 28 degrees C in Leibovitz L-15 medium supplemented with 20% fetal bovine serum without a feeder layer. The ES-like cells were round or polygonal and grew exponentially in culture. The SBES cells exhibited an intense alkaline phosphatase activity and expression of transcription factor Oct 4. The undifferentiated state of these cells was maintained at low seeding densities and the cells formed embryoid bodies when seeded in bacteriological plates. On treatment with all-trans retinoic acid, these cells differentiated into neuron-like cells, muscle cells, and beating cardiomyocytes, indicating their pluripotency. This embryonic ES-like cell line derived from an oviparous fish blastula conserved several peculiar features of viviparous mammalian embryonic stem cell lines. The present study highlights the importance and potential of piscine ES-like cell line for stem cell research without evoking ethical issues and invasive interventions sparing mammalian embryos.

Park, J., et al. (2018). "DSG2 Is a Functional Cell Surface Marker for Identification and Isolation of Human Pluripotent Stem Cells." Stem Cell Reports **11**(1): 115-127.

Pluripotent stem cells (PSCs) represent the most promising clinical source for regenerative medicine. However, given the cellular heterogeneity within cultivation and safety concerns, the development of specific and efficient tools to isolate a pure population and eliminate all residual undifferentiated PSCs from differentiated derivatives is a prerequisite for clinical applications. In this study, we raised a monoclonal antibody and identified its target antigen as desmoglein-2 (DSG2). DSG2 co-localized with human PSC (hPSC)-specific cell surface markers, and its expression was rapidly downregulated upon differentiation. The depletion of DSG2 markedly decreased hPSC proliferation and pluripotency marker expression. In addition, DSG2-negative population in hPSCs exhibited a notable suppression in embryonic

body and teratoma formation. The actions of DSG2 in regulating the self-renewal and pluripotency of hPSCs were predominantly exerted through the regulation of beta-catenin/Slug-mediated epithelial-to-mesenchymal transition. Our results demonstrate that DSG2 is a valuable PSC surface marker that is essential for the maintenance of PSC self-renewal.

Park, J. A., et al. (2012). "High sensitivity of embryonic stem cells to proteasome inhibitors correlates with low expression of heat shock protein and decrease of pluripotent cell marker expression." BMB Rep **45**(5): 299-304.

The ubiquitin-proteasome system is a major proteolytic system for nonlysosomal degradation of cellular proteins. Here, we investigated the response of mouse embryonic stem (ES) cells under proteotoxic stress. Proteasome inhibitors induced expression of heat shock protein 70 (HSP70) in a concentration- and time-dependent manner, and also induced apoptosis of ES cells. Importantly, more apoptotic cells were observed in ES cells compared with other somatic cells. To understand this phenomenon, we further investigated the expression of HSP70 and pluripotent cell markers. HSP70 expression was more significantly increased in somatic cells than in ES cells, and expression levels of pluripotent cell markers such as Oct4 and Nanog were decreased in ES cells. These results suggest that higher sensitivity of ES cells to proteotoxic stress may be related with lower capacity of HSP70 expression and decreased pluripotent cell marker expression, which is essential for the survival of ES cells.

Park, S., et al. (2017). "A Comprehensive, Ethnically Diverse Library of Sickle Cell Disease-Specific Induced Pluripotent Stem Cells." Stem Cell Reports **8**(4): 1076-1085.

Sickle cell anemia affects millions of people worldwide and is an emerging global health burden. As part of a large NIH-funded NextGen Consortium, we generated a diverse, comprehensive, and fully characterized library of sickle-cell-disease-specific induced pluripotent stem cells (iPSCs) from patients of different ethnicities, beta-globin gene (HBB) haplotypes, and fetal hemoglobin (HbF) levels. iPSCs stand to revolutionize the way we study human development, model disease, and perhaps eventually, treat patients. Here, we describe this unique resource for the study of sickle cell disease, including novel haplotype-specific polymorphisms that affect disease severity, as well as for the development of patient-specific therapeutics for this phenotypically diverse disorder. As a complement to this library, and as proof of principle for future cell- and gene-based therapies, we also designed and employed CRISPR/Cas gene

editing tools to correct the sickle hemoglobin (HbS) mutation.

Park, S. W., et al. (2014). "Variable allelic expression of imprinted genes in human pluripotent stem cells during differentiation into specialized cell types in vitro." Biochem Biophys Res Commun **446**(2): 493-498.

Genomic imprinting is an epigenetic phenomenon by which a subset of genes is asymmetrically expressed in a parent-of-origin manner. However, little is known regarding the epigenetic behaviors of imprinted genes during human development. Here, we show dynamic epigenetic changes in imprinted genes in hESCs during in vitro differentiation into specialized cell types. Out of 9 imprinted genes with single nucleotide polymorphisms, mono-allelic expression for three imprinted genes (H19, KCNQ1OT1, and IPW), and bi- or partial-allelic expression for three imprinted genes (OSBPL5, PPP1R9A, and RTL1) were stably retained in H9-hESCs throughout differentiation, representing imprinting stability. Three imprinted genes (KCNK9, ATP10A, and SLC22A3) showed a loss and a gain of imprinting in a lineage-specific manner during differentiation. Changes in allelic expression of imprinted genes were observed in another hESC line during in vitro differentiation. These findings indicate that the allelic expression of imprinted genes may be vulnerable in a lineage-specific manner in human pluripotent stem cells during differentiation.

Parmar, V. M., et al. (2018). "A2E-associated cell death and inflammation in retinal pigmented epithelial cells from human induced pluripotent stem cells." Stem Cell Res **27**: 95-104.

Accumulation of lipofuscin in the retinal pigmented epithelium (RPE) is observed in retinal degenerative diseases including Stargardt disease and age-related macular degeneration. Bis-retinoid N-retinyl-N-retinylidene ethanolamine (A2E) is a major component of lipofuscin. A2E has been implicated in RPE atrophy and retinal inflammation; however, mice with A2E accumulation display only a mild retinal phenotype. In the current study, human iPSC-RPE (hiPSC-RPE) cells were generated from healthy individuals to examine effects of A2E in human RPE cells. hiPSC-RPE cells displayed RPE-specific features, which include expression of RPE-specific genes, tight junction formation and ability to carry out phagocytosis. hiPSC-RPE cells demonstrated cell death and increased VEGF-A production in a time-dependent manner when they were cocultured with 10muM of A2E. PCR array analyses revealed upregulation of 26 and 12 pro-inflammatory cytokines upon A2E and H2O2 exposure respectively, indicating

that A2E and H2O2 can cause inflammation in human retinas. Notably, identified gene profiles were different between A2E- and H2O2- treated hiPSC-RPE cells. A2E caused inflammatory changes observed in retinal degenerative diseases more closely as compared to H2O2. Collectively, these data obtained with hiPSC-RPE cells provide evidence that A2E plays an important role in pathogenesis of retinal degenerative diseases in humans.

Parsons, X. H. (2013). "Constraining the Pluripotent Fate of Human Embryonic Stem Cells for Tissue Engineering and Cell Therapy - The Turning Point of Cell-Based Regenerative Medicine." Br Biotechnol J **3**(4): 424-457.

To date, the lack of a clinically-suitable source of engraftable human stem/progenitor cells with adequate neurogenic potential has been the major setback in developing safe and effective cell-based therapies for regenerating the damaged or lost CNS structure and circuitry in a wide range of neurological disorders. Similarly, the lack of a clinically-suitable human cardiomyocyte source with adequate myocardium regenerative potential has been the major setback in regenerating the damaged human heart. Given the limited capacity of the CNS and heart for self-repair, there is a large unmet healthcare need to develop stem cell therapies to provide optimal regeneration and reconstruction treatment options to restore normal tissues and function. Derivation of human embryonic stem cells (hESCs) provides a powerful in vitro model system to investigate molecular controls in human embryogenesis as well as an unlimited source to generate the diversity of human somatic cell types for regenerative medicine. However, realizing the developmental and therapeutic potential of hESC derivatives has been hindered by the inefficiency and instability of generating clinically-relevant functional cells from pluripotent cells through conventional uncontrollable and incomplete multi-lineage differentiation. Recent advances and breakthroughs in hESC research have overcome some major obstacles in bringing hESC therapy derivatives towards clinical applications, including establishing defined culture systems for de novo derivation and maintenance of clinical-grade pluripotent hESCs and lineage-specific differentiation of pluripotent hESCs by small molecule induction. Retinoic acid was identified as sufficient to induce the specification of neuroectoderm direct from the pluripotent state of hESCs and trigger a cascade of neuronal lineage-specific progression to human neuronal progenitors and neurons of the developing CNS in high efficiency, purity, and neuronal lineage specificity by promoting nuclear translocation of the neuronal specific transcription factor Nurr-1. Similarly, nicotinamide was rendered sufficient to induce the

specification of cardiomesoderm direct from the pluripotent state of hESCs by promoting the expression of the earliest cardiac-specific transcription factor Csx/Nkx2.5 and triggering progression to cardiac precursors and beating cardiomyocytes with high efficiency. This technology breakthrough enables direct conversion of pluripotent hESCs into a large supply of high purity neuronal cells or heart muscle cells with adequate capacity to regenerate CNS neurons and contractile heart muscles for developing safe and effective stem cell therapies. Transforming pluripotent hESCs into fate-restricted therapy derivatives dramatically increases the clinical efficacy of graft-dependent repair and safety of hESC-derived cellular products. Such milestone advances and medical innovations in hESC research allow generation of a large supply of clinical-grade hESC therapy derivatives targeting for major health problems, bringing cell-based regenerative medicine to a turning point.

Parsons, X. H. (2013). "Human Stem Cell Derivatives Retain More Open Epigenomic Landscape When Derived from Pluripotent Cells than from Tissues." J Regen Med **1**(2).

The growing number of identified stem cell derivatives and escalating concerns for safety and efficacy of these cells towards clinical applications have made it increasingly crucial to be able to assess the relative risk-benefit ratio of a given stem cell from a given source for a particular disease. Discerning the intrinsic plasticity and regenerative potential of human stem cell populations might reside in chromatin modifications that shape the respective epigenomes of their derivation routes. Previously, we have generated engraftable human neuronal progenitors direct from pluripotent human embryonic stem cells (hESCs) by small molecule induction (hESC-I hNuPs). Unlike the prototypical neuroepithelial-like nestin-positive human neural stem cells (hNSCs), these in vitro neuroectoderm-derived Nurr1-positive hESC-I hNuPs are a more neuronal lineage-specific and plastic hESC derivative. In this study, the global chromatin landscape changes in pluripotent hESCs and their neuronal lineage-specific derivative hESC-I hNuPs were profiled using genome-wide mapping and compared to CNS tissue-derived hNSCs. This study found that the broad potential of pluripotent hESCs is defined by an epigenome constituted of open conformation of chromatin mediated by a pattern of Oct-4 global distribution that corresponds closely with those of acetylated nucleosomes genome-wide. The epigenomic transition from pluripotency to restriction in lineage choices is characterized by genome-wide increases in histone H3K9 methylation that mediates global chromatin-silencing and somatic identity.

Tissue-resident CNS-derived hNSCs have acquired a substantial number of additional histone H3K9 methylation, therefore, more silenced chromatin. These data suggest that the intrinsic plasticity and regenerative potential of human stem cell derivatives can be differentiated by their epigenomic landscape features, and that human stem cell derivatives retain more open epigenomic landscape, therefore, more developmental potential for scale-up regeneration, when derived from the hESCs in vitro than from the CNS tissue in vivo.

Parsons, X. H. (2016). "Direct Conversion of Pluripotent Human Embryonic Stem Cells Under Defined Culture Conditions into Human Neuronal or Cardiomyocyte Cell Therapy Derivatives." Methods Mol Biol **1307**: 299-318.
Developing novel strategies for well-controlled efficiently directing pluripotent human embryonic stem cells (hESCs) exclusively and uniformly towards clinically relevant cell types in a lineage-specific manner is not only crucial for unveiling the molecular and cellular cues that direct human embryogenesis but also vital to harnessing the power of hESC biology for tissue engineering and cell-based therapies. Conventional hESC differentiation methods require uncontrollable simultaneous multi-lineage differentiation of pluripotent cells, which yield embryoid bodies (EB) or aggregates consisting of a mixed population of cell types of three embryonic germ layers, among which only a very small fraction of cells display targeted differentiation, impractical for commercial and clinical applications. Here, a protocol for lineage-specific differentiation of hESCs, maintained under defined culture systems, direct from the pluripotent stage using small-molecule induction exclusively and uniformly to a neural or a cardiac lineage is described. Lineage-specific differentiation of pluripotent hESCs by small-molecule induction enables well-controlled highly efficient direct conversion of nonfunctional pluripotent hESCs into a large supply of high-purity functional human neuronal or cardiomyocyte cell therapy derivatives for commercial and therapeutic uses.

Pascoal, J. F., et al. (2018). "Three-Dimensional Cell-Based Microarrays: Printing Pluripotent Stem Cells into 3D Microenvironments." Methods Mol Biol **1771**: 69-81.
Cell-based microarrays are valuable platforms for the study of cytotoxicity and cellular microenvironment because they enable high-throughput screening of large sets of conditions at reduced reagent consumption. However, most of the described microarray technologies have been applied to two-dimensional cultures, which do not accurately emulate the in vivo three-dimensional (3D) cell-cell and cell-extracellular matrix interactions.Herein, we describe the methodology for production of alginate- and Matrigel-based 3-D cell microarrays for the study of mouse and human pluripotent stem cells on two different chip-based platforms. We further provide protocols for on-chip proliferation/viability analysis and the assessment of protein expression by immunofluorescence.

Pellett, S., et al. (2015). "Human Induced Pluripotent Stem Cell Derived Neuronal Cells Cultured on Chemically-Defined Hydrogels for Sensitive In Vitro Detection of Botulinum Neurotoxin." Sci Rep **5**: 14566.
Botulinum neurotoxin (BoNT) detection provides a useful model for validating cell-based neurotoxicity screening approaches, as sensitivity is dependent on functionally competent neurons and clear quantitative endpoints are available for correlating results to approved animal testing protocols. Here, human induced pluripotent stem cell (iPSC)-derived neuronal cells were cultured on chemically-defined poly (ethylene glycol) (PEG) hydrogels formed by "thiol-ene" photopolymerization and tested as a cell-based neurotoxicity assay by determining sensitivity to active BoNT/A1. BoNT/A1 sensitivity was comparable to the approved in vivo mouse bioassay for human iPSC-derived neurons and neural stem cells (iPSC-NSCs) cultured on PEG hydrogels or treated tissue culture polystyrene (TCP) surfaces. However, maximum sensitivity for BoNT detection was achieved two weeks earlier for iPSC-NSCs that were differentiated and matured on PEG hydrogels compared to TCP. Therefore, chemically-defined synthetic hydrogels offer benefits over standard platforms when optimizing culture conditions for cell-based screening and achieve sensitivities comparable to an approved animal testing protocol.

Peng, K. Y., et al. (2016). "Human pluripotent stem cell (PSC)-derived mesenchymal stem cells (MSCs) show potent neurogenic capacity which is enhanced with cytoskeletal rearrangement." Oncotarget **7**(28): 43949-43959.
Mesenchymal stem cells (MSCs) are paraxial mesodermal progenitors with potent immunomodulatory properties. Reports also indicate that MSCs can undergo neural-like differentiation, offering hope for use in neurodegenerative diseases. However, ex vivo expansion of these rare somatic stem cells for clinical use leads to cellular senescence. A newer source of MSCs derived from human pluripotent stem cells (PSC) can offer the 'best-of-both-worlds' scenario, abrogating the concern of teratoma formation while preserving PSC proliferative

capacity. PSC-derived MSCs (PSC-MSCs) also represent MSCs at the earliest developmental stage, and we found that these MSCs harbor stronger neuro-differentiation capacity than post-natal MSCs. PSC-MSCs express higher levels of neural stem cell (NSC)-related genes and transcription factors than adult bone marrow MSCs at baseline, and rapidly differentiate into neural-like cells when cultured in either standard neurogenic differentiation medium (NDM) or when the cytoskeletal modulator RhoA kinase (ROCK) is inhibited. Interestingly, when NDM is combined with ROCK inhibition, PSC-MSCs undergo further commitment, acquiring characteristics of post-mitotic neurons including nuclear condensation, extensive dendritic growth, and neuron-restricted marker expression including NeuN, beta-III-tubulin and Doublecortin. Our data demonstrates that PSC-MSCs have potent capacity to undergo neural differentiation and also implicate the important role of the cytoskeleton in neural lineage commitment.

Pera, R. A. (2013). "Status of human germ cell differentiation from pluripotent stem cells." Reprod Fertil Dev **25**(2): 396-404.

Historically, the quality of life of infertile couples has been greatly diminished by the loss of opportunity to conceive. However, beginning with the advent of IVF in the late 1970s, novel clinical interventions have greatly changed the outlook for those with severe forms of infertility. Yet, in cases in which the quality and quantity of germ cells are most compromised, there are few options. In the present paper, the current status of germ cell development from stem cells is reviewed in light of potential utility for basic science and clinical applications.

Peters, D. T., et al. (2016). "Asialoglycoprotein receptor 1 is a specific cell-surface marker for isolating hepatocytes derived from human pluripotent stem cells." Development **143**(9): 1475-1481.

Hepatocyte-like cells (HLCs) are derived from human pluripotent stem cells (hPSCs) in vitro, but differentiation protocols commonly give rise to a heterogeneous mixture of cells. This variability confounds the evaluation of in vitro functional assays performed using HLCs. Increased differentiation efficiency and more accurate approximation of the in vivo hepatocyte gene expression profile would improve the utility of hPSCs. Towards this goal, we demonstrate the purification of a subpopulation of functional HLCs using the hepatocyte surface marker asialoglycoprotein receptor 1 (ASGR1). We analyzed the expression profile of ASGR1-positive cells by microarray, and tested their ability to perform mature hepatocyte functions (albumin and urea secretion, cytochrome activity). By these measures, ASGR1-

positive HLCs are enriched for the gene expression profile and functional characteristics of primary hepatocytes compared with unsorted HLCs. We have demonstrated that ASGR1-positive sorting isolates a functional subpopulation of HLCs from among the heterogeneous cellular population produced by directed differentiation.

Pettinato, G., et al. (2014). "Formation of well-defined embryoid bodies from dissociated human induced pluripotent stem cells using microfabricated cell-repellent microwell arrays." Sci Rep **4**: 7402.

A simple, scalable, and reproducible technology that allows direct formation of large numbers of homogeneous and synchronized embryoid bodies (EBs) of defined sizes from dissociated human induced pluripotent stem cells (hiPSCs) was developed. Non-cell-adhesive hydrogels were used to create round-bottom microwells to host dissociated hiPSCs. No Rho-associated kinase inhibitor (ROCK-i), or centrifugation was needed and the side effects of ROCK-i can be avoided. The key requirement for the successful EB formation in addition to the non-cell-adhesive round-bottom microwells is the input cell density per microwell. Too few or too many cells loaded into the microwells will compromise the EB formation process. In parallel, we have tested our microwell-based system for homogeneous hEB formation from dissociated human embryonic stem cells (hESCs). Successful production of homogeneous hEBs from dissociated hESCs in the absence of ROCK-i and centrifugation was achieved within an optimal range of input cell density per microwell. Both the hiPSC- and hESC-derived hEBs expressed key proteins characteristic of all the three developmental germ layers, confirming their EB identity. This novel EB production technology may represent a versatile platform for the production of homogeneous EBs from dissociated human pluripotent stem cells (hPSCs).

Peyrard, T., et al. (2011). "Banking of pluripotent adult stem cells as an unlimited source for red blood cell production: potential applications for alloimmunized patients and rare blood challenges." Transfus Med Rev **25**(3): 206-216.

The transfusion of red blood cells (RBCs) is now considered a well-settled and essential therapy. However, some difficulties and constraints still occur, such as long-term blood product shortage, blood donor population aging, known and yet unknown transfusion-transmitted infectious agents, growing cost of the transfusion supply chain management, and the inescapable blood group polymorphism barrier. Red blood cells can be now cultured in vitro from human hematopoietic, human embryonic, or human-induced pluripotent stem cells (hiPSCs). The highly promising

hiPSC technology represents a potentially unlimited source of RBCs and opens the door to the revolutionary development of a new generation of allogeneic transfusion products. Assuming that in vitro large-scale cultured RBC production efficiently operates in the near future, we draw here some futuristic but realistic scenarios regarding potential applications for alloimmunized patients and those with a rare blood group. We retrospectively studied a cohort of 16,486 consecutive alloimmunized patients (10-year period), showing 1 to 7 alloantibodies with 361 different antibody combinations. We showed that only 3 hiPSC clones would be sufficient to match more than 99% of the 16,486 patients in need of RBC transfusions. The study of the French National Registry of People with a Rare Blood Phenotype/Genotype (10-year period) shows that 15 hiPSC clones would cover 100% of the needs in patients of white ancestry. In addition, one single hiPSC clone would meet 73% of the needs in alloimmunized patients with sickle cell disease for whom rare cryopreserved RBC units were required. As a result, we consider that a very limited number of RBC clones would be able to not only provide for the need for most alloimmunized patients and those with a rare blood group but also efficiently allow for a policy for alloimmunization prevention in multiply transfused patients.

Phanthong, P., et al. (2017). "Enhancement of beta-Globin Gene Expression in Thalassemic IVS2-654 Induced Pluripotent Stem Cell-Derived Erythroid Cells by Modified U7 snRNA." Stem Cells Transl Med **6**(4): 1059-1069.

The therapeutic use of patient-specific induced pluripotent stem cells (iPSCs) is emerging as a potential treatment of beta-thalassemia. Ideally, patient-specific iPSCs would be genetically corrected by various approaches to treat beta-thalassemia including lentiviral gene transfer, lentivirus-delivered shRNA, and gene editing. These corrected iPSCs would be subsequently differentiated into hematopoietic stem cells and transplanted back into the same patient. In this article, we present a proof of principle study for disease modeling and screening using iPSCs to test the potential use of the modified U7 small nuclear (sn) RNA to correct a splice defect in IVS2-654 beta-thalassemia. In this case, the aberration results from a mutation in the human beta-globin intron 2 causing an aberrant splicing of beta-globin pre-mRNA and preventing synthesis of functional beta-globin protein. The iPSCs (derived from mesenchymal stromal cells from a patient with IVS2-654 beta-thalassemia/hemoglobin (Hb) E) were transduced with a lentivirus carrying a modified U7 snRNA targeting an IVS2-654 beta-globin pre-mRNA

in order to restore the correct splicing. Erythroblasts differentiated from the transduced iPSCs expressed high level of correctly spliced beta-globin mRNA suggesting that the modified U7 snRNA was expressed and mediated splicing correction of IVS2-654 beta-globin pre-mRNA in these cells. Moreover, a less active apoptosis cascade process was observed in the corrected cells at transcription level. This study demonstrated the potential use of a genetically modified U7 snRNA with patient-specific iPSCs for the partial restoration of the aberrant splicing process of beta-thalassemia. Stem Cells Translational Medicine 2017;6:1059-1069.

Picanco-Castro, V., et al. (2014). "Can pluripotent stem cells be used in cell-based therapy?" Cell Reprogram **16**(2): 98-107.

Pluripotent stem cells, both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have the ability to differentiate into several cell types that can be used in drug testing and also in the study and treatment of diseases. These cells can be differentiated by in vitro systems, which may serve as models for human diseases and for cell transplantation. In this review, we address the pluripotent cell types, how to obtain and characterize these cells, and differentiation assays. We also focus on the potential of these cells in clinical trials, and we describe the clinical trials that are underway.

Polinati, P. P., et al. (2015). "Patient-Specific Induced Pluripotent Stem Cell-Derived RPE Cells: Understanding the Pathogenesis of Retinopathy in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency." Invest Ophthalmol Vis Sci **56**(5): 3371-3382.

PURPOSE: Retinopathy is an important manifestation of trifunctional protein (TFP) deficiencies but not of other defects of fatty acid oxidation. The common homozygous mutation in the TFP alpha-subunit gene HADHA (hydroxyacyl-CoA dehydrogenase), c.1528G>C, affects the long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity of TFP and blindness in infancy. The pathogenesis of the retinopathy is unknown. This study aimed to utilize human induced pluripotent stem cell (hiPSC) technology to create a disease model for the disorder, and to derive clues for retinopathy pathogenesis. METHODS: We implemented hiPSC technology to generate LCHAD deficiency (LCHADD) patient-specific retinal pigment epithelial (RPE) monolayers. These patient and control RPEs were extensively characterized for function and structure, as well as for lipid composition by mass spectrometry. RESULTS: The hiPSC-derived RPE monolayers of patients and controls were functional, as they both were able to

phagocytose the photoreceptor outer segments in vitro. Interestingly, the patient RPEs had intense cytoplasmic neutral lipid accumulation, and lipidomic analysis revealed an increased triglyceride accumulation. Further, patient RPEs were small and irregular in shape, and their tight junctions were disorganized. Their ultrastructure showed decreased pigmentation, few melanosomes, and more melanolysosomes. CONCLUSIONS: We demonstrate that the RPE cell model reveals novel early pathogenic changes in LCHADD retinopathy, with robust lipid accumulation, inefficient pigmentation that is evident soon after differentiation, and a defect in forming tight junctions inducing apoptosis. We propose that LCHADD-RPEs are an important model for mitochondrial TFP retinopathy, and that their early pathogenic changes contribute to infantile blindness of LCHADD.

Polo, J. M., et al. (2010). "Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells." Nat Biotechnol **28**(8): 848-855.

Induced pluripotent stem cells (iPSCs) have been derived from various somatic cell populations through ectopic expression of defined factors. It remains unclear whether iPSCs generated from different cell types are molecularly and functionally similar. Here we show that iPSCs obtained from mouse fibroblasts, hematopoietic and myogenic cells exhibit distinct transcriptional and epigenetic patterns. Moreover, we demonstrate that cellular origin influences the in vitro differentiation potentials of iPSCs into embryoid bodies and different hematopoietic cell types. Notably, continuous passaging of iPSCs largely attenuates these differences. Our results suggest that early-passage iPSCs retain a transient epigenetic memory of their somatic cells of origin, which manifests as differential gene expression and altered differentiation capacity. These observations may influence ongoing attempts to use iPSCs for disease modeling and could also be exploited in potential therapeutic applications to enhance differentiation into desired cell lineages.

Pomeshchik, Y., et al. (2015). "Transplanted Human Induced Pluripotent Stem Cell-Derived Neural Progenitor Cells Do Not Promote Functional Recovery of Pharmacologically Immunosuppressed Mice With Contusion Spinal Cord Injury." Cell Transplant **24**(9): 1799-1812.

Improved functional recovery after spinal cord injury by transplantation of induced pluripotent stem cell-derived neural stem/progenitor cells (iPSC-NPCs) has been reported. However, beneficial effects of iPSC-based therapy have so far been produced mostly using genetically immunodeficient rodents. Because of the long time required for generation and characterization of iPSCs, the use of autologous iPSCs for treating patients with acute spinal cord injury (SCI) is not feasible. Therefore, it is of utmost importance to investigate the effect of iPSC-based therapy on functional recovery after SCI using pharmacologically immunosuppressed, immunocompetent animal models. Here we studied the functional outcome following subacute transplantation of human iPSC-derived NPCs into contused mouse spinal cord when tacrolimus was used as an immunosuppressive agent. We show that human iPSC-derived NPCs transplanted into pharmacologically immunosuppressed C57BL/6J mice exhibited poor long-term survival and failed to improve functional recovery after SCI as measured by Basso Mouse Scale (BMS) for locomotion and CatWalk gait analysis when compared to vehicle-treated animals. The scarce effect of iPSC-based therapy observed in the current study may be attributable to insufficient immunosuppressive effect, provided by monotherapy with tacrolimus in combination with immunogenicity of transplanted cells and complex microenvironment of the injured spinal cord. Our results highlight the importance of extensive preclinical studies of transplanted cells before the clinical application of iPSC-based cell therapy is achieved.

Pryzhkova, M. V., et al. (2013). "Patterning Pluripotent Stem Cells at a Single Cell Level." J Biomater Tissue Eng **3**(4): 461-471.

Studies of cell-extracellular matrix (ECM) interactions at a single cell level have drawn interest from scientists around the world. Subcellular ECM micropatterning techniques allow researchers to control cell shape, migration, and spindle orientation during mitosis potentially influencing the stem cell fate. Generally these studies have been limited to somatic cells rather than human pluripotent stem cells (hPSCs) which are capable of enormous differentiation potential. hPSCs require a defined ECM for attachment and express characteristic integrins mediating cell-substrate interactions. hPSCs also rely on cell-cell contacts for survival and to maintain self-renewal properties, but these circumstances also significantly limit hPSC observation at a single cell level. In addition, currently available methods for ECM micropatterning generally require a facility with trained personnel and intricate equipment to produce protein micropatterns. To overcome this problem, we have developed a protocol for vitronectin micropatterning using simple UV/ozone modification of polystyrene. Single hPSCs were able to attach and form characteristic stress fibers and focal adhesions similar to somatic cell types which demonstrate hPSC responsiveness to extracellular adhesive cues. Micropatterned hPSCs were able to be cultured for up

to 48 hours while maintaining expression of pluripotency-associated transcription factor OCT4. Although further studies are necessary, the results of our investigation will potentially have a large impact on cell regenerative medicine and tissue engineering.

Puceat, M. (2012). "[Pluripotent stem cells: a cell model for early cardiac development]." Biol Aujourdhui **206**(1): 25-29.

Mouse embryonic stem cell lines were derived three decades ago and allow the process of transgenesis and in turn the generation of transgenic mice. In the past and still nowadays, these mice as well as more primitive organisms have provided models to study the first cell decisions in the embryo. Derivation of human embryonic stem cells more than a decade ago has provided a similar cell model for human early embryonic development, an issue that could not be addressed for obvious ethical reasons which limit research on human embryos. These cells allow investigating the genetic and epigenetic mechanisms underlying the first cell decisions in the human embryo. Herein, we use cardiogenesis as an example to reveal the potential of these cells to better understand the first steps of cardiac development.

Qi, X., et al. (2016). "Exosomes Secreted by Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Repair Critical-Sized Bone Defects through Enhanced Angiogenesis and Osteogenesis in Osteoporotic Rats." Int J Biol Sci **12**(7): 836-849.

Bone defects caused by trauma, severe infection, tumor resection and skeletal abnormalities are common osteoporotic conditions and major challenges in orthopedic surgery, and there is still no effective solution to this problem. Consequently, new treatments are needed to develop regeneration procedures without side effects. Exosomes secreted by mesenchymal stem cells (MSCs) derived from human induced pluripotent stem cells (hiPSCs, hiPSC-MSC-Exos) incorporate the advantages of both MSCs and iPSCs with no immunogenicity. However, there are no reports on the application of hiPSC-MSC-Exos to enhance angiogenesis and osteogenesis under osteoporotic conditions. HiPSC-MSC-Exos were isolated and identified before use. The effect of hiPSC-MSC-Exos on the proliferation and osteogenic differentiation of bone marrow MSCs derived from ovariectomized (OVX) rats (rBMSCs-OVX) in vitro were investigated. In vivo, hiPSC-MSC-Exos were implanted into critical size bone defects in ovariectomized rats, and bone regeneration and angiogenesis were examined by microcomputed tomography (micro-CT), sequential fluorescent labeling analysis, microfil perfusion and histological and immunohistochemical analysis. The results in vitro showed that hiPSC-MSC-Exos enhanced cell proliferation and alkaline phosphatase (ALP) activity, and up-regulated mRNA and protein expression of osteoblast-related genes in rBMSCs-OVX. In vivo experiments revealed that hiPSC-MSC-Exos dramatically stimulated bone regeneration and angiogenesis in critical-sized calvarial defects in ovariectomized rats. The effect of hiPSC-MSC-Exos increased with increasing concentration. In this study, we showed that hiPSC-MSC-Exos effectively stimulate the proliferation and osteogenic differentiation of rBMSCs-OVX, with the effect increasing with increasing exosome concentration. Further analysis demonstrated that the application of hiPSC-MSC-Exos+beta-TCP scaffolds promoted bone regeneration in critical-sized calvarial defects by enhancing angiogenesis and osteogenesis in an ovariectomized rat model.

Qin, J., et al. (2014). "Cell fusion enhances mesendodermal differentiation of human induced pluripotent stem cells." Stem Cells Dev **23**(23): 2875-2882.

Human induced pluripotent stem cells (iPS cells) resemble embryonic stem cells and can differentiate into cell derivatives of all three germ layers. However, frequently the differentiation efficiency of iPS cells into some lineages is rather poor. Here, we found that fusion of iPS cells with human hematopoietic stem cells (HSCs) enhances iPS cell differentiation. Such iPS hybrids showed a prominent differentiation bias toward hematopoietic lineages but also toward other mesendodermal lineages. Additionally, during differentiation of iPS hybrids, expression of early mesendodermal markers-Brachyury (T), MIX1 Homeobox-Like Protein 1 (MIXL1), and Goosecoid (GSC)-appeared with faster kinetics than in parental iPS cells. Following iPS hybrid differentiation there was a prominent induction of NODAL and inhibition of NODAL signaling blunted mesendodermal differentiation. This indicates that NODAL signaling is critically involved in mesendodermal bias of iPS hybrid differentiation. In summary, we demonstrate that iPS cell fusion with HSCs prominently enhances iPS cell differentiation.

Qin, Y., et al. (2016). "Potential Cancer Prevention and Treatment by Silencing the Killer Cell Immunoglobulin-like Receptor Gene in Natural Killer Cells Derived from Induced Pluripotent Stem Cells." Enliven J Stem Cell Res Regen Med **3**(1).

Cancer immunosurveillance is an important host protection process, monitoring the presence of irregular cells that could potentially transform into tumor cells, effectively clearing the body of

transformed tumor cells at their earliest stages, and thus maintaining regular cellular homeostasis. Natural killer (NK) cells are effector lymphocytes of the innate immune system, playing a critical role in surveillance for tumor cells, while also eliminating virally infected cells. The significance of the anti-tumor role of NK cells was recently further verified by findings that immunosuppression in most cancer patients is not perceptible until late stages. NK cells express the low-affinity Fc-activating receptor, CD16, and the inhibitory receptor, killer cell immunoglobulin-like receptor (KIR). Consequently, activation of NK cells is determined by the balance of inhibitory and activating receptor stimulation. Here, we propose establishing an induced pluripotent stem cell (iPSC)-derived NK cell line with KIR gene knockout or knockdown as a possible regimen to treat and prevent cancer. We further postulate that an optimal mixture of NK iPSCs with and without KIR gene knockout, would reach a maximum antitumor activity, with minimal side effects. We also discuss the possible advantages of KIR-knockout NK iPSCs for adoptive immunotherapy in patients with cancer.

Quang, T., et al. (2014). "Dosage and cell line dependent inhibitory effect of bFGF supplement in human pluripotent stem cell culture on inactivated human mesenchymal stem cells." PLoS One **9**(1): e86031.

Many different culture systems have been developed for expanding human pluripotent stem cells (hESCs and hiPSCs). In general, 4-10 ng/ml of bFGF is supplemented in culture media in feeder-dependent systems regardless of feeder cell types, whereas in feeder-free systems, up to 100 ng/ml of bFGF is required for maintaining long-term culture on various substrates. The amount of bFGF required in native hESCs growth niche is unclear. Here we report using inactivated adipose-derived human mesenchymal stem cells as feeder cells to examine long-term parallel cultures of two hESCs lines (H1 and H9) and one hiPSCs line (DF19-9-7T) in media supplemented with 0, 0.4 or 4 ng/ml of bFGF for up to 23 passages, as well as parallel cultures of H9 and DF19 in media supplemented with 4, 20 or 100 ng/ml bFGF for up to 13 passages for comparison. Across all cell lines tested, bFGF supplement demonstrated inhibitory effect over growth expansion, single cell colonization and recovery from freezing in a dosage dependent manner. In addition, bFGF exerted differential effects on different cell lines, inducing H1 and DF19 differentiation at 4 ng/ml or higher, while permitting long-term culture of H9 at the same concentrations with no apparent dosage effect. Pluripotency was confirmed for all cell lines cultured in 0, 0.4 or 4 ng/ml bFGF excluding H1-4 ng, as well as H9 cultured in 4,

20 and 100 ng/ml bFGF. However, DF19 demonstrated similar karyotypic abnormality in both 0 and 4 ng/ml bFGF media while H1 and H9 were karyotypically normal in 0 ng/ml bFGF after long-term culture. Our results indicate that exogenous bFGF exerts dosage and cell line dependent effect on human pluripotent stem cells cultured on mesenchymal stem cells, and implies optimal use of bFGF in hESCs/hiPSCs culture should be based on specific cell line and its culture system.

Rajaei, B., et al. (2017). "In Vitro Generation of Glucose-Responsive Insulin-Secreting Cells from Pancreatic and Duodenal Homeobox 1-Overexpressing Human-Induced Pluripotent Stem Cell Derived from Diabetic Patient." ASAIO J.

Pancreatic and duodenal homeobox 1 (PDX1), a member of the homeodomain-containing transcription factor family, is a key transcription factor for pancreas development and mature beta-cell function. In this study, induced overexpression of PDX1 resulted in producing susceptible cells for pancreatic differentiation and was well beneficial to enhance beta-cell production, maturation, function, and survival. Induced PDX1 overexpression in harmony with a set of signaling molecules involves in guiding the signaling pathways toward pancreas development, leaded to high-efficient in vitro generation of ectopic insulin-producing cells (IPCs) with the effectively reduced number of polyhormonal cells and increased number of INS single-positive cells. This strategy yielded 85.61% glucose-responsive insulin-positive cells in vitro, which was seven times higher than the basal level, and electron microscopy images revealed the presence of mature beta-cell secretory granules. The generation of glucose-responsive insulin-secreting beta-like cells from human-induced pluripotent stem cells (hiPSCs) in vitro would provide a promising approach to produce an unprecedented cell source for cell transplantation therapy in diabetes without the ethical obstacle of embryonic stem cells and would bypass immune rejection. These cells are an invaluable source for disease modeling, drug discovery, and pharmacogenomics studies as well.

Rajesh, D., et al. (2011). "Human lymphoblastoid B-cell lines reprogrammed to EBV-free induced pluripotent stem cells." Blood **118**(7): 1797-1800.

Generation of patient-specific induced pluripotent cells (iPSCs) holds great promise for regenerative medicine. Epstein-Barr virus immortalized lymphoblastoid B-cell lines (LCLs) can be generated from a minimal amount of blood and are banked worldwide as cellular reference material for immunologic or genetic analysis of pedigreed study populations. We report the generation of iPSCs from 2

LCLs (LCL-iPSCs) via a feeder-free episomal method using a cocktail of transcription factors and small molecules. LCL-derived iPSCs exhibited normal karyotype, expressed pluripotency markers, lost oriP/EBNA-1 episomal vectors, generated teratomas, retained donor identity, and differentiated in vitro into hematopoietic, cardiac, neural, and hepatocyte-like lineages. Significantly, although the parental LCLs express viral EBNA-1 and other Epstein-Barr virus latency-related elements for their survival, their presence was not detectable in LCL-iPSCs. Thus, reprogramming LCLs could offer an unlimited source for patient-specific iPSCs.

Ramasamy, T. S., et al. (2018). "Stem Cells Derived from Amniotic Fluid: A Potential Pluripotent-Like Cell Source for Cellular Therapy?" Curr Stem Cell Res Ther **13**(4): 252-264.
BACKGROUND: Regenerative medicine aims to provide therapeutic treatment for disease or injury, and cell-based therapy is a newer therapeutic approach different from conventional medicine. Ethical issues that rose by the utilisation of human embryonic stem cells (hESC) and the limited capacity of adult stem cells, however, hinder the application of these stem cells in regenerative medicine. Recently, isolation and characterisation of c-kit positive cells from human amniotic fluid, which possess intermediate characteristics between hESCs and adult stem cells, provided a new approach towards realising their promise for fetal and adult regenerative medicine. Despite the number of studies that have been initiated to characterize their molecular signature, research on developing approaches to maintain and enhance their regenerative potential is urgently needed and must be developed. AIM: Thus, this review is focused on understanding their potential uses and factors influencing their pluripotent status in vitro. CONCLUSION: In short, this cell source could be an ideal cellular resource for pluripotent cells for potential applications in allogeneic cellular replacement therapies, fetal tissue engineering, pharmaceutical screening, and in disease modelling.

Ramirez, J. M., et al. (2010). "Human pluripotent stem cells: from biology to cell therapy." World J Stem Cells **2**(2): 24-33.
Human pluripotent stem cells (PSCs), encompassing embryonic stem cells and induced pluripotent stem cells, proliferate extensively and differentiate into virtually any desired cell type. PSCs endow regenerative medicine with an unlimited source of replacement cells suitable for human therapy. Several hurdles must be carefully addressed in PSC research before these theoretical possibilities are translated into clinical applications. These obstacles are: (1) cell proliferation; (2) cell differentiation; (3) genetic integrity; (4) allogenicity; and (5) ethical issues. We discuss these issues and underline the fact that the answers to these questions lie in a better understanding of the biology of PSCs. To contribute to this aim, we have developed a free online expression atlas, Amazonia!, that displays for each human gene a virtual northern blot for PSC samples and adult tissues (http://www.amazonia.transcriptome.eu).

Ramos-Ibeas, P., et al. (2015). "Characterisation of the deleted in azoospermia like (Dazl)-green fluorescent protein mouse model generated by a two-step embryonic stem cell-based strategy to identify pluripotent and germ cells." Reprod Fertil Dev.
The deleted in azoospermia like (Dazl) gene is preferentially expressed in germ cells; however, recent studies indicate that it may have pluripotency-related functions. We generated Dazl-green fluorescent protein (GFP) transgenic mice and assayed the ability of Dazl-driven GFP to mark preimplantation embryo development, fetal, neonatal and adult tissues, and in vitro differentiation from embryonic stem cells (ESCs) to embryoid bodies (EBs) and to primordial germ cell (PGC)-like cells. The Dazl-GFP mice were generated by a two-step ESC-based strategy, which enabled primary and secondary screening of stably transfected clones before embryo injection. During preimplantation embryo stages, GFP was detected from the zygote to blastocyst stage. At Embryonic Day (E) 12.5, GFP was expressed in gonadal ridges and in neonatal gonads of both sexes. In adult mice, GFP expression was found during spermatogenesis from spermatogonia to elongating spermatids and in the cytoplasm of oocytes. However, GFP mRNA was also detected in other tissues harbouring multipotent cells, such as the intestine and bone marrow. Fluorescence was maintained along in vitro Dazl-GFP ESC differentiation to EBs, and in PGC-like cells. In addition to its largely known function in germ cell development, Dazl could have an additional role in pluripotency, supporting these transgenic mice as a valuable tool for the prospective identification of stem cells from several tissues.

Rasmussen, M. L., et al. (2018). "Wnt Signaling and Its Impact on Mitochondrial and Cell Cycle Dynamics in Pluripotent Stem Cells." Genes (Basel) **9**(2).
The core transcriptional network regulating stem cell self-renewal and pluripotency remains an intense area of research. Increasing evidence indicates that modified regulation of basic cellular processes such as mitochondrial dynamics, apoptosis, and cell cycle are also essential for pluripotent stem cell identity and fate decisions. Here, we review evidence for Wnt

regulation of pluripotency and self-renewal, and its connections to emerging features of pluripotent stem cells, including (1) increased mitochondrial fragmentation, (2) increased sensitivity to cell death, and (3) shortened cell cycle. We provide a general overview of the stem cell-specific mechanisms involved in the maintenance of these uncharacterized hallmarks of pluripotency and highlight potential links to the Wnt signaling pathway. Given the physiological importance of stem cells and their enormous potential for regenerative medicine, understanding fundamental mechanisms mediating the crosstalk between Wnt, organelle-dynamics, apoptosis, and cell cycle will be crucial to gain insight into the regulation of stemness.

Ratajczak, M. Z., et al. (2016). "Stem cells and clinical practice: new advances and challenges at the time of emerging problems with induced pluripotent stem cell therapies." Pol Arch Med Wewn **126**(11): 879-890.

Humans, like other species that reproduce sexually, originate from a fertilized oocyte (zygote), which is a totipotent stem cell giving rise to an adult organism. During the process of embryogenesis, stem cells at different levels of the developmental hierarchy establish all 3 germ layers and give rise to tissuecommitted stem cells, which are responsible for rejuvenation of a given tissue or organ. The robustness of the stem cell compartment is one of the major factors that directly impact life quality as well as lifespan. Stem cells continuously replace cells and tissues that are used up during life; however, this replacement occurs at a different pace in various organs. The rapidly developing field of regenerative medicine is taking advantage of these physiological properties of stem cells and is attempting to employ them in clinical settings to regenerate damaged organs (eg, the heart, liver or bone). For this purpose, the stem cells most successfully employed so far are adult tissue-derived stem cells isolated mainly from bone marrow, mobilized peripheral blood, umbilical cord blood, fat tissue, and even myocardial biopsies. At the same time, attempts to employ embryonic stem cells and induced pluripotent stem cells in the clinic have failed due to their genomic instability and the risk of tumor formation. In this review, we will discuss the various potential sources of stem cells that are currently employed in regenerative medicine and the mechanisms that explain their beneficial effects. We will also highlight the preliminary results of clinical trials as well as the emerging problems relating to stem cell therapies in cardiology.

Reboun, M., et al. (2016). "X-Chromosome Inactivation Analysis in Different Cell Types and Induced Pluripotent Stem Cells Elucidates the Disease Mechanism in a Rare Case of Mucopolysaccharidosis Type II in a Female." Folia Biol (Praha) **62**(2): 82-89.

Mucopolysaccharidosis type II (MPS II) is an X-linked lysosomal storage disorder resulting from deficiency of iduronate-2-sulphatase activity. The disease manifests almost exclusively in males; only 16 symptomatic heterozygote girls have been reported so far. We describe the results of X-chromosome inactivation analysis in a 5-year-old girl with clinically severe disease and heterozygous mutation p.Arg468Gln in the IDS gene. X inactivation analysed at three X-chromosome loci showed extreme skewing (96/4 to 99/1) in two patient's cell types. This finding correlated with exclusive expression of the mutated allele. Induced pluripotent stem cells (iPSC) generated from the patient's peripheral blood demonstrated characteristic pluripotency markers, deficiency of enzyme activity, and mutation in the IDS gene. These cells were capable of differentiation into other cell types (cardiomyocytes, neurons). In MPS II iPSC clones, the X inactivation ratio remained highly skewed in culture conditions that led to partial X inactivation reset in Fabry disease iPSC clones. Our data, in accordance with the literature, suggest that extremely skewed X inactivation favouring the mutated allele is a crucial condition for manifestation of MPS II in females. This suggests that the X inactivation status and enzyme activity have a prognostic value and should be used to evaluate MPS II in females. For the first time, we show generation of iPSC from a symptomatic MPS II female patient that can serve as a cellular model for further research of the pathogenesis and treatment of this disease.

Reddy, G. P., et al. (1997). "Cell cycle analysis and synchronization of pluripotent hematopoietic progenitor stem cells." Blood **90**(6): 2293-2299.

Hematopoietic stem cells purified from mouse bone marrow are quiescent with less than 2% of Lin-Hoechst (low)/Rhodamine (low) (Lin- Ho (low)/Rho (low)) and 10% to 15% of Lin-/Sca+ cells in S phase. These cells enter proliferative cycle and progress through G1 and into S phase in the presence of cytokines and 5% heat-inactivated fetal calf serum (HI-FCS). Cytokine-stimulated Lin- Ho (low)/Rho (low) cells took 36 to 40 hours to complete first division and only 12 hours to complete each of 5 subsequent divisions. These cells require 16 to 18 hours to transit through G0/G1 period and 28 to 30 hours to enter into mid-S phase during the first cycle. Up to 56% of Lin- Rho (low)/Ho (low) cells are high-proliferative potential (7 factor-responsive) colony-forming cells (HPP-CFC). At isolation, HPP-CFC are quiescent, but after 28 to 30 hours of culture, greater than 60% are in S phase. Isoleucine-deprivation of Lin- Ho (low)/Rho (low) cells in S phase of first cycle

reversibly blocked them from entering into second cycle. After the release from isoleucine-block, these cells exhibited a G1 period of less than 2 hours and entered into mid-S phase by 12 hours. Thus, the duration of G1 phase of the cells in second cycle is 4 to 5 times shorter than that observed in their first cycle. Similar cell cycle kinetics are observed with Lin-/Sca+ population of bone marrow cells. Stem cell factor (SCF) alone, in the presence of HI-FCS, is as effective as a cocktail of 2 to 7 cytokines in inducing quiescent Lin-/Sca+ cells to enter into proliferative cycle. Aphidicolin treatment reversibly blocked cytokine-stimulated Lin-/Sca+ cells at G1/S boundary, allowing their tight synchrony as they progress through first S phase and enter into second G1. For these cells also, SCF alone is sufficient for their progression through S phase. These studies indicate a very short G1 phase for stem cells induced to proliferate and offer experimental approaches to synchronize murine hematopoietic stem cells.

Rufaihah, A. J., et al. (2013). "Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity." Am J Transl Res **5**(1): 21-35.

Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) are promising for treatment of vascular diseases. However, hiPSC-ECs purified based on CD31 expression are comprised of arterial, venous, and lymphatic subtypes. It is unclear whether hiPSC-ECs are heterogeneous in nature, and whether there may be functional benefits of enriching for specific subtypes. Therefore, we sought to characterize the hiPSC-ECs and enrich for each subtype, and demonstrate whether such enrichment would have functional significance. The hiPSC-ECs were generated from differentiation of hiPSCs using vascular endothelial growth factor (VEGF)-A and bone morphogenetic protein-4. The hiPSC-ECs were purified based on positive expression of CD31. Subsequently, we sought to enrich for each subtype. Arterial hiPSC-ECs were induced using higher concentrations of VEGF-A and 8-bromoadenosine-3':5'-cyclic monophosphate in the media, whereas lower concentrations of VEGF-A favored venous subtype. VEGF-C and angiopoietin-1 promoted the expression of lymphatic phenotype. Upon FACS purification based on CD31+ expression, the hiPSC-EC population was observed to display typical endothelial surface markers and functions. However, the hiPSC-EC population was heterogeneous in that they displayed arterial, venous, and to a lesser degree, lymphatic lineage markers. Upon comparing vascular formation in matrigel plugs in vivo, we observed that arterial enriched hiPSC-ECs formed a more extensive capillary network in this model, by comparison to a heterogeneous population of hiPSC-ECs. This study demonstrates that FACS purification of CD31+ hiPSC-ECs produces a diverse population of ECs. Refining the differentiation methods can enrich for subtype-specific hiPSC-ECs with functional benefits of enhancing neovascularization.

Ruiz, S., et al. (2015). "Limiting replication stress during somatic cell reprogramming reduces genomic instability in induced pluripotent stem cells." Nat Commun **6**: 8036.

The generation of induced pluripotent stem cells (iPSC) from adult somatic cells is one of the most remarkable discoveries in recent decades. However, several works have reported evidence of genomic instability in iPSC, raising concerns on their biomedical use. The reasons behind the genomic instability observed in iPSC remain mostly unknown. Here we show that, similar to the phenomenon of oncogene-induced replication stress, the expression of reprogramming factors induces replication stress. Increasing the levels of the checkpoint kinase 1 (CHK1) reduces reprogramming-induced replication stress and increases the efficiency of iPSC generation. Similarly, nucleoside supplementation during reprogramming reduces the load of DNA damage and genomic rearrangements on iPSC. Our data reveal that lowering replication stress during reprogramming, genetically or chemically, provides a simple strategy to reduce genomic instability on mouse and human iPSC.

Rungsiwiwut, R., et al. (2016). "Human Umbilical Cord Blood-Derived Serum for Culturing the Supportive Feeder Cells of Human Pluripotent Stem Cell Lines." Stem Cells Int **2016**: 4626048.

Although human pluripotent stem cells (hPSCs) can proliferate robustly on the feeder-free culture system, genetic instability of hPSCs has been reported in such environment. Alternatively, feeder cells enable hPSCs to maintain their pluripotency. The feeder cells are usually grown in a culture medium containing fetal bovine serum (FBS) prior to coculture with hPSCs. The use of FBS might limit the clinical application of hPSCs. Recently, human cord blood-derived serum (hUCS) showed a positive effect on culture of mesenchymal stem cells. It is interesting to test whether hUCS can be used for culture of feeder cells of hPSCs. This study was aimed to replace FBS with hUCS for culturing the human foreskin fibroblasts (HFFs) prior to feeder cell preparation. The results showed that HFFs cultured in hUCS-containing medium (HFF-hUCS) displayed fibroblastic features, high proliferation rates, short population doubling times, and normal karyotypes after prolonged culture. Inactivated HFF-hUCS expressed important genes,

including Activin A, FGF2, and TGFbeta1, which have been implicated in the maintenance of hPSC pluripotency. Moreover, hPSC lines maintained pluripotency, differentiation capacities, and karyotypic stability after being cocultured for extended period with inactivated HFF-hUCS. Therefore, the results demonstrated the benefit of hUCS for hPSCs culture system.

Saadai, P., et al. (2013). "Human induced pluripotent stem cell-derived neural crest stem cells integrate into the injured spinal cord in the fetal lamb model of myelomeningocele." J Pediatr Surg **48**(1): 158-163.

BACKGROUND/PURPOSE: Neurological function in patients with myelomeningocele (MMC) is limited even after prenatal repair. Neural crest stem cells (NCSCs) can improve neurological function in models of spinal cord injury. We aimed to evaluate the survival, integration, and differentiation of human NCSCs derived from induced pluripotent stem cells (iPSC-NCSCs) in the fetal lamb model of MMC. METHODS: Human iPSCs derived from skin fibroblasts were differentiated into NCSCs in vitro, mixed with hydrogel, and seeded on nanofibrous scaffolds for surgical transplantation. Fetal lambs (n=2) underwent surgical MMC creation and repair with iPSC-NCSC seeded scaffolds. Gross necropsy and immunohistochemistry were performed at term. RESULTS: IPSC-NCSCs expressed NCSC markers, maintained > 95% viability, and demonstrated neuronal differentiation in vitro. Immunohistochemical analysis of repaired spinal cords thirty days after transplantation demonstrated the co-localization of human nuclear mitotic apparatus protein (NuMA) and Neurofilament M subunit (NFM) in the area of spinal cord injury. No gross tumors were identified. CONCLUSIONS: Human iPSC-NCSCs survived, integrated, and differentiated into neuronal lineage in the fetal lamb model of MMC. This is the first description of human stem cell engraftment in a model of fetal MMC and supports the concept of using NCSCs to address spinal cord damage in MMC.

Sakiyama, R., et al. (2017). "Clinical translation of bioartificial liver support systems with human pluripotent stem cell-derived hepatic cells." World J Gastroenterol **23**(11): 1974-1979.

There is currently a pressing need for alternative therapies to liver transplantation. The number of patients waiting for a liver transplant is substantially higher than the number of transplantable donor livers, resulting in a long waiting time and a high waiting list mortality. An extracorporeal liver support system is one possible approach to overcome this problem. However, the ideal cell source for developing

bioartificial liver (BAL) support systems has yet to be determined. Recent advancements in stem cell technology allow researchers to generate highly functional hepatocyte-like cells from human pluripotent stem cells (hPSCs). In this mini-review, we summarize previous clinical trials with different BAL systems, and discuss advantages of and potential obstacles to utilizing hPSC-derived hepatic cells in clinical-scale BAL systems.

Sakurai, F., et al. (2017). "Human induced-pluripotent stem cell-derived hepatocyte-like cells as an in vitro model of human hepatitis B virus infection." Sci Rep **7**: 45698.

In order to understand the life cycle of hepatitis B virus (HBV) and to develop efficient anti-HBV drugs, a useful in vitro cell culture system which allows HBV infection and recapitulates virus-host interactions is essential; however, pre-existing in vitro HBV infection models are often problematic. Here, we examined the potential of human induced-pluripotent stem (iPS) cell-derived hepatocyte-like cells (iPS-HLCs) as an in vitro HBV infection model. Expression levels of several genes involved in HBV infection, including the sodium taurocholate cotransporting polypeptide (NTCP) gene, were gradually elevated as the differentiation status of human iPS cells proceeded to iPS-HLCs. The mRNA levels of these genes were comparable between primary human hepatocytes (PHHs) and iPS-HLCs. Following inoculation with HBV, we found significant production of HBV proteins and viral RNAs in iPS-HLCs. The three major forms of the HBV genome were detected in iPS-HLCs by Southern blotting analysis. Anti-HBV agents entecavir and Myrcludex-B, which are a nucleoside analogue reverse transcriptase inhibitor and a synthetic pre-S1 peptide, respectively, significantly inhibited HBV infection in iPS-HLCs. These data demonstrate that iPS-HLCs can be used as a promising in vitro HBV infection model.

Salewski, R. P., et al. (2010). "Are induced pluripotent stem cells the future of cell-based regenerative therapies for spinal cord injury?" J Cell Physiol **222**(3): 515-521.

Despite advances in medical and surgical care, current clinical therapies for spinal cord injury (SCI) are limited. During the last two decades, the search for new therapies has been revolutionized by the discovery of stem cells, inspiring scientists and clinicians to search for stem cell-based reparative approaches for many disorders, including neurotrauma. Cell-based therapies using embryonic and adult stem cells in animal models of these disorders have provided positive outcome results. However, the availability of clinically suitable cell sources for

human application has been hindered by both technical and ethical issues. The recent discovery of induced pluripotent stem (iPS) cells holds the potential to revolutionize the field of regenerative medicine by offering the option of autologous transplantation, thus eliminating the issue of host rejection. Herein, we will provide the rationale for the use of iPS cells in SCI therapies. In this review, we will evaluate the recent advancements in the field of iPS cells including their capacity for differentiation toward neural lineages that may allow iPS cells transplantation in cell-based therapy for spinal cord repair.

Salewski, R. P., et al. (2015). "Transplantation of Induced Pluripotent Stem Cell-Derived Neural Stem Cells Mediate Functional Recovery Following Thoracic Spinal Cord Injury Through Remyelination of Axons." Stem Cells Transl Med **4**(7): 743-754.

UNLABELLED:: Neural stem cells (NSCs) from embryonic or fetal/adult tissue sources have shown considerable promise in regenerative strategies for traumatic spinal cord injury (SCI). However, there are limitations with their use related to the availability, immunogenicity, and uncertainty of the mechanisms involved. To address these issues, definitive NSCs derived from induced pluripotent stem (iPS) cells generated using a nonviral, piggyBac transposon approach, were investigated. Committed NSCs were generated from iPS cells using a free-floating neurosphere methodology previously described by our laboratory. To delineate the mechanism of action, specifically the role of exogenous myelination, NSCs derived from wildtype (wt) and nonmyelinating Shiverer (shi) iPS cell lines were used following thoracic SCI with subacute intraspinal transplantation. Behavioral, histological, and electrophysiological outcomes were analyzed to assess the effectiveness of this treatment. The wt- and shi-iPS-NSCs were validated and shown to be equivalent except in myelination capacity. Both iPS-NSC lines successfully integrated into the injured spinal cord and predominantly differentiated to oligodendrocytes, but only the wt-iPS-NSC treatment resulted in a functional benefit. The wt-iPS-dNSCs, which exhibited the capacity for remyelination, significantly improved neurobehavioral function (Basso Mouse Scale and CatWalk), histological outcomes, and electrophysiological measures of axonal function (sucrose gap analysis) compared with the nonmyelinating iPS-dNSCs and cell-free controls. In summary, we demonstrated that iPS cells can generate translationally relevant NSCs for applications in SCI. Although NSCs have a diverse range of functions in the injured spinal cord, remyelination is the predominant mechanism of recovery following thoracic SCI. SIGNIFICANCE: Gain-of-function/loss-of-function techniques were used to examine the mechanistic importance of graft-derived remyelination following thoracic spinal cord injury (SCI). The novel findings of this study include the first use of neural stem cells (NSCs) from induced pluripotent stem cells (iPSCs) derived using the clonal neurosphere expansion conditions, for the treatment of SCI, the first characterization and in vivo application of iPSCs from Shiverer mouse fibroblasts, and the first evidence of the importance of remyelination by pluripotent-sourced NSCs for SCI repair and regeneration.

Salomonis, N., et al. (2016). "Integrated Genomic Analysis of Diverse Induced Pluripotent Stem Cells from the Progenitor Cell Biology Consortium." Stem Cell Reports **7**(1): 110-125.

The rigorous characterization of distinct induced pluripotent stem cells (iPSC) derived from multiple reprogramming technologies, somatic sources, and donors is required to understand potential sources of variability and downstream potential. To achieve this goal, the Progenitor Cell Biology Consortium performed comprehensive experimental and genomic analyses of 58 iPSC from ten laboratories generated using a variety of reprogramming genes, vectors, and cells. Associated global molecular characterization studies identified functionally informative correlations in gene expression, DNA methylation, and/or copy-number variation among key developmental and oncogenic regulators as a result of donor, sex, line stability, reprogramming technology, and cell of origin. Furthermore, X-chromosome inactivation in PSC produced highly correlated differences in teratoma-lineage staining and regulator expression upon differentiation. All experimental results, and raw, processed, and metadata from these analyses, including powerful tools, are interactively accessible from a new online portal at https://www.synapse.org to serve as a reusable resource for the stem cell community.

Sambathkumar, R., et al. (2018). "Pluripotent Stem Cell-Derived Pancreatic Progenitors and beta-Like Cells for Type 1 Diabetes Treatment." Physiology (Bethesda) **33**(6): 394-402.

In this review, we focus on the processes guiding human pancreas development and provide an update on methods to efficiently generate pancreatic progenitors (PPs) and beta-like cells in vitro from human pluripotent stem cells (hPSCs). Furthermore, we assess the strengths and weaknesses of using PPs and beta-like cell for cell replacement therapy for the treatment of Type 1 diabetes with respect to cell manufacturing, engrafting, functionality, and safety. Finally, we discuss the identification and use of specific cell surface markers to generate safer

populations of PPs for clinical translation and to study the development of PPs in vivo and in vitro.

Sanchez-Freire, V., et al. (2014). "Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells." J Am Coll Cardiol **64**(5): 436-448.

BACKGROUND: Human-induced pluripotent stem cells (iPSCs) are a potentially unlimited source for generation of cardiomyocytes (iPSC-CMs). However, current protocols for iPSC-CM derivation face several challenges, including variability in somatic cell sources and inconsistencies in cardiac differentiation efficiency. OBJECTIVES: This study aimed to assess the effect of epigenetic memory on differentiation and function of iPSC-CMs generated from somatic cell sources of cardiac versus noncardiac origins. METHODS: Cardiac progenitor cells (CPCs) and skin fibroblasts from the same donors were reprogrammed into iPSCs and differentiated into iPSC-CMs via embryoid body and monolayer-based differentiation protocols. RESULTS: Differentiation efficiency was found to be higher in CPC-derived iPSC-CMs (CPC-iPSC-CMs) than in fibroblast-derived iPSC-CMs (Fib-iPSC-CMs). Gene expression analysis during cardiac differentiation demonstrated up-regulation of cardiac transcription factors in CPC-iPSC-CMs, including NKX2-5, MESP1, ISL1, HAND2, MYOCD, MEF2C, and GATA4. Epigenetic assessment revealed higher methylation in the promoter region of NKX2-5 in Fib-iPSC-CMs compared with CPC-iPSC-CMs. Epigenetic differences were found to dissipate with increased cell passaging, and a battery of in vitro assays revealed no significant differences in their morphological and electrophysiological properties at early passage. Finally, cell delivery into a small animal myocardial infarction model indicated that CPC-iPSC-CMs and Fib-iPSC-CMs possess comparable therapeutic capabilities in improving functional recovery in vivo. CONCLUSIONS: This is the first study to compare differentiation of iPSC-CMs from human CPCs versus human fibroblasts from the same donors. The authors demonstrate that although epigenetic memory improves differentiation efficiency of cardiac versus noncardiac somatic cell sources in vitro, it does not contribute to improved functional outcome in vivo.

Santosa, M. M., et al. (2015). "Knowledge Gaps in Rodent Pancreas Biology: Taking Human Pluripotent Stem Cell-Derived Pancreatic Beta Cells into Our Own Hands." Front Endocrinol (Lausanne) **6**: 194.

In the field of stem cell biology and diabetes, we and others seek to derive mature and functional human pancreatic beta cells for disease modeling and cell replacement therapy. Traditionally, knowledge gathered from rodents is extended to human pancreas developmental biology research involving human pluripotent stem cells (hPSCs). While much has been learnt from rodent pancreas biology in the early steps toward Pdx1(+) pancreatic progenitors, much less is known about the transition toward Ngn3(+) pancreatic endocrine progenitors. Essentially, the later steps of pancreatic beta cell development and maturation remain elusive to date. As a result, the most recent advances in the stem cell and diabetes field have relied upon combinatorial testing of numerous growth factors and chemical compounds in an arbitrary trial-and-error fashion to derive mature and functional human pancreatic beta cells from hPSCs. Although this hit-or-miss approach appears to have made some headway in maturing human pancreatic beta cells in vitro, its underlying biology is vaguely understood. Therefore, in this mini-review, we discuss some of these late-stage signaling pathways that are involved in human pancreatic beta cell differentiation and highlight our current understanding of their relevance in rodent pancreas biology. Our efforts here unravel several novel signaling pathways that can be further studied to shed light on unexplored aspects of rodent pancreas biology. New investigations into these signaling pathways are expected to advance our knowledge in human pancreas developmental biology and to aid in the translation of stem cell biology in the context of diabetes treatments.

Sarafian, R., et al. (2018). "Monitoring cell line identity in collections of human induced pluripotent stem cells." Stem Cell Res **28**: 66-70.

The ability to reprogram somatic cells into induced pluripotent stem cells (hiPSCs) has led to the generation of large collections of cell lines from thousands of individuals with specific phenotypes, many of which will be shared among different research groups as invaluable tools for biomedical research. As hiPSC-based research involves extensive culture of many cell lines, the issue periodic cell line identification is particularly important to ensure that cell line identity remains accurate. Here we analyzed the different commercially available genotyping methods considering ease of in-house genotyping, cost and informativeness, and applied one of them in our workflow for hiPSC generation. We show that the chosen STR method was able to establish a unique DNA profile for each of the 35 individuals/hiPSC lines at the examined sites, as well as identify two discrepancies resulting from inadvertently exchanged samples. Our results highlight the importance of hiPSC line genotyping by an in-house method that allows periodic cell line identification and demonstrate

that STR is a useful approach to supplement less frequent karyotyping and epigenetic evaluations.

Sasaki, K., et al. (2015). "Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells." Cell Stem Cell **17**(2): 178-194.

Mechanisms underlying human germ cell development are unclear, partly due to difficulties in studying human embryos and lack of suitable experimental systems. Here, we show that human induced pluripotent stem cells (hiPSCs) differentiate into incipient mesoderm-like cells (iMeLCs), which robustly generate human primordial germ cell-like cells (hPGCLCs) that can be purified using the surface markers EpCAM and INTEGRINalpha6. The transcriptomes of hPGCLCs and primordial germ cells (PGCs) isolated from non-human primates are similar, and although specification of hPGCLCs and mouse PGCs rely on similar signaling pathways, hPGCLC specification transcriptionally activates germline fate without transiently inducing eminent somatic programs. This includes genes important for naive pluripotency and repression of key epigenetic modifiers, concomitant with epigenetic reprogramming. Accordingly, BLIMP1, which represses somatic programs in mice, activates and stabilizes a germline transcriptional circuit and represses a default neuronal differentiation program. Together, these findings provide a foundation for understanding and reconstituting human germ cell development in vitro.

Sato, M., et al. (2017). "Regeneration of cervical reserve cell-like cells from human induced pluripotent stem cells (iPSCs): A new approach to finding targets for cervical cancer stem cell treatment." Oncotarget **8**(25): 40935-40945.

Cervical reserve cells are epithelial progenitor cells that are pathologically evident as the origin of cervical cancer. Thus, investigating the characteristics of cervical reserve cells could yield insight into the features of cervical cancer stem cells (CSCs). In this study, we established a method for the regeneration of cervical reserve cell-like properties from human induced pluripotent stem cells (iPSCs) and named these cells induced reserve cell-like cells (iRCs). Approximately 70% of iRCs were positive for the reserve cell markers p63, CK5 and CK8. iRCs also expressed the SC junction markers CK7, AGR2, CD63, MMP7 and GDA. While iRCs expressed neither ERalpha nor ERbeta, they expressed CA125. These data indicated that iRCs possessed characteristics of cervical epithelial progenitor cells. iRCs secreted higher levels of several inflammatory cytokines such as macrophage migration inhibitory factor (MIF), soluble intercellular adhesion molecule 1 (sICAM-1)

and C-X-C motif ligand 10 (CXCL-10) compared with normal cervical epithelial cells. iRCs also expressed human leukocyte antigen-G (HLA-G), which is an important cell-surface antigen for immune tolerance and carcinogenesis. Together with the fact that cervical CSCs can originate from reserve cells, our data suggested that iRCs were potent immune modulators that might favor cervical cancer cell survival. In conclusion, by generating reserve cell-like properties from iPSCs, we provide a new approach that may yield new insight into cervical cancer stem cells and help find new oncogenic targets.

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.

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 H_2O_2-induced injury. iPSCs were induced into NSCs, iPSC-derived NSCs were treated with 50 muM Sal B for 24.5 h and 500 muM H_2O_2 for 24 h. The resulting effects were examined by flow cytometry analysis, quantitative reverse-transcription polymerase chain reaction, and western blotting. Upon H_2O_2 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 H_2O_2. These results suggest that Sal B protects iPSC-derived NSCs against H_2O_2-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.

Spitzhorn, L. S., et al. (2018). "Transplanted human pluripotent stem cell-derived mesenchymal stem cells support liver regeneration in Gunn rats." Stem Cells Dev.

Gunn rats bear a mutation within the uridine diphosphate glucuronosyltransferase-1A1 (Ugt1a1) gene resulting in high serum bilirubin levels as seen in Crigler-Najjar syndrome. In the present study, the Gunn rat was used as an animal model for heritable liver dysfunction. Human mesenchymal stem cells (iMSC) derived from embryonic stem cells (H1) and induced pluripotent stem cells were transplanted into Gunn rats after partial hepatectomy. The iMSCs engrafted and survived in the liver for up to 2 months. The transplanted iMSCs differentiated into functional hepatocytes as evidenced by partially suppressed hyperbilirubinemia and expression of multiple human-specific hepatocyte markers such as Albumin, hepatocyte nuclear factor 4alpha, UGT1A1, Cytokeratin 18, bile salt export pump, multidrug resistance protein 2, Na/taurocholate-cotransporting polypeptide and alpha-Fetoprotein. These findings imply that transplanted human iMSCs can contribute to liver regeneration in vivo and thus represent a promising tool for the treatment of inherited liver diseases.

Srinivasan, G., et al. (2018). "An integrated biomanufacturing platform for the large-scale expansion and neuronal differentiation of human pluripotent stem cell-derived neural progenitor cells." Acta Biomater **74**: 168-179.

Human pluripotent stem cell derived neural progenitor cells (hNPCs) have the unique properties of long-term in vitro expansion as well as differentiation into the various neurons and supporting cell types of

the central nervous system (CNS). Because of these characteristics, hNPCs have tremendous potential in the modeling and treatment of various CNS diseases and disorders. However, expansion and neuronal differentiation of hNPCs in quantities necessary for these applications is not possible with current two dimensional (2-D) approaches. Here, we used a fully defined peptide substrate as the basis for a microcarrier (MC)-based suspension culture system. Several independently derived hNPC lines were cultured on MCs for multiple passages as well as efficiently differentiated to neurons. Finally, this MC-based system was used in conjunction with a low shear rotating wall vessel (RWV) bioreactor for the integrated, large-scale expansion and neuronal differentiation of hNPCs. Overall, this fully defined and scalable biomanufacturing system will facilitate the generation of hNPCs and their neuronal derivatives in quantities necessary for basic and translational applications. STATEMENT OF SIGNIFICANCE: In this work, we developed a microcarrier (MC)-based culture system that allows for the expansion and neuronal differentiation of human pluripotent stem cell-derived neural progenitor cells (hNPCs) under defined conditions. In turn, this MC approach was implemented in a rotating wall vessel (RWV) bioreactor for the large-scale expansion and neuronal differentiation of hNPCs. This work is of significance as it overcomes current limitations of conventional two dimensional (2-D) culture systems to enable the generation of hNPCs and their neuronal derivatives in quantities required for downstream applications in disease modeling, drug screening, and regenerative medicine.

Stebbins, M. J., et al. (2018). "Activation of RARalpha, RARgamma, or RXRalpha Increases Barrier Tightness in Human Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells." Biotechnol J **13**(2).

The blood-brain barrier (BBB) is critical to central nervous system (CNS) health. Brain microvascular endothelial cells (BMECs) are often used as in vitro BBB models for studying BBB dysfunction and therapeutic screening applications. Human pluripotent stem cells (hPSCs) can be differentiated to cells having key BMEC barrier and transporter properties, offering a renewable, scalable source of human BMECs. hPSC-derived BMECs have previously been shown to respond to all-trans retinoic acid (RA), and the goal of this study was to identify the stages at which differentiating human induced pluripotent stem cells (iPSCs) respond to activation of RA receptors (RARs) to impart BBB phenotypes. Here the authors identified that RA application to iPSC-derived BMECs at days 6-8 of differentiation led to a

substantial elevation in transendothelial electrical resistance and induction of VE-cadherin expression. Specific RAR agonists identified RARalpha, RARgamma, and RXRalpha as receptors capable of inducing barrier phenotypes. Moreover, RAR/RXRalpha costimulation elevated VE-cadherin expression and improved barrier fidelity to levels that recapitulated the effects of RA. This study elucidates the roles of RA signaling in iPSC-derived BMEC differentiation, and identifies directed agonist approaches that can improve BMEC fidelity for drug screening studies while also distinguishing potential nuclear receptor targets to explore in BBB dysfunction and therapy.

Stebbins, M. J., et al. (2016). "Differentiation and characterization of human pluripotent stem cell-derived brain microvascular endothelial cells." Methods **101**: 93-102.

The blood-brain barrier (BBB) is a critical component of the central nervous system (CNS) that regulates the flux of material between the blood and the brain. Because of its barrier properties, the BBB creates a bottleneck to CNS drug delivery. Human in vitro BBB models offer a potential tool to screen pharmaceutical libraries for CNS penetration as well as for BBB modulators in development and disease, yet primary and immortalized models respectively lack scalability and robust phenotypes. Recently, in vitro BBB models derived from human pluripotent stem cells (hPSCs) have helped overcome these challenges by providing a scalable and renewable source of human brain microvascular endothelial cells (BMECs). We have demonstrated that hPSC-derived BMECs exhibit robust structural and functional characteristics reminiscent of the in vivo BBB. Here, we provide a detailed description of the methods required to differentiate and functionally characterize hPSC-derived BMECs to facilitate their widespread use in downstream applications.

Stover, A. E., et al. (2016). "Culturing Human Pluripotent and Neural Stem Cells in an Enclosed Cell Culture System for Basic and Preclinical Research." J Vis Exp (112).

This paper describes how to use a custom manufactured, commercially available enclosed cell culture system for basic and preclinical research. Biosafety cabinets (BSCs) and incubators have long been the standard for culturing and expanding cell lines for basic and preclinical research. However, as the focus of many stem cell laboratories shifts from basic research to clinical translation, additional requirements are needed of the cell culturing system. All processes must be well documented and have exceptional requirements for sterility and

reproducibility. In traditional incubators, gas concentrations and temperatures widely fluctuate anytime the cells are removed for feeding, passaging, or other manipulations. Such interruptions contribute to an environment that is not the standard for cGMP and GLP guidelines. These interruptions must be minimized especially when cells are utilized for therapeutic purposes. The motivation to move from the standard BSC and incubator system to a closed system is that such interruptions can be made negligible. Closed systems provide a work space to feed and manipulate cell cultures and maintain them in a controlled environment where temperature and gas concentrations are consistent. This way, pluripotent and multipotent stem cells can be maintained at optimum health from the moment of their derivation all the way to their eventual use in therapy.

Stover, A. E. and P. H. Schwartz (2011). "Adaptation of human pluripotent stem cells to feeder-free conditions in chemically defined medium with enzymatic single-cell passaging." Methods Mol Biol **767**: 137-146.

This protocol describes the culture of human pluripotent stem cells (PSCs) under feeder-free conditions in a commercially available, chemically defined, growth medium, using Matrigel as a substrate and the enzyme solution Accutase for single-cell passaging. This system is strikingly different from traditional PSC culture, where the cells are co-cultured with feeder cells and in medium containing serum replacement. PSCs cultured in this new system have a different morphology than those cultured on feeder cells but retain their characteristic pluripotency. This feeder-free PSC culture system is conceptually similar to feeder-free systems that use mouse embryonic fibroblast (MEF)-conditioned medium (MEF-CM) and Matrigel substratum. Instead of MEF-CM, a very complex and undefined medium, this new system uses StemPro SFM, a chemically defined medium that permits enzymatic passaging with Accutase to disaggregate the colonies into single cells. Accutase passaging has been used in conjunction with Stempro in our hands for 20+ passages without detectable karyotypic abnormalities. We will also review techniques for adapting cultures previously grown on MEFs, routine passaging of the cells, and cryopreservation.

Sugawara, T., et al. (2012). "Investigating cellular identity and manipulating cell fate using induced pluripotent stem cells." Stem Cell Res Ther **3**(2): 8.

Induced pluripotent stem (iPS) cells, obtained from reprogramming somatic cells by ectopic expression of a defined set of transcription factors or chemicals, are expected to be used as differentiated cells for drug screening or evaluations of drug toxicity and cell replacement therapies. As pluripotent stem cells, iPS cells are similar to embryonic stem (ES) cells in morphology and marker expression. Several types of iPS cells have been generated using combinations of reprogramming molecules and/or small chemical compounds from different types of tissues. A comprehensive approach, such as global gene or microRNA expression analysis and whole genomic DNA methylation profiling, has demonstrated that iPS cells are similar to their embryonic counterparts. Considering the substantial variation among iPS cell lines reported to date, the safety and therapeutic implications of these differences should be thoroughly evaluated before they are used in cell therapies. Here, we review recent research defining the concept of standardization for iPS cells, their ability to differentiate and the identity of the differentiated cells.

Sugita, S., et al. (2015). "Inhibition of T-cell activation by retinal pigment epithelial cells derived from induced pluripotent stem cells." Invest Ophthalmol Vis Sci **56**(2): 1051-1062.

PURPOSE: The purpose of this study was to determine whether human retinal pigment epithelial (RPE) cells from induced pluripotent stem (iPS) cells could inhibit T-cell activation in vitro. METHODS: Cultured iPS-derived RPE (iPS-RPE) cells were established from fresh skin tissues or dental pulp cells obtained from healthy donors or a retinal patient after informed consent was obtained. To confirm expression of the specific markers on iPS and iPS-RPE cells, immunohistochemistry, quantitative RT-PCR (qRT-PCR), and flow cytometry were performed. Target T cells were obtained from peripheral blood mononuclear cells of healthy donors. Target T cells were assessed for proliferation by incorporation of bromodeoxyuridine or carboxyfluorescein succinimidyl ester for production of cytokines such as IFN-gamma. Expression of TGFbeta and other candidate molecules by iPS-RPE cells was evaluated with flow cytometry, ELISA, multiplex cytokine array, immunohistochemistry, and qRT-PCR. RESULTS: The RPE cells we established from iPS cells had many characteristics of mature RPE cells but no characteristics of pluripotent stem cells. Cultured iPS-RPE cells inhibited cell proliferation and production of IFN-gamma by activated CD4(+) T cells. In some bystander T cells, iPS-derived RPE cells induced CD25(+)Foxp3(+) regulatory T cells in vitro. Induced pluripotent stem-RPE cells constitutively expressed TGFbeta and suppressed activation of T cells via soluble TGFbeta, because TGFbeta-downregulated iPS-RPE cells did not inhibit this T-cell activation.

CONCLUSIONS: Cultured iPS-derived retinal cells fully suppress T-cell activation. Transplantation of iPS-RPE cells into the eye might be a therapy for retinal disorders.

Sugita, S., et al. (2018). "Natural Killer Cell Inhibition by HLA-E Molecules on Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelial Cells." Invest Ophthalmol Vis Sci **59**(5): 1719-1731.

Purpose: To determine whether human induced pluripotent stem (iPS) cell-derived retinal pigment epithelial (RPE) cells (iPS-RPE) can suppress natural killer (NK) cell activation. Methods: iPS-RPE cells were cocultured with peripheral blood mononuclear cells (PBMCs) or purified NK cells from healthy donors after stimulation with cytokines. To confirm expression of NK cell-specific markers, flow cytometry and quantitative RT-PCR (qRT-PCR) were performed. NK cells (or PBMCs) cocultured with iPS-RPE cells were assessed for proliferation by Ki-67 expression with flow cytometry, and NK suppression by RPE cells was assessed for granzyme B production with ELISA. Human leukocyte antigen (HLA) expression including HLA-E on iPS-RPE cells was evaluated with flow cytometry and qRT-PCR. The effect of HLA-E downregulation was also investigated using small interfering RNA (siRNA) systems. Following iPS-RPE cell transplantation in vivo, we evaluated NK cell invasion in the retina with immunohistochemistry. Results: Activated NK cells expressed NK-related markers such as CD16, CD56, and CD11b, and NK cells produced cytotoxic agents such as granzyme B, perforin, and TNF-alpha. Human iPS-RPE cells inhibited cell proliferation and production of these cytotoxic agents by activated NK cells in vitro. iPS-RPE cells constitutively expressed HLA-E and suppressed NK cell activation through an interaction between HLA-E and CD94/NKG2A. Moreover, immunohistochemical evaluation of monkey RPE transplantation into in vivo immune rejection models showed no NK cell invasion in the retina in allografts or xenografts except for one xenografted eye. Conclusions: Cultured iPS cell-derived RPE cells greatly suppress NK cell activation. Thus, NK cells might be inactivated when exposed to this type of retinal cell.

Takayama, K., et al. (2017). "Generation of safe and therapeutically effective human induced pluripotent stem cell-derived hepatocyte-like cells for regenerative medicine." Hepatol Commun **1**(10): 1058-1069.

Hepatocyte-like cells (HLCs) differentiated from human induced pluripotent stem (iPS) cells are expected to be applied for regenerative medicine. In

this study, we attempted to generate safe and therapeutically effective human iPS-HLCs for hepatocyte transplantation. First, human iPS-HLCs were generated from a human leukocyte antigen-homozygous donor on the assumption that the allogenic transplantation might be carried out. Highly efficient hepatocyte differentiation was performed under a feeder-free condition using human recombinant laminin 111, laminin 511, and type IV collagen. The percentage of asialoglycoprotein receptor 1-positive cells was greater than 80%, while the percentage of residual undifferentiated cells was approximately 0.003%. In addition, no teratoma formation was observed even at 16 weeks after human iPS-HLC transplantation. Furthermore, harmful genetic somatic single-nucleotide substitutions were not observed during the hepatocyte differentiation process. We also developed a cryopreservation protocol for hepatoblast-like cells without negatively affecting their hepatocyte differentiation potential by programming the freezing temperature. To evaluate the therapeutic potential of human iPS-HLCs, these cells (1 x 10(6) cells/mouse) were intrasplenically transplanted into acute liver injury mice treated with 3 mL/kg CCl4 only once and chronic liver injury mice treated with 0.6 mL/kg CCl4 twice weekly for 8 weeks. By human iPS-HLC transplantation, the survival rate of the acute liver injury mice was significantly increased and the liver fibrosis level of chronic liver injury mice was significantly decreased. Conclusion: We were able to generate safe and therapeutically effective human iPS-HLCs for hepatocyte transplantation. (Hepatology Communications 2017;1:1058-1069).

Takayama, K. and H. Mizuguchi (2017). "Generation of human pluripotent stem cell-derived hepatocyte-like cells for drug toxicity screening." Drug Metab Pharmacokinet **32**(1): 12-20.

Because drug-induced liver injury is one of the main reasons for drug development failures, it is important to perform drug toxicity screening in the early phase of pharmaceutical development. Currently, primary human hepatocytes are most widely used for the prediction of drug-induced liver injury. However, the sources of primary human hepatocytes are limited, making it difficult to supply the abundant quantities required for large-scale drug toxicity screening. Therefore, there is an urgent need for a novel unlimited, efficient, inexpensive, and predictive model which can be applied for large-scale drug toxicity screening. Human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are able to replicate indefinitely and differentiate into most of the body's cell types, including hepatocytes. It is expected that hepatocyte-like cells generated from human

ES/iPS cells (human ES/iPS-HLCs) will be a useful tool for drug toxicity screening. To apply human ES/iPS-HLCs to various applications including drug toxicity screening, homogenous and functional HLCs must be differentiated from human ES/iPS cells. In this review, we will introduce the current status of hepatocyte differentiation technology from human ES/iPS cells and a novel method to predict drug-induced liver injury using human ES/iPS-HLCs.

Takei, H., et al. (2018). "Skewed megakaryopoiesis in human induced pluripotent stem cell-derived haematopoietic progenitor cells harbouring calreticulin mutations." Br J Haematol **181**(6): 791-802.

Somatic mutations in the calreticulin (CALR) gene have been found in most patients with JAK2- and MPL-unmutated Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs). It has recently been shown that mutant CALR constitutively activates the thrombopoietin receptor MPL and, thus, plays a causal role in the development of MPNs. However, the roles of mutant CALR in human haematopoietic cell differentiation remain predominantly elusive. To examine the impact of the 5-base insertion mutant CALR gene (Ins5) on haematopoietic cell differentiation, we generated induced pluripotent stem cells from an essential thrombocythaemia (ET) patient harbouring a CALR-Ins5 mutation and from a healthy individual (WT). Megakaryopoiesis was more prominent in Ins5-haematopoietic progenitor cells (Ins5-HPCs) than in WT-HPCs, implying that the system recapitulates megakaryocytosis observed in the bone marrow of CALR-mutant ET patients. Ins5-HPCs exhibited elevated expression levels of GATA1 and GATA2, suggesting a premature commitment to megakaryocytic differentiation in progenitor cells. We also demonstrated that 3-hydroxy anagrelide markedly perturbed megakaryopoiesis, but not erythropoiesis. Collectively, we established an in vitro model system that recapitulates megakaryopoiesis caused by mutant CALR. This system can be used to validate therapeutic compounds for MPN patients harbouring CALR mutations and in detailed studies on mutant CALR in human haematological cell differentiation.

Talavera-Adame, D., et al. (2016). "Effective endothelial cell and human pluripotent stem cell interactions generate functional insulin-producing beta cells." Diabetologia **59**(11): 2378-2386.

AIMS/HYPOTHESIS: Endothelial cells (ECs) play an essential role in pancreatic organogenesis. We hypothesise that effective in vitro interactions between human microvascular endothelial cells (HMECs) and human pluripotent stem cells (hPSCs) results in the generation of functional pancreatic beta cells.

METHODS: Embryoid bodies (EBs) derived from hPSCs were cultured alone (controls) or with ECs in collagen gels. Subsequently, cells were analysed for pancreatic beta cell markers, and then isolated and expanded. Insulin secretion in response to glucose was evaluated in vitro by static and dynamic (perifusion) assays, and in vivo by EB transplantation into immunodeficient mice. RESULTS: Co-cultured EBs had a higher expression of mature beta cells markers and enhanced insulin secretion in vitro, compared with controls. In mice, transplanted EBs had higher levels of human C-peptide secretion with a significant reduction in hyperglycaemia after the selective destruction of native pancreatic beta cells. In addition, there was significant in vitro upregulation of bone morphogenetic proteins 2 and 4 (BMP-2, 4) in co-cultured cells, compared with controls. CONCLUSIONS/INTERPRETATION: ECs provide essential signalling in vitro, such as activation of the BMP pathway, for derivation of functional insulin-producing beta cells from hPSCs.

Talavera-Adame, D., et al. (2016). "Erratum to: Effective endothelial cell and human pluripotent stem cell interactions generate functional insulin-producing beta cells." Diabetologia **59**(12): 2730.

Talbot, N. C., et al. (2017). "Bovine trophectoderm cells induced from bovine fibroblasts with induced pluripotent stem cell reprogramming factors." Mol Reprod Dev **84**(6): 468-485.

Thirteen independent induced bovine trophectoderm (iBT) cell lines were established by reprogramming bovine fetal liver-derived fibroblasts after viral-vector transduction with either six or eight factors, including POU5F1 (OCT4), KLF4, SOX2, MYC, NANOG, LIN28, SV40 large T antigen, and hTERT. Light- and electron-microscopy analysis showed that the iBT cells had epithelial cell morphology typical of bovine trophectoderm cells. Reverse-transcription-PCR assays indicated that all of the cell lines expressed interferon-tau (IFNT) at passages 1 or 2. At later passages (>/= passage 8), however, immunoblot and antiviral activity assays revealed that more than half of the iBT cell lines had stopped expressing IFNT. Messenger RNAs specific to trophectoderm differentiation and function were found in the iBT cell lines, and 2-dimensional-gel analysis for cellular proteins showed an expression pattern similar to that of trophectoderm cell lines derived from bovine blastocysts. Integration of some of the human reprogramming factors, including POU5F1, KLF4, SOX2, MYC, NANOG, and LIN28, were detected by PCR, but their transcription was mostly absent in the iBT cell lines. Gene expression assessment of endogenous bovine reprogramming factor orthologs

revealed endogenous bLIN28 and bMYC transcripts in all; bSOX2 and bNANOG in none; and bKLF4 and bPOU5F1 in less than half of the iBT cell lines. These results demonstrate that bovine trophectoderm can be induced via reprogramming factor expression from bovine liver-derived fibroblasts, although other fibroblast populations-e.g., derived from fetal thigh tissue-may produce similar results, albeit at lower frequencies.

Tamaoki, N., et al. (2010). "Dental pulp cells for induced pluripotent stem cell banking." J Dent Res **89**(8): 773-778.

Defined sets of transcriptional factors can reprogram human somatic cells to induced pluripotent stem (iPS) cells. However, many types of human cells are not easily accessible to minimally invasive procedures. Here we evaluated dental pulp cells (DPCs) as an optimal source of iPS cells, since they are easily obtained from extracted teeth and can be expanded under simple culture conditions. From all 6 DPC lines tested with the conventional 3 or 4 reprogramming factors, iPS cells were effectively established from 5 DPC lines. Furthermore, determination of the HLA types of 107 DPC lines revealed 2 lines homozygous for all 3 HLA loci and showed that if an iPS bank is established from these initial pools, the bank will cover approximately 20% of the Japanese population with a perfect match. Analysis of these data demonstrates the promising potential of DPC collections as a source of iPS cell banks for use in regenerative medicine.

Tamo, L., et al. (2018). "Generation of an alveolar epithelial type II cell line from induced pluripotent stem cells." Am J Physiol Lung Cell Mol Physiol.

Differentiation of primary alveolar type II epithelial cells (AEC type II) to AEC type I in culture is a major barrier in the study of the alveolar epithelium in vitro. The establishment of an AEC type II cell line derived from induced pluripotent stem cells (iPSC) represents a novel opportunity to study alveolar epithelial cell biology, for instance in the context of lung injury, fibrosis and repair. In the present study, we generated long lasting AECII from iPSC (iPSC-AEC). Long lasting iPSC-AECII (LL)-iPSC-AECII) displayed morphological characteristics of AECII including growth in a cobble stone monolayer, the presence of lamellar bodies and microvilli as shown by electron microscopy. Also, (LL)-iPSC-AECII expressed AEC type II proteins such as cytokeratin, surfactant protein C and lysotracker DND 26 (a marker for lamellar bodies). Furthermore, the (LL)-iPSC-AECII exhibited functional properties of AECII by an increase of transepithelial electrical resistance

(TERR) over time, secretion of inflammatory mediators in biologically relevant quantities (interleukin-6, interleukin-8) and efficient in vitro alveolar epithelial wound repair. Consistent with the AECII phenotype, the cell line showed the ability to uptake and release surfactant protein B, to secrete phospholipids and to differentiate into AEC type I. In summary, we established a long lasting, but finite AEC type II cell line derived from iPSC as a novel cellular model to study alveolar epithelial cell biology in lung health and disease.

Tan, H. K., et al. (2014). "Human finger-prick induced pluripotent stem cells facilitate the development of stem cell banking." Stem Cells Transl Med **3**(5): 586-598.

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients can be a good model for studying human diseases and for future therapeutic regenerative medicine. Current initiatives to establish human iPSC (hiPSC) banking face challenges in recruiting large numbers of donors with diverse diseased, genetic, and phenotypic representations. In this study, we describe the efficient derivation of transgene-free hiPSCs from human finger-prick blood. Finger-prick sample collection can be performed on a "do-it-yourself" basis by donors and sent to the hiPSC facility for reprogramming. We show that single-drop volumes of finger-prick samples are sufficient for performing cellular reprogramming, DNA sequencing, and blood serotyping in parallel. Our novel strategy has the potential to facilitate the development of large-scale hiPSC banking worldwide.

Tan, R. P., et al. (2018). "Integration of induced pluripotent stem cell-derived endothelial cells with polycaprolactone/gelatin-based electrospun scaffolds for enhanced therapeutic angiogenesis." Stem Cell Res Ther **9**(1): 70.

BACKGROUND: Induced pluripotent stem-cell derived endothelial cells (iPSC-ECs) can be generated from any somatic cell and their iPSC sources possess unlimited self-renewal. Previous demonstration of their proangiogenic activity makes them a promising cell type for treatment of ischemic injury. As with many other stem cell approaches, the low rate of in-vivo survival has been a major limitation to the efficacy of iPSC-ECs to date. In this study, we aimed to increase the in-vivo lifetime of iPSC-ECs by culturing them on electrospun polycaprolactone (PCL)/gelatin scaffolds, before quantifying the subsequent impact on their proangiogenic function. METHODS: iPSC-ECs were isolated and stably transfected with a luciferase reporter to facilitate quantification of cell numbers and non-invasive imaging in-vivo PCL/gelatin scaffolds were

engineered using electrospinning to obtain woven meshes of nanofibers. iPSC-ECs were cultured on scaffolds for 7 days. Subsequently, cell growth and function were assessed in vitro followed by implantation in a mouseback subcutaneous model for 7 days. RESULTS: Using a matrix of conditions, we found that scaffold blends with ratios of PCL:gelatin of 70:30 (PG73) spun at high flow rates supported the greatest levels of iPSC-EC growth, retention of phenotype, and function in vitro. Implanting iPSC-ECs seeded on PG73 scaffolds in vivo improved their survival up to 3 days, compared to cells directly injected into control wounds, which were no longer observable within 1 h. Enhanced engraftment improved blood perfusion, observed through non-invasive laser Doppler imaging. Immunohistochemistry revealed a corresponding increase in host angiogenic mechanisms characterized by the enhanced recruitment of macrophages and the elevated expression of proangiogenic cytokines vascular endothelial growth factor and placental growth factor. CONCLUSIONS: Knowledge of these mechanisms combined with a deeper understanding of the scaffold parameters influencing this function provides the groundwork for optimizing future iPSC-EC therapies utilizing engraftment platforms. The development of combined scaffold and iPSC-EC therapies could ultimately improve therapeutic angiogenesis and the treatment of ischemic injury.

Tashiro, S., et al. (2018). "High cell density suppresses BMP4-induced differentiation of human pluripotent stem cells to produce macroscopic spatial patterning in a unidirectional perfusion culture chamber." J Biosci Bioeng **126**(3): 379-388.

Spatial pattern formation is a critical step in embryogenesis. Bone morphogenetic protein 4 (BMP4) and its inhibitors are major factors for the formation of spatial patterns during embryogenesis. However, spatial patterning of the human embryo is unclear because of ethical issues and isotropic culture environments resulting from conventional culture dishes. Here, we utilized human pluripotent stem cells (hiPSCs) and a simple anisotropic (unidirectional perfusion) culture chamber, which creates unidirectional conditions, to measure the cell community effect. The influence of cell density on BMP4-induced differentiation was explored during static culture using a conventional culture dish. Immunostaining of the early differentiation marker SSEA-1 and the mesendoderm marker BRACHYURY revealed that high cell density suppressed differentiation, with small clusters of differentiated and undifferentiated cells formed. Addition of five-fold higher concentration of BMP4 showed similar results, suggesting that suppression was not caused by

depletion of BMP4 but rather by high cell density. Quantitative RT-PCR array analysis showed that BMP4 induced multi-lineage differentiation, which was also suppressed under high-density conditions. We fabricated an elongated perfusion culture chamber, in which proteins were transported unidirectionally, and hiPSCs were cultured with BMP4. At low density, the expression was the same throughout the chamber. However, at high density, SSEA-1 and BRACHYURY were expressed only in upstream cells, suggesting that some autocrine/paracrine factors inhibited the action of BMP4 in downstream cells to form the spatial pattern. Human iPSCs cultured in a perfusion culture chamber might be useful for studying in vitro macroscopic pattern formation in human embryogenesis.

Tateno, H., et al. (2017). "Development of a practical sandwich assay to detect human pluripotent stem cells using cell culture media." Regen Ther **6**: 1-8.

Human pluripotent stem cells are considered to be ideal cell sources for regenerative medicine, but their clinical and industrial application is hindered by their tumorigenic potential. Previously we have identified a pluripotent stem cell-specific lectin rBC2LCN recognizing podocalyxin as a cell surface ligand. More recently, podocalyxin was found to be a soluble ligand of rBC2LCN that is secreted specifically from human pluripotent stem cells into cell culture media. Taking advantage of this phenomenon, we have previously developed a sandwich assay targeting the soluble podocalyxin using rBC2LCN as a capturing probe and another lectin rABA as an overlay probe to detect human pluripotent stem cells residing in cell therapy products derived from human pluripotent stem cells. A drawback to this, however, was that cell culture media containing fetal bovine serum was found to cause a substantial background signal to the sandwich assay. To reduce the background and increase the sensitivity, we screened different overlay probes to detect the soluble podocalyxin. Among them, an anti-keratan sulfate monoclonal antibody called R-10G showed the highest sensitivity and provided a low background signal to fetal bovine serum. The established sandwich assay using rBC2LCN and R-10G was proved to be powerful, which allowed the high-sensitive detection of human induced pluripotent stem cells residing among clinical-grade cardiomyocytes and neural stem cells, both derived from human induced pluripotent stem cells. The developed method has a possibility to be a standard technology to detect human induced pluripotent stem cells resided in various types of cell therapy products.

Tateno, H., et al. (2014). "Live-cell imaging of human pluripotent stem cells by a novel lectin probe rBC2LCN." Methods Mol Biol **1200**: 313-318.

We performed comprehensive glycome analysis of a large set of human pluripotent stem cells (hPSCs) using a high-density lectin microarray. We found that a recombinant lectin, rBC2LCN, binds exclusively to all of the undifferentiated hPSCs tested, but not to differentiated somatic cells. rBC2LCN can be used for both the staining and sorting of fixed and live hPSCs. rBC2LCN could serve as a novel detection reagent for hPSCs, particularly given that rBC2LCN is cost effective and, unlike conventional antibodies which require mammalian cells for their production, is easy to produce in a large amount (0.1 g/L) in an Escherichia coli expression system. Here we describe protocols for the fluorescence staining of fixed and live hPSCs and their detection by flow cytometry.

Taura, A., et al. (2014). "Effects of mouse utricle stromal tissues on hair cell induction from induced pluripotent stem cells." BMC Neurosci **15**: 121.

BACKGROUND: Hair cells are important for maintaining our sense of hearing and balance. However, they are difficult to regenerate in mammals once they are lost. Clarification of the molecular mechanisms underlying inner ear disorders is also impeded by the anatomical limitation of experimental access to the human inner ear. Therefore, the generation of hair cells, possibly from induced pluripotent stem (iPS) cells, is important for regenerative therapy and studies of inner ear diseases. RESULTS: We generated hair cells from mouse iPS cells using an established stepwise induction protocol. First, iPS cells were differentiated into the ectodermal lineage by floating culture. Next, they were treated with basic fibroblast growth factor to induce otic progenitor cells. Finally, the cells were co-cultured with three kinds of mouse utricle tissues: stromal tissue, stromal tissue + sensory epithelium, and the extracellular matrix of stromal tissue. Hair cell-like cells were successfully generated from iPS cells using mouse utricle stromal tissues. However, no hair cell-like cells with hair bundle-like structures were formed using other tissues. CONCLUSIONS: Hair cell-like cells were induced from mouse iPS cells using mouse utricle stromal tissues. Certain soluble factors from mouse utricle stromal cells might be important for induction of hair cells from iPS cells.

Tchao, J., et al. (2013). "Engineered Human Muscle Tissue from Skeletal Muscle Derived Stem Cells and Induced Pluripotent Stem Cell Derived Cardiac Cells." Int J Tissue Eng **2013**: 198762.

During development, cardiac and skeletal muscle share major transcription factors and sarcomere proteins which were generally regarded as specific to either cardiac or skeletal muscle but not both in terminally differentiated adult cardiac or skeletal muscle. Here, we investigated whether artificial muscle constructed from human skeletal muscle derived stem cells (MDSCs) recapitulates developmental similarities between cardiac and skeletal muscle. We constructed 3-dimensional collagen-based engineered muscle tissue (EMT) using MDSCs (MDSC-EMT) and compared the biochemical and contractile properties with EMT using induced pluripotent stem (iPS) cell-derived cardiac cells (iPS-EMT). Both MDSC-EMT and iPS-EMT expressed cardiac specific troponins, fast skeletal muscle myosin heavy chain, and connexin-43 mimicking developing cardiac or skeletal muscle. At the transcriptional level, MDSC-EMT and iPS-EMT upregulated both cardiac and skeletal muscle-specific genes and expressed Nkx2.5 and Myo-D proteins. MDSC-EMT displayed intracellular calcium ion transients and responses to isoproterenol. Contractile force measurements of MDSC-EMT demonstrated functional properties of immature cardiac and skeletal muscle in both tissues. Results suggest that the EMT from MDSCs mimics developing cardiac and skeletal muscle and can serve as a useful in vitro functioning striated muscle model for investigation of stem cell differentiation and therapeutic options of MDSCs for cardiac repair.

Teichert, A. M., et al. (2014). "The neural stem cell lineage reveals novel relationships among spermatogonial germ stem cells and other pluripotent stem cells." Stem Cells Dev **23**(7): 767-778.

The embryonic stem cell (ESC) derived from the inner cell mass is viewed as the core pluripotent cell (PC) type from which all other cell types emanate. This familiar perspective derives from an embryological time line in which PCs are ordered according to their time of appearance. However, this schema does not take into account their potential for interconversion, thereby excluding this critical quality of PCs. The persistence of bona fide pluripotent adult stem cells has garnered increasing attention in recent years. Adult pluripotent spermatogonial germ stem cells (aSGSCs) arise from primordial germ cells (pGCs) that emerge from the epiblast during gastrulation. Adult definitive neural stem cells (dNSCs) arise clonally from pluripotent embryonic primitive neural stem cells (pNSCs), which can also be derived clonally from ESCs. To test for stem cell-type convertibility, we employed differentiation in the clonal lineage from ESCs to pNSCs to dNSCs, and revealed the relationships and lineage positioning among various PC populations, including spermatogonial germ cells (aSGSCs), epiblast-derived stem cells (Epi-SCs) and the bFGF, Activin, and BIO-

derived stem cell (FAB-SC). Adult, murine aSGSCs assumed a 'pseudo-ESC' state in vitro, and then differentiated into dNSCs, but not pNSCs. Similarly, Epi-SCs and FAB-SCs only gave rise to dNSCs and not to pNSCs. The results of these experiments suggest a new pluripotency lineage model describing the relationship (s) among PCs that better reflects the transitions between these cell types in vitro.

Telugu, B. P., et al. (2011). "Leukemia inhibitory factor (LIF)-dependent, pluripotent stem cells established from inner cell mass of porcine embryos." J Biol Chem **286**(33): 28948-28953.

The pig is important for agriculture and as an animal model in human and veterinary medicine, yet despite over 20 years of effort, there has been a failure to generate pluripotent stem cells analogous to those derived from mouse embryos. Here we report the production of leukemia inhibitory factor-dependent, so-called naive type, pluripotent stem cells from the inner cell mass of porcine blastocysts by up-regulating expression of KLF4 and POU5F1. The alkaline phosphatase-positive colonies resulting from reprogramming resemble mouse embryonic stem cells in colony morphology, cell cycle interval, transcriptome profile, and expression of pluripotent markers, such as POU5F1, SOX2, and surface marker SSEA1. They are dependent on leukemia inhibitory factor signaling for maintenance of pluripotency, can be cultured over extended passage, and have the ability to form teratomas. These cells derived from the inner cell mass of pig blastocysts are clearly distinct from the FGF2-dependent "primed" induced pluripotent stem cells described recently from porcine mesenchymal cells. The data are consistent with the hypothesis that the up-regulation of KLF4, as well as POU5F1, is required to create and stabilize the naive pluripotent state and may explain why the derivation of embryonic stem cells from pigs and other ungulates has proved so difficult.

Templin, C., et al. (2012). "Transplantation and tracking of human-induced pluripotent stem cells in a pig model of myocardial infarction: assessment of cell survival, engraftment, and distribution by hybrid single photon emission computed tomography/computed tomography of sodium iodide symporter transgene expression." Circulation **126**(4): 430-439.

BACKGROUND: Evaluation of novel cellular therapies in large-animal models and patients is currently hampered by the lack of imaging approaches that allow for long-term monitoring of viable transplanted cells. In this study, sodium iodide symporter (NIS) transgene imaging was evaluated as an approach to follow in vivo survival, engraftment, and distribution of human-induced pluripotent stem cell (hiPSC) derivatives in a pig model of myocardial infarction. METHODS AND RESULTS: Transgenic hiPSC lines stably expressing a fluorescent reporter and NIS (NIS (pos)-hiPSCs) were established. Iodide uptake, efflux, and viability of NIS (pos)-hiPSCs were assessed in vitro. Ten (+/-2) days after induction of myocardial infarction by transient occlusion of the left anterior descending artery, catheter-based intramyocardial injection of NIS (pos)-hiPSCs guided by 3-dimensional NOGA mapping was performed. Dual-isotope single photon emission computed tomographic/computed tomographic imaging was applied with the use of (123)I to follow donor cell survival and distribution and with the use of (99m)TC-tetrofosmin for perfusion imaging. In vitro, iodide uptake in NIS (pos)-hiPSCs was increased 100-fold above that of nontransgenic controls. In vivo, viable NIS (pos)-hiPSCs could be visualized for up to 15 weeks. Immunohistochemistry demonstrated that hiPSC-derived endothelial cells contributed to vascularization. Up to 12 to 15 weeks after transplantation, no teratomas were detected. CONCLUSIONS: This study describes for the first time the feasibility of repeated long-term in vivo imaging of viability and tissue distribution of cellular grafts in large animals. Moreover, this is the first report demonstrating vascular differentiation and long-term engraftment of hiPSCs in a large-animal model of myocardial infarction. NIS (pos)-hiPSCs represent a valuable tool to monitor and improve current cellular treatment strategies in clinically relevant animal models.

Thakar, N. Y., et al. (2015). "RELB Alters Proliferation of Human Pluripotent Stem Cells via IMP3- and LIN28-Mediated Modulation of the Expression of IGF2 and Other Cell-Cycle Regulators." Stem Cells Dev **24**(16): 1888-1900.

The molecular mechanisms that orchestrate the exit from pluripotency, cell cycle progression, and lineage-specific differentiation in human pluripotent stem cells (hPSCs) are poorly understood. RELB, a key protein in the noncanonical nuclear factor-kappaB (NFkappaB) signaling pathway, was previously implicated in controlling the switch between human embryonic stem cell (hESC) proliferation and differentiation. Here, we show that RELB enhances the proliferation of hESCs and human-induced pluripotent stem cells (hiPSCs) without affecting their pluripotency. We demonstrate that RELB does this by interacting with two RNA-binding proteins LIN28A and IMP3 (IGF2 mRNA-binding protein 3); further, these interactions control mRNA levels and protein expression of insulin-like growth factor 2 (IGF2) and key cell-cycle genes. Finally, after stress, these

proteins co-localize in stress granules in hESCs and iPSCs. Our data identify RELB as a novel regulator of hPSC proliferation, and suggest a new function for RELB, in addition to its widely accepted role as a transcription factor, that involves recruitment of IMP3 and LIN28 to the cytosolic mRNA translation-control domains for post-transcriptional modulation of IGF2 and cell-cycle gene expression.

Theka, I., et al. (2013). "Rapid generation of functional dopaminergic neurons from human induced pluripotent stem cells through a single-step procedure using cell lineage transcription factors." Stem Cells Transl Med **2**(6): 473-479.

Current protocols for in vitro differentiation of human induced pluripotent stem cells (hiPSCs) to generate dopamine (DA) neurons are laborious and time-expensive. In order to accelerate the overall process, we have established a fast protocol by expressing the developmental transcription factors ASCL1, NURR1, and LMX1A. With this method, we were able to generate mature and functional dopaminergic neurons in as few as 21 days, skipping all the intermediate steps for inducting and selecting embryoid bodies and rosette-neural precursors. Strikingly, the resulting neuronal conversion process was very proficient, with an overall efficiency that was more than 93% of all the coinfected cells. hiPSC-derived DA neurons expressed all the critical molecular markers of the DA molecular machinery and exhibited sophisticated functional features including spontaneous electrical activity and dopamine release. This one-step protocol holds important implications for in vitro disease modeling and is particularly amenable for exploitation in high-throughput screening protocols.

Thoma, E. C., et al. (2016). "Establishment of a translational endothelial cell model using directed differentiation of induced pluripotent stem cells from Cynomolgus monkey." Sci Rep **6**: 35830.

Due to their broad differentiation potential, pluripotent stem cells (PSCs) offer a promising approach for generating relevant cellular models for various applications. While human PSC-based cellular models are already advanced, similar systems for non-human primates (NHPs) are still lacking. However, as NHPs are the most appropriate animals for evaluating the safety of many novel pharmaceuticals, the availability of in vitro systems would be extremely useful to bridge the gap between cellular and animal models. Here, we present a NHP in vitro endothelial cell system using induced pluripotent stem cells (IPSCs) from Cynomolgus monkey (Macaca fascicularis). Based on an adapted protocol for human IPSCs, we directly differentiated macaque IPSCs into

endothelial cells under chemically defined conditions. The resulting endothelial cells can be enriched using immuno-magnetic cell sorting and display endothelial marker expression and function. RNA sequencing revealed that the differentiation process closely resembled vasculogenesis. Moreover, we showed that endothelial cells derived from macaque and human IPSCs are highly similar with respect to gene expression patterns and key endothelial functions, such as inflammatory responses. These data demonstrate the power of IPSC differentiation technology to generate defined cell types for use as translational in vitro models to compare cell type-specific responses across species.

Tolosa, L., et al. (2016). "Clinical Application of Pluripotent Stem Cells: An Alternative Cell-Based Therapy for Treating Liver Diseases?" Transplantation **100**(12): 2548-2557.

The worldwide shortage of donor livers for organ and hepatocyte transplantation has prompted the search for alternative therapies for intractable liver diseases. Cell-based therapy is envisaged as a useful therapeutic option to recover and stabilize the lost metabolic function for acute liver failure, end-stage and congenital liver diseases, or for those patients who are not considered eligible for organ transplantation. In recent years, research to identify alternative and reliable cell sources for transplantation that can be derived by reproducible methods has been encouraged. Human pluripotent stem cells (PSCs), which comprise both embryonic and induced PSCs, may offer many advantages as an alternative to hepatocytes for liver cell therapy. Their capacity for expansion, hepatic differentiation and self-renewal make them a promising source of unlimited numbers of hepatocyte-like cells for treating and repairing damaged livers. Immunogenicity and tumorigenicity of human PSCs remain the bottleneck for successful clinical application. However, recent advances made to develop disease-corrected hepatocyte-like cells from patients' human-induced PSCs by gene editing have opened up many potential gateways for the autologous treatment of hereditary liver diseases, which may likely reduce the risk of rejection and the need for lifelong immunosuppression. Well-defined methods to reduce the expression of oncogenic genes in induced PSCs, including protocols for their complete and safe hepatic differentiation, should be established to minimize the tumorigenicity of transplanted cells. On top of this, such new strategies are currently being rigorously tested and validated in preclinical studies before they can be safely transferred to clinical practice with patients.

Tomokiyo, A., et al. (2017). "Generation of Neural Crest-Like Cells From Human Periodontal Ligament Cell-Derived Induced Pluripotent Stem Cells." J Cell Physiol **232**(2): 402-416.

Neural crest cells (NCC) hold great promise for tissue engineering, however the inability to easily obtain large numbers of NCC is a major factor limiting their use in studies of regenerative medicine. Induced pluripotent stem cells (iPSC) are emerging as a novel candidate that could provide an unlimited source of NCC. In the present study, we examined the potential of neural crest tissue-derived periodontal ligament (PDL) iPSC to differentiate into neural crest-like cells (NCLC) relative to iPSC generated from a non-neural crest derived tissue, foreskin fibroblasts (FF). We detected high HNK1 expression during the differentiation of PDL and FF iPSC into NCLC as a marker for enriching for a population of cells with NCC characteristics. We isolated PDL iPSC- and FF iPSC-derived NCLC, which highly expressed HNK1. A high proportion of the HNK1-positive cell populations generated, expressed the MSC markers, whilst very few cells expressed the pluripotency markers or the hematopoietic markers. The PDL and FF HNK1-positive populations gave rise to smooth muscle, neural, glial, osteoblastic and adipocytic like cells and exhibited higher expression of smooth muscle, neural, and glial cell-associated markers than the PDL and FF HNK1-negative populations. Interestingly, the HNK1-positive cells derived from the PDL-iPSC exhibited a greater ability to differentiate into smooth muscle, neural, glial cells and adipocytes, than the HNK1-positive cells derived from the FF-iPSC. Our work suggests that HNK1-enriched NCLC from neural crest tissue-derived iPSC more closely resemble the phenotypic and functional hallmarks of NCC compared to the HNK1-low population and non-neural crest iPSC-derived NCLC. J. Cell. Physiol. 232: 402-416, 2017. (c) 2016 Wiley Periodicals, Inc.

Tomotsune, D., et al. (2016). "Enrichment of Pluripotent Stem Cell-Derived Hepatocyte-Like Cells by Ammonia Treatment." PLoS One **11**(9): e0162693.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are potential resources for the regeneration of defective organs, including the liver. However, some obstacles must be overcome before this becomes reality. Undifferentiated cells that remain following differentiation have teratoma-forming potential. Additionally, practical applications require a large quantity of differentiated cells, so the differentiation process must be economical. Here we describe a DNA microarray-based global analysis of the gene expression profiles of differentiating human pluripotent stem cells. We identified differences and commonalities among six human pluripotent stem cell lines: the hESCs KhES1, KhES2, KhES3, and H1, and the iPSCs 201B7 and 243G1. Embryoid bodies (EBs) formed without requiring supplementation with inducing factors. EBs also expressed some liver-specific metabolic genes including the ammonia-metabolizing enzymes glutamine synthetase and carbamoyl-phosphate synthase 1. Real-time PCR analysis revealed hepatocyte-like differentiation of EBs treated with ammonia in Lanford medium. Analysis of DNA microarray data suggested that hepatocyte-like cells were the most abundant population in ammonia-treated cells. Furthermore, expression levels of undifferentiated pluripotent stem cell markers were drastically reduced, suggesting a reduced teratoma-forming capacity. These results indicate that treatment of EBs with ammonia in Lanford medium may be an effective inducer of hepatic differentiation in absence of expensive inducing factors.

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.

Vale, K. L. D., et al. (2017). "The Effects of Photobiomodulation Delivered by Light-Emitting Diode on Stem Cells from Human Exfoliated Deciduous Teeth: A Study on the Relevance to Pluripotent Stem Cell Viability and Proliferation." Photomed Laser Surg **35**(12): 659-665.

OBJECTIVE: Photobiomodulation (PBM) can modulate the proliferation of some types of stem cells. However, few reports have addressed the effects of PBM delivered by light-emitting diode (LED) on stem cells obtained from the pulp tissue of deciduous teeth. The aim of the present study was to investigate the effect of PBM delivered by red LED (630 nm, 75 mW, 37 mW/cm (2)) with different radiant exposures on the cell cycle, mitochondrial membrane potential, and senescence of stem cells from human exfoliated deciduous teeth (SHED). MATERIALS AND METHODS: Cultures were irradiated with LED (2, 4, 8, 16, and 32 J/cm (2)). After 24 h, the cell cycle and mitochondrial membrane potential of the cultures were evaluated using flow cytometry. Nonirradiated cultures served as control. RESULTS: Cultures irradiated with 16 J/cm (2) had higher percentages of cells in the synthesis phase than control cultures (p < 0.05), and no significant differences were found regarding the percentage of cells with viable mitochondria between irradiated and control cultures. No significant difference in cell senescence was found between control cultures and cultures irradiated with 2

or 16 J/cm (2). CONCLUSIONS: LED irradiation at 630 nm (37 mW/cm (2), 75 mW) with radiant exposure of 16 J/cm (2) was capable of inducing a proliferative response in stem cells from the pulp tissue of deciduous teeth without affecting mitochondrial function or inducing senescence.

Vallier, L., et al. (2009). "Signaling pathways controlling pluripotency and early cell fate decisions of human induced pluripotent stem cells." Stem Cells **27**(11): 2655-2666.

Human pluripotent stem cells from embryonic origins and those generated from reprogrammed somatic cells share many characteristics, including indefinite proliferation and a sustained capacity to differentiate into a wide variety of cell types. However, it remains to be demonstrated whether both cell types rely on similar mechanisms to maintain their pluripotent status and to control their differentiation. Any differences in such mechanisms would suggest that reprogramming of fibroblasts to generate induced pluripotent stem cells (iPSCs) results in novel states of pluripotency. In that event, current methods for expanding and differentiating human embryonic stem cells (ESCs) might not be directly applicable to human iPSCs. However, we show here that human iPSCs rely on activin/nodal signaling to control Nanog expression and thereby maintain pluripotency, thus revealing their mechanistic similarity to human ESCs. We also show that growth factors necessary and sufficient for achieving specification of human ESCs into extraembryonic tissues, neuroectoderm, and mesendoderm also drive differentiation of human iPSCs into the same tissues. Importantly, these experiments were performed in fully chemically defined medium devoid of factors that could obscure analysis of developmental mechanisms or render the resulting tissues incompatible with future clinical applications. Together these data reveal that human iPSCs rely on mechanisms similar to human ESCs to maintain their pluripotency and to control their differentiation, showing that these pluripotent cell types are functionally equivalent.

Vanova, T., et al. (2017). "Tyrosine Kinase Expressed in Hepatocellular Carcinoma, TEC, Controls Pluripotency and Early Cell Fate Decisions of Human Pluripotent Stem Cells via Regulation of Fibroblast Growth Factor-2 Secretion." Stem Cells **35**(9): 2050-2059.

Human pluripotent stem cells (hPSC) require signaling provided by fibroblast growth factor (FGF) receptors. This can be initiated by the recombinant FGF2 ligand supplied exogenously, but hPSC further support their niche by secretion of endogenous FGF2. In this study, we describe a role of tyrosine kinase

expressed in hepatocellular carcinoma (TEC) kinase in this process. We show that TEC-mediated FGF2 secretion is essential for hPSC self-renewal, and its lack mediates specific differentiation. Following both short hairpin RNA- and small interfering RNA-mediated TEC knockdown, hPSC secretes less FGF2. This impairs hPSC proliferation that can be rescued by increasing amounts of recombinant FGF2. TEC downregulation further leads to a lower expression of the pluripotency markers, an improved priming towards neuroectodermal lineage, and a failure to develop cardiac mesoderm. Our data thus demonstrate that TEC is yet another regulator of FGF2-mediated hPSC pluripotency and differentiation. Stem Cells 2017;35:2050-2059.

Varga, N., et al. (2011). "Mesenchymal stem cell like (MSCl) cells generated from human embryonic stem cells support pluripotent cell growth." Biochem Biophys Res Commun **414**(3): 474-480.

Mesenchymal stem cell like (MSCl) cells were generated from human embryonic stem cells (hESC) through embryoid body formation, and isolated by adherence to plastic surface. MSCl cell lines could be propagated without changes in morphological or functional characteristics for more than 15 passages. These cells, as well as their fluorescent protein expressing stable derivatives, efficiently supported the growth of undifferentiated human embryonic stem cells as feeder cells. The MSCl cells did not express the embryonic (Oct4, Nanog, ABCG2, PODXL, or SSEA4), or hematopoietic (CD34, CD45, CD14, CD133, HLA-DR) stem cell markers, while were positive for the characteristic cell surface markers of MSCs (CD44, CD73, CD90, CD105). MSCl cells could be differentiated toward osteogenic, chondrogenic or adipogenic directions and exhibited significant inhibition of mitogen-activated lymphocyte proliferation, and thus presented immunosuppressive features. We suggest that cultured MSCl cells can properly model human MSCs and be applied as efficient feeders in hESC cultures.

Varun, D., et al. (2017). "A robust vitronectin-derived peptide for the scalable long-term expansion and neuronal differentiation of human pluripotent stem cell (hPSC)-derived neural progenitor cells (hNPCs)." Acta Biomater **48**: 120-130.

Despite therapeutic advances, neurodegenerative diseases and disorders remain some of the leading causes of mortality and morbidity in the United States. Therefore, cell-based therapies to replace lost or damaged neurons and supporting cells of the central nervous system (CNS) are of great therapeutic interest. To that end, human pluripotent stem cell (hPSC) derived neural progenitor cells (hNPCs) and their

neuronal derivatives could provide the cellular 'raw material' needed for regenerative medicine therapies for a variety of CNS disorders. In addition, hNPCs derived from patient-specific hPSCs could be used to elucidate the underlying mechanisms of neurodegenerative diseases and identify potential drug candidates. However, the scientific and clinical application of hNPCs requires the development of robust, defined, and scalable substrates for their long-term expansion and neuronal differentiation. In this study, we rationally designed a vitronectin-derived peptide (VDP) that served as an adhesive growth substrate for the long-term expansion of several hNPC lines. Moreover, VDP-coated surfaces allowed for the directed neuronal differentiation of hNPC at levels similar to cells differentiated on traditional extracellular matrix protein-based substrates. Overall, the ability of VDP to support the long-term expansion and directed neuronal differentiation of hNPCs will significantly advance the future translational application of these cells in treating injuries, disorders, and diseases of the CNS.

Veraitch, O., et al. (2013). "Human induced pluripotent stem cell-derived ectodermal precursor cells contribute to hair follicle morphogenesis in vivo." J Invest Dermatol **133**(6): 1479-1488.

Well-orchestrated epithelial-mesenchymal interactions are crucial for hair follicle (HF) morphogenesis. In this study, ectodermal precursor cells (EPCs) with the capacity to cross talk with hair-inductive dermal cells were generated from human induced pluripotent stem cells (hiPSCs) and assessed for HF-forming ability in vivo. EPCs derived from three hiPSC lines generated with 4 or 3 factors (POU5F1, SOX2, KLF4 +/- MYC) mostly expressed keratin 18, a marker of epithelial progenitors. When cocultured with human dermal papilla (DP) cells, a 4 factor 201B7 hiPSC-EPC line upregulated follicular keratinocyte (KC) markers more significantly than normal human adult KCs (NHKCs) and other hiPSC-EPC lines. DP cells preferentially increased DP biomarker expression in response to this line. Interestingly, 201B7 hiPSCs were shown to be ectodermal/epithelial prone, and the derived EPCs were putatively in a wingless-type MMTV integration site family (WNT)-activated state. Importantly, co-transplantation of 201B7 hiPSC-EPCs, but not NHKCs, with trichogenic mice dermal cells into immunodeficient mice resulted in HF formation.

Veraitch, O., et al. (2017). "Induction of hair follicle dermal papilla cell properties in human induced pluripotent stem cell-derived multipotent LNGFR (+)THY-1(+) mesenchymal cells." Sci Rep **7**: 42777.

The dermal papilla (DP) is a specialised mesenchymal component of the hair follicle (HF) that plays key roles in HF morphogenesis and regeneration. Current technical difficulties in preparing trichogenic human DP cells could be overcome by the use of highly proliferative and plastic human induced pluripotent stem cells (hiPSCs). In this study, hiPSCs were differentiated into induced mesenchymal cells (iMCs) with a bone marrow stromal cell phenotype. A highly proliferative and plastic LNGFR (+)THY-1(+) subset of iMCs was subsequently programmed using retinoic acid and DP cell activating culture medium to acquire DP properties. The resultant cells (induced DP-substituting cells [iDPSCs]) exhibited up-regulated DP markers, interacted with human keratinocytes to up-regulate HF related genes, and when co-grafted with human keratinocytes in vivo gave rise to fibre structures with a hair cuticle-like coat resembling the hair shaft, as confirmed by scanning electron microscope analysis. Furthermore, iDPSCs responded to the clinically used hair growth reagent, minoxidil sulfate, to up-regulate DP genes, further supporting that they were capable of, at least in part, reproducing DP properties. Thus, LNGFR (+)THY-1(+) iMCs may provide material for HF bioengineering and drug screening for hair diseases.

Verpelli, C., et al. (2013). "Comparative neuronal differentiation of self-renewing neural progenitor cell lines obtained from human induced pluripotent stem cells." Front Cell Neurosci **7**: 175.

Most human neuronal disorders are associated with genetic alterations that cause defects in neuronal development and induce precocious neurodegeneration. In order to fully characterize the molecular mechanisms underlying the onset of these devastating diseases, it is important to establish in vitro models able to recapitulate the human pathology as closely as possible. Here we compared three different differentiation protocols for obtaining functional neurons from human induced pluripotent stem cells (hiPSCs): human neural progenitors (hNPs) obtained from hiPSCs were differentiated by co-culturing them with rat primary neurons, glial cells or simply by culturing them on matrigel in neuronal differentiation medium, and the differentiation level was compared using immunofluorescence, biochemical and electrophysiological methods. We show that the differentiated neurons displayed distinct maturation properties depending on the protocol used and the faster morphological and functional maturation was obtained when hNPs were co-cultured with rat primary neurons.

Vignesh, S., et al. (2018). "Fabrication of micropatterned alginate-gelatin and k-carrageenan

hydrogels of defined shapes using simple wax mould method as a platform for stem cell/induced Pluripotent Stem Cells (iPSC) culture." Int J Biol Macromol **112**: 737-744.

Micropatterning techniques involve soft lithography, which is laborious, expensive and restricted to a narrow spectrum of biomaterials. In this work we report, first time employment of patterned wax moulds for generation of micropatterned alginate-gelatin and kappa-carrageenan (kappa-CRG) hydrogel systems by a novel, simple and cost effective method. We generated and characterized uniform and reproducible micropatterned hydrogels of varying sizes and shapes such as square projections, square grooves, and circular grids and crisscrossed hillocks. The rheological analysis showed that kappa-carrageenan hydrogels had higher gel strength when compared to alginate-gelatin hydrogels. Human Mesenchymal stem cells (hMSCs) and Human Induced Pluripotent Stem Cells (hiPSCs) were found to be cytocompatible with these hydrogels. This micropatterned hydrogel system may have potential application in tissue engineering and also in understanding the basic biology behind the stem cell/iPSC fate.

Villa-Diaz, L. G., et al. (2013). "Concise review: The evolution of human pluripotent stem cell culture: from feeder cells to synthetic coatings." Stem Cells **31**(1): 1-7.

Current practices to maintain human pluripotent stem cells (hPSCs), which include induced pluripotent stem cells and embryonic stem cells, in an undifferentiated state typically depend on the support of feeder cells such as mouse embryonic fibroblasts (MEFs) or an extracellular matrix such as Matrigel. Culture conditions that depend on these undefined support systems limit our ability to interpret mechanistic studies aimed at resolving how hPSCs interact with their extracellular environment to remain in a unique undifferentiated state and to make fate-changing lineage decisions. Likewise, the xenogeneic components of MEFs and Matrigel ultimately hinder our ability to use pluripotent stem cells to treat debilitating human diseases. Many of these obstacles have been overcome by the development of synthetic coatings and bioreactors that support hPSC expansion and self-renewal within defined culture conditions that are free from xenogeneic contamination. The establishment of defined culture conditions and synthetic matrices will facilitate studies to more precisely probe the molecular basis of pluripotent stem cell self-renewal and differentiation. When combined with three-dimensional cultures in bioreactors, these systems will also enable large-scale expansion for future clinical applications.

Vlismas, A., et al. (2016). "Microarray Analyses Reveal Marked Differences in Growth Factor and Receptor Expression Between 8-Cell Human Embryos and Pluripotent Stem Cells." Stem Cells Dev **25**(2): 160-177.

Previous microarray analyses of RNAs from 8-cell (8C) human embryos revealed a lack of cell cycle checkpoints and overexpression of core circadian oscillators and cell cycle drivers relative to pluripotent human stem cells [human embryonic stem cells/induced pluripotent stem (hES/iPS)] and fibroblasts, suggesting growth factor independence during early cleavage stages. To explore this possibility, we queried our combined microarray database for expression of 487 growth factors and receptors. Fifty-one gene elements were overdetected on the 8C arrays relative to hES/iPS cells, including 14 detected at least 80-fold higher, which annotated to multiple pathways: six cytokine family (CSF1R, IL2RG, IL3RA, IL4, IL17B, IL23R), four transforming growth factor beta (TGFB) family (BMP6, BMP15, GDF9, ENG), one fibroblast growth factor (FGF) family [FGF14(FH4)], one epidermal growth factor member (GAB1), plus CD36, and CLEC10A. 8C-specific gene elements were enriched (73%) for reported circadian-controlled genes in mouse tissues. High-level detection of CSF1R, ENG, IL23R, and IL3RA specifically on the 8C arrays suggests the embryo plays an active role in blocking immune rejection and is poised for trophectoderm development; robust detection of NRG1, GAB1, -2, GRB7, and FGF14(FHF4) indicates novel roles in early development in addition to their known roles in later development. Forty-four gene elements were underdetected on the 8C arrays, including 11 at least 80-fold under the pluripotent cells: two cytokines (IFITM1, TNFRSF8), five TGFBs (BMP7, LEFTY1, LEFTY2, TDGF1, TDGF3), two FGFs (FGF2, FGF receptor 1), plus ING5, and WNT6. The microarray detection patterns suggest that hES/iPS cells exhibit suppressed circadian competence, underexpression of early differentiation markers, and more robust expression of generic pluripotency genes, in keeping with an artificial state of continual uncommitted cell division.

Wada, H., et al. (2011). "Successful differentiation to T cells, but unsuccessful B-cell generation, from B-cell-derived induced pluripotent stem cells." Int Immunol **23**(1): 65-74.

Forced expression of certain transcription factors in somatic cells results in generation of induced pluripotent stem (iPS) cells, which differentiate into various cell types. We investigated T-cell and B-cell lineage differentiation from iPS cells in vitro. To

evaluate the impact of iPS cell source, murine splenic B-cell-derived iPS (B-iPS) cells were generated after retroviral transduction of four transcription factors (Oct4, Sox2, Klf4 and c-Myc). B-iPS cells were identical to embryonic stem (ES) cells and mouse embryonic fibroblast (MEF)-derived iPS cells in morphology, ES cell marker expression as well as teratoma and chimera mouse formation. Both B-iPS and MEF-derived iPS cells differentiated into lymphocytes in OP9 co-culture systems. Both efficiently differentiated into T-cell lineage that produced IFN-gamma on T-cell receptor stimulation. However, iPS cells including B-iPS cells were relatively resistant to B-cell lineage differentiation. One of the reasons of the failure of B-cell lineage differentiation seemed due to a defect of Pax5 expression in the differentiated cells. Therefore, current in vitro differentiation systems using iPS cells are sufficient for inducing T-cell but not B-cell lineage.

Wakao, S., et al. (2012). "Isolation of adult human pluripotent stem cells from mesenchymal cell populations and their application to liver damages." Methods Mol Biol **826**: 89-102.

We have found a novel type of pluripotent stem cells, Multilineage-differentiating stress enduring (Muse) cells that can be isolated from mesenchymal cell populations. Muse cells are characterized by stress tolerance, expression of pluripotency markers, self-renewal, and the ability to differentiate into endodermal-, mesodermal-, and ectodermal-lineage cells from a single cell, demonstrating that they are pluripotent stem cells. They can be isolated as cells positive for stage-specific embryonic antigen-3, a human pluripotent stem cell marker. Here, we introduce the isolation method for Muse cells and the effect of transplantation of these cells on chronic liver diseases.

Wakao, S., et al. (2012). "Regenerative Effects of Mesenchymal Stem Cells: Contribution of Muse Cells, a Novel Pluripotent Stem Cell Type that Resides in Mesenchymal Cells." Cells **1**(4): 1045-1060.

Mesenchymal stem cells (MSCs) are easily accessible and safe for regenerative medicine. MSCs exert trophic, immunomodulatory, anti-apoptotic, and tissue regeneration effects in a variety of tissues and organs, but their entity remains an enigma. Because MSCs are generally harvested from mesenchymal tissues, such as bone marrow, adipose tissue, or umbilical cord as adherent cells, MSCs comprise crude cell populations and are heterogeneous. The specific cells responsible for each effect have not been clarified. The most interesting property of MSCs is that, despite being adult stem cells that belong to the mesenchymal tissue lineage, they are able to differentiate into a broad spectrum of cells beyond the boundary of mesodermal lineage cells into ectodermal or endodermal lineages, and repair tissues. The broad spectrum of differentiation ability and tissue-repairing effects of MSCs might be mediated in part by the presence of a novel pluripotent stem cell type recently found in adult human mesenchymal tissues, termed multilineage-differentiating stress enduring (Muse) cells. Here we review recently updated studies of the regenerative effects of MSCs and discuss their potential in regenerative medicine.

Wakui, T., et al. (2017). "Method for evaluation of human induced pluripotent stem cell quality using image analysis based on the biological morphology of cells." J Med Imaging (Bellingham) **4**(4): 044003.

We propose an image analysis method for quality evaluation of human pluripotent stem cells based on biologically interpretable features. It is important to maintain the undifferentiated state of induced pluripotent stem cells (iPSCs) while culturing the cells during propagation. Cell culture experts visually select good quality cells exhibiting the morphological features characteristic of undifferentiated cells. Experts have empirically determined that these features comprise prominent and abundant nucleoli, less intercellular spacing, and fewer differentiating cellular nuclei. We quantified these features based on experts' visual inspection of phase contrast images of iPSCs and found that these features are effective for evaluating iPSC quality. We then developed an iPSC quality evaluation method using an image analysis technique. The method allowed accurate classification, equivalent to visual inspection by experts, of three iPSC cell lines.

Weist, R., et al. (2018). "Differential Expression of Cholinergic System Components in Human Induced Pluripotent Stem Cells, Bone Marrow-Derived Multipotent Stromal Cells, and Induced Pluripotent Stem Cell-Derived Multipotent Stromal Cells." Stem Cells Dev **27**(3): 166-183.

The components of the cholinergic system are evolutionary very old and conserved molecules that are expressed in typical spatiotemporal patterns. They are involved in signaling in the nervous system, whereas their functions in nonneuronal tissues are hardly understood. Stem cells present an attractive cellular system to address functional issues. This study therefore compared human induced pluripotent stem cells (iPSCs; from cord blood endothelial cells), mesenchymal stromal cells derived from iPSCs (iPSC-MSCs), and bone marrow-derived MSCs (BM-MSCs) from up to 33 different human donors with respect to gene expressions of components of the cholinergic system. The status of cells was identified and

characterized by the detection of cell surface antigens using flow cytometry. Acetylcholinesterase expression in iPSCs declined during their differentiation into MSCs and was comparably low in BM-MSCs. Butyrylcholinesterase was present in iPSCs, increased upon transition from the three-dimensional embryoid body phase into monolayer culture, and declined upon further differentiation into iPSC-MSCs. In BM-MSCs a notable butyrylcholinesterase expression could be detected in only four donors, but was elusive in other patient-derived samples. Different nicotinic acetylcholine receptor subunits were preferentially expressed in iPSCs and during early differentiation into iPSC-MSCs, low expression was detected in iPS-MSCs and in BM-MSCs.

West, M. D., et al. (2016). "Adult Versus Pluripotent Stem Cell-Derived Mesenchymal Stem Cells: The Need for More Precise Nomenclature." Curr Stem Cell Rep **2**: 299-303.

The complexity of human pluripotent stem cell (hPSC) fate represents both opportunity and challenge. In theory, all somatic cell types can be differentiated from hPSCs, opening the door to many opportunities in transplant medicine. However, such clinical applications require high standards of purity and identity, that challenge many existing protocols. This underscores the need for increasing precision in the description of cell identity during hPSC differentiation. We highlight one salient example, namely, the numerous published reports of hPSC-derived mesenchymal stem cells (MSCs). We suggest that many of these reports likely represent an improper use of certain cluster of differentiation (CD) antigens in defining bone marrow-derived MSCs. Instead, most such hPSC-derived mesenchymal cells are likely a complex mixture of embryonic anlagen, primarily of diverse mesodermal and neural crest origins, making precise identification, reproducible manufacture, and uniform differentiation difficult to achieve. We describe a potential path forward that may provide more precision in nomenclature, and cells with higher purity and identity for potential therapeutic use.

Wheeler, H. E., et al. (2015). "Modeling chemotherapeutic neurotoxicity with human induced pluripotent stem cell-derived neuronal cells." PLoS One **10**(2): e0118020.

There are no effective agents to prevent or treat chemotherapy-induced peripheral neuropathy (CIPN), the most common non-hematologic toxicity of chemotherapy. Therefore, we sought to evaluate the utility of human neuron-like cells derived from induced pluripotent stem cells (iPSCs) as a means to study CIPN. We used high content imaging measurements of neurite outgrowth phenotypes to compare the changes that occur to iPSC-derived neuronal cells among drugs and among individuals in response to several classes of chemotherapeutics. Upon treatment of these neuronal cells with the neurotoxic drug paclitaxel, vincristine or cisplatin, we identified significant differences in five morphological phenotypes among drugs, including total outgrowth, mean/median/maximum process length, and mean outgrowth intensity ($P < 0.05$). The differences in damage among drugs reflect differences in their mechanisms of action and clinical CIPN manifestations. We show the potential of the model for gene perturbation studies by demonstrating decreased expression of TUBB2A results in significantly increased sensitivity of neurons to paclitaxel (0.23 +/- 0.06 decrease in total neurite outgrowth, $P = 0.011$). The variance in several neurite outgrowth and apoptotic phenotypes upon treatment with one of the neurotoxic drugs is significantly greater between than within neurons derived from four different individuals ($P < 0.05$), demonstrating the potential of iPSC-derived neurons as a genetically diverse model for CIPN. The human neuron model will allow both for mechanistic studies of specific genes and genetic variants discovered in clinical studies and for screening of new drugs to prevent or treat CIPN.

White, M. P., et al. (2013). "Limited gene expression variation in human embryonic stem cell and induced pluripotent stem cell-derived endothelial cells." Stem Cells **31**(1): 92-103.

Recent evidence suggests human embryonic stem cell (hESC) and induced pluripotent stem (iPS) cell lines have differences in their epigenetic marks and transcriptomes, yet the impact of these differences on subsequent terminally differentiated cells is less well understood. Comparison of purified, homogeneous populations of somatic cells derived from multiple independent human iPS and ES lines will be required to address this critical question. Here, we report a differentiation protocol based on embryonic development that consistently yields large numbers of endothelial cells (ECs) derived from multiple hESCs or iPS cells. Mesoderm differentiation of embryoid bodies was maximized, and defined growth factors were used to generate KDR (+) EC progenitors. Magnetic purification of a KDR (+) progenitor subpopulation resulted in an expanding, homogeneous pool of ECs that expressed EC markers and had functional properties of ECs. Comparison of the transcriptomes revealed limited gene expression variability between multiple lines of human iPS-derived ECs or between lines of ES- and iPS-derived ECs. These results demonstrate a method to generate large numbers of pure human EC progenitors and differentiated ECs from pluripotent stem cells and

suggest individual lineages derived from human iPS cells may have significantly less variance than their pluripotent founders.

Whitt, J., et al. (2016). "Induced pluripotent stem cell-derived mesenchymal stem cells: A leap toward personalized therapies." Curr Stem Cell Res Ther **11**(2): 141-148.

Mesenchymal Stem/stromal cell (MSCs) transplantation procedures have been used since the 1960's to treat leukemia and other diseases, but due to the risks involved only patients with life threatening illnesses were typically subjected to the transplantation procedure until the last decade. Recent advancements in transplantation techniques have made it more feasible to use it for non-life-threatening diseases. However, the potential uses for stem cells are still limited by their rarity, and, in the case of allogeneic transplants, graft-vs.-host complications. An evolving alternative to conventional stem cell therapies is induced pluripotent stem-cell derived mesenchymal stem/stromal cells (iPSC- MSCs), which have a multi-lineage potential comparable to conventionally acquired MSCs with the added benefit of being less immunoreactive. However there are still many hurdles left to be overcome before they can be used regularly for personalized therapies. This review will focus on recent advancements that have been made regarding the role MSCs play in tumor development and the potential uses iPSC-MSCs may have in future cancer treatment.

Wilson, H. K., et al. (2015). "Exploring the effects of cell seeding density on the differentiation of human pluripotent stem cells to brain microvascular endothelial cells." Fluids Barriers CNS **12**: 13.

BACKGROUND: Brain microvascular-like endothelial cells (BMECs) derived from human pluripotent stem cells (hPSCs) have significant promise as tools for drug screening and studying the structure and function of the BBB in health and disease. The density of hPSCs is a key factor in regulating cell fate and yield during differentiation. Prior reports of hPSC differentiation to BMECs have seeded hPSCs in aggregates, leading to non-uniform cell densities that may result in differentiation heterogeneity. Here we report a singularized-cell seeding approach compatible with hPSC-derived BMEC differentiation protocols and evaluate the effects of initial hPSC seeding density on the subsequent differentiation, yield, and blood-brain barrier (BBB) phenotype. METHODS: A range of densities of hPSCs was seeded and differentiated, with the resultant endothelial cell yield quantified via VE-cadherin flow cytometry. Barrier phenotype of purified hPSC-derived BMECs was measured via

transendothelial electrical resistance (TEER), and purification protocols were subsequently optimized to maximize TEER. Expression of characteristic vascular markers, tight junction proteins, and transporters was confirmed by immunocytochemistry and quantified by flow cytometry. P-glycoprotein and MRP-family transporter activity was assessed by intracellular accumulation assay. RESULTS: The initial hPSC seeding density of approximately 30,000 cells/cm (2) served to maximize the yield of VE-cadherin+ BMECs per input hPSC. BMECs displayed the highest TEER (>2,000 Omega x cm (2)) within this same range of initial seeding densities, although optimization of the BMEC purification method could minimize the seeding density dependence for some lines. Localization and expression levels of tight junction proteins as well as efflux transporter activity were largely independent of hPSC seeding density. Finally, the utility of the singularized-cell seeding approach was demonstrated by scaling the differentiation and purification process down from 6-well to 96-well culture without impacting BBB phenotype. CONCLUSIONS: Given the yield and barrier dependence on initial seeding density, the singularized-cell seeding approach reported here should enhance the reproducibility and scalability of hPSC-derived BBB models, particularly for the application to new pluripotent stem cell lines.

Wong, J. C., et al. (2014). "The epigenetic bivalency of core pancreatic beta-cell transcription factor genes within mouse pluripotent embryonic stem cells is not affected by knockdown of the polycomb repressive complex 2, SUZ12." PLoS One **9**(5): e97820.

This study assesses changes in activator and repressor modifications to histones associated with the core transcription factor genes most highly upregulated or downregulated in pancreatic beta-cells relative to expression in an embryonic stem cell line. Epigenetic analysis of the Oct4, Utf1, Nanog and Sox2 (pluripotency) and Pdx1, Nkx6.1, Nkx2.2 and MafA (pancreatic beta-cells) transcription factor genes in embryonic stem cells and a beta-cell line (MIN6) showed the pluripotency genes were enriched for active (histone 3 trimethylated at lysine 4 and histone 3 acetylated at lysine 9) and depleted of repressor modifications (histone 3 trimethylated at lysine 27 and histone 3 trimethylated at lysine 9) around the transcription start site in mouse embryonic stem cells (D3), and this was reversed in MIN6 cells. The beta-cell transcription factors were bivalently enriched for activating (histone 3 trimethylated at lysine 4) and repressor (histone 3 trimethylated at lysine 27) modifications in embryonic stem cells but were monovalent for the activator modification (histone 3

trimethylated at lysine 4) in the beta-cells. The polycomb repressor complex 2 acts as a histone 3 lysine 27 methylase and an essential component of this complex, SUZ12, was enriched at the beta-cell transcription factors in embryonic stem cells and was reduced MIN6. Knock-down of SUZ12 in embryonic stem cells, however, did not reduce the level of histone 3 trimethylated at lysine 27 at beta-cell transcription factor loci or break the transcriptional repression of these genes in embryonic stem cells. This study shows the reduction in the total SUZ12 level was not a sufficient cause of the resolution of the epigenetic bivalency of beta-cell transcription factors in embryonic stem cells.

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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