Embryonic Stem Cell Research Literatures

Mark Herbert, PhD

39-06 Main Street, Flushing, Queens, New York 11354, USA, ma8080@gmail.com

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

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Key words: stem cell; embryonic; life; research; literature

Introduction

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

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

Abboud, N., et al. (2017). "Culture conditions have an impact on the maturation of traceable, transplantable mouse embryonic stem cell-derived otic progenitor cells." J Tissue Eng Regen Med **11**(9): 2629-2642.

The generation of replacement inner ear hair cells (HCs) remains a challenge and stem cell therapy holds the potential for developing therapeutic solutions to hearing and balance disorders. Recent developments have made significant strides in producing mouse otic progenitors using cell culture techniques to initiate HC differentiation. However, no consensus has been reached as to efficiency and therefore current methods remain unsatisfactory. In order to address these issues, we compare the generation of otic and HC progenitors from embryonic stem (ES) cells in two cell culture systems: suspension vs. adherent conditions. In the present study, an ES cell line derived from an Atoh1-green fluorescent protein (GFP) transgenic mouse was used to track the generation of otic progenitors, initial HCs and to compare these two differentiation systems. We used a two-step short-term differentiation method involving an induction period of 5 days during which ES cells were cultured in the presence of Wnt/transforming growth factor TGF-beta inhibitors and insulin-like growth factor IGF-1 to suppress mesoderm and reinforce presumptive ectoderm and otic lineages. The generated embryoid bodies were then differentiated in medium containing basic fibroblast growth factor (bFGF) for an additional 5 days using either suspension or adherent culture methods. Upon completion of differentiation, quantitative polymerase chain reaction analysis and immunostaining monitored the expression of otic/HC progenitor lineage markers. The results indicate that cells differentiated in suspension cultures produced cells expressing otic progenitor/HC markers at a higher efficiency compared with the production of these cell types within adherent cultures. Furthermore, we demonstrated that a fraction of these cells can incorporate into ototoxin-injured mouse postnatal cochlea explants and express MYO7A after transplantation. Copyright (c) 2016 John Wiley & Sons, Ltd.

Abd Jalil, A., et al. (2017). "Vitamin E-Mediated Modulation of Glutamate Receptor Expression in an Oxidative Stress Model of Neural Cells Derived from Embryonic Stem Cell Cultures." Evid Based Complement Alternat Med **2017**: 6048936.

Glutamate is the primary excitatory neurotransmitter in the central nervous system. Excessive concentrations of glutamate in the brain can be excitotoxic and cause oxidative stress, which is associated with Alzheimer's disease. In the present study, the effects of vitamin E in the form of tocotrienol-rich fraction (TRF) and alpha-tocopherol

(alpha-TCP) in modulating the glutamate receptor and neuron injury markers in an in vitro model of oxidative stress in neural-derived embryonic stem (ES) cell cultures were elucidated. A transgenic mouse ES cell line (46C) was differentiated into a neural lineage in vitro via induction with retinoic acid. These cells were then subjected to oxidative stress with a significantly high concentration of glutamate. Measurement of reactive oxygen species (ROS) was performed after inducing glutamate excitotoxicity, and recovery from this toxicity in response to vitamin E was determined. The gene expression levels of glutamate receptors and neuron-specific enolase were elucidated using real-time PCR. The results reveal that neural cells derived from 46C cells and subjected to oxidative stress exhibit downregulation of NMDA, kainate receptor, and NSE after posttreatment with different concentrations of TRF and alpha-TCP, a sign of neurorecovery. Treatment of either TRF or alpha-TCP reduced the levels of ROS in neural cells subjected to glutamate-induced oxidative stress; these results indicated that vitamin E is a potent antioxidant.

Abdelalim, E. M. (2013). "Molecular mechanisms controlling the cell cycle in embryonic stem cells." Stem Cell Rev **9**(6): 764-773.

Embryonic stem (ES) cells are originated from the inner cell mass of a blastocyst stage embryo. They can proliferate indefinitely, maintain an undifferentiated state (self-renewal), and differentiate into any cell type (pluripotency). ES cells have an unusual cell cycle structure, consists mainly of S phase cells, a short G1 phase and absence of G1/S checkpoint. Cell division and cell cycle progression are controlled by mechanisms ensuring the accurate transmission of genetic information from generation to generation. Therefore, control of cell cycle is a complicated process, involving several signaling pathways. Although great progress has been made on the molecular mechanisms involved in the regulation of ES cell cycle, many regulatory mechanisms remain unknown. This review summarizes the current knowledge about the molecular mechanisms regulating the cell cycle of ES cells and describes the relationship existing between cell cycle progression and the self-renewal.

Abdelbaset-Ismail, A., et al. (2016). "Vitamin D3 stimulates embryonic stem cells but inhibits migration and growth of ovarian cancer and teratocarcinoma cell lines." J Ovarian Res **9**: 26.

BACKGROUND: Deficiency in Vitamin D3 (cholecalciferol) may predispose to some malignancies, including gonadal tumors and in experimental models vitamin D3 has been proven to inhibit the growth of cancer cells. To learn more about the potential role of vitamin D3 in cancerogenesis, we evaluated the expression and functionality of the vitamin D receptor (VDR) and its role in metastasis of ovarian cancer cells and of murine and human teratocarcinoma cell lines. METHODS: In our studies we employed murine embrynic stem cells (ESD3), murine (P19) and human (NTERA-2) teratocarcimona cells lines, human ovarian cancer cells (A2780) as well as purified murine and human purified very small embryonic like stem cells (VSELs). We evaluated expression of Vitamin D3 receptor (VDR) in these cells as well as effect of vitamin D3 exposure on cell proliferation and migration. RESULTS: We here provide also more evidence for the role of vitamin D3 in germline-derived malignancies, and this evidence supports the proposal that vitamin D3 treatment inhibits growth and metastatic potential of several germline-derived malignancies. We also found that the ESD3 murine immortalized embryonic stem cell line and normal, pluripotent, germline-marker-positive very small embryonic-like stem cells (VSELs) isolated from adult tissues are stimulated by vitamin D3, which suggests that vitamin D3 affects the earliest stages of embryogenesis. CONCLUSIONS: We found that however all normal and malignant germ-line derived cells express functional VDR, Vitamin D3 differently affects their proliferation and migration. We postulate that while Vitamin D3 as anticancer drug inhibits proliferation of malignant cells, it may protect normal stem cells that play an important role in development and tissue/organ regeneration.

Abdelhady, S., et al. (2013). "Erg channel is critical in controlling cell volume during cell cycle in embryonic stem cells." PLoS One **8**(8): e72409.

The cell cycle progression in mouse embryonic stem cells (mESCs) is controlled by ion fluxes that alter cell volume [1]. This suggests that ion fluxes might control dynamic changes in morphology over the cell cycle, such as rounding up of the cell at mitosis. However, specific channels regulating such dynamic changes and the possible interactions with actomyosin complex have not been clearly identified. Following RNAseq transcriptome analysis of cell cycle sorted mESCs, we found that expression of the K (+) ion channel Erg1 peaked in G1 cell cycle phase, which was confirmed by immunostaining. Inhibition of Erg channel activity caused loss of G1 phase cells via non-apoptotic cell death. Cells first lost the ability of membrane blebbing, a typical feature of cultured embryonic stem cells. Continued Erg inhibition further increased cell volume and the cell eventually ruptured. In addition, atomic force measurements on live cells revealed a decreased cortical stiffness after treatment, suggesting alterations in actomyosin organization. When the intracellular osmotic pressure was

experimentally decreased by hypertonic solution or block of K (+) ion import via the Na, K-ATPase, cell viability was restored and cells acquired normal volume and blebbing activity. Our results suggest that Erg channels have a critical function in K (+) ion homeostasis of mESCs over the cell cycle, and that cell death following Erg inhibition is a consequence of the inability to regulate cell volume.

Aberdam, D. (2008). "Epidermal stem cell fate: what can we learn from embryonic stem cells?" Cell Tissue Res **331**(1): 103-107.

Because of its constant renewal and high propensity for repair, the epidermis is, together with the gut and the hematopoietic system, a tissue of choice to explore stem cell biology. Previous research over many years has revealed the complexity of the epidermis: the heterogeneity of the stem cell compartment, with its rare, slowly cycling, multipotent, hair-follicle, "bulge" stem cells and the more restricted interfollicular, follicle-matrix, and sebaceous-gland stem cells, which in turn generate the large pool of transit-amplifying progeny. Stem cell activity has been used for some considerable time to repair skin injuries, but ex-vivo keratinocyte amplification has its limitations, and grafted skin homeostasis is not totally satisfactory. Human embryonic stem cells raise the hope that the understanding of the developmental steps leading to the generation of epidermal stem cells and the characterization of the key signaling pathways involved in skin morphogenesis (such as p63) will be translated into therapeutic benefit. Our recent results suggest the feasibility not only of identifying but also of amplifying human ES cells, early ectodermal progenitors with an intact multipotent potential that might improve the quality and functionality of grafts, provided that preclinical in vivo studies confirm our expectations from in vitro analysis.

Abraham, J. and C. Keller (2010). "Renal stem cell biology starts to take spherical shape. Commentary on: Lusis et al., Isolation of clonogenic, long-term self renewing embryonic renal stem cells." Stem Cell Res **5**(1): 1-3.

The nephron is the fundamental unit of renal function, yet the ability of the kidney to regenerate nephrons after birth is limited. Due to the medical and financial impact of chronic and end stage renal disease, an improved understanding of nephron formation is necessary if regenerative or cell therapy are to be a feasible alternative to dialysis or renal transplant. In the study presented by Lusis et al., the presence of metanephric mesenchymal stem cells is definitively demonstrated. However, these "Nephrospheres" have characteristics of mesenchymal stem cells and substantially lack the ability to undergo an epithelial-to-mesenchyme transition or to form epithelial elements otherwise necessary for building the constituent cells of the nephron. Nevertheless, this newly isolated cell population opens many opportunities to investigate the consequences of normal and aberrant nephrogenesis, including Wilm's tumor.

Abu Khamidakh, A. E., et al. (2018). "Wound healing of human embryonic stem cell-derived retinal pigment epithelial cells is affected by maturation stage." Biomed Eng Online **17**(1): 102.

BACKGROUND: Wound healing of retinal pigment epithelium (RPE) is a complex process that may take place in common age-related macular degeneration eye disease. The purpose of this study was to evaluate whether wounding and wound healing has an effect on Ca (2+) dynamics in human embryonic stem cell (hESC)-RPEs cultured different periods of time. METHODS: The 9-day-cultured or 28-day-cultured hESC-RPEs from two different cell lines were wounded and the dynamics of spontaneous and mechanically induced intracellular Ca (2+) activity was measured with live-cell Ca (2+) imaging either immediately or 7 days after wounding. The healing time and speed were analyzed with time-lapse bright field microscopy. The Ca (2+) activity and healing speed were analysed with image analysis. In addition the extracellular matrix deposition was assessed with confocal microscopy. RESULTS: The Ca (2+) dynamics in hESC-RPE monolayers differed depending on the culture time: 9-day-cultured cells had higher number of cells with spontaneous Ca (2+) activity close to freshly wounded edge compared to control areas, whereas in 28-day-cultured cells there was no difference in wounded and control areas. The 28-day-cultured, wounded and 7-day-healed hESC-RPEs produced wide-spreading intercellular Ca (2+) waves upon mechanical stimulation, while in controls propagation was restricted. Most importantly, both wave spreading and spontaneous Ca (2+) activity of cells within the healed area, as well as the cell morphology of 28-day-cultured, wounded and thereafter 7-day-healed areas resembled the 9-day-cultured hESC-RPEs. CONCLUSIONS: This acquired knowledge about Ca (2+) dynamics of wounded hESC-RPE monolayers is important for understanding the dynamics of RPE wound healing, and could offer a reliable functionality test for RPE cells. The data presented in here suggests that assessment of Ca (2+) dynamics analysed with image analysis could be used as a reliable non-invasive functionality test for RPE cells.

Adler, E. D., et al. (2009). "In vivo detection of embryonic stem cell-derived cardiovascular progenitor

cells using Cy3-labeled Gadofluorine M in murine myocardium." JACC Cardiovasc Imaging **2**(9): 1114-1122.

OBJECTIVES: The aim of the current study is to test the ability to label and detect murine embryonic stem cell-derived cardiovascular progenitor cells (ES-CPC) with cardiac magnetic resonance (CMR) using the novel contrast agent Gadofluorine M-Cy3 (GdFM-Cy3). BACKGROUND: Cell therapy shows great promise for the treatment of cardiovascular disease. An important limitation to previous clinical studies is the inability to accurately identify transplanted cells. GdFM-Cy3 is a lipophilic paramagnetic contrast agent that contains a perfluorinated side chain and an amphiphilic character that allows for micelle formation in an aqueous solution. Previous studies reported that it is easily taken up and stored within the cytosol of mesenchymal stem cells, thereby allowing for paramagnetic cell labeling. Investigators in our laboratory have recently developed techniques for the robust generation of ES-CPC. We reasoned that GdFM-Cy3 would be a promising agent for the in vivo detection of these cells after cardiac cell transplantation. METHODS: ES-CPC were labeled with GdFM-Cy3 by incubation. In vitro studies were performed to assess the impact of GdFM-Cy3 on cell function and survival. A total of 500,000 GdFM-Cy3-labeled ES-CPC or control ES-CPC were injected into the myocardium of mice with and without myocardial infarction. Mice were imaged (9.4-T) before and over a 2-week time interval after stem cell transplantation. Mice were then euthanized, and their hearts were sectioned for fluorescence microscopy. RESULTS: In vitro studies demonstrated that GdFM-Cy3 was easily transfectable, nontoxic, stayed within cells after labeling, and could be visualized using CMR and fluorescence microscopy. In vivo studies confirmed the efficacy of the agent for the detection of cells transplanted into the hearts of mice after myocardial infarction. A correspondence between CMR and histology was observed. CONCLUSIONS: The results of the current study suggest that it is possible to identify and potentially track GdFM-Cy3-labeled ES-CPC in murine infarct models via CMR.

Aflatoonian, B., et al. (2009). "In vitro post-meiotic germ cell development from human embryonic stem cells." Hum Reprod **24**(12): 3150-3159.

BACKGROUND: Investigating the mechanisms of human primordial germ cell (PGC) and gamete development are important for understanding the causes of infertility and effects of environmental chemicals on reproductive development. However, there are practical and ethical difficulties associated with obtaining human tissue in early development. The aim of this study was to investigate whether human embryonic stem cell-hESC-generated germ cells could provide an in vitro model of gamete development. METHOD: Human ESCs were differentiated as embryoid bodies (EBs) in vitro. Gene and protein marker expression profiles of EBs in different periods of culture were analysed by quantitative polymerase chain reaction (Q-PCR) and immunolocalization to monitor germ cell development. Secretion of hormones involved in germ cell maturation was measured, to detect the existence of a germ cell niche within EBs. RESULTS: Q-PCR revealed gene expression profiles consistent with PGC formation and germ cell development. A small population of post-meiotic spermatid cells were identified using sperm-specific antibodies (Protamine 1 and 1.97). Although gene expression profiles characteristic of oocyte development and follicle-like structures were detected, a committed oocyte with extra-cellular zona pellucida was not recognized with zona pellucida-specific monoclonal antibody. CONCLUSIONS: hESCs can form PGCs and post-meiotic spermatids in vitro, however, there remains doubt about oocyte development. Levels of steroid hormones produced by EBs were significant when compared with known values for a similar quantity of human testis, suggesting that hESC may intrinsically create a favourable hormonal niche for spermatogenesis.

Ahmad, S., et al. (2007). "Differentiation of human embryonic stem cells into corneal epithelial-like cells by in vitro replication of the corneal epithelial stem cell niche." Stem Cells **25**(5): 1145-1155.

Human embryonic stem cells (hESCs) are pluripotent cells capable of differentiating into any cell type of the body. It has long been known that the adult stem cell niche is vital for the maintenance of adult stem cells. The cornea at the front of the eye is covered by a stratified epithelium that is renewed by stem cells located at its periphery in a region known as the limbus. These so-called limbal stem cells are maintained by factors within the limbal microenvironment, including collagen IV in basement membrane and limbal fibroblasts in the stroma. Because this niche is very specific to the stem cells (rather than to the more differentiated cells) of the corneal epithelium, it was hypothesized that replication of these factors in vitro would result in hESC differentiation into corneal epithelial-like cells. Indeed, here we show that culturing of hESC on collagen IV using medium conditioned by the limbal fibroblasts results in the loss of pluripotency and differentiation into epithelial-like cells. Further differentiation results in the formation of terminally differentiated epithelial-like cells not only of the

cornea but also of skin. Scanning electron microscopy shows that some differences exist between hESC-derived and adult limbal epithelial-like cells, necessitating further investigation using in vivo animal models of limbal stem cell deficiency. Such a model of hESC differentiation is useful for understanding the early events of epithelial lineage specification and to the eventual potential application of epithelium differentiated from hESC for clinical conditions of epithelial stem cell loss. Disclosure of potential conflicts of interest is found at the end of this article.

Ahn, J. I., et al. (2004). "Temporal expression changes during differentiation of neural stem cells derived from mouse embryonic stem cell." J Cell Biochem **93**(3): 563-578.

Temporal analysis in gene expression during differentiation of neural stem cells (NSCs) was performed by using in-house microarrays composed of 10,368 genes. The changes in mRNA level were measured during differentiation day 1, 2, 3, 6, 12, and 15. Out of 10,368 genes analyzed, 259 genes were up-regulated or down-regulated by 2-fold or more at least at one time-point during differentiation, and were classified into six clusters based on their expression patterns by K-means clustering. Clusters characterized by gradual increase have large numbers of genes involved in transport and cell adhesion; those which showed gradual decrease have much of genes in nucleic acid metabolism, cell cycle, transcription factor, and RNA processing. In situ hybridization (ISH) validated microarray data and it also showed that Fox M1, cyclin D2, and CDK4 were highly expressed in CNS germinal zones and ectonucleotide pyrophosphatase/phosphodiesterase 2 (Enpp2) was highly expressed in choroid plexus where stem/progenitor cells are possibly located. Together, this clustering analysis of expression patterns of functionally classified genes may give insight into understanding of CNS development and mechanisms of NSCs proliferation and differentiation.

Ahn, J. S., et al. (2010). "Identification of differentially expressed genes in human embryonic stem cell-derived endothelial cells using suppression subtractive hybridization." Stem Cells Dev **19**(8): 1249-1256.

Human embryonic stem cells (hESCs) are pluripotent, self-renewing cells derived from the inner cell mass of human blastocysts. During normal development, hESCs differentiate into 3 germ layers. Cellular lineages differentiated from hESCs express a set of genes that are exclusive to these specialized cells. Therefore, we hypothesized that endothelial cells derived from hESCs would express genes specific to endothelial cells. We previously isolated endothelial cells from human embryonic stem cells (hESC-ECs) using fluorescence-activated cell sorter (FACS). The aim of the current study was to identify genes associated with hESC-derived endothelial-like cells. Using suppression subtractive hybridization (SSH), we identified a set of genes specific to cells differentiated from hESC-ECs. We obtained 113 clones of expressed sequences that were more abundant in hESC-ECs compared with hESCs. Based on the NCBI GenBank database, 56 of these clones were known genes, 13 clones corresponded to nucleotides, 2 clones showed homology with chromosome sequences, and 42 clones showed no significant homology with any nucleotide sequences. These identified genes are related to cytoskeleton and cell adhesion, development, heat shock protein, metabolism, signal transduction, and transcription/nuclear-specific proteins. We anticipate that further study of these genes will provide crucial insights into their specific roles in the development of endothelial cells from hESCs.

Aiba, K., et al. (2009). "Defining developmental potency and cell lineage trajectories by expression profiling of differentiating mouse embryonic stem cells." DNA Res **16**(1): 73-80.

Biologists rely on morphology, function and specific markers to define the differentiation status of cells. Transcript profiling has expanded the repertoire of these markers by providing the snapshot of cellular status that reflects the activity of all genes. However, such data have been used only to assess relative similarities and differences of these cells. Here we show that principal component analysis of global gene expression profiles map cells in multidimensional transcript profile space and the positions of differentiating cells progress in a stepwise manner along trajectories starting from undifferentiated embryonic stem (ES) cells located in the apex. We present three 'cell lineage trajectories', which represent the differentiation of ES cells into the first three lineages in mammalian development: primitive endoderm, trophoblast and primitive ectoderm/neural ectoderm. The positions of the cells along these trajectories seem to reflect the developmental potency of cells and can be used as a scale for the potential of cells. Indeed, we show that embryonic germ cells and induced pluripotent cells are mapped near the origin of the trajectories, whereas mouse embryo fibroblast and fibroblast cell lines are mapped near the far end of the trajectories. We suggest that this method can be used as the non-operational semi-quantitative definition of cell differentiation status and developmental potency. Furthermore, the global expression profiles of cell lineages provide a framework for the future study of in vitro and in vivo cell differentiation.

Aktug, H., et al. (2016). "Comparison of cell cycle components, apoptosis and cytoskeleton-related molecules and therapeutic effects of flavopiridol and geldanamycin on the mouse fibroblast, lung cancer and embryonic stem cells." Tumour Biol **37**(9): 12423-12440.

Similarities and differences in the cell cycle components, apoptosis and cytoskeleton-related molecules among mouse skin fibroblast cells (MSFs), mouse squamous cell lung carcinomas (SqCLCs) and mouse embryonic stem cells (mESCs) are important determinants of the behaviour and differentiation capacity of these cells. To reveal apoptotic pathways and to examine the distribution and the role of cell cycle-cell skeleton comparatively would necessitate tumour biology and stem cell biology to be assessed together in terms of oncogenesis and embryogenesis. The primary objectives of this study are to investigate the effects of flavopiridol, a cell cycle inhibitor, and geldanamycin, a heat shock protein inhibitor on mouse somatic, tumour and embryonic stem cells, by specifically focusing on alterations in cytoskeletal proteins, cell polarity and motility as well as cell cycle regulators. To meet these objectives, expression of several genes, cell cycle analysis and immunofluorescence staining of intracellular cytoskeletal molecules were performed in untreated and flavopiridol- or geldanamycin-treated cell lines. Cytotoxicity assays showed that SqCLCs are more sensitive to flavopiridol than MSFs and mESCs. Keratin-9 and keratin-2 expressions increased dramatically whereas cell cycle regulatory genes decreased significantly in the flavopiridol-treated MSFs. Flavopiridol-treated SqCLCs displayed a slight increase in several cell cytoskeleton regulatory genes as well as cell cycle regulatory genes. However, gene expression profiles of mESCs were not affected after flavopiridol treatment except the Cdc2a. Cytotoxic concentrations of geldanamycin were close to each other for all cell lines. Cdkn1a was the most increased gene in the geldanamycin-treated MSFs. However, expression levels of cell cytoskeleton-associated genes were increased dramatically in the geldanamycin-treated SqCLCs. Our results revealing differences in molecular mechanisms between embryogenesis and carcinogenesis may prove crucial in developing novel therapeutics that specifically target cancer cells.

Akyash, F., et al. (2017). "Human embryonic stem cells and good manufacturing practice: Report of a 1- day workshop held at Stem Cell Biology Research Center, Yazd, 27(th) April 2017." Int J Reprod Biomed (Yazd) **15**(5): 255-256.

This report explains briefly the minutes of a 1-day workshop entitled; "human embryonic stem cells (hESCs) and good manufacturing practice (GMP)" held by Stem Cell Biology Research Center based in Yazd Reproductive Sciences Institute at Shahid Sadoughi University of Medical Sciences, Yazd, Iran on 27(th) April 2017. In this workshop, in addition to the practical sessions, Prof. Harry D. Moore from Centre for Stem Cell Biology, University of Sheffield, UK presented the challenges and the importance of the biotechnology of clinical-grade human embryonic stem cells from first derivation to robust defined culture for therapeutic applications.

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.

Almstrup, K., et al. (2006). "From embryonic stem cells to testicular germ cell cancer-- should we be concerned?" Int J Androl **29**(1): 211-218.

Since the discovery of testicular carcinoma in situ (CIS) -- the precursor cell for the vast majority of germ cell tumours -- it has been proposed that CIS cells could be derived from transformed primordial germ cells or gonocytes. Here, we review recent discoveries not only substantiating that initial hypothesis but also indicating that CIS cells have a striking phenotypic similarity to embryonic stem cells (ESC). Many cancers have been proposed to originate from tissue-specific stem cells [so-called 'cancer stem cells' (CSC)] and we argue that CIS may be a very good example of a CSC, but with exceptional features due to the retention of embryonic pluripotency. In addition, considering the fact that pre-invasive CIS

cells are transformed from early fetal cells, possibly due to environmentally induced alterations of the niche, we discuss potential risks linked to the uncontrolled therapeutic use of ESC.

Amano, T., et al. (2001). "Full-term development of enucleated mouse oocytes fused with embryonic stem cells from different cell lines." Reproduction **121**(5): 729-733.

The developmental potential of enucleated mouse oocytes receiving embryonic stem cells from ten lines with either the same or different genetic backgrounds using the cell fusion method was examined in vitro and in vivo. The development of nuclear-transferred oocytes into blastocysts was high (34-88%). However, there was no clear correlation between development into blastocysts after nuclear transfer and the chimaera formation rate of embryonic stem cells. The development into live young was low (1-3%) in all cell lines and 14 of 19 young died shortly after birth. Most of the live young had morphological abnormalities. Of the five remaining mice, two died at days 23 and 30 after birth, but the other three mice are still active at days 359 (mouse 1) and 338 (mice 4 and 5) after birth, with normal fertility. However, the reasons for the abnormalities and postnatal death of embryonic stem cell-derived mice are unknown.

Ambasudhan, R., et al. (2014). "Potential for cell therapy in Parkinson's disease using genetically programmed human embryonic stem cell-derived neural progenitor cells." J Comp Neurol **522**(12): 2845-2856.

Neural transplantation is a promising strategy for restoring dopaminergic dysfunction and modifying disease progression in Parkinson's disease (PD). Human embryonic stem cells (hESCs) are a potential resource in this regard because of their ability to provide a virtually limitless supply of homogenous dopaminergic progenitors and neurons of appropriate lineage. The recent advances in developing robust cell culture protocols for directed differentiation of hESCs to near pure populations of ventral mesencephalic (A9-type) dopaminergic neurons has heightened the prospects for PD cell therapy. Here, we focus our review on current state-of-the-art techniques for harnessing hESC-based strategies toward development of a stem cell therapeutic for PD. Importantly, we also briefly describe a novel genetic-programming approach that may address many of the key challenges that remain in the field and that may hasten clinical translation.

Amir, H., et al. (2017). "Spontaneous Single-Copy Loss of TP53 in Human Embryonic Stem Cells Markedly Increases Cell Proliferation and Survival." Stem Cells **35**(4): 872-885.

Genomic aberrations have been identified in many human pluripotent stem cell (hPSC) cultures. Commonly observed duplications in portions of chromosomes 12p and 17q have been associated with increases in genetic instability and resistance to apoptosis, respectively. However, the phenotypic consequences related to sporadic mutations have not been evaluated to date. Here, we report on the effects of a single-copy deletion of the chr17p13.1 region, a sporadic mutation that spontaneously arose independently in several subclones of a human embryonic stem cell culture. Compared to cells with two normal copies of chr17p13.1 ("wild-type"), the cells with a single-copy deletion of this region ("mutant") displayed a selective advantage when exposed to stressful conditions, and retained a higher percentage of cells expressing the pluripotency marker POU5F1/OCT4 after 2 weeks of in vitro differentiation. Knockdown of TP53, which is a gene encompassed by the deleted region, in wild-type cells mimicked the chr17p13.1 deletion phenotype. Thus, sporadic mutations in hPSCs can have phenotypic effects that may impact their utility for clinical applications. Stem Cells 2017;35:872-885.

Amirpour, N., et al. (2012). "Differentiation of human embryonic stem cell-derived retinal progenitors into retinal cells by Sonic hedgehog and/or retinal pigmented epithelium and transplantation into the subretinal space of sodium iodate-injected rabbits." Stem Cells Dev **21**(1): 42-53.

Transplantation of retinal cells has recently provided a promising therapeutic approach for retinal degeneration. Here, we differentiated initially retinal progenitors (RPs) from adherent feeder-free human embryonic stem cells (hESCs) with the use of defined media supplemented with a specific combination of growth factors. The differentiated RPs highly (>80%) expressed related molecular features that included Six3 at an early stage in addition to Crx, Rx, Pax6, Otx2, and Chx10 at later stage. Next, we examined the induction of photoreceptors by Shh and/or the coculture of rabbit retinal pigmented epithelium with hESCs-derived RPs. The differentiation of retinal cells was demonstrated by protein and gene expression in all groups. However, S-Opsin, a cone photoreceptor marker, had higher expression in the presence of Shh, whereas expressions of Gli and Hes1 decreased in the same group. Finally, hESC-derived RPs were treated with Shh transplanted into the subretinal space of sodium iodate-injected albino-type adult rabbits and analyzed 4 weeks later. Transplanted retinal cells survived, migrated into retinal layers, and restored a small but significant B-wave. The grafted cells

expressed photoreceptor markers, S-Opsin and Rhodopsin. Our results indicate that putative hESC-derived retinal cells express related genes and proteins. Further, our results show that retinal-like cells can be useful replacements for photoreceptors in retinal diseases.

Amit, M., et al. (2005). "No evidence for infection of human embryonic stem cells by feeder cell-derived murine leukemia viruses." Stem Cells **23**(6): 761-771.

Until recently, culture and expansion of nondifferentiated human embryonic stem cells (hESCs) depended on coculture with murine embryonic fibroblasts. Because mice are known to harbor a variety of pathogens, such culture conditions implicate the risk of xenozoonoses. Among these pathogens, endogenous retroviruses, including murine leukemia viruses (MuLVs), are of special importance. It is well known that some strains cause pathogenic (e.g., leukemic) effects and that xenotropic, polytropic, and amphotropic MuLVs are able to infect human cells. In view of potential clinical applications of hESC lines, it is therefore imperative to investigate potential infection of hESCs by mouse feeder cell-derived viruses. As a first step towards a comprehensive infection risk assessment, we have analyzed embryonic fibroblasts derived from different mouse strains for expression and release of xenotropic, polytropic, and amphotropic MuLVs. Moreover, several hESC lines have been investigated for expression of specific receptors for xenotropic/polytropic MuLVs, as well as for MuLV infection and expression. Evidence for expression of humantropic MuLVs was found in cultures of mouse embryonic fibroblasts (MEFs). Moreover, expression of specific receptors for xenotropic/ polytropic MuLV on human HEK293 and hESC lines and infection after coculture with an MuLV-producing mink cell line could be demonstrated. In contrast, no evidence of MuLV transmission from MEFs to human HEK293 cells or to the hESC lines I-3, I-6, I-8, and H-9 has been obtained. Our results suggest that recently established hESC lines are free of MuLV infections despite long-term close contact with MEFs.

Anand, S., et al. (2013). "Quiescent very small embryonic-like stem cells resist oncotherapy and can restore spermatogenesis in germ cell depleted mammalian testis." Stem Cells Dev.

Adult mouse and human testes harbor relatively quiescent, pluripotent very small embryonic-like stem cells (VSELs), in addition to actively dividing spermatogonial stem cells (SSCs). Here we report that various oncotherapy regimens in human cancer patients (n=7) and busulphan treatment (25mg/Kg body weight) in eight weeks old male mice (n=15) selectively affects actively dividing SSCs, spermatogonia, haploid germ cells and somatic microenvironment resulting in germ cell aplasia, whereas VSELs are unaffected and persist in otherwise germ cell depleted testis. Testicular VSELs are 2-5 microm in size, have high nucleo-cytoplasmic ratio, SCA-1+/CD45-/LIN- (mice), CD133+/CD45-/LIN- (human survivors of childhood cancer) and express various pluripotent transcripts including OCT-4A. SCA-1 sorted cells from busulphan treated mice testes in vitro formed small clusters suggestive of self-renewal and differentiation into progenitors, which divide rapidly. Inter-tubular random injections of syngeneic Sertoli cells (105 cells per testis, n=14) or bone marrow derived mesenchymal cells (104 cells per testis, n=16) into the germ cell depleted busulphan treated mice testes, were able to restore spermatogenesis from persisting VSELs. Transplanted Sertoli or mesenchymal cells possibly were a source of growth factors essential for VSELs differentiation. Since sperm formation occurred in situ, various epigenetic concerns associated with the 'synthetic gametes' may be eliminated in our approach. Ability of mesenchymal cells to restore spermatogenesis may benefit existing azoospermic survivors of childhood cancer who were otherwise deprived of testicular tissue cryopreservation prior to oncotherapy. Further studies are warranted to delineate the underlying mechanisms and to study quality and potential of sperm generated by this approach.

Anand, T., et al. (2011). "Buffalo (Bubalus bubalis) embryonic stem cell-like cells and preimplantation embryos exhibit comparable expression of pluripotency-related antigens." Reprod Domest Anim **46**(1): 50-58.

In this study, inner cell mass (ICM) cells were isolated from in vitro produced buffalo blastocysts and were cultured on mitomycin-C treated buffalo foetal fibroblast feeder layer for producing embryonic stem (ES) cells. Among different sources (hatched vs expanded blastocysts) or methods (enzymatic vs mechanical), mechanical isolation of ICM from hatched blastocysts resulted in the highest primary colony formation rate and the maximum passage number up to which ES cells survived. Putative ES cells expressed alkaline phosphatase and exhibited a normal karyotype up to passage 7. Putative ES cells and embryos at 2- to 4-cell, 8- to 16-cell, morula and blastocyst stages strongly expressed stage-specific embryonic antigen (SSEA)-4 but lacked expressions of SSEA-1 and SSEA-3. Putative ES cells also expressed tumour rejection antigen (TRA)-1-60, TRA-1-81 and Oct4. Whereas in all early embryonic stages, TRA-1-60 was observed only in the periplasmic space, and

TRA-1-81 expression was observed as small spots at a few places inside the embryos, both these markers were expressed by ICM. Oct4 expression, which was observed at all the embryonic stages and also in the trophectoderm, was the strongest in the ICM. Buffalo putative ES cells possess a unique pluripotency-related surface antigen phenotype, which resembles that of the ICM.

Ando, Y., et al. (2017). "Can Human Embryonic Stem Cell-Derived Stromal Cells Serve a Starting Material for Myoblasts?" Stem Cells Int **2017**: 7541734.

A large number of myocytes are necessary to treat intractable muscular disorders such as Duchenne muscular dystrophy with cell-based therapies. However, starting materials for cellular therapy products such as myoblasts, marrow stromal cells, menstrual blood-derived cells, and placenta-derived cells have a limited lifespan and cease to proliferate in vitro. From the viewpoints of manufacturing and quality control, cells with a long lifespan are more suitable as a starting material. In this study, we generated stromal cells for future myoblast therapy from a working cell bank of human embryonic stem cells (ESCs). The ESC-derived CD105(+) cells with extensive in vitro proliferation capability exhibited myogenesis and genetic stability in vitro. These results imply that ESC-derived CD105(+) cells are another cell source for myoblasts in cell-based therapy for patients with genetic muscular disorders. Since ESCs are immortal, mesenchymal stromal cells generated from ESCs can be manufactured at a large scale in one lot for pharmaceutical purposes.

Andressen, C., et al. (2001). "Nestin-specific green fluorescent protein expression in embryonic stem cell-derived neural precursor cells used for transplantation." Stem Cells **19**(5): 419-424.

Expression of the enhanced green fluorescent protein (EGFP) under control of a thymidine kinase promoter/nestin second intron was specifically detected in nestin immunoreactive neural precursor cells after selection of murine embryonic stem (ES) cells in chemically defined medium. Allowing differentiation in vitro, the capacity of these cells to give rise to astroglia, oligodendroglia, and neurones was investigated. After intracerebral transplantation, long-lasting integration of precursor cells into the host tissue was observed, serving as a pool for successive neuronal and glial differentiation. EGFP expression by ES cell-derived neural precursor cells may be a valuable tool to optimize protocols for maintenance and expansion of these cells in vitro as well as in vivo after intracerebral transplantation. In addition, preparative fluorescence-activated cell sorting of EGFP-labeled neural precursor cells should be useful for standardization of a donor cell population for cell replacement therapies.

Anzai, H., et al. (1999). "Self-renewal and differentiation of a basic fibroblast growth factor-dependent multipotent hematopoietic cell line derived from embryonic stem cells." Dev Growth Differ **41**(1): 51-58.

Despite the accumulation of informat on on the origin of hematopoietic stem cells, it is still unclear how these cells are generated in ontogeny. Isolation of cell lines equivalent to early embryonic hematopoietic progenitor cells can be helpful. A multipotent hematopoietic progenitor cell line, A-6, was isolated from H-1 embryonic stem (ES) cells. The self-renewal of A-6 cells was supported by basic-fibroblast growth factor (b-FGF) and their differentiation into definitive erythroid cells, granulocytes and macrophages was induced after co-culture with ST-2 stromal cells. A-6 cells were positive for the surface markers of hematopoietic stem cell, c-kit, CD31, CD34, Flt3/Flk2, PgP-1, and HSA, but were negative for that of the differentiated cells. Reverse transcription-polymerase chain reaction analysis showed that A-6 cells produced mRNA from SCL/tal-1 and GATA-2 genes. Among various cytokines examined, on y stem cell factor (SCF) and Flt3/Flk2 ligand (FL) supported the proliferation of A-6 cells instead of b-FGF. The FL, as well as b-FGF, supported the self-renewal of A-6 cells, whereas SCF induced differentiation into myeloid cells. A-6 cells will be useful for the characterization of hematopoietic progenitor cells derived from ES cells and provide a model system to realize the control mechanisms between self-renewal and different ation of hematopoietic stem cells.

Aoki, H., et al. (2006). "Embryonic stem cells that differentiate into RPE cell precursors in vitro develop into RPE cell monolayers in vivo." Exp Eye Res **82**(2): 265-274.

A culture system to generate eye-like structures consisting of lens, neural retina, and retinal pigmented epithelium (RPE) cells from undifferentiated embryonic stem cells has been established. Precursors of RPE cells that differentiated in the cultures were responsive to Wnt2b signaling and identified retrospectively to form secondary colonies consisting of only RPE-like cells in eye-like structures. These transplanted eye-like structures were capable of populating the developing chick eye as neuronal retina and RPE cells. The outgrowth of a single cell layer of mature RPE cells from the grafted eye-like structures confirmed the existence of precursors for RPE cells. These results suggest that the eye-like structures resulted from the normal developmental pathway

responsible for generating eyes in vivo. If a functional effect of these cells can be established, such eye-like structures may be potentially used to establish therapy models for various eye diseases.

Aoyama, M., et al. (2010). "Resistance to chemotherapeutic agents and promotion of transforming activity mediated by embryonic stem cell-expressed Ras (ERas) signal in neuroblastoma cells." Int J Oncol **37**(4): 1011-1016.

Neuroblastoma is a common childhood tumor derived from neural crest precursor cells. In the present study, we investigated the expression and function of embryonic stem cell-expressed Ras (ERas), a novel Ras family protein previously reported as the specific expression gene in embryonic stem cells (ES cells), in neuroblastoma cell lines. Our results showed that the expressions of ERas were detected in neuroblastoma cell lines by RT-PCR and Western blotting. Therefore, we transfected a full length ERas expression vector into the neuroblastoma cell line SH-SY5Y, which has weak endogenous expression of ERas, and obtained clones with higher levels of expression. Overexpression of ERas did not increase the growth rate of the ERas transfectants but promoted their transforming activity. The ERas transfectants were more resistant to all the chemotherapy agents than the parental cell line. The ability of ERas to rescue cells from the toxic effect of chemotherapeutic agents was inhibited by the phosphatidylinositol 3'-kinase (PI3K) inhibitor PD294002. These results show that the ERas/PI3K pathway may provide resistance to chemotherapy and promote transforming activity in neuroblastoma.

Aparicio, J. G., et al. (2017). "Temporal expression of CD184(CXCR4) and CD171(L1CAM) identifies distinct early developmental stages of human retinal ganglion cells in embryonic stem cell derived retina." Exp Eye Res **154**: 177-189.

Human retinal ganglion cells (RGCs) derived from pluripotent stem cells (PSCs) have anticipated value for human disease study, drug screening, and therapeutic applications; however, their full potential remains underdeveloped. To characterize RGCs in human embryonic stem cell (hESC) derived retinal organoids we examined RGC markers and surface antigen expression and made comparisons to human fetal retina. RGCs in both tissues exhibited CD184 and CD171 expression and distinct expression patterns of the RGC markers BRN3 and RBPMS. The retinal progenitor cells (RPCs) of retinal organoids expressed CD184, consistent with its expression in the neuroblastic layer in fetal retina. In retinal organoids CD184 expression was enhanced in RGC competent RPCs and high CD184 expression was retained on post-mitotic RGC precursors; CD171 was detected on maturing RGCs. The differential expression timing of CD184 and CD171 permits identification and enrichment of RGCs from retinal organoids at differing maturation states from committed progenitors to differentiating neurons. These observations will facilitate molecular characterization of PSC-derived RGCs during differentiation, critical knowledge for establishing the veracity of these in vitro produced cells. Furthermore, observations made in the retinal organoid model closely parallel those in human fetal retina further validating use of retinal organoid to model early retinal development.

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

Induced pluripotent stem cells hold great promise for regenerative medicine but point mutations have been identified in these cells and have raised serious concerns about their safe use. We generated nuclear transfer embryonic stem cells (ntESCs) from both mouse embryonic fibroblasts (MEFs) and tail-tip fibroblasts (TTFs) and by whole genome sequencing found fewer mutations compared with iPSCs generated by retroviral gene transduction. Furthermore, TTF-derived ntESCs showed only a very small number of point mutations, approximately 80% less than the number observed in iPSCs generated using retrovirus. Base substitution profile analysis confirmed this greatly reduced number of point mutations. The point mutations in iPSCs are therefore not a Yamanaka factor-specific phenomenon but are intrinsic to genome reprogramming. Moreover, the dramatic reduction in point mutations in ntESCs suggests that most are not essential for genome reprogramming. Our results suggest that it is feasible to reduce the point mutation frequency in iPSCs by optimizing various genome reprogramming conditions. We conducted whole genome sequencing of ntES cells derived from MEFs or TTFs. We thereby succeeded in establishing TTF-derived ntES cell lines with far fewer point mutations. Base substitution profile analysis of these clones also indicated a reduced point mutation frequency, moving from a transversion-predominance to a transition-predominance. Stem Cells 2017;35:1189-1196.

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

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

Arnhold, S., et al. (2000). "Differentiation of green fluorescent protein-labeled embryonic stem cell-derived neural precursor cells into Thy-1-positive neurons and glia after transplantation into adult rat striatum." J Neurosurg **93**(6): 1026-1032.

OBJECT: The aim of this investigation was to assess new information concerning the capacity of transplanted embryonic stem cell (ESC)-derived neuronal cells to migrate into host brain and to evaluate these cells as a possible source for cell replacement therapy in neurodegenerative disorders such as Parkinson's disease (PD). METHODS: The authors investigated the ability of ESC-derived neural precursor cells to migrate and differentiate in a host striatum by using a D3-derived ESC clone that was transfected stably with a chicken beta-actin cytomegalovirus enhancer-driven green fluorescent protein (GFP)-labeled construct. This procedure allowed easy monitoring of all transplanted cells because of the green fluorescent labeling of donor cells. This approach also afforded easy estimation of cell integration and simultaneous observation of the entire transplanted cell population in relation to immunocytochemically identified neuronal and glial differentiation. After selection of nestin-positive neural precursor cells in a synthetic medium, they were implanted into the striatum of male adult Wistar rats. Their integration was analyzed on morphological studies performed 3 days to 4 weeks posttransplantation. CONCLUSIONS: The investigators found that after transplantation, a subpopulation of GFP-labeled cells differentiated into various neural morphological types that were positive for the mouse-specific Thy-1 antigen, which is known be expressed on neurons, as well as being positive for the astroglial marker glial fibrillary acidic protein. Moreover, GFP-expressing cells that were negative for either of these markers remained close to the injection site, presumably representing other derivatives of the neural lineage. Together, these findings contribute to basic research regarding future transplantation strategies in neurodegenerative diseases such as PD.

Arpornmaeklong, P., et al. (2009). "Phenotypic characterization, osteoblastic differentiation, and bone regeneration capacity of human embryonic stem cell-derived mesenchymal stem cells." Stem Cells Dev **18**(7): 955-968.

To enhance the understanding of differentiation patterns and bone formation capacity of hESCs, we determined (1) the temporal pattern of osteoblastic differentiation of human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs), (2) the influence of a three-dimensional matrix on the osteogenic differentiation of hESC-MSCs in long-term culture, and (3) the bone-forming capacity of osteoblast-like cells derived from hESC-MSCs in calvarial defects. Incubation of hESC-MSCs in osteogenic medium induced osteoblastic differentiation of hESC-MSCs into mature osteoblasts in a similar chronological pattern to human bone marrow stromal cells and primary osteoblasts. Osteogenic differentiation was enhanced by culturing the cells on three-dimensional collagen scaffolds. Fluorescent-activated cell sorting of alkaline phosphatase expressing cells was used to obtain an enriched osteogenic cell population for in vivo transplantation. The identification of green fluorescence protein and expression of human-specific nuclear antigen in osteocytes in newly formed bone verified the role of transplanted human cells in the bone regeneration process. The current cell culture model and osteogenic cell enrichment method could provide large numbers of osteoprogenitor cells for analysis of differentiation patterns and cell transplantation to regenerate skeletal defects.

Arpornmaeklong, P., et al. (2010). "Expansion and characterization of human embryonic stem cell-derived osteoblast-like cells." Cell Reprogram **12**(4): 377-389.

Human embryonic stem cells (hESCs) have the potential to serve as a repository of cells for the replacement of damaged or diseased tissues and organs. However, to use hESCs in clinically relevant scenarios, a large number of cells are likely to be required. The aim of this study was to demonstrate an alternative cell culture method to increase the quantity of osteoblast-like cells directly derived from hESCs (hESCs-OS). Undifferentiated hESCs were directly cultivated and serially passaged in osteogenic medium (hESC-OS), and exhibited similar expression patterns of osteoblast-related genes to osteoblast-like cells derived from mesenchymal stem cells derived from hESCs (hESCs-MSCs-OS) and human bone marrow stromal cells (hBMSCs-OS). In comparison to hESCs-MSCs-OS, the hESCs-OS required a shorter expansion time to generate a homogenous population of osteoblast-like cells that did not contain contaminating undifferentiated hESCs. Identification of human specific nuclear antigen (HuNu) in the newly formed bone in calvarial defects verified the role of the transplanted hESCs-OS as active bone forming cells in vivo. Taken together, this study suggests that osteoblast-like cells directly derived from hESCs have the potential to serve as an alternative source of osteoprogenitors for bone tissue engineering strategies.

Arumugam, S. B., et al. (2011). "Detection of embryonic stem cell markers in adult human adipose tissue-derived stem cells." Indian J Pathol Microbiol **54**(3): 501-508.

BACKGROUND: Bone marrow transplantation is already an established therapy, which is now widely used in medicine to treat leukemia, lymphoma, and several inherited blood disorders. The culture of multilineage cells from easily available adipose tissue is another source of multipotent mesenchymal stem cells, and is referred to as adipose tissue-derived stem cells (ADSCs). While ADSCs are being used to treat various conditions, some lacuna exists regarding the specific proteins in these. It was therefore decided to analyze the specific proteins of embryonic cells in ADSCs. AIMS: To analyze the specific protein of embryonic stem cells (ESCs) in ADSCs. MATERIALS AND METHODS: Adult human adipose tissue-derived stem cells (ADSCs) were harvested from 13 patients after obtaining patients' consent. The specific markers of ESCs included surface proteins CD10, CD13, CD44, CD59, CD105, and CD166, and further nucleostemin, (NS) NANOG, peroxisome proliferator-activated receptor-ggamma, collagen type 1 (Coll1), alkaline phosphate, (ALP) osteocalcin (OC), and core binding factor 1 (Cbfa1) were analyzed using by reverse transcription-polymerase chain reaction, (RT-PCR) immunofluorescence (IF), and western blot. RESULTS: All the proteins were expressed distinctly, except CD13 and OC. CD13 was found individually with different expressions, and OC expression was discernable. CONCLUSIONS: Although the ESC with its proven self-renewal capacity and pluripotency seems appropriate for clinical use, the recent work on ADSCs suggests that these adult stem cells would be a valuable source for future biotechnology, especially since there is a relative ease of procurement.

Auerbach, W. and T. M. DeChiara (2017). "Injecting Embryonic Stem Cells into Eight-Cell-Stage Mouse Embryos." Cold Spring Harb Protoc **2017**(9): pdb prot094367.

In this protocol, eight-cell-stage precompaction embryos from outbred mouse strains are used for the injection of hybrid or inbred embryonic stem (ES) cells. This process often leads to generation of fully ES cell-derived so-called F0 mice (VelociMice). Postinjection culture of embryos is necessary to achieve the highest ratio of fully ES cell-derived mice and high-degree chimeras. Typically, 50 embryos are injected per ES cell clone.

Bahena, I., et al. (2014). "Role of Mael in early oogenesis and during germ-cell differentiation from embryonic stem cells in mice in vitro." Zygote **22**(4): 513-520.

In a previous study, we have identified a set of conserved spermatogenic genes whose expression is restricted to testis and ovary and that are developmentally regulated. One of these genes, the transcription factor Mael, has been reported to play an essential role in mouse spermatogenesis. Nevertheless, the role of Mael in mouse oogenesis has not been defined. In order to analyse the role of Mael in mouse oogenesis, the expression of this gene was blocked during early oogenesis in mouse in vitro using RNAi technology. In addition, the role of Mael during differentiation of embryonic stem cells (ESC) into germ cells in vitro was analysed. Results show that downregulation of Mael by a specific short interfering RNA disrupted fetal oocyte growth and differentiation in fetal ovary explants in culture and the expression of several germ-cell markers in ESC during their differentiation. These results suggest that there is an important role for Mael in early oogenesis and during germ-cell differentiation from embryonic stem cells in mouse in vitro.

Bahrami, S. B., et al. (2011). "Temporal changes in Hox gene expression accompany endothelial cell

differentiation of embryonic stem cells." Cell Adh Migr **5**(2): 133-141.

In pluripotent embryonic stem cells (ESCs), expression of the Hox master regulatory transcription factors that play essential roles in organogenesis, angiogenesis, and maintenance of differentiated tissues, is globally suppressed. We investigated whether differentiation of endothelial cells (ECs) from mouse ESCs was accompanied by activation of distinct Hox gene expression profiles. Differentiation was observed within 3 days, as indicated by the appearance of cells expressing specific endothelial marker genes (Flk-1+ /VE-Cadherin+). Expression of HoxA3 and HoxD3, which drive adult endothelial cell invasion and angiogenesis, peaked at day 3 and declined thereafter, whereas expression of HoxA5 and HoxD10, which maintain a mature quiescent EC phenotype, was low at day 3, but increased over time. The temporal and reciprocal changes in HoxD3 and HoxA5 expression were accompanied by corresponding changes in expression of established downstream target genes including integrin beta3 and Thrombospondin-2. Our results indicate that differentiation and maturation of ECs derived from cultured ESCs mimic changes in Hox gene expression that accompany maturation of immature angiogenic endothelium into differentiated quiescent endothelium in vivo.

Bai, H., et al. (2012). "Bcl-xL enhances single-cell survival and expansion of human embryonic stem cells without affecting self-renewal." Stem Cell Res **8**(1): 26-37.

Robust expansion and genetic manipulation of human embryonic stem cells (hESCs) and induced-pluripotent stem (iPS) cells are limited by poor cell survival after enzymatic dissociation into single cells. Although inhibition of apoptosis is implicated for the single-cell survival of hESCs, the protective role of attenuation of apoptosis in hESC survival has not been elucidated. Bcl-xL is one of several anti-apoptotic proteins, which are members of the Bcl-2 family of proteins. Using an inducible system, we ectopically expressed Bcl-xL gene in hESCs, and found a significant increase of hESC colonies in the single-cell suspension cultures. Overexpression of Bcl-xL in hESCs decreased apoptotic caspase-3(+) cells, suggesting attenuation of apoptosis in hESCs. Without altering the kinetics of pluripotent gene expression, the efficiency to generate embryoid bodies (EBs) in vitro and the formation of teratoma in vivo were significantly increased in Bcl-xL-overexpressing hESCs after single-cell dissociation. Interestingly, the number and size of hESC colonies from cluster cultures were not affected by Bcl-xL overexpression. Several genes of extracellular matrix and adhesion molecules were upregulated by Bcl-xL in hESCs without single-cell dissociation, suggesting that Bcl-xL regulates adhesion molecular expression independent of cell dissociation. In addition, the gene expressions of FAS and several TNF signaling mediators were downregulated by Bcl-xL. These data support a model in which Bcl-xL promotes cell survival and increases cloning efficiency of dissociated hESCs without altering hESC self-renewal by i) attenuation of apoptosis, and ii) upregulation of adhesion molecules to facilitate cell-cell or cell-matrix interactions.

Bai, Q., et al. (2015). "Temporal analysis of genome alterations induced by single-cell passaging in human embryonic stem cells." Stem Cells Dev **24**(5): 653-662.

Simplified culture conditions are essential for large-scale drug screening and medical applications of human pluripotent stem cells (hPSCs). However, hPSCs [ie, human embryonic stem cells (hESCs), and human induced pluripotent stem cells (iPSCs) are prone to genomic instability, a phenomenon that is highly influenced by the culture conditions. Enzymatic dissociation, a cornerstone of large-scale hPSC culture systems, has been reported to be deleterious, but the extent and the timeline of the genomic alterations induced by this passaging technique are still unclear. We prospectively monitored three hESC lines that were initially derived and cultured on human feeders and passaged mechanically before switching to enzymatic single-cell passaging. We show that karyotype abnormalities and copy number variations are not restricted to long-term culture, but can occur very rapidly, within five passages after switching hESCs to enzymatic dissociation. Subchromosomal abnormalities preceded or accompanied karyotype abnormalities and were associated with increased occurrence of DNA double-strand breaks. Our results indicate that enzymatic single-cell passaging can be highly deleterious to the hPSC genome, even when used only for a limited period of time. Moreover, hPSC culture techniques should be reappraised by complementing the routine karyotype analysis with more sensitive techniques, such as microarrays, to detect subchromosomal abnormalities.

Bak, X. Y., et al. (2011). "Human embryonic stem cell-derived mesenchymal stem cells as cellular delivery vehicles for prodrug gene therapy of glioblastoma." Hum Gene Ther **22**(11): 1365-1377.

Mesenchymal stem cells (MSCs) possess tumor-tropic properties and consequently have been used to deliver therapeutic agents for cancer treatment. Their potential in cancer therapy highlights the need for a consistent and renewable source for the production of uniform human MSCs suitable for clinical applications.

In this study, we seek to investigate whether human embryonic stem cells can be used as a cell source to fulfill this goal. We generated MSC-like cells from two human embryonic stem cell lines, HuES9 and H1, and observed that MSC-like cells derived from human embryonic stem cells were able to migrate into human glioma intracranial xenografts after being injected into the cerebral hemisphere contralateral to the tumor inoculation site. We engineered these cells with baculoviral and lentiviral vectors, respectively, for transient and stable expression of the herpes simplex virus thymidine kinase gene. In tumor-bearing mice the engineered MSC-like cells were capable of inhibiting tumor growth and prolonging survival in the presence of ganciclovir after they were injected either directly into the xenografts or into the opposite hemisphere. Our findings suggest that human embryonic stem cell-derived MSCs may be a viable and attractive alternative for large-scale derivation of targeting vehicles for cancer therapy.

Balconi, G., et al. (2000). "Development of endothelial cell lines from embryonic stem cells: A tool for studying genetically manipulated endothelial cells in vitro." Arterioscler Thromb Vasc Biol **20**(6): 1443-1451.

Totipotent embryonic stem cells can be induced to differentiate to endothelium in vitro. This may be a useful tool for obtaining cultures of genetically manipulated endothelial cells because embryonic stem cells are relatively easy to transfect and are commonly used for gene inactivation experiments in mice. However, embryonic stem cell-derived endothelial cells could not be easily separated from embryoid bodies and maintained in culture. In this study, we describe the isolation and characterization of immortalized endothelial cell lines obtained from embryonic stem cells differentiated in vitro. The cell lines were analyzed for expression of endothelial cell markers, including growth factor receptors and adhesion molecules, and compared with endothelial cells obtained from the yolk sac, the embryo proper, or the heart microcirculation of the adult. We propose that this approach may be useful for obtaining endothelial cells carrying gene mutations that are lethal at very early stages of development.

Ballabeni, A., et al. (2011). "Cell cycle adaptations of embryonic stem cells." Proc Natl Acad Sci U S A **108**(48): 19252-19257.

ES cells proliferate with very short gap phases yet maintain their capacity to differentiate. It had been thought that the levels of cyclins and other substrates of ubiquitin ligase APC/C remain nearly constant and Cdk activity remains constitutively high in mouse ES cells. Here we demonstrate that APC/C (anaphase-promoting complex/cyclosome) enzyme is active in ES cells but attenuated by high levels of the Emi1 (early mitotic inhibitor-1) protein. Despite the presence of high Cdk activity during the G1 phase, chromatin can be effectively licensed for DNA replication and fast entry into the S phase can still occur. High Cdk activity during S-G2-M phases produces high levels of the DNA replication factor Cdt1, and this leads to efficient Mcm proteins loading on chromatin after mitotic exit. Although disturbing the usual balance between Cdk activity and APC/C activity found in somatic cells, a few key adaptations allow normal progression of a very rapid cell cycle.

Bandi, S. and R. Akkina (2008). "Human embryonic stem cell (hES) derived dendritic cells are functionally normal and are susceptible to HIV-1 infection." AIDS Res Ther **5**: 1.

BACKGROUND: Human embryonic stem (hES) cells hold considerable promise for cell replacement and gene therapies. Their remarkable properties of pluripotency, self-renewal, and tractability for genetic modification potentially allows for the production of sizeable quantities of therapeutic cells of the hematopoietic lineage. Dendritic cells (DC) arise from CD34+ hematopoietic progenitor cells (HPCs) and are important in many innate and adaptive immune functions. With respect to HIV-1 infection, DCs play an important role in the efficient capture and transfer of the virus to susceptible cells. With an aim of generating DCs from a renewable source for HIV-1 studies, here we evaluated the capacity of hES cell derived CD34+ cells to give rise to DCs which can support HIV-1 infection. RESULTS: Undifferentiated hES cells were cultured on S17 mouse bone marrow stromal cell layers to derive CD34+ HPCs which were subsequently grown in specific cytokine differentiation media to promote the development of DCs. The hES derived DCs (hES-DC) were subjected to phenotypic and functional analyses and compared with DCs derived from fetal liver CD34+ HPC (FL-DC). The mature hES-DCs displayed typical DC morphology consisting of veiled stellate cells. The hES-DCs also displayed characteristic phenotypic surface markers CD1a, HLA-DR, B7.1, B7.2, and DC-SIGN. The hES-DCs were found to be capable of antigen uptake and stimulating naive allogeneic CD4+ T cells in a mixed leukocyte reaction assay. Furthermore, the hES-DCs supported productive HIV-1 viral infection akin to standard DCs. CONCLUSION: Phenotypically normal and functionally competent DCs that support HIV-1 infection can be derived from hES cells. hES-DCs can now be exploited in applied immunology and HIV-1 infection studies. Using gene therapy approaches, it is now possible to generate HIV-1 resistant DCs from

anti-HIV gene transduced hES-CD34+ hematopoietic progenitor cells.

Banerjee, S. and M. Bacanamwo (2010). "DNA methyltransferase inhibition induces mouse embryonic stem cell differentiation into endothelial cells." Exp Cell Res **316**(2): 172-180.

Understanding endothelial cell (EC) differentiation is a step forward in tissue engineering, controlling angiogenesis, and endothelial dysfunction. We hypothesized that epigenetic activation of EC lineage specification genes is an important mediator of embryonic stem cell (ESC) differentiation into EC. Mouse ESC was differentiated by removing leukemia inhibitory factor (LIF) from the maintenance media in the presence or absence of the specific DNA methyltransferase (DNMT) inhibitor 5'-aza-2'-deoxycytidine (aza-dC). Expression of EC specification and marker genes was monitored by quantitative PCR, western, immunocytochemistry, and flow cytometry. Functionality of differentiated EC was assessed by angiogenesis assay. The methylation status in the proximal promoter CpGs of the mediators of EC differentiation VEGF-A, BMP4, and EPAS-1 as well as of the mature EC marker VE-cadherin was determined by bisulfite sequencing. ESC differentiation resulted in repression of OCT4 expression in both the absence and presence of aza-dC treatment. However, significant increase in angiogenesis and expression of the mediators of EC differentiation and EC-specific genes was only observed in aza-dC-treated cells. The DNMT inhibition-mediated increase in EC specification and marker gene expression was not associated with demethylation of these genes. These studies suggest that DNMT inhibition is an efficient inducer of EC differentiation from ESC.

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.

Barbuti, A., et al. (2009). "Molecular composition and functional properties of f-channels in murine embryonic stem cell-derived pacemaker cells." J Mol Cell Cardiol **46**(3): 343-351.

Mouse embryonic stem cells (mESCs) differentiate into all cardiac phenotypes, and thus represent an important potential source for cardiac regenerative therapies. Here we characterize the molecular composition and functional properties of "funny" (f-) channels in mESC-derived pacemaker cells. Following differentiation, a fraction of mESC-derived myocytes exhibited action potentials characterized by a slow diastolic depolarization and expressed the I (f) current. I (f) plays an important role in the pacemaking mechanism of these cells since ivabradine (3 microM), a specific f-channel inhibitor, inhibited I (f) by about 50% and slowed rate by about 25%. Analysis of I (f) kinetics revealed the presence of two populations of cells, one expressing a fast- and one a slow-activating I (f); the two components are present both at early and late stages of differentiation and had also distinct activation curves. Immunofluorescence analysis revealed that HCN1 and HCN4 are the only isoforms of the pacemaker channel expressed in these cells. Rhythmic cells responded to beta-adrenergic and muscarinic agonists: isoproterenol (1 microM) accelerated and acetylcholine (0.1 microM) slowed spontaneous rate by about 50 and 12%, respectively. The same agonists caused quantitatively different effects on I (f): isoproterenol shifted

activation curves by about 5.9 and 2.7 mV and acetylcholine by -4.0 and -2.0 mV in slow and fast I (f)-activating cells, respectively. Accordingly, beta1- and beta2-adrenergic, and M2-muscarinic receptors were detected in mESC-derived myocytes. Our data show that mESC-derived pacemaker cells functionally express proteins which underlie generation and modulation of heart rhythm, and can therefore represent a potential cell substrate for the generation of biological pacemakers.

Barta, T., et al. (2013). "Cell cycle regulation in human embryonic stem cells: links to adaptation to cell culture." Exp Biol Med (Maywood) **238**(3): 271-275.

Cell cycle represents not only a tightly orchestrated mechanism of cell replication and cell division but it also plays an important role in regulation of cell fate decision. Particularly in the context of pluripotent stem cells or multipotent progenitor cells, regulation of cell fate decision is of paramount importance. It has been shown that human embryonic stem cells (hESCs) show unique cell cycle characteristics, such as short doubling time due to abbreviated G1 phase; these properties change with the onset of differentiation. This review summarizes the current understanding of cell cycle regulation in hESCs. We discuss cell cycle properties as well as regulatory machinery governing cell cycle progression of undifferentiated hESCs. Additionally, we provide evidence that long-term culture of hESCs is accompanied by changes in cell cycle properties as well as configuration of several cell cycle regulatory molecules.

Basford, C. L., et al. (2012). "The functional and molecular characterisation of human embryonic stem cell-derived insulin-positive cells compared with adult pancreatic beta cells." Diabetologia **55**(2): 358-371.

AIMS/HYPOTHESIS: Using a novel directed differentiation protocol, we recently generated up to 25% insulin-producing cells from human embryonic stem cells (hESCs) (insulin (+) cells). At this juncture, it was important to functionally and molecularly characterise these hESC-derived insulin (+) cells and identify key differences and similarities between them and primary beta cells. METHODS: We used a new reporter hESC line with green fluorescent protein (GFP) cDNA targeted to the INS locus by homologous recombination (INS (GFP/w)) and an untargeted hESC line (HES2). INS (GFP/w) allowed efficient identification and purification of GFP-producing (INS:GFP (+)) cells. Insulin (+) cells were examined for key features of adult beta cells using microarray, quantitative PCR, secretion assays, imaging and electrophysiology. RESULTS: Immunofluorescent staining showed complete co-localisation of insulin with GFP; however, cells were often multihormonal, many with granules containing insulin and glucagon. Electrophysiological recordings revealed variable K (ATP) and voltage-gated Ca (2+) channel activity, and reduced glucose-induced cytosolic Ca (2+) uptake. This translated into defective glucose-stimulated insulin secretion but, intriguingly, appropriate glucagon responses. Gene profiling revealed differences in global gene expression between INS:GFP (+) cells and adult human islets; however, INS:GFP (+) cells had remarkably similar expression of endocrine-lineage transcription factors and genes involved in glucose sensing and exocytosis. CONCLUSIONS/INTERPRETATION: INS:GFP (+) cells can be purified from differentiated hESCs, providing a superior source of insulin-producing cells. Genomic analyses revealed that INS:GFP (+) cells collectively resemble immature endocrine cells. However, insulin (+) cells were heterogeneous, a fact that translated into important functional differences within this population. The information gained from this study may now be used to generate new iterations of functioning beta cells that can be purified for transplant.

Batista, P. J., et al. (2014). "m (6)A RNA modification controls cell fate transition in mammalian embryonic stem cells." Cell Stem Cell **15**(6): 707-719.

N6-methyl-adenosine (m (6)A) is the most abundant modification on messenger RNAs and is linked to human diseases, but its functions in mammalian development are poorly understood. Here we reveal the evolutionary conservation and function of m (6)A by mapping the m (6)A methylome in mouse and human embryonic stem cells. Thousands of messenger and long noncoding RNAs show conserved m (6)A modification, including transcripts encoding core pluripotency transcription factors. m (6)A is enriched over 3' untranslated regions at defined sequence motifs and marks unstable transcripts, including transcripts turned over upon differentiation. Genetic inactivation or depletion of mouse and human Mettl3, one of the m (6)A methylases, led to m (6)A erasure on select target genes, prolonged Nanog expression upon differentiation, and impaired ESC exit from self-renewal toward differentiation into several lineages in vitro and in vivo. Thus, m (6)A is a mark of transcriptome flexibility required for stem cells to differentiate to specific lineages.

Becker, K. A., et al. (2006). "Self-renewal of human embryonic stem cells is supported by a shortened G1 cell cycle phase." J Cell Physiol **209**(3): 883-893.

Competency for self-renewal of human embryonic stem (ES) cells is linked to pluripotency. However, there is a critical paucity of fundamental parameters of human ES cell division. In this study we show that human ES cells (H1 and H9; NIH-designated WA01 and WA09) rapidly proliferate due to a very short overall cell cycle (15-16 h) compared to somatic cells (e.g., normal diploid IMR90 fibroblasts and NT-2 teratocarcinoma cells). The human ES cell cycle maintains the four canonical cell cycle stages G1, S, G2, and M, but the duration of G1 is dramatically shortened. Bromodeoxyuridine (BrdU) incorporation and FACS analysis demonstrated that 65% of asynchronously growing human ES cells are in S phase. Immunofluorescence microscopy studies detecting BrdU labeled mitotic chromosomes, Ki67 domains, and p220(NPAT) containing Cajal bodies revealed that the durations of the S (approximately 8 h), G2 (approximately 4 h), and M phases (approximately 1 h) are similar in ES and somatic cells. We determined that human ES cells remain viable after synchronization with either nocodazole or the anti-tumor drug Paclitaxel (taxol) and have an abbreviated G1 phase of only 2.5-3 h that is significantly shorter than in somatic cells. Molecular analyses using quantitative RT-PCR demonstrate that human ES cells and somatic cells express similar cell cycle markers. However, among cyclins and cyclin-dependent kinases (CDKs), we observed high mRNA levels for the G1-related CDK4 and cyclin D2 genes. We conclude that human ES cells exhibit unique G1 cell cycle kinetics and use CDK4/cyclin D2 related mechanisms to attain competency for DNA replication.

Becker, K. A., et al. (2007). "Establishment of histone gene regulation and cell cycle checkpoint control in human embryonic stem cells." J Cell Physiol **210**(2): 517-526.

Rapid self-renewal of human embryonic stem (ES) cells (NIH designation WA01 and WA09) is accommodated by an abbreviated cell cycle due to a reduction in the G1 phase. Thus, molecular mechanisms operative in ES cells may expedite the cellular commitment to progress into S phase to initiate replication of DNA and biosynthesis of histone proteins to form new chromatin. Here we show that the selective cell cycle regulated expression of individual histone H4 gene copies, which is typical for somatic cell types, is already firmly established in human ES cells. This early establishment of H4 gene regulation, which is E2F independent, is consistent with co-expression of the cognate transcriptional regulators HiNF-P and p220(NPAT). Human ES cells differ from somatic cells in the expression of members of the E2F family and RB-related pocket proteins (p105(RB1), p107(RBL1), and p130(RBL2/RB2)) that control expression of genes encoding enzymes for nucleotide metabolism and DNA synthesis. Human ES cells rapidly and robustly (>200-fold) induce the cyclin dependent kinase (CDK) inhibitor p21(WAF1/CIP1) upon gamma-irradiation. This DNA damage response promptly reduces histone gene expression as well as mRNA levels for HiNF-P and p220(NPAT) and causes accumulation of unprocessed histone H4 precursor RNAs. Furthermore, while E2F4, E2F5 and p130(RBL2/RB2) are the major E2F and pocket protein mRNAs in actively proliferating ES cells, expression levels of E2F5, E2F6, and p105(RB1) are most strongly elevated during cell cycle arrest in cells responding to DNA damage. Our data suggest that the brief G1 phase of ES cells is supported by a potent p21(WAF1/CIP1) related DNA damage response that functions through several mechanisms to rapidly inhibit cell cycle progression. This response may alter the E2F/pocket protein combinations that control E2F dependent genes and block H4 gene expression by inhibiting histone-specific transcription factors and processing of histone gene transcripts, as well as by destabilizing histone mRNAs.

Behroozi, F., et al. (2018). "Smart liposomal drug delivery for treatment of oxidative stress model in human embryonic stem cell-derived retinal pigment epithelial cells." Int J Pharm **548**(1): 62-72.

Oxidative stress has been implicated in the progression of age-related macular degeneration (AMD). Treatment with antioxidants seems to delay progression of AMD. In this study, we suggested an antioxidant delivery system based on redox-sensitive liposome composed of phospholipids and a diselenide centered alkyl chain. Dynamic light scattering assessment indicated that the liposomes had an average size of 140nm with a polydispersity index below 0.2. The percentage of encapsulation efficiency of the liposomes was calculated by high-performance liquid chromatography. The carriers were loaded with N-acetyl cysteine as a model antioxidant drug. We demonstrated responsiveness of the nanocarrier and its efficiency in drug delivery in an oxidative stress model of human embryonic stem cell-derived retinal pigment epithelial (hESC-RPE) cells. The modeled cells treated with diselenide containing liposomes loaded with 10mM NAC, showed a better therapeutic effect with a cell metabolic activity of 90%, which was significantly higher compared to insensitive liposomes or NAC treated groups ($P<0.05$). In addition, the expression of oxidative-sensitive gene markers in diselenide containing liposomes groups were improved. Our results demonstrated fabricated smart liposomes opens new opportunity for targeted treatment of retinal degeneration.

Bel, A., et al. (2010). "Composite cell sheets: a further step toward safe and effective myocardial regeneration by cardiac progenitors derived from embryonic stem cells." Circulation **122**(11 Suppl): S118-123.

BACKGROUND: The safety and efficacy of myocardial regeneration using embryonic stem cells are limited by the risk of teratoma and the high rate of cell death. METHODS AND RESULTS: To address these issues, we developed a composite construct made of a sheet of adipose tissue-derived stroma cells and embryonic stem cell-derived cardiac progenitors. Ten Rhesus monkeys underwent a transient coronary artery occlusion followed, 2 weeks later, by the open-chest delivery of the composite cell sheet over the infarcted area or a sham operation. The sheet was made of adipose tissue-derived stroma cells grown from a biopsy of autologous adipose tissue and cultured onto temperature-responsive dishes. Allogeneic Rhesus embryonic stem cells were committed to a cardiac lineage and immunomagnetically sorted to yield SSEA-1(+) cardiac progenitors, which were then deposited onto the cell sheet. Cyclosporine was given for 2 months until the animals were euthanized. Preimplantation studies showed that the SSEA-1(+) progenitors expressed cardiac markers and had lost pluripotency. After 2 months, there was no teratoma in any of the 5 cell-treated monkeys. Analysis of >1500 histological sections showed that the SSEA-1(+) cardiac progenitors had differentiated into cardiomyocytes, as evidenced by immunofluorescence and real-time polymerase chain reaction. There were also a robust engraftment of autologous adipose tissue-derived stroma cells and increased angiogenesis compared with the sham animals. CONCLUSIONS: These data collected in a clinically relevant nonhuman primate model show that developmentally restricted SSEA-1(+) cardiac progenitors appear to be safe and highlight the benefit of the epicardial delivery of a construct harboring cells with a cardiomyogenic differentiation potential and cells providing them the necessary trophic support.

De Repentigny, Y. and R. Kothary (2010). "Production of mouse chimeras by injection of embryonic stem cells into the perivitelline space of one-cell stage embryos." Transgenic Res **19**(6): 1137-1144.

Generation of mouse chimeras is useful for the elucidation of gene function. In the present report, we describe a new technique for the production of chimeras by injection of R1 embryonic stem (ES) cells into the perivitelline space of one-cell stage mouse embryos. One-cell embryos are injected with 2-6 ES cells into the perivitelline space under the zona pellucida without laser-assistance. Our embryo culture experiments reveal that ES cells injected at the one-cell stage embryo start to be incorporated into the blastomeres beginning at the 8-cell stage and form a chimeric blastocyst after 4 days. We have used this approach to successfully produce a high rate of mouse chimeras in two different mouse genetic backgrounds permitting the establishment of germ line transmitters. This method allows for the earlier introduction of ES cells into mouse embryos, and should free up the possibility of using frozen one-cell embryos for this purpose.

De Silva, M. G., et al. (2006). "Gene expression changes during step-wise differentiation of embryonic stem cells along the inner ear hair cell pathway." Acta Otolaryngol **126**(11): 1148-1157.

CONCLUSION: Our study outlines an alternative approach for the selection and investigation of genes involved in inner ear function. OBJECTIVE: To gain understanding of the gene pathways involved in the development of the normal cochlea. MATERIALS AND METHODS: Microarray technology currently offers the most efficient approach to investigate gene expression and identify pathways involved in cell differentiation. Epidermal growth factor (EGF) induces cultures derived from the organ of Corti to proliferate and produce new hair cells. Since pluripotent embryonic stem (ES) cells have the capacity to generate all tissues, we induced murine ES cells to differentiate towards ectodermal and neuroectodermal cell types and from there investigated their commitment towards the hair cell lineage in the presence of EGF. Cells were collected at three points along the differentiation pathway and their expression profiles were determined using the Soares NMIE mouse inner ear cDNA library printed in microarray format. RESULTS: Three genes up-regulated after addition of EGF (serine (or cysteine) proteinase inhibitor, clade H, member 1 (Serpinh1), solute carrier family 2 (facilitated glucose transporter), member 10 (Slc2a10) and secreted acidic cysteine-rich glycoprotein (Sparc)) were selected for further analysis and characterization. Of the three genes, Serpinh1 and Slc2a10 have never been implicated in the hearing process.

De Smedt, A., et al. (2008). "Optimisation of the cell cultivation methods in the embryonic stem cell test results in an increased differentiation potential of the cells into strong beating myocard cells." Toxicol In Vitro **22**(7): 1789-1796.

In order to support drug research in the selection process for non-embryotoxic pharmaceutical compounds, a screening method for embryotoxicity is needed. The murine embryonic stem cell test (EST) is

a validated in vitro test based on two permanent mouse cell lines and delivering results in 10-days. Implementation of this test within our laboratory, revealed variability in the differentiation potential of the embryonic stem cells and, as a consequence, a lot of assays needed to be rejected due the fact the acceptance criteria were not reached. In order to gain a better yield of contracting myocardial cells, we used (1) a stringent control of the cell growth during subcultivation and a standardised hanging drop culture method and (2) a non-enzymatic cell harvest instead of a trypsin/EDTA cell harvest. Implementing of these cell culture modifications resulted in a decreased variability in the size of embryonic bodies, an increase of the number of acceptable tests and a significant increase of the differentiation potential of embryonic cells into strong beating myocardium, which made scoring less time consuming. Testing of 6 reference compounds in the optimized EST showed that the cell culture modifications did not changed the in vitro classification.

de Waard, H., et al. (2008). "Cell-type-specific consequences of nucleotide excision repair deficiencies: Embryonic stem cells versus fibroblasts." DNA Repair (Amst) **7**(10): 1659-1669.

Pluripotent embryonic stem cells (ES cells) are the precursors of all different cell types comprising the organism. Since persistent DNA damage in this cell type might lead to mutations that cause huge malformations in the developing organism, genome caretaking is of prime importance. We first compared the sensitivity of wild type mouse embryonic fibroblasts (MEFs) and ES cells for various genotoxic agents and show that ES cells are more sensitive to treatment with UV-light, gamma-rays and mitomycin C than MEFs. We next investigated the contribution of the transcription-coupled (TC-NER) and global genome (GG-NER) sub-pathways of nucleotide excision repair (NER) in protection of ES cells, using cells from mouse models for the NER disorders xeroderma pigmentosum (XP) and Cockayne syndrome (CS). TC-NER-deficient Csb (-/-) and GG-NER/TC-NER-defective Xpa (-/-) MEFs are hypersensitive to UV, whereas GG-NER-deficient Xpc (-/-) MEFs attribute intermediate UV sensitivity. The observed UV-hypersensitivity in Csb (-/-) and Xpa (-/-) MEFs correlates with increased apoptosis. In contrast, Xpa (-/-) and Xpc (-/-) ES cells are highly UV-sensitive, while a Csb deficiency only causes a mild increase in UV-sensitivity. Surprisingly, a UV-induced hyperapoptotic response is mainly observed in Xpa (-/-) ES cells, suggesting a different mechanism of apoptosis induction in ES cells, mainly triggered by damage in the global genome rather than in transcribed genes (as in MEFs). Moreover, we show a pronounced S-phase delay in Xpa (-/-) and Xpc (-/-) ES cells, which might well function as a safeguard mechanism for heavily damaged ES cells in case the apoptotic response fails. Although Xpa (-/-) and Xpc (-/-) ES cells are totally NER-defective or GG-NER-deficient respectively, mutation induction upon UV is similar compared to wild type ES cells indicating that the observed apoptotic and cell cycle responses are indeed sufficient to protect against proliferation of damaged cells. In conclusion, we show a double safeguard mechanism in ES cells against NER-type of damages, which mainly relies on damage detection in the global genome.

Debeb, B. G., et al. (2010). "Characterizing cancer cells with cancer stem cell-like features in 293T human embryonic kidney cells." Mol Cancer **9**: 180.

BACKGROUND: Since the first suggestion of prospectively identifiable cancer stem cells in solid tumors, efforts have been made to characterize reported cancer stem cell surrogates in existing cancer cell lines, and cell lines rich with these surrogates have been used to screen for cancer stem cell targeted agents. Although 293T cells were derived from human embryonic kidney, transplantation of these cells into the mammary fat pad yields aggressive tumors that self-renew as evidenced by serial xenograft passages through transplantation. Herein we fully characterize cancer stem cell-like features in 293T human embryonic kidney cells. RESULTS: 293T cells can be readily cultured and passaged as spheres in serum-free stem cell promoting culture conditions. Cells cultured in vitro as three-dimensional spheres (3D) were shown to contain higher ALDH1 and CD44+/CD24- population compared to monolayer cells. These cells were also resistant to radiation and upregulate stem cell survival signaling including beta-catenin, Notch1 and Survivin in response to radiation. Moreover, 3D spheres generated from the 293T cells have increased expression of mesenchymal genes including vimentin, n-cadherin, zeb1, snail and slug as well as pro-metastatic genes RhoC, Tenascin C and MTA1. In addition, microRNAs implicated in self-renewal and metastases were markedly reduced in 3D spheres. CONCLUSIONS: 293T cells exhibit a cancer stem cell-like phenotype when cultured as 3D spheres and represent an important research tool for studying the molecular and biological mechanisms of cancer stem cells and for testing and developing novel targets for cancer therapy.

Delacroix, L., et al. (2010). "Cell-specific interaction of retinoic acid receptors with target genes in mouse embryonic fibroblasts and embryonic stem cells." Mol Cell Biol **30**(1): 231-244.

All-trans retinoic acid (RA) induces transforming growth factor beta (TGF-beta)-dependent autocrine growth of mouse embryonic fibroblasts (MEFs). We have used chromatin immunoprecipitation to map 354 RA receptor (RAR) binding loci in MEFs, most of which were similarly occupied by the RAR alpha and RAR gamma receptors. Only a subset of the genes associated with these loci are regulated by RA, among which are several critical components of the TGF-beta pathway. We also show RAR binding to a novel series of target genes involved in cell cycle regulation, transformation, and metastasis, suggesting new pathways by which RA may regulate proliferation and cancer. Few of the RAR binding loci contained consensus direct-repeat (DR)-type elements. The majority comprised either degenerate DRs or no identifiable DRs but anomalously spaced half sites. Furthermore, we identify 462 RAR target loci in embryonic stem (ES) cells and show that their occupancy is cell type specific. Our results also show that differences in the chromatin landscape regulate the accessibility of a subset of more than 700 identified loci to RARs, thus modulating the repertoire of target genes that can be regulated and the biological effects of RA.

Deleu, S., et al. (2009). "Human cystic fibrosis embryonic stem cell lines derived on placental mesenchymal stromal cells." Reprod Biomed Online **18**(5): 704-716.

This study describes the production of two new human embryonic stem cell (hESC) lines affected by cystic fibrosis. These cell lines are heterozygous compounds, each a carrier of the DF508 mutations associated either with E585X or with 3849+10 kb C-->T. The derivation process was performed on irradiated human placental mesenchymal stromal cells and designed to minimize contact with xeno-components. This new source of feeder cells is easy to obtain and devoid of ethical concerns. The cells have a great capacity to proliferate which reduces the need for continuous preparation of new feeder cell lines. In addition, three normal hESC lines were obtained in the same conditions. The five stem cell lines retained hESC-specific features, including an unlimited and undifferentiated proliferation capacity, marker expression and the maintenance of stable karyotype. They also demonstrated pluripotency in vitro, forming cell lineages of the three germ layers, as indicated by immunolocalization of beta-tubulin, alpha-fetoprotein and actin. These new genetic cell lines represent an important in-vitro tool to study the physiological processes underlying this genetic disease, drug screening, and tissue engineering.

Denham, M., et al. (2012). "Glycogen synthase kinase 3beta and activin/nodal inhibition in human embryonic stem cells induces a pre-neuroepithelial state that is required for specification to a floor plate cell lineage." Stem Cells **30**(11): 2400-2411.

The floor plate is one of the major organizers of the developing nervous system through its secretion of sonic hedgehog (Shh). Although the floor plate is located within the neural tube, the derivation of the floor plate during development is still debatable and some studies suggest that floor plate cells are specified by Shh in a temporarily restricted window different to neuroepithelial cells. Using human embryonic stem cells (hESC) as a model of neurogenesis, we sought to determine how floor plate cells may be temporarily specified by SHH signaling during human embryogenesis. We found that inhibition of both GSK3beta and activin/nodal pathways in hESC induces a cellular state of SOX2+/PAX6- expression, we describe as "pre-neuroepithelial." Exposure of SHH during this pre-neuroepithelial period causes the expression of GLI transcription factors to function as activators and consequently upregulate expression of the floor plate marker, FOXA2, while also supressing PAX6 expression to inhibit neuroepithelial fate. FOXA2+ cells were able to efficiently generate mesencephalic dopaminergic neurons, a floor plate derivative. Overall, this study demonstrates a highly efficient system for generating floor plate cells from hESC and, most importantly, reveals that specification of floor plate cells is temporally dependent, whereby it occurs prior to the onset of PAX6 expression, within a pre-neuroepithelial stage.

Denker, H. W. (2006). "Potentiality of embryonic stem cells: an ethical problem even with alternative stem cell sources." J Med Ethics **32**(11): 665-671.

The recent discussions about alternative sources of human embryonic stem cells (White Paper of the US President's Council on Bioethics, 2005), while stirring new interest in the developmental potential of the various abnormal embryos or constructs proposed as such sources, also raise questions about the potential of the derived embryonic stem cells. The data on the developmental potential of embryonic stem cells that seem relevant for ethical considerations and aspects of patentability are discussed. Particular attention is paid to the meaning of "totipotency, omnipotency and pluripotency" as illustrated by a comparison of the developmental potential of three-dimensional clusters of blastomeres (morula), embryonic stem cells, somatic or (adult) stem cells or other somatic (non-stem) cells. This paper focuses on embryoid bodies and on direct cloning by tetraploid complementation. Usage and patenting of these cells cannot be considered to be ethically sound as long as

totipotency and tetraploid complementability of embryonic stem cells are not excluded for the specific cell line in question. Testing this poses an ethical problem in itself and needs to be discussed in the future.

Desai, N., et al. (2013). "Development of a xeno-free non-contact co-culture system for derivation and maintenance of embryonic stem cells using a novel human endometrial cell line." J Assist Reprod Genet **30**(5): 609-615.

PURPOSE: Mouse embryonic fibroblast feeder layers (MEF) have conventionally been used to culture and maintain the pluripotency of embryonic stem cells (ESC). This study explores the potential of using a novel human endometrial cell line to develop a non-xeno, non-contact co-culture system for ESC propagation and derivation. Such xeno-free systems may prove essential for the establishment of clinical grade human ESC lines suitable for therapeutic application. METHODS: A novel line of human endometrial cells were seeded in a 6-well dish. Filter inserts containing mouse ESCs were placed on these wells and passaged 2-3 times per week. Inner cell masses derived from mouse blastocysts were also cultured on transwells in the presence of the feeder layer. In both cases, staining for SSEA-1, SOX-2, OCT-4 and alkaline phosphatase were used to monitor the retention of stem cells. RESULTS: ESC colonies retained their stem cell morphology and attributes for over 120 days in culture and 44 passages to date. Inner cell mass derived ESC cultures were maintained in a pluripotent state for 45 days, through 6 passages with retention of all stem cell characteristics. The stem cell colonies expressed stem cell specific markers SSEA-1, Sox 2, Oct-4 and alkaline phosphatase. Upon removal of the human feeder layer, there was a distinct change in cell morphology within the colonies and evidence of ESC differentiation. CONCLUSIONS: Human feeder layers offer a simple path away from the use of MEF feeder cells or MEF conditioned medium for ESC culture. Furthermore, indirect co-culture using porous membranes to separate the two cell types can prevent contamination of stem cell preparations with feeder cells during passaging.

Desai, N., et al. (2011). "Vitrification of mouse embryo-derived ICM cells: a tool for preserving embryonic stem cell potential?" J Assist Reprod Genet **28**(2): 93-99.

PURPOSE: Vitrification technology presents new opportunities for preservation of embryo derived stem cells without first establishing a viable ESC line. This study tests the feasibility of cryopreserving ICM cells using vitrification. MATERIALS AND METHODS: ICMs from mouse embryos were isolated and vitrified in HSV straws or on cryoloops. Upon warming, the vitrified ICMs were cultured and observed for attachment and morphology. Colonies were passaged every 3-6 days. ICMs and ICM-derived ESC colonies were tested for expression of stem cell specific markers. RESULTS: ICMs vitrified on both the cryoloop and the HSV straw had high survival rates. ICM derived ESCs remained undifferentiated for several passages and demonstrated expression of typical stem cell markers; SSEA-1, Sox-2, Oct 4 and alkaline phosphatase. CONCLUSION: This is the first report on successful vitrification of isolated ICMs and the subsequent derivation of ESC colonies. Vitrification of isolated ICMs is a novel approach for preservation of the "stem cell source" material.

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.

Di Giorgio, F. P., et al. (2008). "Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation." Cell Stem Cell **3**(6): 637-648.

It has been proposed that human embryonic stem cells could be used to provide an inexhaustible supply of differentiated cell types for the study of disease processes. Although methods for differentiating embryonic stem cells into specific cell types have become increasingly sophisticated, the utility of the resulting cells for modeling disease has not been determined. We have asked whether specific neuronal subtypes produced from human embryonic stem cells can be used to investigate the mechanisms leading to

neural degeneration in amyotrophic lateral sclerosis (ALS). We show that human spinal motor neurons, but not interneurons, are selectively sensitive to the toxic effect of glial cells carrying an ALS-causing mutation in the SOD1 gene. Our findings demonstrate the relevance of these non-cell-autonomous effects to human motor neurons and more broadly demonstrate the utility of human embryonic stem cells for studying disease and identifying potential therapeutics.

Dihazi, H., et al. (2011). "Multipotent adult germline stem cells and embryonic stem cells functional proteomics revealed an important role of eukaryotic initiation factor 5A (Eif5a) in stem cell differentiation." J Proteome Res **10**(4): 1962-1973.

Multipotent adult germline stem cells (maGSCs) are pluripotent cells that can be differentiated into somatic cells of the three primary germ layers. To highlight the protein profile changes associated with stem cell differentiation, retinoic acid (RA) treated mouse stem cells (maGSCs and ESCs) were compared to nontreated stem cells. 2-DE and DIGE reference maps were created, and differentially expressed proteins were further processed for identification. In both stem cell types, the RA induced differentiation resulted in an alteration of 36 proteins of which 18 were down-regulated and might be potential pluripotency associated proteins, whereas the other 18 proteins were up-regulated. These might be correlated to stem cell differentiation. Surprisingly, eukaryotic initiation factor 5A (Eif5a), a protein which is essential for cell proliferation and differentiation, was significantly down-regulated under RA treatment. A time-dependent investigation of Eif5a showed that the RA treatment of stem cells resulted in a significant up-regulation of the Eif5a in the first 48 h followed by a progressive down-regulation thereafter. This effect could be blocked by the hypusination inhibitor ciclopirox olamine (CPX). The alteration of Eif5a hypusination, as confirmed by mass spectrometry, exerts an antiproliferative effect on ESCs and maGSCs in vitro, but does not affect the cell pluripotency. Our data highlights the important role of Eif5a and its hypusination for stem cell differentiation and proliferation.

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.

Dutta, R., et al. (2011). "A comparative study on efficiency of adult fibroblast, putative embryonic stem cell and lymphocyte as donor cells for production of handmade cloned embryos in goat and characterization of putative ntES cells obtained from these embryos." Theriogenology **76**(5): 851-863.

The main purpose of the experiment was to compare the efficiency of three cell types, namely adult fibroblast, putative embryonic stem (ES) cell, and lymphocyte, as donor cells for somatic cell nuclear transfer by handmade cloning in goats. The outcome clearly shows that putative embryonic stem cells, with a cleavage and blastocyst production rate of 74.69% +/- 3.92 and 39.75% +/- 3.86, respectively, performs better in comparison to adult fibroblast cell and lymphocyte. Between adult fibroblast cell and lymphocyte no statistically significant difference exists at $P < 0.05$. An overall cleavage and blastocyst formation rate of 67.41% +/- 3.92 and 26.96% +/- 3.86 was obtained using adult fibroblast donor cells. The study establishes beyond doubt the reprogrammability of lymphocyte by handmade cloning (HMC) protocol with a cleavage and blastocyst production rate of 56.47% +/- 3.92 and 24.70% +/- 3.86, respectively. PCR analysis of highly polymorphic 286 bp fragment of MHC II DRB genes of cloned embryos and three donor cells were performed to verify the cloned embryos. The amplified PCR products were subjected to SSCP to confirm their genetic identity. The karyotyping of the cloned embryos showed normal chromosomal status as expected in goat. Significantly, in the second stage of the experiment, the produced

cloned embryos were successfully used to derive ntES-like cells. The rate of primary colony formation rate was 62.50% +/- 4.62 for fibroblast donor cell derived embryos. The same was 60.60% +/- 4.62 for putative ES donor cell derived embryos and 66.66% +/- 4.62 for lymphocyte donor cell derived embryos, respectively. The putative ntES colonies were positively characterized for alkaline phosphatase, Oct-4, TRA-1-60, TRA-1-81, Sox-2, and Nanog by Immunocytochemistry and Reverse Transcription PCR. To further validate the stem ness, the produced putative ntES colonies were differentiated to embryoid bodies. Immunocytochemistry revealed that embryoid bodies expressed NESTIN specific for ectodermal lineage; GATA-4 for endodermal lineage and smooth muscle actin-I, and troponin-I specific for mesodermal lineage. The study has established an efficient protocol for putative ntES cell derivation from HMC embryos. It could be of substantial significance as patient specific ntES cells have proven therapeutic significance.

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.

Eckardt, S., et al. (2008). "In vivo and in vitro differentiation of uniparental embryonic stem cells into hematopoietic and neural cell types." Organogenesis **4**(1): 33-41.

The biological role of genomic imprinting in adult tissue is central to the consideration of transplanting uniparental embryonic stem (ES) cell-derived tissues. We have recently shown that both maternal (parthenogenetic/gynogenetic) and paternal (androgenetic) uniparental ES cells can differentiate, both in vivo in chimeras and in vitro, into adult-repopulating hematopoietic stem and progenitor cells. This suggests that, at least in some tissues, the presence of two maternal or two paternal genomes does not interfere with stem cell function and tissue homeostasis in the adult. Here, we consider implications of the contribution of uniparental cells to hematopoiesis and to development of other organ systems, notably neural tissue for which consequences of genomic imprinting are associated with a known bias in development and behavioral disorders. Our findings so far indicate that there is little or no limit to the differentiation potential of uniparental ES cells outside the normal developmental paradigm. As a potentially donor MHC-matching source of tissue, uniparental transplants may provide not only a clinical resource but also a unique tool to investigate aspects of genomic imprinting in adults.

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.

Jahandideh, S., et al. (2018). "Anti-inflammatory effects of human embryonic stem cell-derived mesenchymal stem cells secretome preconditioned with diazoxide, trimetazidine and MG-132 on LPS-induced systemic inflammation mouse model." Artif Cells Nanomed Biotechnol: 1-10.

Systemic inflammatory response syndrome is a complex pathophysiologic and immunologic response to an insult. Sepsis is a life-threatening condition happening when the body's response to infection causes injury to its own tissues and organs. Stem cell therapy is a new approach to modulate immune responses. Mesenchymal stem cells (MSCs) establish a regenerative niche by secreting secretome and modulating immune responses. MSC secretome can be leveraged for therapeutic applications if production of secretary molecules were optimized. Pharmacological preconditioning using small molecules can increase survival of MSCs after transplantation. The aim of this study was to investigate the effect of secretome of human embryonic-derived mesenchymal stem cells (hESC-MSCs) preconditioned with MG-132,Trimetazidine (TMZ) and Diazoxide (DZ) on immunomodulatory efficiency of these cells in Lipo polysaccharide (LPS) challenged mice models. Mice were injected intraperitoneally with LPS and groups of animals were intraperitoneally given 1 ml 30x secretome 6 h after LPS injection. Serum levels of biochemical parameters were then measured by an auto analyser and serum inflammatory cytokine levels were analysed using commercially available RayBio Mouse Inflammation Antibody Array. Ultimately, histopathology and survival studies were conducted. The results showed that TMZ and DZ-conditioned medium significantly increasing the survival and improvement of histopathological score. We found that MG-132-conditioned medium failed to show significant outcomes. This study demonstrated that human MSC secretome has the potential to control inflammation.

Jain, A. K., et al. (2012). "p53 regulates cell cycle and microRNAs to promote differentiation of human embryonic stem cells." PLoS Biol **10**(2): e1001268.

Multiple studies show that tumor suppressor p53 is a barrier to dedifferentiation; whether this is strictly due to repression of proliferation remains a subject of debate. Here, we show that p53 plays an active role in promoting differentiation of human embryonic stem cells (hESCs) and opposing self-renewal by regulation of specific target genes and microRNAs. In contrast to mouse embryonic stem cells, p53 in hESCs is maintained at low levels in the nucleus, albeit in a deacetylated, inactive state. In response to retinoic acid, CBP/p300 acetylates p53 at lysine 373, which leads to dissociation from E3-ubiquitin ligases HDM2 and TRIM24. Stabilized p53 binds CDKN1A to establish a G (1) phase of cell cycle without activation of cell death pathways. In parallel, p53 activates expression of miR-34a and miR-145, which in turn repress stem cell factors OCT4, KLF4, LIN28A, and SOX2 and prevent backsliding to pluripotency. Induction of p53 levels is a key step: RNA-interference-mediated knockdown of p53 delays differentiation, whereas depletion of negative regulators of p53 or ectopic expression of p53 yields spontaneous differentiation of hESCs, independently of retinoic acid. Ectopic expression of p53R175H, a mutated form of p53 that does not bind DNA or regulate transcription, failed to induce differentiation. These studies underscore the importance of a p53-regulated network in determining the human stem cell state.

Jakobs, P. M., et al. (1999). "Embryonic stem cells can be used to construct hybrid cell lines containing a single, selectable murine chromosome." Mamm Genome **10**(4): 381-384.

Microcell-mediated chromosome transfer is a useful technique for the study of gene function, gene regulation, gene mapping, and functional cloning in mammalian cells. Complete panels of donor cell lines, each containing a different human chromosome, have been developed. These donor cell lines contain a single human chromosome marked with a dominant selectable gene in a rodent cell background. However, a similar panel does not exist for murine chromosomes. To produce mouse monochromosomal donor hybrids, we have utilized embryonic stem (ES) cells with targeted gene disruptions of known chromosomal location as starting material. ES cells with mutations in aprt, fyn, and myc were utilized to generate monochromosomal hybrids with neomycin phosphotransferase-marked murine Chr 8, 10, or 15 respectively in a hamster or rat background. This same methodology can be used to generate a complete panel of marked mouse chromosomes for somatic cell genetic experimentaion.

Jamaladdin, S., et al. (2014). "Histone deacetylase (HDAC) 1 and 2 are essential for accurate cell division and the pluripotency of embryonic stem cells." Proc Natl Acad Sci U S A **111**(27): 9840-9845.

Histone deacetylases 1 and 2 (HDAC1/2) form the core catalytic components of corepressor complexes that modulate gene expression. In most cell types, deletion of both Hdac1 and Hdac2 is required to generate a discernible phenotype, suggesting their activity is largely redundant. We have therefore

generated an ES cell line in which Hdac1 and Hdac2 can be inactivated simultaneously. Loss of HDAC1/2 resulted in a 60% reduction in total HDAC activity and a loss of cell viability. Cell death is dependent upon cell cycle progression, because differentiated, nonproliferating cells retain their viability. Furthermore, we observe increased mitotic defects, chromatin bridges, and micronuclei, suggesting HDAC1/2 are necessary for accurate chromosome segregation. Consistent with a critical role in the regulation of gene expression, microarray analysis of Hdac1/2-deleted cells reveals 1,708 differentially expressed genes. Significantly for the maintenance of stem cell self-renewal, we detected a reduction in the expression of the pluripotent transcription factors, Oct4, Nanog, Esrrb, and Rex1. HDAC1/2 activity is regulated through binding of an inositol tetraphosphate molecule (IP4) sandwiched between the HDAC and its cognate corepressor. This raises the important question of whether IP4 regulates the activity of the complex in cells. By rescuing the viability of double-knockout cells, we demonstrate for the first time (to our knowledge) that mutations that abolish IP4 binding reduce the activity of HDAC1/2 in vivo. Our data indicate that HDAC1/2 have essential and pleiotropic roles in cellular proliferation and regulate stem cell self-renewal by maintaining expression of key pluripotent transcription factors.

James, D., et al. (2010). "Expansion and maintenance of human embryonic stem cell-derived endothelial cells by TGFbeta inhibition is Id1 dependent." Nat Biotechnol **28**(2): 161-166.

Previous efforts to differentiate human embryonic stem cells (hESCs) into endothelial cells have not achieved sustained expansion and stability of vascular cells. To define vasculogenic developmental pathways and enhance differentiation, we used an endothelial cell-specific VE-cadherin promoter driving green fluorescent protein (GFP) (hVPr-GFP) to screen for factors that promote vascular commitment. In phase 1 of our method, inhibition of transforming growth factor (TGF)beta at day 7 of differentiation increases hVPr-GFP (+) cells by tenfold. In phase 2, TGFbeta inhibition maintains the proliferation and vascular identity of purified endothelial cells, resulting in a net 36-fold expansion of endothelial cells in homogenous monolayers, which exhibited a transcriptional profile of Id1(high)VEGFR2(high)VE-cadherin (+) ephrinB2(+). Using an Id1-YFP hESC reporter line, we showed that TGFbeta inhibition sustains Id1 expression in hESC-derived endothelial cells and that Id1 is required for increased proliferation and preservation of endothelial cell commitment. Our approach provides a serum-free method for differentiation and long-term maintenance of hESC-derived endothelial cells at a scale relevant to clinical application.

Jaramillo, M. and I. Banerjee (2012). "Endothelial cell co-culture mediates maturation of human embryonic stem cell to pancreatic insulin producing cells in a directed differentiation approach." J Vis Exp (61).

Embryonic stem cells (ESC) have two main characteristics: they can be indefinitely propagated in vitro in an undifferentiated state and they are pluripotent, thus having the potential to differentiate into multiple lineages. Such properties make ESCs extremely attractive for cell based therapy and regenerative treatment applications. However for its full potential to be realized the cells have to be differentiated into mature and functional phenotypes, which is a daunting task. A promising approach in inducing cellular differentiation is to closely mimic the path of organogenesis in the in vitro setting. Pancreatic development is known to occur in specific stages, starting with endoderm, which can develop into several organs, including liver and pancreas. Endoderm induction can be achieved by modulation of the nodal pathway through addition of Activin A in combination with several growth factors. Definitive endoderm cells then undergo pancreatic commitment by inhibition of sonic hedgehog inhibition, which can be achieved in vitro by addition of cyclopamine. Pancreatic maturation is mediated by several parallel events including inhibition of notch signaling; aggregation of pancreatic progenitors into 3-dimentional clusters; induction of vascularization; to name a few. By far the most successful in vitro maturation of ESC derived pancreatic progenitor cells have been achieved through inhibition of notch signaling by DAPT supplementation. Although successful, this results in low yield of the mature phenotype with reduced functionality. A less studied area is the effect of endothelial cell signaling in pancreatic maturation, which is increasingly being appreciated as an important contributing factor in in-vivo pancreatic islet maturation. The current study explores such effect of endothelial cell signaling in maturation of human ESC derived pancreatic progenitor cells into insulin producing islet-like cells. We report a multi-stage directed differentiation protocol where the human ESCs are first induced towards endoderm by Activin A along with inhibition of PI3K pathway. Pancreatic specification of endoderm cells is achieved by inhibition of sonic hedgehog signaling by Cyclopamine along with retinoid induction by addition of Retinoic Acid. The final stage of maturation is induced by endothelial cell signaling achieved by a co-culture configuration. While several endothelial cells have been tested in the

co-culture, herein we present our data with rat heart microvascular endothelial Cells (RHMVEC), primarily for the ease of analysis.

Jaramillo, M., et al. (2015). "Endothelial cells mediate islet-specific maturation of human embryonic stem cell-derived pancreatic progenitor cells." Tissue Eng Part A **21**(1-2): 14-25.

It is well recognized that in vitro differentiation of embryonic stem cells (ESC) can be best achieved by closely recapitulating the in vivo developmental niche. Thus, implementation of directed differentiation strategies has yielded encouraging results in the area of pancreatic islet differentiation. These strategies have concentrated on direct addition of chemical signals, however, other aspect of the developmental niche are yet to be explored. During development, pancreatic progenitor (PP) cells grow as an epithelial sheet, which aggregates with endothelial cells (ECs) during the final stages of maturation. Several findings suggest that the interactions with EC play a role in pancreatic development. In this study, we recapitulated this phenomenon in an in vitro environment by maturing the human ESC (hESC)-derived PP cells in close contact with ECs. We find that co-culture with different ECs (but not fibroblast) alone results in pancreatic islet-specific differentiation of hESC-derived PP cells even in the absence of additional chemical induction. The differentiated cells responded to exogenous glucose levels by enhanced C-peptide synthesis. The co-culture system aligned well with endocrine development as determined by comprehensive analysis of involved signaling pathways. By recapitulating cell-cell interaction aspects of the developmental niche we achieved a differentiation model that aligns closely with islet organogenesis.

Javaherian, A. and A. Kriegstein (2009). "A stem cell niche for intermediate progenitor cells of the embryonic cortex." Cereb Cortex **19 Suppl 1**: i70-77.

The excitatory neurons of the mammalian cerebral cortex arise from asymmetric divisions of radial glial cells in the ventricular zone and symmetric division of intermediate progenitor cells (IPCs) in the subventricular zone (SVZ) of the embryonic cortex. Little is known about the microenvironment in which IPCs divide or whether a stem cell niche exists in the SVZ of the embryonic cortex. Recent evidence suggests that vasculature may provide a niche for adult stem cells but its role in development is less clear. We have investigated the vasculature in the embryonic cortex during neurogenesis and find that IPCs are spatially and temporally associated with blood vessels during cortical development. Intermediate progenitors mimic the pattern of capillaries suggesting patterns of angiogenesis and neurogenesis are coordinated during development. More importantly, we find that IPCs divide near blood vessel branch points suggesting that cerebral vasculature establishes a stem cell niche for intermediate progenitors in the SVZ. These data provide novel evidence for the presence of a neurogenic niche for intermediate progenitors in the embryonic SVZ and suggest blood vessels are important for proper patterning of neurogenesis.

Lam, M. L., et al. (2011). "Embryonic stem cell-derived cardiomyocytes harbor a subpopulation of niche-forming Sca-1+ progenitor cells." Mol Cell Biochem **349**(1-2): 69-76.

The adult mammalian heart is known to contain a population of cardiac progenitor cells. It has not been unambiguously determined, however, whether these cells form as part of the developmental program of the heart or migrate there by way of the circulatory system. This study was done in order to determine the origin of this population of cells. A population of cardiomyocytes was established from mouse embryonic stem (ES) cells using a genetic selection technique. In order to determine whether cardiac progenitor cells exist within this ES cell-derived cardiomyocyte population, the cells were analyzed by fluorescence activated cell sorting (FACS) using an antibody directed against stem cell antigen-1 (Sca-1). We observed that approximately 4% of the cardiomyocyte population was composed of Sca-1(+) cells. When the Sca-1(+) cells were isolated by magnetic cell sorting and differentiated as cellular aggregates, contractions were observed in 100% of the aggregates. Gene expression studies using quantitative RT-PCR showed that these cells expressed terminally differentiated cardiac-specific genes. When three-dimensional cellular aggregates were formed from ES cell-derived cardiomyocytes co-cultured with adult HL-1 cardiomyocytes, the Sca-1(+) cells were found to "sort out" and form niches within the cell aggregates. Our data demonstrate that cardiac progenitor cells in the adult heart originate as part of the developmental program of the heart and that Sca-1(+) progenitor cells can provide an important in vitro model system to study the formation of cellular niches in the heart.

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.

Lange, S., et al. (2009). "Platelet-derived growth factor BB stimulates vasculogenesis of embryonic stem cell-derived endothelial cells by calcium-mediated generation of reactive oxygen species." Cardiovasc Res **81**(1): 159-168.

AIMS: Platelet-derived growth factor BB (PDGF-BB) has been assigned a critical role in vascular growth and recruitment of perivascular mural cells. The purpose of the present study is to investigate the signalling events underlying the stimulation of vasculogenesis of mouse embryonic stem (ES) cells by PDGF-BB. METHODS AND RESULTS: PDGF-BB increased vascular sprouting and branching of capillary-like structures in embryoid bodies as evaluated by computer-assisted analysis of CD31-positive cell structures. It also activated extracellular-regulated kinase 1,2 (ERK1,2) and c-Jun N-terminal kinase but not p38 mitogen-activated protein kinase or PI 3-kinase. Microfluorometric analysis of fluo-4 fluorescence revealed that treatment with PDGF-BB raised intracellular Ca (2+) levels in differentiating ES cells expressing the PDGF receptor beta, an effect that was abolished in the presence of the intracellular Ca (2+) chelator BAPTA. Furthermore, PDGF-BB raised reactive oxygen species (ROS) levels in embryoid bodies as evaluated using the redox-sensitive dye H (2)DCF-DA. ROS generation was blunted in the presence of the NADPH oxidase inhibitors diphenylen iodonium (DPI) and apocynin as well as in the presence of BAPTA, suggesting that ROS generation is regulated by intracellular Ca (2+) transients. The stimulation of vasculogenesis of ES cells upon treatment with PDGF-BB was significantly inhibited by the ERK1,2 inhibitor U0126, the NADPH oxidase inhibitors DPI, apocynin, 4-(2-aminoethyl)benzenesulfonylfluoride and VAS2870, the free radical scavengers vitamin E, and N-(2-mercaptopropionyl)glycin as well as by BAPTA. CONCLUSION: Our data demonstrate that the pro-vasculogenic effects of PDGF-BB are mediated by Ca (2+)-induced ROS generation, resulting in the activation of an ERK1,2-mediated signal transduction cascade.

Larbi, A., et al. (2012). "The HOXB4 homeoprotein promotes the ex vivo enrichment of functional human embryonic stem cell-derived NK cells." PLoS One **7**(6): e39514.

Human embryonic stem cells (hESCs) can be induced to differentiate into blood cells using either co-culture with stromal cells or following human embryoid bodies (hEBs) formation. It is now well established that the HOXB4 homeoprotein promotes the expansion of human adult hematopoietic stem cells (HSCs) but also myeloid and lymphoid progenitors. However, the role of HOXB4 in the development of hematopoietic cells from hESCs and particularly in the generation of hESC-derived NK-progenitor cells remains elusive. Based on the ability of HOXB4 to passively enter hematopoietic cells in a system that comprises a co-culture with the MS-5/SP-HOXB4 stromal cells, we provide evidence that HOXB4 delivery promotes the enrichment of hEB-derived precursors that could differentiate into fully mature and functional NK. These hEB-derived NK cells enriched by HOXB4 were characterized according to their CMH class I receptor expression, their cytotoxic arsenal, their expression of IFNgamma and CD107a after stimulation and their lytic activity. Furthermore our study provides new insights into the gene expression profile of hEB-derived cells exposed to HOXB4 and shows the emergence of CD34(+)CD45RA (+) precursors from hEBs indicating the lymphoid specification of hESC-derived hematopoietic precursors. Altogether, our results outline the effects of HOXB4 in combination with stromal cells in the development of NK cells from hESCs and suggest the potential use of HOXB4 protein for NK-cell enrichment from pluripotent stem cells.

Maeda, I., et al. (2013). "Max is a repressor of germ cell-related gene expression in mouse embryonic stem cells." Nat Commun **4**: 1754.

Embryonic stem cells and primordial germ cells (PGCs) express many pluripotency-associated genes, but embryonic stem cells do not normally undergo conversion into primordial germ cells. Thus, we predicted that there is a mechanism that represses primordial germ cell-related gene expression in embryonic stem cells. Here we identify genes involved in this putative mechanism, by using an embryonic stem cell line with a Vasa reporter in an RNA interference screen of transcription factor genes expressed in embryonic stem cells. We identify five genes that result in the expression of Vasa when silenced. Of these, Max is the most striking. Transcriptome analysis reveals that Max knockdown in embryonic stem cells results in selective, global derepression of germ cell-specific genes. Max interacts with histone H3K9 methyltransferases and associates with the germ cell-specific genes in embryonic stem cells. In addition, Max knockdown results in a decrease in histone H3K9 dimethylation at their promoter regions. We propose that Max is part of protein complex that acts as a repressor of germ cell-related genes in embryonic stem cells.

Mahfuz Chowdhury, M., et al. (2012). "Induction of alternative fate other than default neuronal fate of embryonic stem cells in a membrane-based two-chambered microbioreactor by cell-secreted BMP4." Biomicrofluidics **6**(1): 14117-1411713.

Cell-secreted soluble factor signaling in a diffusion dominant microenvironment plays an important role on early stage differentiation of pluripotent stem cells invivo. In this study, we utilized a membrane-based two-chambered microbioreactor (MB) to differentiate mouse embryonic stem cells (mESCs) in a diffusion dominant microenvironment of the top chamber while providing enough nutrient through the bottom chamber. Speculating that accumulated FGF4 in the small top chamber will augment neuronal differentiation in the MB culture, we first differentiated mESCs for 8 days by using a chemically optimized culture medium for neuronal induction. However, comparison of cellular morphology and expression of neuronal markers in the MB with that in the 6-well plate (6WP) indicated relatively lower neuronal differentiation in the MB culture. Therefore, to investigate whether microenvironment in the MB facilitates non-neuronal differentiation, we differentiated mESCs for 8 days by using chemically defined basal medium. In this case, differentiated cell morphology differed markedly between the MB and 6WP cultures: epithelial sheet-like morphology in the MB, whereas rosette morphology in the 6WP. Expression of markers from the three germ layers indicated lower neuronal but higher meso- and endo-dermal differentiation of mESCs in the MB than the 6WP culture. Moreover, among various cell-secreted soluble factors, BMP4 expression was remarkably upregulated in the MB culture. Inhibition of BMP4 signaling demonstrated that enhanced effect of upregulated BMP4 was responsible for the prominent meso- and endo-dermal differentiation in the MB. However, in the 6WP, downregulated BMP4 had a minimal influence on the differentiation behavior. Our study demonstrated utilization of a microbioreactor to modulate the effect of cell-secreted soluble factors by autoregulation and thereby inducing alternative self-capability of mESCs. Understanding and implementation of autoregulation of soluble factors similar to this study will lead to the development of robust culture systems to control ESC behavior.

Mahmood, A., et al. (2011). "In vitro differentiation and maturation of human embryonic stem cell into multipotent cells." Stem Cells Int **2011**: 735420.

Human embryonic stem cells (hESCs), which have the potential to generate virtually any differentiated progeny, are an attractive cell source for transplantation therapy, regenerative medicine, and tissue engineering. To realize this potential, it is essential to be able to control ESC differentiation and to direct the development of these cells along specific pathways. Basic science in the field of embryonic development, stem cell differentiation, and tissue engineering has offered important insights into key pathways and scaffolds that regulate hESC differentiation, which have produced advances in modeling gastrulation in culture and in the efficient induction of endoderm, mesoderm, ectoderm, and many of their downstream derivatives. These findings have lead to identification of several pathways controlling the differentiation of hESCs into mesodermal derivatives such as myoblasts, mesenchymal cells, osteoblasts, chondrocytes, adipocytes, as well as hemangioblastic derivatives. The next challenge will be to demonstrate the functional utility of these cells, both in vitro and in preclinical models of bone and vascular diseases.

Maj, M., et al. (2015). "The cell cycle- and insulin-signaling-inhibiting miRNA expression pattern of very small embryonic-like stem cells contributes to their quiescent state." Exp Biol Med (Maywood) **240**(8): 1107-1111.

Murine Oct4(+), very small embryonic-like stem cells (VSELs), are a quiescent stem cell population that requires a supportive co-culture layer to

proliferate and/or to differentiate in vitro. Gene expression studies have revealed that the quiescence of these cells is due to changes in expression of parentally imprinted genes, including genes involved in cell cycle regulation and insulin and insulin-like growth factor signaling (IIS). To investigate the role of microRNAs (miRNAs) in VSEL quiescence, we performed miRNA studies in highly purified VSELs and observed a unique miRNA expression pattern in these cells. Specifically, we observed significant differences in the expression of certain miRNA species (relative to a reference cell population), including (i) miRNA-25_1 and miRNA-19 b, whose downregulation has the effect of upregulating cell cycle checkpoint genes and (ii) miRNA-675-3 p and miRNA-675-5 p, miRNA-292-5 p, miRNA-184, and miRNA-125 b, whose upregulation attenuates IIS. These observations are important for understanding the biology of these cells and for developing efficient ex vivo expansion strategies for VSELs isolated from adult tissues.

Makoolati, Z., et al. (2016). "In vitro germ cell differentiation from embryonic stem cells of mice: induction control by BMP4 signalling." Biosci Rep **36**(6).

The present study aims to confirm and analyse germ cell-related patterns and specific gene expressions at a very early stage of cell commitment. Following the XY cytogenetic confirmation of the CCE mouse embryonic stem cells (mESCs) line, cells were cultured to form embryoid bodies (EBs). Expression pattern assessment of the mouse vasa homologue (Mvh), Stra8, alpha6 and beta1 integrin genes in ESC and 1-3-day-old EB showed that all genes except alpha6 integrin were expressed in the ESC. The mean calibration of Mvh, Stra8 and alpha6 integrin expression significantly increased upon EB formation compared with the ESCs. During mouse embryogenesis, the signalling of bone morphogenetic protein (BMP) is essential for germ-line formation. To investigate its role in germ-line induction in vitro, mESCs were cultured as 1-day-old EB aggregates with BMP4 for 4 days in STO co-culture systems, in the presence and absence of 5 ng/ml BMP4. At the end of the culture period, colony assay (number and diameter) was performed and the viability percentage and proliferation rate was determined. There were no significant statistical differences in the abovementioned criteria between these two groups. Moreover, the expression of Mvh, alpha6 and beta1 integrins, Stra8 and Piwil2 genes was evaluated in co-culture groups. The molecular results of co-culture groups showed higher-but insignificant-Piwil2 and significant alpha6 integrin expressions in BMP4 treated co-culture systems. These results confirmed that the EB system and the presence of BMP4 in a STO co-culture system improve the differentiation of ESCs to germ cell.

Makoolati, Z., et al. (2016). "Proliferation in culture of primordial germ cells derived from embryonic stem cell: induction by retinoic acid." Biosci Rep **36**(6).

An in vitro system that supports primordial germ cells (PGCs) survival and proliferation is useful for enhancement of these cells and efficient transplantation in infertility disorders. One approach is cultivation of PGCs under proper conditions that allow self-renewal and proliferation of PGCs. For this purpose, we compared the effects of different concentrations of retinoic acid (RA), and the effect of PGCs co-culture (Co-C) with SIM mouse embryo-derived thioguanine- and ouabain-resistant (STO) cells on the proliferation of embryonic stem cells (ESCs)-derived PGCs. One-day-old embryoid body (EB) was cultured for 4 days in simple culture system in the presence of 5 ng/ml bone morphogenetic protein-4 (BMP4) (SCB group) for PGC induction. For PGC enrichment, ESCs-derived germ cells were cultured for 7 days in the presence of different doses (0-5 muM) of RA, both in the simple and STO Co-C systems. At the end of the culture period, viability and proliferation rates were assessed and expression of mouse vasa homologue (Mvh), alpha6 integrin, beta1 integrin, stimulated by retinoic acid 8 (Stra8) and piwi (Drosophila)-like 2 (Piwil2) was evaluated using quantitative PCR. Also, the inductive effects were investigated immunocytochemically with Mvh and cadherin1 (CDH1) on the selected groups. Immunocytochemistry/PCR results showed higher expression of Mvh, the PGC-specific marker, in 3 muM RA concentrations on the top of the STO feeder layer. Meanwhile, assessment of the Stra8 mRNA and CDH1 protein, the specific makers for spermatogonia, showed no significant differences between groups. Based on the results, it seems that in the presence of 3 muM RA on top of the STO feeder layer cells, the majority of the cells transdifferentiated into germ cells were PGCs.

Makoolati, Z., et al. (2017). "Embryonic stem cell derived germ cells induce spermatogenesis after transplantation into the testes of an adult mouse azoospermia model." Clin Sci (Lond) **131**(18): 2381-2395.

The present study aimed to: (i) identify the exogenous factors that allow in vitro differentiation of mouse spermatogonial stem cells (SSCs) from embryonic stem cells (ESCs); (ii) evaluate the effects of Sertoli cells in SSC enrichment; and (iii) assess the success of transplantation using in vitro differentiated

SSCs in a mouse busulfan-treated azoospermia model. A 1-day-old embryoid body (EB) received 5 ng/ml of bone morphogenetic protein 4 (BMP4) for 4 days, 3 microM retinoic acid (RA) in a SIM mouse embryo-derived thioguanine and ouabain resistant (STO) co-culture system for 7 days, and was subsequently co-cultured for 2 days with Sertoli cells in the presence or absence of a leukaemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF) and RA composition, and in the presence of these factors in simple culture medium. Higher viability, proliferation and germ cell gene expression were seen in the presence of the LIF, bFGF and RA composition, on top of Sertoli cells. Immunocytochemistry results showed higher CDH1 expression in this group. Sertoli co-culture had no effects on SSC proliferation. Eight weeks after transplantation, injected cells were observed at the base of the seminiferous tubules and in the recipient testes. The number of spermatogonia and the mass of the testes were higher in transplanted testes relative to the control group. It seems that transplantation of these cells can be useful in infertility treatment.

Maltsev, V. A., et al. (1993). "Embryonic stem cells differentiate in vitro into cardiomyocytes representing sinusnodal, atrial and ventricular cell types." Mech Dev **44**(1): 41-50.

Pluripotent embryonic stem cells (ESC, ES cells) of line D3 were differentiated in vitro and via embryo-like aggregates (embryoid bodies) of defined cell number into spontaneously beating cardiomyocytes. By using RT-PCR technique, alpha- and beta-cardiac myosin heavy chain (MHC) genes were found to be expressed in embryoid bodies of early to terminal differentiation stages. The exclusive expression of the beta-cardiac MHC gene detected in very early differentiated embryoid bodies proved to be dependent on the number of ES cells developing in the embryoid body. Cardiomyocytes enzymatically isolated from embryoid body outgrowths at different stages of development were further characterized by immunocytological and electrophysiological techniques. All cardiomyocytes appeared to be positive in immunofluorescence assays with monoclonal antibodies against cardiac-specific alpha-cardiac MHC, as well as muscle-specific sarcomeric myosin heavy chain and desmin. The patch-clamp technique allowed a more detailed characterization of the in vitro differentiated cardiomyocytes which were found to represent phenotypes corresponding to sinusnode, atrium or ventricle of the heart. The cardiac cells of early differentiated stage expressed pacemaker-like action potentials similar to those described for embryonic cardiomyocytes. The action potentials of terminally differentiated cells revealed shapes, pharmacological characteristics and hormonal regulation inherent to adult sinusnodal, atrial or ventricular cells. In cardiomyocytes of intermediate differentiation state, action potentials of very long duration (0.3-1 s) were found, which may represent developmentally controlled transitions between different types of action potentials. Therefore, the presented ES cell differentiation system permits the investigation of commitment and differentiation of embryonic cells into the cardiomyogenic lineage in vitro.

Manabe, K., et al. (2004). "Developmental changes of Ni (2+) sensitivity and automaticity in Nkx2.5-positive cardiac precursor cells from murine embryonic stem cell." Circ J **68**(7): 724-726.

BACKGROUND: It is controversial which subtypes of T type Ca (2+) channels are implicated in automaticity of cardiac cells during the embryonic period. METHOD AND RESULTS: The effect of Ni (2+) on the automaticity of Nkx2.5-positive cardiac precursor cells sorted from embryonic stem cells during their differentiation was examined using patch clamp techniques. Although 40 micromol/L Ni (2+), which is enough to block Ni (2+)sensitive T type-Ca (2+) channels, decreased the spontaneous beating rate in all cells in the early and intermediate stage, Ni (2+) did not show any effects on the automaticity of 50% of the cells in the late stage. CONCLUSION: These results indicate that Ni (2+)-sensitive T-type Ca (2+) channels expressed in the Nkx2.5-positive cardiac precursor cells are developmentally regulated.

Manceur, A., et al. (2007). "Flow cytometric screening of cell-penetrating peptides for their uptake into embryonic and adult stem cells." Anal Biochem **364**(1): 51-59.

There is an increasing appreciation of the potential of cell-penetrating peptides (CPPs) as vectors to deliver peptides, proteins, and DNA into cells. However, the absolute and relative efficacy of various CPPs for applications targeting stem cells and primary cells is unclear. In this study, we have developed a two-step loading method and a flow cytometric assay to systematically compare the cellular uptake of five CPPs into embryonic stem cells, neurospheres (NSs), primary bone marrow hematopoietic progenitor (Sca-1(+)Lin (-)) cells, and hematopoietic cell lines (TF-1, K562, and FDCP Mix). The series of CPPs tested included three arginine-rich peptides; one was derived from HIV transactivator of transcription (TAT), one was derived from Antennapedia (Antp), and the third was a synthetic peptide known as protein transduction domain 4 (PTD4). Two hydrophobic peptides were also tested; one was derived from Kaposi fibroblast growth factor (K-FGF), and one was derived from PreS2 surface antigen of hepatitis B virus (PreS2-

TLM). Our results indicate, for the first time, that arginine-rich CPPs can internalize into primary NSs and bone marrow Sca-1(+)Lin (-) cells. In addition, in all cell types examined, the uptake of arginine-rich CPPs is significantly greater than that of hydrophobic peptides.

Mandal, A., et al. (2016). "Long-term culture and cryopreservation does not affect the stability and functionality of human embryonic stem cell-derived hepatocyte-like cells." In Vitro Cell Dev Biol Anim **52**(2): 243-251.

Human embryonic stem cells (hESCs) are predicted to be an unlimited source of hepatocytes which can pave the way for applications such as cell replacement therapies or as a model of human development or even to predict the hepatotoxicity of drug compounds. We have optimized a 23-d differentiation protocol to generate hepatocyte-like cells (HLCs) from hESCs, obtaining a relatively pure population which expresses the major hepatic markers and is functional and mature. The stability of the HLCs in terms of hepato-specific marker expression and functionality was found to be intact even after an extended period of in vitro culture and cryopreservation. The hESC-derived HLCs have shown the capability to display sensitivity and an alteration in the level of CYP enzyme upon drug induction. This illustrates the potential of such assays in predicting the hepatotoxicity of a drug compound leading to advancement of pharmacology.

Mandenius, C. F., et al. (2011). "Toward preclinical predictive drug testing for metabolism and hepatotoxicity by using in vitro models derived from human embryonic stem cells and human cell lines - a report on the Vitrocellomics EU-project." Altern Lab Anim **39**(2): 147-171.

Drug-induced liver injury is a common reason for drug attrition in late clinical phases, and even for post-launch withdrawals. As a consequence, there is a broad consensus in the pharmaceutical industry, and within regulatory authorities, that a significant improvement of the current in vitro test methodologies for accurate assessment and prediction of such adverse effects is needed. For this purpose, appropriate in vivo-like hepatic in vitro models are necessary, in addition to novel sources of human hepatocytes. In this report, we describe recent and ongoing research toward the use of human embryonic stem cell (hESC)-derived hepatic cells, in conjunction with new and improved test methods, for evaluating drug metabolism and hepatotoxicity. Recent progress on the directed differentiation of human embryonic stem cells to the functional hepatic phenotype is reported, as well as the development and adaptation of bioreactors and toxicity assay technologies for the testing of hepatic cells. The aim of achieving a testing platform for metabolism and hepatotoxicity assessment, based on hESC-derived hepatic cells, has advanced markedly in the last 2-3 years. However, great challenges still remain, before such new test systems could be routinely used by the industry. In particular, we give an overview of results from the Vitrocellomics project (EU Framework 6) and discuss these in relation to the current state-of-the-art and the remaining difficulties, with suggestions on how to proceed before such in vitro systems can be implemented in industrial discovery and development settings and in regulatory acceptance.

Mani, V., et al. (2008). "Serial in vivo positive contrast MRI of iron oxide-labeled embryonic stem cell-derived cardiac precursor cells in a mouse model of myocardial infarction." Magn Reson Med **60**(1): 73-81.

Myocardial regeneration with stem-cell transplantation is a possible treatment option to reverse deleterious effects that occur after myocardial infarction. Since little is known about stem cell survival after transplantation, developing techniques for "tracking" cells would be desirable. Iron-oxide-labeled stem cells have been used for in vivo tracking using MRI but produce negative contrast images that are difficult to interpret. The aim of the current study was to test a positive contrast MR technique using reduced z-gradient rephasing (GRASP) to aid in dynamically tracking stem cells in an in vivo model of mouse myocardial infraction. Ferumoxides and protamine sulfate were complexed and used to magnetically label embryonic stem cell-derived cardiac-precursor-cells (ES-CPCs). A total of 500,000 ES-CPCs were injected in the border zone of infarcted mice and MR imaging was performed on a 9.4T scanner using T (2)*-GRE sequences (negative contrast) and positive contrast GRASP technique before, 24 hours, and 1 week after ES-CPC implantation. Following imaging, mice were sacrificed for histology and Perl's staining was used to confirm iron within myocardium. Good correlation was observed between signal loss seen on conventional T (2)* images, bright areas on GRASP, and the presence of iron on histology. This demonstrated the feasibility of in vivo stem cell imaging with positive contrast MRI.

Manley, N. C., et al. (2017). "Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cells: Preclinical Efficacy and Safety in Cervical Spinal Cord Injury." Stem Cells Transl Med **6**(10): 1917-1929.

Cervical spinal cord injury (SCI) remains an important research focus for regenerative medicine given the potential for severe functional deficits and the current lack of treatment options to augment neurological recovery. We recently reported the preclinical safety data of a human embryonic cell-derived oligodendrocyte progenitor cell (OPC) therapy that supported initiation of a phase I clinical trial for patients with sensorimotor complete thoracic SCI. To support the clinical use of this OPC therapy for cervical injuries, we conducted preclinical efficacy and safety testing of the OPCs in a nude rat model of cervical SCI. Using the automated TreadScan system to track motor behavioral recovery, we found that OPCs significantly improved locomotor performance when administered directly into the cervical spinal cord 1 week after injury, and that this functional improvement was associated with reduced parenchymal cavitation and increased sparing of myelinated axons within the injury site. Based on large scale biodistribution and toxicology studies, we show that OPC migration is limited to the spinal cord and brainstem and did not cause any adverse clinical observations, toxicities, allodynia, or tumors. In combination with previously published efficacy and safety data, the results presented here supported initiation of a phase I/IIa clinical trial in the U.S. for patients with sensorimotor complete cervical SCI. Stem Cells Translational Medicine 2017;6:1917-1929.

Manton, K. J., et al. (2010). "A chimeric vitronectin: IGF-I protein supports feeder-cell-free and serum-free culture of human embryonic stem cells." Stem Cells Dev **19**(9): 1297-1305.

The therapeutic use of human embryonic stem (hES) cells is severely limited by safety concerns regarding their culture in media containing animal-derived or nondefined factors and on animal-derived feeder cells. Thus, there is a pressing need to develop culture techniques that are xeno-free, fully defined, and synthetic. Our laboratory has discovered that insulin-like growth factor (IGF) and vitronectin (VN) bind to each other resulting in synergistic short-term functional effects in several cell types, including keratinocytes and breast epithelial cells. We have further refined this complex into a single chimeric VN:IGF-I protein that functionally mimics the effects obtained upon binding of IGF-I to VN. The aim of the current study was to determine whether hES cells can be serially propagated in feeder-cell-free and serum-free conditions using medium containing our novel chimeric VN:IGF-I protein. Here we demonstrate that hES cells can be serially propagated and retain their undifferentiated state in vitro for up to 35 passages in our feeder-cell-free, serum-free, chemically defined media. We have utilized real-time polymerase chain reaction (PCR), immunofluorescence, and fluorescence-activated cell sorter (FACS) analysis to show that the hES cells have maintained an undifferentiated phenotype. In vitro differentiation assays demonstrated that the hES cells retain their pluripotent potential and the karyotype of the hES cells remains unchanged. This study demonstrates that the novel, fully defined, synthetic VN:IGF-I chimera-containing medium described herein is a viable alternative to media containing serum, and that in conjunction with laminin-coated plates facilitates feeder-cell-free and serum-free growth of hES.

Mantsoki, A., et al. (2016). "Gene expression variability in mammalian embryonic stem cells using single cell RNA-seq data." Comput Biol Chem **63**: 52-61.

BACKGROUND: Gene expression heterogeneity contributes to development as well as disease progression. Due to technological limitations, most studies to date have focused on differences in mean expression across experimental conditions, rather than differences in gene expression variance. The advent of single cell RNA sequencing has now made it feasible to study gene expression heterogeneity and to characterise genes based on their coefficient of variation. METHODS: We collected single cell gene expression profiles for 32 human and 39 mouse embryonic stem cells and studied correlation between diverse characteristics such as network connectivity and coefficient of variation (CV) across single cells. We further systematically characterised properties unique to High CV genes. RESULTS: Highly expressed genes tended to have a low CV and were enriched for cell cycle genes. In contrast, High CV genes were co-expressed with other High CV genes, were enriched for bivalent (H3K4me3 and H3K27me3) marked promoters and showed enrichment for response to DNA damage and DNA repair. CONCLUSIONS: Taken together, this analysis demonstrates the divergent characteristics of genes based on their CV. High CV genes tend to form co-expression clusters and they explain bivalency at least in part.

Marchetto, M. C., et al. (2008). "Non-cell-autonomous effect of human SOD1 G37R astrocytes on motor neurons derived from human embryonic stem cells." Cell Stem Cell **3**(6): 649-657.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron death. ALS can be induced by mutations in the superoxide dismutase 1 gene (SOD1). Evidence for the non-cell-autonomous nature of ALS emerged from the observation that wild-type glial cells extended the survival of SOD1 mutant motor neurons in chimeric

mice. To uncover the contribution of astrocytes to human motor neuron degeneration, we cocultured hESC-derived motor neurons with human primary astrocytes expressing mutated SOD1. We detected a selective motor neuron toxicity that was correlated with increased inflammatory response in SOD1-mutated astrocytes. Furthermore, we present evidence that astrocytes can activate NOX2 to produce superoxide and that effect can be reversed by antioxidants. We show that NOX2 inhibitor, apocynin, can prevent the loss of motor neurons caused by SOD1-mutated astrocytes. These results provide an assay for drug screening using a human ALS in vitro astrocyte-based cell model.

Mariappan, I., et al. (2015). "Enriched Cultures of Retinal Cells From BJNhem20 Human Embryonic Stem Cell Line of Indian Origin." Invest Ophthalmol Vis Sci **56**(11): 6714-6723.

PURPOSE: To test the retinal differentiation potential and to establish an optimized protocol for enriching retinal cells from an Indian origin, human embryonic stem cell (hESC) line, BJNhem20. METHODS: The BJNhem20 cells were cultured and expanded under feeder-free culture conditions. Differentiation was initiated by embryoid body (EB) formation and were cultured on Matrigel in neural induction medium (NIM) for 1 week and further maintained in retinal differentiation medium (RDM). After 1 month, the neuro-retinal progenitor clusters located at the center of pigmented retinal patches were picked and cultured as suspended neurospheres in RDM for 3 days and subsequently on Matrigel in neuro-retinal medium. The mildly pigmented, immature retinal pigmented epithelial (RPE) cells were picked separately and cultured on Matrigel in RPE medium (RPEM). After 1 week, the confluent neuro-retinal and RPE cultures were maintained in RDM for 2 to 3 months and characterized by immunofluorescence and RT-PCR. RESULTS: The BJNhem20 cells efficiently differentiated into both neuro-retinal and RPE cells. The early retinal progenitors expressed Nestin, GFAP, Pax6, Rx, MitfA, Chx10, and Otx2. Neuro-retinal cells expressed the neural markers, Map2, beta-III tubulin, acetylated tubulin and photoreceptor-specific markers, Crx, rhodopsin, recoverin, calbindin, PKC, NeuroD1, RLBP1, rhodopsin kinase, PDE6A, and PDE6C. Mature RPE cells developed intense pigmentation within 3 months and showed ZO-1 and Phalloidin staining at cell-cell junctions and expressed RPE65, tyrosinase, bestrophin1, Mertk, and displayed phagocytic activity. CONCLUSIONS: This study confirms the retinal differentiation potential of BJNhem20 cells and describes an optimized protocol to generate enriched populations of neuro-retinal and RPE cells.

Maric, D., et al. (2003). "Prospective cell sorting of embryonic rat neural stem cells and neuronal and glial progenitors reveals selective effects of basic fibroblast growth factor and epidermal growth factor on self-renewal and differentiation." J Neurosci **23**(1): 240-251.

We directly isolated neural stem cells and lineage-restricted neuronal and glial progenitors from the embryonic rat telencephalon using a novel strategy of surface labeling and fluorescence-activated cell sorting. Neural stem cells, which did not express surface epitopes characteristic of differentiation or apoptosis, were sorted by negative selection. These cells predominantly expressed fibroblast growth factor receptor type 1 (FGFR-1), and a minority exhibited basic fibroblast growth factor (bFGF), whereas few expressed epidermal growth factor receptor (EGFR) or EGF. Clonal analyses revealed that these cells primarily self-renewed without differentiating in bFGF-containing medium, whereas few survived or expanded in EGF-containing medium. Culturing of neural stem cells in bFGF- and EGF-containing medium permitted both self-renewal and differentiation into neuronal, astroglial, and oligodendroglial phenotypes. In contrast, lineage-restricted progenitors were directly sorted by positive selection using a combination of surface epitopes identifying neuronal or glial phenotypes or both. These cells were also primarily FGFR-1(+), with few EGFR (+), and most expanded and progressed along their expected lineages in bFGF-containing medium but not in EGF-containing medium. Ca (2+) imaging of self-renewing neural stem cells cultured in bFGF-containing medium revealed that bFGF, but not EGF, induced cytosolic Ca (2+) (Ca (2+)c) responses in these cells, whereas in bFGF- and EGF-containing medium, both bFGF and EGF evoked Ca (2+)c signals only in differentiating progeny of these cells. The results demonstrate that bFGF, but not EGF, sustains a calcium-dependent self-renewal of neural stem cells and early expansion of lineage-restricted progenitors, whereas together the two growth factors permit the initial commitment of neural stem cells into neuronal and glial phenotypes.

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.

Markoulaki, S., et al. (2008). "Somatic cell nuclear transfer and derivation of embryonic stem cells in the mouse." Methods **45**(2): 101-114.

Addressing the fundamental questions of nuclear equivalence in somatic cells has fascinated scientists for decades and has resulted in the development of somatic cell nuclear transfer (SCNT) or animal cloning. SCNT involves the transfer of the nucleus of a somatic cell into the cytoplasm of an egg whose own chromosomes have been removed. In the mouse, SCNT has not only been successfully used to address the issue of nuclear equivalence, but has been used as a model system to test the hypothesis that embryonic stem cells (ESCs) derived from NT blastocysts have the potential to correct--through genetic manipulations--degenerative diseases. This paper aims to provide a comprehensive description of SCNT in the mouse and the derivation of ESCs from blastocysts generated by this technique. SCNT is a very challenging and inefficient procedure because it is technically complex, it bypasses the normal events of gamete interactions and egg activation, and it depends on adequate reprogramming of the somatic cell nucleus in vivo. Improvements in any or all those aspects may enhance the efficiency and applicability of SCNT. ESC derivation from SCNT blastocysts, on the other hand, requires the survival of only a few successfully reprogrammed cells, which have the capacity to proliferate indefinitely in vitro, maintain correct genetic and epigenetic status, and differentiate into any cell type in the body--characteristics that are essential for transplantation therapy or any other in vivo application.

Markoullis, K., et al. (2009). "Mycoplasma contamination of murine embryonic stem cells affects cell parameters, germline transmission and chimeric progeny." Transgenic Res **18**(1): 71-87.

Murine embryonic stem cells (mESCs) inoculated at passage P13 with the mycoplasma species M. hominis, M. fermentans and M. orale and cultured over 20 passages showed reduced growth rate and viability ($P < 0.0001$) compared to control mESCs. Spectral karyotypic analysis of mycoplasma-infected mESCs showed a number of non-clonal chromosomal aberrations which increased with the duration of infection. The differentiation status of the infected mESCs was most affected at passage P13+6 where the infection was strongest and 46.3% of the mESCs expressed both POU5F1 and SSEA-1 markers whereas 84.8% of control mESCs expressed both markers. The percentage of germline chimeras from mycoplasma-infected mESCs was examined after blastocyst injection and embryo transfer to suitable recipients at different passages and, compared to the respective control group, was most affected at passage P13+5 (50% vs. 90%; $P < 0.07$). Further reductions were obtained at the same passage in the percentage of litters born (50% vs. 100%; $P < 0.07$) and in the percentage of pups born (22% vs. 45%; $P < 0.001$). Thirty three chimeras (39.8%) obtained from blastocyst injection with mycoplasma-infected mESCs showed reduced body weight ($P < 0.0001$), nasal discharge, osteoarthropathia, and cachexia. Flow cytometric analysis of plasma from chimeras produced with mycoplasma-infected mESCs revealed statistically significant differences in the proportions of T-cells and increased levels of IgG1 ($P < 0.001$), IgG2a ($P < 0.05$) and IgM ($P < 0.05$), anti-DNA antibodies ($P < 0.05$) and rheumatoid factor ($P < 0.01$). The present data indicate that mycoplasma contamination of mESCs affects various cell parameters, germline transmission, and postnatal development of the resulting chimeras.

Martin, C. H., et al. (2008). "Differences in lymphocyte developmental potential between human embryonic stem cell and umbilical cord blood-derived hematopoietic progenitor cells." Blood **112**(7): 2730-2737.

Hematopoietic progenitor cells derived from human embryonic stem cells (hESCs) develop into diverse mature hematopoietic lineages, including lymphocytes. Whereas functional natural killer (NK) cells can be efficiently generated in vitro from hESC-derived CD34(+) cells, studies of T- and B-cell development from hESCs have been much more limited. Here, we demonstrate that despite expressing functional Notch-1, CD34(+) cells from hESCs did not derive T cells when cocultured with OP9 cells expressing Delta-like 1, or in fetal thymus organ culture. hESC-derived CD34(+) cells also did not produce B cells in vitro. In contrast, CD34(+) cells isolated from UCB routinely generated T and B cells when cultured in the same conditions. Notably, both

undifferentiated hESCs, and sorted hESC-derived populations with hematopoietic developmental potential exhibited constitutive expression of ID family genes and of transcriptional targets of stem cell factor-induced signaling. These pathways both inhibit T-cell development and promote NK-cell development. Together, these results demonstrate fundamental differences between hESC-derived hematopoietic progenitors and analogous primary human cells. Therefore, hESCs can be more readily supported to differentiate into certain cell types than others, findings that have important implications for derivation of defined lineage-committed populations from hESCs.

Massumi, M., et al. (2016). "An Abbreviated Protocol for In Vitro Generation of Functional Human Embryonic Stem Cell-Derived Beta-Like Cells." PLoS One **11**(10): e0164457.

The ability to yield glucose-responsive pancreatic beta-cells from human pluripotent stem cells in vitro will facilitate the development of the cell replacement therapies for the treatment of Type 1 Diabetes. Here, through the sequential in vitro targeting of selected signaling pathways, we have developed an abbreviated five-stage protocol (25-30 days) to generate human Embryonic Stem Cell-Derived Beta-like Cells (ES-DBCs). We showed that Geltrex, as an extracellular matrix, could support the generation of ES-DBCs more efficiently than that of the previously described culture systems. The activation of FGF and Retinoic Acid along with the inhibition of BMP, SHH and TGF-beta led to the generation of 75% NKX6.1+/NGN3+ Endocrine Progenitors. The inhibition of Notch and tyrosine kinase receptor AXL, and the treatment with Exendin-4 and T3 in the final stage resulted in 35% mono-hormonal insulin positive cells, 1% insulin and glucagon positive cells and 30% insulin and NKX6.1 co-expressing cells. Functionally, ES-DBCs were responsive to high glucose in static incubation and perifusion studies, and could secrete insulin in response to successive glucose stimulations. Mitochondrial metabolic flux analyses using Seahorse demonstrated that the ES-DBCs could efficiently metabolize glucose and generate intracellular signals to trigger insulin secretion. In conclusion, targeting selected signaling pathways for 25-30 days was sufficient to generate ES-DBCs in vitro. The ability of ES-DBCs to secrete insulin in response to glucose renders them a promising model for the in vitro screening of drugs, small molecules or genes that may have potential to influence beta-cell function.

Mathieu, J., et al. (2011). "HIF induces human embryonic stem cell markers in cancer cells." Cancer Res **71**(13): 4640-4652.

Low oxygen levels have been shown to promote self-renewal in many stem cells. In tumors, hypoxia is associated with aggressive disease course and poor clinical outcomes. Furthermore, many aggressive tumors have been shown to display gene expression signatures characteristic of human embryonic stem cells (hESC). We now tested whether hypoxia might be responsible for the hESC signature observed in aggressive tumors. We show that hypoxia, through hypoxia-inducible factor (HIF), can induce an hESC-like transcriptional program, including the induced pluripotent stem cell (iPSC) inducers, OCT4, NANOG, SOX2, KLF4, cMYC, and microRNA-302 in 11 cancer cell lines (from prostate, brain, kidney, cervix, lung, colon, liver, and breast tumors). Furthermore, nondegradable forms of HIFalpha, combined with the traditional iPSC inducers, are highly efficient in generating A549 iPSC-like colonies that have high tumorigenic capacity. To test potential correlation between iPSC inducers and HIF expression in primary tumors, we analyzed primary prostate tumors and found a significant correlation between NANOG-, OCT4-, and HIF1alpha-positive regions. Furthermore, NANOG and OCT4 expressions positively correlated with increased prostate tumor Gleason score. In primary glioma-derived CD133 negative cells, hypoxia was able to induce neurospheres and hESC markers. Together, these findings suggest that HIF targets may act as key inducers of a dynamic state of stemness in pathologic conditions.

Matsumoto, M., et al. (2008). "Potential of embryonic stem cell-derived neurons for synapse formation with auditory hair cells." J Neurosci Res **86**(14): 3075-3085.

Recent studies have indicated that embryonic stem cells (ESCs) can be a source for the replacement of spiral ganglion neurons (SGNs), auditory primary neurons, and neurite projections from ESC-derived neurons to auditory sensory epithelia. However, the potential of ESC-derived neurons for synapse formation with auditory hair cells (HCs) has not been elucidated. The present study therefore aimed to examine the ability of ESC-derived neurons to form synaptic connections with HCs in vitro. Mouse ESC-derived neural progenitors expressing enhanced green fluorescence protein (EGFP) were cocultured with explants of cochlea sensory epithelia obtained from postnatal day 3 mice. After a 7-day culture, neurites of ESC-derived neurons predominantly elongated toward inner hair cells (IHCs), which play a crucial role in sound transmission to SGNs. Immunohistochemical analyses revealed the expression of synapsin 1 and synaptophysin in the nerve endings of ESC-derived neurons adjacent to IHCs, indicating the formation of synaptic connections. Transmission electron

microscopy demonstrated synaptic contacts between nerve endings of ESC-derived neurons and IHCs. The present findings show that ESC-derived neurons can make synaptic connections with IHCs.

Matsunaga, Y., et al. (2008). "Activation of antigen-specific cytotoxic T lymphocytes by beta 2-microglobulin or TAP1 gene disruption and the introduction of recipient-matched MHC class I gene in allogeneic embryonic stem cell-derived dendritic cells." J Immunol **181**(9): 6635-6643.

A method for the genetic modification of dendritic cells (DC) was previously established based on the in vitro differentiation of embryonic stem (ES) cells to DC (ES-DC). The unavailability of human ES cells genetically identical to the patients will be a problem in the future clinical application of this technology. This study attempted to establish a strategy to overcome this issue. The TAP1 or beta (2)-microglobulin (beta (2)m) gene was disrupted in 129 (H-2(b))-derived ES cells and then expression vectors for the H-2K (d) or beta (2)m-linked form of K (d) (beta2m-K (d)) were introduced, thus resulting in two types of genetically engineered ES-DC, TAP1(-/-)/K (d) ES-DC and beta (2)m (-/-)/beta (2)m-K (d) ES-DC. As intended, both of the transfectant ES-DC expressed K (d) but not the intrinsic H-2(b) haplotype-derived MHC class I. Beta (2)m (-/-)/beta (2)m-K (d) and TAP1(-/-)/K (d) ES-DC were not recognized by pre-activated H-2(b)-reactive CTL and did not prime H-2(b) reactive CTL in vitro or in vivo. Beta (2)m (-/-)/beta (2)m-K (d) ES-DC and TAP1(-/-)/K (d) ES-DC had a survival advantage in comparison to beta (2)m (+/-)/beta (2)m-K (d) ES-DC and TAP1(+/+)/K (d) ES-DC, when transferred into BALB/c mice. K (d)-restricted RSV-M2-derived peptide-loaded ES-DC could prime the epitope-specific CTL upon injection into the BALB/c mice, irrespective of the cell surface expression of intrinsic H-2(b) haplotype-encoded MHC class I. Beta (2)m (-/-)/beta (2)m-K (d) ES-DC were significantly more efficient in eliciting immunity against RSV M2 protein-expressing tumor cells than beta (2)m (+/-)/beta (2)m-K (d) ES-DC. The modification of the beta (2)m or TAP gene may therefore be an effective strategy to resolve the problem of HLA class I allele mismatch between human ES or induced pluripotent stem cells and the recipients to be treated.

Matsuoka, H., et al. (2007). "Semi-quantitative expression and knockdown of a target gene in single-cell mouse embryonic stem cells by high performance microinjection." Biotechnol Lett **29**(3): 341-350.

Interactions of multiple genes and associated factors are involved in the differentiation and de-differentiation of embryonic stem (ES) cells. Quantitative analysis of these genes and factors is essential for the elucidation of their mechanism. To meet this requirement, we have investigated various experimental conditions for high performance microinjection into mouse ES cells. A speedy and rhythmic operation was found to be important and was accomplished robotically by using a single-cell manipulation technique and XY-address registrable culture dishes. Among many experimental parameters, the tip size of an injection capillary, the pressure condition, and the DNA concentration in the injection capillary were of critical significance. Their optimum values were 0.5-0.8 microm, 0.7 kgf/cm (2) for 30 ms, and 1-100 ng/microl, respectively. Under these conditions, semi-quantitative control of the EGFP gene expression in mouse ES cells and its knockdown was successfully demonstrated.

Matsuyoshi, H., et al. (2004). "Enhanced priming of antigen-specific CTLs in vivo by embryonic stem cell-derived dendritic cells expressing chemokine along with antigenic protein: application to antitumor vaccination." J Immunol **172**(2): 776-786.

Dendritic cell (DC)-based immunotherapy is regarded as a promising means for anti-cancer therapy. The efficiency of T cell-priming in vivo by transferred DCs should depend on their encounter with T cells. In the present study, we attempted to improve the capacity of DCs to prime T cells in vivo by genetic modification to express chemokine with a T cell-attracting property. For genetic modification of DCs, we used a recently established method to generate DCs from mouse embryonic stem cells. We generated double-transfectant DCs expressing a chemokine along with a model Ag (OVA) by sequential transfection of embryonic stem cells, and then induced differentiation to DCs. We comparatively evaluated the effect of three kinds of chemokines; secondary lymphoid tissue chemokine (SLC), monokine induced by IFN-gamma (Mig), and lymphotactin (Lptn). All three types of double transfectant DCs primed OVA-specific CTLs in vivo more efficiently than did DCs expressing only OVA, and the coexpression of SLC or Lptn was more effective than that of Mig. Immunization with DCs expressing OVA plus SLC or Mig provided protection from OVA-expressing tumor cells more potently than did immunization with OVA alone, and SLC was more effective than Mig. In contrast, coexpression of Lptn gave no additive effect on protection from the tumor. Collectively, among the three chemokines, expression of SLC was the most effective in enhancing antitumor immunity by transferred DCs in vivo. The findings provide useful information for the development of a potent DC-based cellular immunotherapy.

Mattei, C., et al. (2018). "Generation of Neural Organoids from Human Embryonic Stem Cells Using the Rotary Cell Culture System: Effects of Microgravity on Neural Progenitor Cell Fate." Stem Cells Dev **27**(12): 848-857.

Progress in aeronautics and spaceflight technologies requires in parallel further research on how microgravity may affect human tissue. To date, little is known about the effects of microgravity on human development. In this study we used the rotary cell culture system to investigate whether microgravity supports the generation and maintenance of neural organoids derived from human embryonic stem cells (hESCs) as a model of human brain development. Our results show that although neural organoids could be generated and maintained in microgravity conditions, there were changes in expression of rostral-caudal neural patterning genes and cortical markers compared to organoids generated in standard conditions. This phenomenon was also observed in hESC-derived cortical organoids exposed to microgravity for relatively shorter periods. These results are one of the first for analyzing human neurogenesis in a microgravity environment.

Matveeva, N. M., et al. (2015). "Generation of mouse chimeras with high contribution of tetraploid embryonic stem cells and embryonic stem cell-fibroblast hybrid cells." Methods Mol Biol **1313**: 61-71.

The in vitro long-term cultivation of embryonic stem (ES) cells derived from pre-implantation embryos offers the unique possibility of combining ES cells with pre-implantation embryos to generate chimeras, thus facilitating the creation of a bridge between in vitro and in vivo investigations. Genomic manipulation using ES cells and homologous recombination is one of the most outstanding scientific achievements, resulting in the generation of animals with desirable genome modifications. As such, the generation of ES cells with different ploidy via cell fusion also deserves much attention because this approach allows for the production of chimeras that contain somatic cells with various ploidy. Therefore, this is a powerful tool that can be used to study the role of polyploidy in the normal development of mammals.

Maya-Espinosa, G., et al. (2015). "Mouse embryonic stem cell-derived cells reveal niches that support neuronal differentiation in the adult rat brain." Stem Cells **33**(2): 491-502.

A neurogenic niche can be identified by the proliferation and differentiation of its naturally residing neural stem cells. However, it remains unclear whether "silent" neurogenic niches or regions suitable for neural differentiation, other than the areas of active neurogenesis, exist in the adult brain. Embryoid body (EB) cells derived from embryonic stem cells (ESCs) are endowed with a high potential to respond to specification and neuralization signals of the embryo. Hence, to identify microenvironments in the postnatal and adult rat brain with the capacity to support neuronal differentiation, we transplanted dissociated EB cells to conventional neurogenic and non-neurogenic regions. Our results show a neuronal differentiation pattern of EB cells that was dependent on the host region. Efficient neuronal differentiation of EB cells occurred within an adjacent region to the rostral migratory stream. EB cell differentiation was initially patchy and progressed toward an even distribution along the graft by 15-21 days post-transplantation, giving rise mostly to GABAergic neurons. EB cells in the striatum displayed a lower level of neuronal differentiation and derived into a significant number of astrocytes. Remarkably, when EB cells were transplanted to the striatum of adult rats after a local ischemic stroke, increased number of neuroblasts and neurons were observed. Unexpectedly, we determined that the adult substantia nigra pars compacta, considered a non-neurogenic area, harbors a robust neurogenic environment. Therefore, neurally uncommitted cells derived from ESCs can detect regions that support neuronal differentiation within the adult brain, a fundamental step for the development of stem cell-based replacement therapies.

Mazzilli, J. L., et al. (2017). "Derivation and characterization of the human embryonic stem cell line CR-4: Differentiation to human retinal pigment epithelial cells." Stem Cell Res **18**: 37-40.

The CR-4 human embryonic stem cell line was derived from the inner cell mass of a developing blastocyst. This cell line has been adapted to grow in feeder-free conditions and is especially well-suited for differentiation to retinal pigment epithelium. The line demonstrates a normal human 46,XX female karyotype. Pluripotency was assessed through qRT-PCR for expression of NANOG, OCT-4, and SOX-2. A teratoma assay was performed and results were positive for all three germ layers. Testing for Mycoplasma was negative.

McCabe, K. L., et al. (2015). "Efficient Generation of Human Embryonic Stem Cell-Derived Corneal Endothelial Cells by Directed Differentiation." PLoS One **10**(12): e0145266.

AIM: To generate human embryonic stem cell derived corneal endothelial cells (hESC-CECs) for transplantation in patients with corneal endothelial dystrophies. MATERIALS AND METHODS: Feeder-free hESC-CECs were generated by a directed

differentiation protocol. hESC-CECs were characterized by morphology, expression of corneal endothelial markers, and microarray analysis of gene expression. RESULTS: hESC-CECs were nearly identical morphologically to primary human corneal endothelial cells, expressed Zona Occludens 1 (ZO-1) and Na+/K+ATPasealpha1 (ATPA1) on the apical surface in monolayer culture, and produced the key proteins of Descemet's membrane, Collagen VIIIalpha1 and VIIIalpha2 (COL8A1 and 8A2). Quantitative PCR analysis revealed expression of all corneal endothelial pump transcripts. hESC-CECs were 96% similar to primary human adult CECs by microarray analysis. CONCLUSION: hESC-CECs are morphologically similar, express corneal endothelial cell markers and express a nearly identical complement of genes compared to human adult corneal endothelial cells. hESC-CECs may be a suitable alternative to donor-derived corneal endothelium.

McCloskey, K. E., et al. (2005). "Use of embryonic stem cell-derived endothelial cells as a cell source to generate vessel structures in vitro." Tissue Eng **11**(3-4): 497-505.

Embryonic stem (ES) cells could potentially serve as an excellent cell source for various applications in regenerative medicine and tissue engineering. Our laboratory is particularly interested in generating a reproducible endothelial cell source for the development of prevascularized materials for tissue/organ reconstruction. After developing methods to isolate highly purified (>96%) proliferating populations of endothelial cells from mouse embryonic stem cells, we tested their ability to form three-dimensional (3-D) vascular structures in vitro. The ES cell-derived endothelial cells were embedded in 3-D collagen gel constructs with rat tail collagen type I (2 mg/mL) at a concentration of 10(6) cells/mL of gel. The gels were observed daily with a phase-contrast microscope to analyze the time course for endothelial cell assembly. The first vessels were observed between days 3 and 5 after gel construct formation. The number and complexity of structures steadily increased, reaching a maximum before beginning to regress. By 2 weeks, all vessel-like structures had regressed back to single cells. Histology and fluorescent images of the vessel-like structures verified that tube structures were multicellular and could develop patent lumens. We have shown that endothelial cells derived, purified and expanded in vitro from ES cells sustain an important endothelial cell function, the ability to undergo vasculogenesis in collagen gels, indicating that endothelial products derived in vitro from stem cells could be useful in regenerative medicine applications.

McCloskey, K. E., et al. (2006). "Embryonic stem cell-derived endothelial cells may lack complete functional maturation in vitro." J Vasc Res **43**(5): 411-421.

Stem cell therapies will only become clinically relevant if the stem cells differentiated in vitro function as their in vivo counterparts. Here, we employed our previously developed techniques for deriving endothelial cells (>96% purity) from mouse embryonic stem cells (ESC) and compared these with mouse aortic endothelial cells (MAEC) obtained from thoracic aortas. Immunocytochemical analysis of ESC-derived endothelial cells (EC) demonstrates that both cell types are positive for the EC markers endothelial nitric oxide synthase (eNOS), Flk-1, Flt-1, vascular endothelial cadherin (VEcad), platelet-endothelial cell adhesion molecule-1 (PECAM-1), and CD34. However, ESC-derived EC express slightly lower levels of PECAM-1 and VE-cadherin, and significantly lower levels of acetylated low-density lipoprotein (LDL) uptake and von Willebrand factor. Although ESC-derived EC do express VE-cadherin, the VE-cadherin in the ESC-derived EC did not localize as well at the cell-cell junctions as in the MAEC. Interestingly, ESC-derived EC express much greater levels of the endothelial and hematopoietic stem cell marker CD34 and vasculogenic and angiogenic sprouting than MAEC. These results indicate that ESC-derived EC share some key characteristics of 'mature' EC, while retaining markers of alternate phenotypes including immature endothelium.

McElroy, S. L. and R. A. Reijo Pera (2008). "Preparation of mouse embryonic fibroblast feeder cells for human embryonic stem cell culture." CSH Protoc **2008**: pdb prot5041.

INTRODUCTIONEmbryonic stem cells (ESCs) are derived from the inner cell mass of day 5-6 blastocysts. ESCs are pluripotent, meaning that they are able to differentiate into all derivatives of the three primary germ layers (ectoderm, endoderm, and mesoderm). In order to maintain the undifferentiated status of human ESCs (hESCs), feeder cells are used to provide both a suitable attachment substrate and critical soluble factors. Since the first hESC lines were established on mouse embryonic fibroblasts (MEFs), mitotically inactivated MEFs have commonly been used for supporting the culture of undifferentiated hESCs. Some previous studies suggest that MEFs may support hESC growth better than the human feeder cells typically isolated from post-natal tissues. This protocol describes a method for isolation and irradiation of MEFs for use in hESC culture.

McHugh, P. C., et al. (2008). "Proteomic analysis of embryonic stem cell-derived neural cells exposed to the antidepressant paroxetine." J Neurosci Res **86**(2): 306-316.

Antidepressant drugs can have significant effects on the mood of a patient suffering from major depression or other disorders. The pharmacological actions of these drugs generally affect the uptake or metabolism of the neurotransmitters serotonin, noradrenalin, and, to a lesser extent, dopamine. However, many aspects of antidepressant action are not understood. We conducted a proteomic analysis in a neuronal cell culture model in an attempt to identify molecules important to the operation of pathways functionally relevant to antidepressant action. The model involved generating cultures containing mixed neural and glial cells by controlled differentiation of mouse embryonic stem cells, followed by exposure to 1 microM paroxetine for 14 days. After antidepressant exposure, we observed increased expression or modification of sepiapterin reductase (SPR), heat shock protein 9A, RAS and EF-hand domain containing, and protein disulfide isomerase associated 3 and decreased expression or modification of creatine kinase, actin, prohibitin, a T-cell receptor alpha chain, defensin-related cryptdin 5, and the intermediate filament proteins glial fibrillary acidic protein and vimentin. SPR, the most strongly up-regulated protein observed, controls production of tetrahydrobiopterin, an essential cofactor for the synthesis of many neurotransmitters including serotonin, making it a plausible and intriguing candidate protein for involvement in mood control and antidepressant drug action. SPR and the other proteins identified may represent links to molecular processes of importance to mood dysregulation and control, and their respective genes may be novel candidates for the study of antidepressant pharmacogenetics.

McWhir, J., et al. (1996). "Selective ablation of differentiated cells permits isolation of embryonic stem cell lines from murine embryos with a non-permissive genetic background." Nat Genet **14**(2): 223-226.

Embryonic stem (ES) cells enable the engineering of precise modifications to the mouse genome by gene targeting. Although there are reports of cultured cell contributions to chimaeras in golden hamster, rat and pig, definitive ES cell lines which contribute to the germline have not been demonstrated in any species but mouse. Among mouse strains, genetic background strongly affects the efficiency of ES isolation, and almost all ES lines in use are derived from strain 129 (refs 1,4,5) or, less commonly, C57BL/6 (refs 6-8). The CBA strain is refractory to ES isolation and there are no published reports of CBA-derived ES lines. Hence, CBA mice may provide a convenient model of ES isolation in other species. In ES derivation it is critical that the primary explant be cultured for a sufficient time to allow multiplication of ES cell progenitors, yet without allowing extensive differentiation. Thus, differences in ES derivation between mouse strains may reflect differences in the control of ES progenitor cells by other lineages within the embryo. Here we describe a strategy to continuously remove differentiated cells by drug selection, which generates germline competent ES lines from genotypes that are non-permissive in the absence of selection.

Meamar, R., et al. (2010). "Toxicity of ecstasy (MDMA) towards embryonic stem cell-derived cardiac and neural cells." Toxicol In Vitro **24**(4): 1133-1138.

"Ecstasy" or methylenedioxymethamphetamine (MDMA) is primarily a recreational drug commonly used during the child bearing period, thus, there is a major concern regarding the embryonic and fetal toxicity of this drug. Here, we report the cardio- and neuro-toxic effects of MDMA on beating embryoid bodies (EBs) and neural cell-containing EBs derived from mouse embryonic stem cell (ESCs). Based on our linear discriminate function, MDMA is considered to be a moderate or weak teratogen. Moreover, the generation of EBs with neural cell morphology and the expression of MAP2, a mature neuron marker, decrease more when MDMA is administered during the EB formation stage rather than post-plated EBs. In addition, the ID50 (inhibition of differentiation) of EBs with neural cell morphology is less than cardiac cells. In conclusion, MDMA causes a marked reduction in beating cardiomyocytes and neurons in ESC cultures, and this drug has a more potent toxicity on neural rather than cardiac cell differentiation.

Medine, C. N., et al. (2008). Robust generation of hepatocyte-like cells from human embryonic stem cell populations. StemBook. Cambridge (MA).

Despite progress in modelling human drug toxicity, many compounds fail during clinical trials due to unpredicted side effects. The cost of clinical studies are substantial, therefore it is essential that more predictive toxicology screens are developed and deployed early on in drug development (Greenhough et al 2010). Human hepatocytes represent the current gold standard model for evaluating drug toxicity, but are a limited resource that exhibit variable function. Therefore, the use of immortalised cell lines and animal tissue models are routinely employed due to their abundance. While both sources are informative, they are limited by poor function, species variability and/or instability in culture (Dalgetty et al 2009).

Pluripotent stem cells (PSCs) are an attractive alternative source of human hepatocyte like cells (HLCs) (Medine et al 2010). PSCs are capable of self renewal and differentiation to all somatic cell types found in the adult and thereby represent a potentially inexhaustible source of differentiated cells. We have developed a procedure that is simple, highly efficient, amenable to automation and yields functional human HLCs (Hay et al 2008; Fletcher et al 2008; Hannoun et al 2010; Payne et al 2011 and Hay et al 2011). We believe our technology will lead to the scalable production of HLCs for drug discovery, disease modeling, the construction of extra-corporeal devices and possibly cell based transplantation therapies.

Medine, C. N., et al. (2011). "Robust generation of hepatocyte-like cells from human embryonic stem cell populations." J Vis Exp (56): e2969.

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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.

Mehat, M. S., et al. (2018). "Transplantation of Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial Cells in Macular Degeneration." Ophthalmology.

PURPOSE: Transplantation of human embryonic stem cell (hESC)-derived retinal pigment epithelial (RPE) cells offers the potential for benefit in macular degeneration. Previous trials have reported improved visual acuity (VA), but lacked detailed analysis of retinal structure and function in the treated area. DESIGN: Phase 1/2 open-label dose-escalation trial to evaluate safety and potential efficacy (clinicaltrials.gov identifier, NCT01469832). PARTICIPANTS: Twelve participants with advanced Stargardt disease (STGD1), the most common cause of macular degeneration in children and young adults. METHODS: Subretinal transplantation of up to 200 000 hESC-derived RPE cells with systemic immunosuppressive therapy for 13 weeks. MAIN OUTCOME MEASURES: The primary end points were the safety and tolerability of hESC-derived RPE cell administration. We also investigated evidence of the survival of transplanted cells and measured retinal structure and function using microperimetry and spectral-domain OCT. RESULTS: Focal areas of subretinal hyperpigmentation developed in all participants in a dose-dependent manner in the recipient retina and persisted after withdrawal of systemic immunosuppression. We found no evidence of uncontrolled proliferation or inflammatory responses. Borderline improvements in best-corrected VA in 4 participants either were unsustained or were matched by a similar improvement in the untreated contralateral eye. Microperimetry demonstrated no evidence of benefit at 12 months in the 12 participants. In one instance at the highest dose, localized retinal thinning and reduced sensitivity in the area of hyperpigmentation suggested the potential for harm. Participant-reported quality of life using the 25-item National Eye Institute Visual Function Questionnaire indicated no significant change. CONCLUSIONS: Subretinal hyperpigmentation is consistent with the survival of viable transplanted hESC-derived RPE cells, but may reflect released pigment in their absence. The findings demonstrate the value of detailed analysis of spatial correlation of retinal structure and function in determining with appropriate sensitivity the impact

of cell transplantation and suggest that intervention in early stage of disease should be approached with caution. Given the slow rate of progressive degeneration at this advanced stage of disease, any protection against further deterioration may be evident only after a more extended period of observation.

Menchon, C., et al. (2011). "The cell cycle inhibitor p27Kip (1) controls self-renewal and pluripotency of human embryonic stem cells by regulating the cell cycle, Brachyury and Twist." Cell Cycle **10**(9): 1435-1447.

The continued turn over of human embryonic stem cells (hESC) while maintaining an undifferentiated state is dependent on the regulation of the cell cycle. Here we asked the question if a single cell cycle gene could regulate the self-renewal or pluripotency properties of hESC. We identified that the protein expression of the p27(Kip) (1) cell cycle inhibitor is low in hESC cells and increased with differentiation. By adopting a gain and loss of function strategy we forced or reduced its expression in undifferentiating conditions to define its functional role in self-renewal and pluripotency. Using undifferentiation conditions, overexpression of p27(Kip) (1) in hESC lead to a G (1)phase arrest with an enlarged and flattened hESC morphology and consequent loss of self-renewal ability. Loss of p27(Kip) (1) caused an elongated/scatter cell-like phenotype involving up-regulation of Brachyury and Twist gene expression. We demonstrate the novel finding that p27(Kip) (1) protein occupies the Twist1 gene promoter and manipulation of p27(Kip) (1) by gain and loss of function is associated with Twist gene expression changes. These results define p27(Kip) (1) expression levels as critical for self-renewal and pluripotency in hESC and suggest a role for p27(Kip) (1) in controlling an epithelial to mesenchymal transition (EMT) in hESC.

Menendez, P., et al. (2006). "Human embryonic stem cells: A journey beyond cell replacement therapies." Cytotherapy **8**(6): 530-541.

Success in the derivation of human embryonic stem cell (hESC) lines has opened up a new area of research in biomedicine. Human ESC not only raise hope for cell replacement therapies but also provide a potential novel system to better understand early human normal development, model human abnormal development and disease, and perform drug-screening and toxicity studies. The realization of these potentials, however, depends on expanding our knowledge about the cellular and molecular mechanisms that regulate self-renewal and lineage specification. Here, we briefly highlight the potential applications of hESC and review how flow cytometry has contributed to the initial characterization of both undifferentiated hESC cultures and hematopoietic development arising from hESC. We envision that a combination of state-of-the-art technologies, including cytomics, proteomics and genomics, will be instrumental in moving the field forward, ultimately lending invaluable knowledge to research areas such as human embryology, oncology and immunology.

Menendez, P., et al. (2004). "Retroviral transduction of hematopoietic cells differentiated from human embryonic stem cell-derived CD45(neg)PFV hemogenic precursors." Mol Ther **10**(6): 1109-1120.

Human embryonic stem cells (hESCs) provide a unique opportunity to study molecular mechanisms that regulate specification of the hematopoietic lineage in the human. Exploitation of this model using transgenic strategies depends on the ability to target cells of the hematopoietic lineage effectively and establish stable transgene expression. Here, a recently defined subpopulation of endothelial-like precursors derived from hESCs that is exclusively responsible for hematopoietic cell fate (CD45(neg)PFV) is shown to express GALVR-1 receptor and be efficiently transduced with GALV-pseudotyped retrovirus. Retroviral transduction, measured by enhanced green fluorescent protein, of hESC-derived CD45(+) cells differentiated from isolated CD45(neg)PFV precursors was 26.5 +/- 13% with 5.6 +/- 4% of these cells coexpressing CD34. An average of 17.5% of clonogenic hematopoietic progenitors derived from CD45(neg)PFV precursors expressed the retroviral transgene. Addition of serum to cultures after retroviral exposure supported transgene expression in resulting hematopoietic cells derived from hemogenic CD45(neg)PFV precursors. Our study represents the first report to demonstrate that retroviral transduction systems, similar to those used currently in clinical gene therapy protocols, are capable of efficient transduction of hematopoietic progenitors derived from hESCs.

Meng, G., et al. (2008). "A novel method for generating xeno-free human feeder cells for human embryonic stem cell culture." Stem Cells Dev **17**(3): 413-422.

Long-term cultures of human embryonic stem (hES) cells require a feeder layer for maintaining cells in an undifferentiated state and increasing karyotype stability. In routine hES cell culture, mouse embryonic fibroblast (MEF) feeders and animal component-containing media (FBS or serum replacement) are commonly used. However, the use of animal materials increases the risk of transmitting pathogens to hES cells and therefore is not optimal for use in cultures intended for human transplantation. There are other

limitations with conventional feeder cells, such as MEFs, which have a short lifespan and can only be propagated five to six passages before senescing. Several groups have investigated maintaining existing hES cell lines and deriving new hES cell lines on human feeder layers. However, almost all of these human source feeder cells employed in previous studies were derived and cultured in animal component conditions. Even though one group previously reported the derivation and culture of human foreskin fibroblasts (HFFs) in human serum-containing medium, this medium is not optimal because HFFs routinely undergo senescence after 10 passages when cultured in human serum. In this study we have developed a completely animal-free method to derive HFFs from primary tissues. We demonstrate that animal-free (AF) HFFs do not enter senescence within 55 passages when cultured in animal-free conditions. This methodology offers alternative and completely animal-free conditions for hES cell culture, thus maintaining hES cell morphology, pluripotency, karyotype stability, and expression of pluripotency markers. Moreover, no difference in hES cell maintenance was observed when they were cultured on AF-HFFs of different passage number or independent derivations.

Mengarelli, I., et al. (2016). "Use of Multicolor Flow Cytometry for Isolation of Specific Cell Populations Deriving from Differentiated Human Embryonic Stem Cells." Methods Mol Biol **1307**: 191-203.

Flow Cytometry-Sorting (FCM-Sorting) is a technique commonly used to identify and isolate specific types of cells from a heterogeneous population of live cells. Here we describe a multicolor flow cytometry technique that uses five distinct cell surface antigens to isolate four live populations with different surface antigen profiles. These profiles were used to help distinguishing between neural and nonneural (the lens) ectoderm derivatives within a highly heterogenous population of differentiating human embryonic stem cells (hESC).

Merkely, B., et al. (2015). "Signaling via PI3K/FOXO1A pathway modulates formation and survival of human embryonic stem cell-derived endothelial cells." Stem Cells Dev **24**(7): 869-878.

Vascular derivatives of human embryonic stem cells (hESC) are being developed as sources of tissue-specific cells for organ regeneration. However, identity of developmental pathways that modulate the specification of endothelial cells is not known yet. We studied phosphatidylinositol 3-kinase (PI3K)-Forkhead box O transcription factor 1A (FOXO1A) pathways during differentiation of hESC toward endothelial lineage and on proliferation, maturation, and cell death of hESC-derived endothelial cells (hESC-EC). During differentiation of hESC, expression of FOXO1A transcription factor was linked to the expression of a cluster of angiogenesis- and vascular remodeling-related genes. PI3K inhibitor LY294002 activated FOXO1A and induced formation of CD31(+) hESC-EC. In contrast, differentiating hESC with silenced FOXO1A by small interfering RNA (siRNA) showed lower mRNA levels of CD31 and angiopoietin2. LY294002 decreased proliferative activity of purified hESC-EC, while FOXO1A siRNA increased their proliferation. LY294002 inhibits migration and tube formation of hESC-EC; in contrast, FOXO1A siRNA increased in vitro tube formation activity of hESC-EC. After in vivo conditioning of cells in athymic nude rats, cells retain their low FOXO1A expression levels. PI3K/FOXO1A pathway is important for function and survival of hESC-EC and in the regulation of endothelial cell fate. Understanding these properties of hESC-EC may help in future applications for treatment of injured organs.

Merle, N., et al. (2012). "ATAD3B is a human embryonic stem cell specific mitochondrial protein, re-expressed in cancer cells, that functions as dominant negative for the ubiquitous ATAD3A." Mitochondrion **12**(4): 441-448.

Here we report on the identification of a human pluripotent embryonic stem cell (hESC) specific mitochondrial protein that is re-expressed in cancer cells, ATAD3B. ATAD3B belongs to the AAA+ ATPase ATAD3 protein family of mitochondrial proteins specific to multicellular eukaryotes. Using loss- and gain-of-function approaches, we show that ATAD3B associates with the ubiquitous ATAD3A species, negatively regulates the interaction of ATAD3A with matrix nucleoid complexes and contributes to a mitochondria fragmentation phenotype. We conclude that ATAD3B is a negative regulator of ATAD3A and may function as an adaptor of mitochondrial homeostasis and metabolism in hESCs and cancer cells.

Metallo, C. M., et al. (2008). "The response of human embryonic stem cell-derived endothelial cells to shear stress." Biotechnol Bioeng **100**(4): 830-837.

An important physiological function of vascular endothelial cells is to detect and respond to physical stimuli. While many efforts have been made to derive endothelial cells from human embryonic stem cells (hESCs), the ability of these derivatives to respond to mechanical forces has yet to be ascertained. hESC-derived endothelial cells (hEECs) were obtained by coculturing hESCs with OP9 stromal cells. Here we applied physiologic levels of shear stress to hEECs in

a parallel plate flow chamber and observed changes in cell morphology and gene expression, comparing the response to that of human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells (HMVECs). Shear induced hEECs to elongate and align in the direction of flow, and their overall transcriptional response to shear was similar to the primary cells tested. In response to shear in hEECs, COX2 and MMP1 were upregulated four- and threefold, MCP1 and VCAM1 expression decreased over fivefold, and ICAM1 and TPA were downregulated almost threefold. TGFbeta1 and SOD2 transcription exhibited no change under the conditions tested. Additionally, preshearing of hEECs mitigated TNFalpha-induced VCAM1 surface expression. These findings suggest that hEECs are capable of functionally responding to changes in fluid shear stress by modulating gene expression and cell morphology.

Mochizuki, H., et al. (2011). "Effect of glucose concentration during embryoid body (EB) formation from mouse embryonic stem cells on EB growth and cell differentiation." J Biosci Bioeng **111**(1): 92-97.

Embryoid body (EB) formation is an important step in the differentiation of pluripotent stem cells. Although glucose concentration is physiologically maintained at 5.5mM (low glucose; LG) in vivo, a medium containing 25 mM glucose (high glucose; HG) has been widely used for forming EBs in vitro. In this study, we investigated the effect of glucose concentration during EB formation from mouse embryonic stem (ES) cells on EB growth and cell differentiation. EBs were formed under various glucose concentrations: 40, 25, 5.5, and 0mM. Cells aggregated to form EBs regardless of glucose concentration, but 0mM glucose was not appropriate for supporting EB growth as compared with 25 mM glucose. The EBs that formed in the presence of 5.5mM glucose (LG-EBs) were similar both in terms of appearance and decreased expression levels of undifferentiated-ES-cell-marker genes to the EBs that formed in the presence of 25 mM glucose (HG-EBs). However, there was a difference in the propensity for cell differentiation between LG-EBs and HG-EBs. In directed differentiation cultures of EBs into cardiomyocytes and neuronal cells, the HG-EBs more efficiently generated beating cardiac muscle, and the LG-EBs more specifically generated betaIII-tubulin-positive cells. These findings demonstrate that the high-glucose (25 mM) condition was not necessary for EB formation in mouse ES cells, whereas the glucose concentration during EB formation affects the propensity for cell differentiation in the attachment cultures of formed EBs. The physiological low-glucose (5.5mM) condition was suitable for forming EBs directed toward neuronal cell differentiation in mouse ES cells.

Moghadam, F. H., et al. (2009). "Transplantation of primed or unprimed mouse embryonic stem cell-derived neural precursor cells improves cognitive function in Alzheimerian rats." Differentiation **78**(2-3): 59-68.

Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by progressive and irreversible decline of memory. Neuropathological features include the progressive degeneration of cholinergic neurons in the forebrain cholinergic projection system especially nucleus basalis of Meynert (nbM). New cell therapeutic approaches for the replacement of degenerated cells are being researched. The aim of this study was to investigate the production of cholinergic neurons from mouse embryonic stem cells (ESCs) and potential for utilizing ESC-derived neuronal precursor cells (NPCs) and primed NPCs (PNPCs) for cell restorative therapy in a rodent model of AD. NPCs were produced by growth factor-mediated selection under serum-free conditions and differentiated better into cholinergic neurons when NPCs primed with Shh (approximately 22%) in comparison with different cholinergic promoting factors. Behavioral assessment of unilateral nbM ibotenic acid-lesioned rats by Morris water maze and spatial probe test revealed a significant behavioral improvement in memory deficits following transplantation with NPCs and/or PNPCs. Immunohistochemical analysis revealed that the majority (approximately 70%) of the NPCs and/or PNPCs retained neuronal phenotype and approximately 40% of them had a cholinergic cell phenotype following transplantation with no tumor formation, indicating that these may be safe for transplantation. This experimental study has important implications as it suggests that the transplantation of mouse ESC-derived NPCs and/or following commitment to a cholinergic cell phenotype can promote behavioral recovery in a rodent model of AD.

Mohammadi Ghahhari, N., et al. (2018). "Secretome of Aggregated Embryonic Stem Cell-Derived Mesenchymal Stem Cell Modulates the Release of Inflammatory Factors in Lipopolysaccharide-Induced Peripheral Blood Mononuclear Cells." Iran Biomed J **22**(4): 237-245.

Background: Bone marrow mesenchymal stem cells (BM-MSCs) have emerged as a potential therapy for various inflammatory diseases. Because of some limitations, several recent studies have suggested the use of embryonic stem cell-derived MSCs (ESC-MSCs) as an alternative for BM-MSCs. Some of the therapeutic effects of the ESC-MSCs are related to the

secretion of a broad array of cytokines and growth factors, known as secretome. Harnessing this secretome for therapeutic applications requires the optimization of production of secretary molecules. It has been shown that aggregation of MSCs into 3D spheroids, as a preconditioning strategy, can enhance immunomodulatory potential of such cells. In this study, we investigated the effect of secretome derived from human ESC-MSCs (hESC-MSCs) spheroids on secretion of IL-1beta, IL-10, and tumor necrosis factor alpha (TNF-alpha) from lipopolysaccharide (LPS)-induced peripheral blood mononuclear cells (PBMCs). Methods: In the present study, after immunophenotyping and considering mesodermal differentiation of hESC-MSCs, the cells were non-adherently grown to prepare 3D aggregates, and then conditioned medium or secretome was extracted from the cultures. Afterwards, the anti-inflammatory effects of the secretome were assessed in an in vitro model of inflammation. Results: Results from this study showed that aggregate-prepared secretome from hESC-MSCs was able to significantly decrease the secretion of TNF-alpha (301.7 +/- 5.906, $p < 0.0001$) and IL-1beta (485.2 +/- 48.38, $p < 0.001$) from LPS-induced PBMCs as the indicators of inflammation, in comparison with adherent culture-prepared secretome (TNF-alpha: 166.6 +/- 8.04, IL-1beta: 125.2 +/- 2.73). Conclusion: Our study indicated that cell aggregation can be an appropriate strategy to increase immunomodulatory characteristics of hESC-MSCs.

Mohib, K., et al. (2012). "Embryonic stem cell-derived factors inhibit T effector activation and induce T regulatory cells by suppressing PKC-theta activation." PLoS One **7**(3): e32420.

Embryonic stem cells (ESCs) possess immune privileged properties and have the capacity to modulate immune activation. However, the mechanisms by which ESCs inhibit immune activation remain mostly unknown. We have previously shown that ESC-derived factors block dendritic cell maturation, thereby indirectly affecting T cell activation. Here, we show that ESC-derived factors also directly affect T cell activation. We provide the first demonstration that ESC-derived factors significantly down-regulated the expressions of IL-2 and IFN-gamma, while markedly up-regulating the expression of IL-10, TGF-beta, and Treg transcription factor Foxp3 in CD4+ CD25+ T cells. Furthermore, ESC-derived factors robustly suppressed T cell proliferation in response to the protein kinase C-theta (PKC-theta) activator phorbol 12-myristate 13-acetate (PMA). Western blot analysis indicated that ESC-derived factors prevented PKC-theta phosphorylation without influencing total PKC-theta levels. Moreover, IkappaB-alpha degradation was abrogated, confirming absence of PKC-theta activity. The impact of ESC-derived factors on PKC-theta activation appeared to be specific since other upstream T cell signaling components were not affected. In conclusion, ESCs appear to directly impact T cell activation and polarization by negatively regulating the PKC-theta pathway.

Mohib, K., et al. (2010). "Human embryonic stem cell-extracts inhibit the differentiation and function of monocyte-derived dendritic cells." Stem Cell Rev **6**(4): 611-621.

Embryonic stem cells (ESC) possess inherent properties of immune privilege with the capacity to evade allogeneic immune responses. Moreover, ESCs have been shown to prevent immune activation in response to third party antigen presenting cells in vitro and have the capacity to promote allograft survival in vivo. However, clinical use of human ESCs to treat immunological disorders may risk teratoma or ectopic tissue formation. Here, we show that cellular extracts from both human and mouse ESCs retain the immune modulatory properties of intact cells. ESC-extracts that contained 12-24 mug of total protein effectively prevented T cell proliferation in allogeneic mixed lymphocyte reactions (MLR), whereas control fibroblast extracts did not affect proliferation. Cellular mechanisms underlying hESC extract-mediated immune modulation involve the maturation of monocyte derived dendritic cells (mDC). hESC extract-treated mDCs had reduced surface expression of co-stimulatory and maturation markers CD80, HLA-DR and CD83 and secreted lower levels of IL12p40. Accordingly, hESC extract-treated DCs were found to be poor stimulators of purified allogeneic T cells compared to those DCs treated with vehicle or fibroblast extracts. Our results demonstrate that ESC extracts retain the immune modulatory properties of ESCs and for the first time demonstrates that ESC derived factors can inhibit human mDC maturation and function.

Mokhber Dezfouli, M. R., et al. (2019). "Hydrocortisone Promotes Differentiation of Mouse Embryonic Stem Cell-Derived Definitive Endoderm toward Lung Alveolar Epithelial Cells." Cell J **20**(4): 469-476.

Objective: The ability to generate lung alveolar epithelial type II (ATII) cells from pluripotent stem cells (PSCs) enables the study of lung development, regenerative medicine, and modeling of lung diseases. The establishment of defined, scalable differentiation methods is a step toward this goal. This study intends to investigate the competency of small molecule induced mouse embryonic stem cell-derived definitive endoderm (mESC-DE) cells towards ATII cells.

Materials and Methods: In this experimental study, we designed a two-step differentiation protocol. mESC line Royan B20 (RB20) was induced to differentiate into DE (6 days) and then into ATII cells (9 days) by using an adherent culture method. To induce differentiation, we treated the mESCs for 6 days in serum-free differentiation (SFD) media and induced them with 200 nM small molecule inducer of definitive endoderm 2 (IDE2). For days 7-15 (9 days) of induction, we treated the resultant DE cells with new differentiation media comprised of 100 ng/ml fibroblast growth factor (FGF2) (group F), 0.5 mug/ml hydrocortisone (group H), and A549 conditioned medium (A549 CM) (group CM) in SFD media. Seven different combinations of factors were tested to assess the efficiencies of these factors to promote differentiation. The expressions of DE- and ATII-specific markers were investigated during each differentiation step. Results: Although both F and H (alone and in combination) promoted differentiation through ATII-like cells, the highest percentage of surfactant protein C (SP-C) expressing cells (~37%) were produced in DE-like cells treated by F+H+CM. Ultrastructural analyses also confirmed the presence of lamellar bodies (LB) in the ATII-like cells. Conclusion: These results suggest that hydrocortisone can be a promoting factor in alveolar fate differentiation of IDE2-induced mESC-DE cells. These cells have potential for drug screening and cell-replacement therapies.

Moller, C., et al. (2007). "Bcl-2 and Bcl-XL are indispensable for the late phase of mast cell development from mouse embryonic stem cells." Exp Hematol **35**(3): 385-393.

OBJECTIVE: The aim of this study was to determine the importance of the prosurvival factors Bcl-2 and Bcl-XL for mast cell development and survival. METHODS: bcl-x (-/-) and bcl-2(-/-) mouse embryonic stem cells were maintained in medium supplemented with either interleukin (IL)-3 or IL-3 in combination with stem cell factor (SCF) to favor mast cell development. The development of Bcl-2 family deficient embryonic stem cell-derived mast cells (ESMCs) was monitored and Bcl-2 family gene expression and cell numbers were analyzed. RESULTS: Deficiency in either bcl-x or bcl-2 totally inhibited the development of ESMCs when IL-3 alone was used as a mast cell growth factor. Intriguingly, when IL-3 was used in combination with SCF, the ESMCs developed normally the first 2 weeks but thereafter the cell numbers dropped drastically. The remaining ESMCs express mouse mast cell protease 1, suggesting a mucosal-like phenotype. ESMCs lacking bcl-x or bcl-2 exhibited strong expression of A1, another prosurvival Bcl-2 family member. CONCLUSION: For the first time we provide direct evidence that both bcl-x and bcl-2 are indispensable for mast cell survival during the late phase of their development.

Mombaerts, P., et al. (1991). "Creation of a large genomic deletion at the T-cell antigen receptor beta-subunit locus in mouse embryonic stem cells by gene targeting." Proc Natl Acad Sci U S A **88**(8): 3084-3087.

Recently it has become possible to introduce predesigned mutations into a given gene in the mouse germ line by homologous recombination in embryonic stem cells. The mutations are usually introduced by inserting the neomycin phosphotransferase gene into an exon of a particular gene. Here we describe an extension of this method that can result in at least a 15-kilobase-long deletion. The deletion created in the present work encompasses one of the two diversity gene segments of the mouse T-cell receptor beta-subunit locus, 10 out of the 12 joining gene segments, and both constant gene segments. This strategy is a valuable alternative to sequential targeting of multiple genes forming a gene cluster, could simplify the construction of plasmids to be used for targeting, and could be the solution for inactivating small genes that have eluded conventional targeting approaches.

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.

Montacir, H., et al. (2017). "The Cell-Surface N-Glycome of Human Embryonic Stem Cells and Differentiated Hepatic Cells thereof." Chembiochem **18**(13): 1234-1241.

Human embryonic stem cells (hESCs) are pluripotent stem cells that offer a wide range of applications in regenerative medicine. In addition, they have been proposed as an appropriate alternative source of hepatocytes. In this work, hESCs were differentiated into definitive endodermal cells (DECs), followed by maturation into hepatocyte-like cells (HLCs). Their cell-surface N-glycome was profiled and also compared with that of primary human hepatocytes (PHHs). Undifferentiated hESCs contained large amounts of high-mannose N-glycans. In contrast, complex-type N-glycans such as asialylated or monosialylated biantennary and triantennary N-glycans were dominant in HLCs, and fully galactosylated structures were significantly more abundant than in undifferentiated hESCs. The cell-surface N-glycosylation of PHHs was more biologically processed than that of HLCs, with bisialylated biantennary and trisialylated triantennary structures predominant. This is the first report of the cell surface N-glycome of PHHs and of HLCs being directly generated from hESCs without embryoid body formation.

Moon, S. H., et al. (2011). "A system for treating ischemic disease using human embryonic stem cell-derived endothelial cells without direct incorporation." Biomaterials **32**(27): 6445-6455.

Despite studies on the use of human embryonic stem cell (hESC) derivatives to treat ischemic diseases, there are technical safety issues that have yet to be resolved. Herein, we sought to develop a method for using secreted angiogenic factors from hESC-derived endothelial cell derivatives (hESC-ECs), while avoiding direct cell incorporation, to reduce tumorigenesis or unidentified side effects of injected cells in vivo. Multicellular aggregation of hESC-ECs (MA-hESC-ECs) increases survivability, and encapsulation in Matrigel (EnMA-hESC-ECs) blocks the cells' in vivo migration. To examine the therapeutic effects of EnMA-hESC-ECs, we implanted both forms of hESC-ECs in a mouse model of hindlimb ischemia. Treatment with EnMA-hESC-ECs suppressed limb loss and tissue damage with no noticeable side effects, such as tumorigenesis or teratoma formation, and the Matrigel implant could be easily removed after the procedure. Thus, MA and the encapsulation system are effective techniques for utilizing humoral factors secreted by hESC derivatives that aid in the survivability and safety. We expect these results to contribute to the thriving stem cell field by improving the bioavailability of hESC derivatives for regenerative medicine.

Moore, J. C., et al. (2005). "Human embryonic stem cells: genetic manipulation on the way to cardiac cell therapies." Reprod Toxicol **20**(3): 377-391.

Almost 7 years after their first derivation from human embryos, a pressing urgency to deliver the promises of therapies based on human embryonic stem cells (hESC) has arisen. Protocols have been developed to support long-term growth of undifferentiated cells and partially direct differentiation to specific cell lineages. The stage has almost been set for the next step: transplantation in animal models of human disease. Here, we review the state-of-the-art with respect to the transplantation of embryonic stem cell-derived heart cells in animals.

One problem affecting progress in this area and functional analysis in vivo in general, is the availability of genetically marked hESC. There are only a few cell lines that express reporter genes ubiquitously, and none is associated with particular lineages; a major hurdle has been the resistance of hESC to established infection and chemical transfection methodologies to introduce ectopic genes. The methods that have been successful are reviewed. We also describe the processes for generating a new, genetically-modified hESC line that constitutively expresses GFP as well as some of its characteristics, including its ability to form cardiomyocytes with electrophysiological properties of ventricular-like cells.

Moore, K. E., et al. (2006). "Alternative sources of adult stem cells: a possible solution to the embryonic stem cell debate." Gend Med **3**(3): 161-168.

The complex moral and ethical debate surrounding the definition of the origins of human life, together with conflicting current and proposed legislation on state and federal levels, is hindering the course of research into the therapeutic uses of human embryonic stem cells. However, newly identified sources of adult stem cells, free from many of the ethical and legal concerns attached to embryonic stem cell research, may offer great promise for the advancement of medicine. These alternative sources may alleviate the need to resolve the stem cell debate before further therapeutic benefits of stem cell research can be realized. While legislation and ethics evolve to address the legal and moral issues of embryonic stem cell research, innovative researchers will continue to search for and find real and present solutions for cell-based therapies using adult stem cells.

Moore, R., et al. (2014). "Cell adhesion and sorting in embryoid bodies derived from N- or E-cadherin deficient murine embryonic stem cells." Biol Open **3**(2): 121-128.

The primitive endoderm epithelial structure in mouse blastocysts forms following cell differentiation and subsequent sorting, and this two-step process can be reproduced in vitro using an embryoid body model. We found that in the chimeric embryoid bodies consisting of paired wildtype and E-cadherin null ES cells, the wildtype sorted to the center and were enveloped by the less adhesive E-cadherin null cells, in accord with Steinberg's hypothesis. However, wildtype and N-cadherin null ES cells intermixed and did not segregate, a situation that may be explained by Albert Harris' modified principle, which incorporates the unique properties of living cells. Furthermore, in chimeric embryoid bodies composed of N-cadherin and E-cadherin null ES cells, the two weakly interacting cell types segregated but did not envelop one another. Lastly, the most consistent and striking observation was that differentiated cells sorted to the surface and formed an enveloping layer, regardless of the relative cell adhesive affinity of any cell combination, supporting the hypothesis that the ability of the differentiated cells to establish apical polarity is the determining factor in surface sorting and positioning.

Morey, L., et al. (2015). "Polycomb Regulates Mesoderm Cell Fate-Specification in Embryonic Stem Cells through Activation and Repression Mechanisms." Cell Stem Cell **17**(3): 300-315.

Polycomb complexes (PRC1 and PRC2) are essential regulators of epigenetic gene silencing in embryonic and adult stem cells. Emerging evidence suggests that the core subunit composition regulates distinct biological processes, yet little is known about the mechanistic underpinnings of how differently composed Polycomb complexes instruct and maintain cell fate. Here we find that Mel18, also known as Pcgf2 and one of six Pcgf paralogs, uniquely regulates PRC1 to specify mesoderm cell fate in embryonic stem cells. Mechanistically, Mel18 functions as a classical Polycomb protein during early cardiac mesoderm differentiation by repressing pluripotency, lineage specification, late cardiac differentiation, and negative regulators of the BMP pathway. However, Mel18 also positively regulates expression of key mesoderm transcription factors, revealing an unexpected function of Mel18 in gene activation during cardiac differentiation. Taken together, our findings reveal that Mel18 is required to specify PRC1 function in both a context- and stage-specific manner.

Mori, T., et al. (2017). "Novel phototoxicity assay using human embryonic stem cell-derived retinal pigment epithelial cells." Toxicology **378**: 1-9.

Some chemicals are harmful in to light-exposed tissues such as skin and eyes. The 3T3 Neutral Red Uptake Phototoxicity Test has been validated and adopted by the Organization of Economic and Community Development (OECD) as a method of evaluating chemical phototoxicity using mouse 3T3 fibroblasts. However, the high rate of false positive results associated with this test eventually led to increased laboratory animal usage. Although the eye is vulnerable to light damage because of constant exposure to environmental radiation, few approaches are available to predict ocular phototoxicity in humans. Here, we propose a tier one test that identifies the potential ocular phototoxicity of chemical substances. Using a three-dimensional culture technique, human embryonic stem cells (hESCs) were differentiated to retinal pigment epithelial cell (RPE) precursors. The precursors after prolonged treatment with FBS formed

a uniform hexagonal lattice of cells with well-developed tight junctions and time-dependent elevation of melanin content and RPE maturation marker levels. Hierarchical clustering of gene transcripts revealed that hESC-derived RPEs were very similar to tissue-derived adult RPEs. Interestingly, there were a high percentage of chemicals eliciting a positive response in 3T3 cells and negative in hESC-derived RPEs under the experimental conditions used in the phototoxicity test. The response to treatment of hESC-derived RPEs with these negative chemicals became positive at a higher dose of UVA irradiation; however, the biological responses to these chemicals differed between the two cells. Taken together, we conclude that hESC-derived RPEs are novel tool for future toxicological and mechanistic studies of ocular phototoxicity in humans.

Morizane, A., et al. (2002). "Optimal conditions for in vivo induction of dopaminergic neurons from embryonic stem cells through stromal cell-derived inducing activity." J Neurosci Res **69**(6): 934-939.

A method of inducing dopamine (DA) neurons from mouse embryonic stem (ES) cells by stromal cell-derived inducing activity (SDIA) was previously reported. When transplanted, SDIA-induced DA neurons integrate into the mouse striatum and remain positive for tyrosine hydroxylase (TH) expression. In the present study, to optimize the transplantation efficiency, we treated mouse ES cells with SDIA for various numbers of days (8-14 days). SDIA-treated ES cell colonies were isolated by papain treatment and then grafted into the 6-hydroxydopamine (6-OHDA)-lesioned mouse striatum. The ratio of the number of surviving TH-positive cells to the total number of grafted cells was highest when ES cells were treated with SDIA for 12 days before transplantation. This ratio revealed that grafting cell colonies was more efficient for obtaining TH-positive cells in vivo than grafting cell suspensions. When we grafted a cell suspension of 2 x 10(5), 2 x 10(4), or 2 x 10(3) cells into the 6-OHDA-lesioned mouse striatum, we observed only a few surviving TH-positive cells. In conclusion, inducing DA neurons from mouse ES cells by SDIA for 12 days and grafting cell colonies into mouse striatum was the most effective method for the survival of TH-positive neurons in vivo.

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.

Mostafavi-Pour, Z., et al. (2012). "Expression of a2, a5 and a6 subunits of integrin in de-differentiated NIH3T3 cells by cell-free extract of embryonic stem cells." Mol Biol Rep **39**(7): 7339-7346.

Generation of patient specific stem cells is among the ultimate goals in regenerative medicine. Such a cell needs to be functional when it transplants. Interaction between the matrix proteins and integrin adjust many cells' function such as adhesion, migration, cell cycle and self renewal in stem cells. In this study, NIH3T3 cells were dedifferentiated by mouse Embryonic Stem Cell (mESC) extract. The expression of pluripotency markers as well as a2, a5 and a6 integrin subunits were determined. NIH3T3 cells treated with mESC extract showed noticeable changes in cell morphology as early as day 2 post-treatment forming colonies similar to typical mESC morphology by day 8, after three passages. Alkaline phosphatase (ALP) assay and immunocytochemistry staining were performed for the induced reprogrammed cells. The results indicated that these colonies showed the ALP activity and they express Sox2 and Nanog. RT-PCR

revealed that the colonies also express Oct3/4. NIH3T3 cells, ESC and reprogrammed cells expressed a2 integrin. a5 integrin expression was greatest in reprogrammed cells followed by the expression of this integrin in NIH3T3 which in turn was more than in ESC. a6A integrin was expressed in NIH3T3 cells while a6B integrin was expressed in ESC and in very low quantity was expressed in reprogrammed cells. These data provide evidence for both the generation of ES like cells from differentiated somatic cells and the expression profile of integrins after de-differentiation by mESC extract.

Motohashi, T., et al. (2007). "Multipotent cell fate of neural crest-like cells derived from embryonic stem cells." Stem Cells **25**(2): 402-410.

Neural crest cells migrate throughout the embryo and differentiate into diverse derivatives: the peripheral neurons, cranial mesenchymal cells, and melanocytes. Because the neural crest cells have critical roles in organogenesis, detailed elucidation of neural crest cell differentiation is important in developmental biology. We recently reported that melanocytes could be induced from mouse ESCs. Here, we improved the culture system and showed the existence of neural crest-like precursors. The addition of retinoic acid to the culture medium reduced the hematopoiesis and promoted the expression of the neural crest marker genes. The colonies formed contained neural crest cell derivatives: neurons and glial cells, together with melanocytes. This suggested that neural crest-like cells assuming multiple cell fates had been generated in these present cultures. To isolate the neural crest-like cells, we analyzed the expression of c-Kit, a cell-surface protein expressed in the early stage of neural crest cells in vivo. The c-Kit-positive (c-Kit (+)) cells appeared as early as day 9 of the culture period and expressed the transcriptional factors Sox10 and Snail, which are expressed in neural crest cells. When the c-Kit (+) cells were separated from the cultures and recultured, they frequently formed colonies containing neurons, glial cells, and melanocytes. Even a single c-Kit (+) cell formed colonies that contained these three cell types, confirming their multipotential cell fate. The c-Kit (+) cells were also capable of migrating along neural crest migratory pathways in vivo. These results indicate that the c-Kit (+) cells isolated from melanocyte-differentiating cultures of ESCs are closely related to neural crest cells.

Motomura, Y., et al. (2006). "Embryonic stem cell-derived dendritic cells expressing glypican-3, a recently identified oncofetal antigen, induce protective immunity against highly metastatic mouse melanoma, B16-F10." Cancer Res **66**(4): 2414-2422.

We have recently established a method to generate dendritic cells from mouse embryonic stem cells. By introducing exogenous genes into embryonic stem cells and subsequently inducing differentiation to dendritic cells (ES-DC), we can now readily generate transfectant ES-DC expressing the transgenes. A previous study revealed that the transfer of genetically modified ES-DC expressing a model antigen, ovalbumin, protected the recipient mice from a challenge with an ovalbumin-expressing tumor. In the present study, we examined the capacity of ES-DC expressing mouse homologue of human glypican-3, a recently identified oncofetal antigen expressed in human melanoma and hepatocellular carcinoma, to elicit protective immunity against glypican-3-expressing mouse tumors. CTLs specific to multiple glypican-3 epitopes were primed by the in vivo transfer of glypican-3-transfectant ES-DC (ES-DC-GPC3). The transfer of ES-DC-GPC3 protected the recipient mice from subsequent challenge with B16-F10 melanoma, naturally expressing glypican-3, and with glypican-3-transfectant MCA205 sarcoma. The treatment with ES-DC-GPC3 was also highly effective against i.v. injected B16-F10. No harmful side effects, such as autoimmunity, were observed for these treatments. The depletion experiments and immunohistochemical analyses suggest that both CD8+ and CD4+ T cells contributed to the observed antitumor effect. In conclusion, the usefulness of glypican-3 as a target antigen for antimelanoma immunotherapy was thus shown in the mouse model using the ES-DC system. Human dendritic cells expressing glypican-3 would be a promising means for therapy of melanoma and hepatocellular carcinoma.

Mountford, P., et al. (1998). "Maintenance of pluripotential embryonic stem cells by stem cell selection." Reprod Fertil Dev **10**(7-8): 527-533.

As gastrulation proceeds, pluripotential stem cells with the capacity to contribute to all primary germ layers disappear from the mammalian embryo. The extinction of pluripotency also occurs during the formation of embryoid bodies from embryonic stem (ES) cells. In this report we show that if the initial differentiated progeny are removed from ES cell aggregates, further differentiation does not proceed and the stem cell population persists and expands. Significantly, the presence of even minor populations of differentiated cells lead to the complete loss of stem cells from the cultures. This finding implies that the normal elimination of pluripotent cells is dictated by inductive signals provided by differentiated progeny. We have exploited this observation to develop a strategy for the isolation of pluripotential cells. This approach, termed stem cell selection, may have

widespread applicability to the derivation and propagation of stem cells.

Mousa, S. A., et al. (2010). "Stress resistant human embryonic stem cells as a potential source for the identification of novel cancer stem cell markers." Cancer Lett **289**(2): 208-216.

Cancer stem cells are known for their inherent resistance to therapy. Here we investigated whether normal stem cells with acquired resistance to stress can be used to identify novel markers of cancer stem cells. For this, we generated a human embryonic stem cell line resistant to Trichostatin A and analyzed changes in its gene expression. The resistant cells over-expressed various genes associated with tumor aggressiveness, many of which are also expressed in the CD133+ glioma cancer stem cells. These findings suggest that stress-resistant stem cells generated in vitro may be useful for the discovery of novel markers of cancer stem cells.

Mueller, D., et al. (2005). "Transplanted human embryonic germ cell-derived neural stem cells replace neurons and oligodendrocytes in the forebrain of neonatal mice with excitotoxic brain damage." J Neurosci Res **82**(5): 592-608.

Stem cell therapy is a hope for the treatment of some childhood neurological disorders. We examined whether human neural stem cells (hNSCs) replace lost cells in a newborn mouse model of brain damage. Excitotoxic lesions were made in neonatal mouse forebrain with the N-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QA). QA induced apoptosis in neocortex, hippocampus, striatum, white matter, and subventricular zone. This degeneration was associated with production of cleaved caspase-3. Cells immunopositive for inducible nitric oxide synthase were present in damaged white matter and subventricular zone. Three days after injury, mice received brain parenchymal or intraventricular injections of hNSCs derived from embryonic germ (EG) cells. Human cells were prelabeled in vitro with DiD for in vivo tracking. The locations of hNSCs within the mouse brain were determined through DiD fluorescence and immunodetection of human-specific nestin and nuclear antigen 7 days after transplantation. hNSCs survived transplantation into the lesioned mouse brain, as evidenced by human cell markers and DiD fluorescence. The cells migrated away from the injection site and were found at sites of injury within the striatum, hippocampus, thalamus, and white matter tracts and at remote locations in the brain. Subsets of grafted cells expressed neuronal and glial cell markers. hNSCs restored partially the complement of striatal neurons in brain-damaged mice. We conclude that human EG cell-derived NSCs can engraft successfully into injured newborn brain, where they can survive and disseminate into the lesioned areas, differentiate into neuronal and glial cells, and replace lost neurons. (c) 2005 Wiley-Liss, Inc.

Mummery, C. L. (2002). "[Human embryonic stem cells: possibilities for future cell transplantation therapy]." Tijdschr Diergeneeskd **127**(6): 189-191.

Human embryonic stem cells are of great importance, and Parkinson's disease is given as an example of a condition that could benefit from the development of stem cell-based transplantation therapies. The reason for this is fairly obvious: the disease is caused by the loss of only one cell type from the brain that has one major function, namely the production of dopamine. Replacement of these cells should in principle cure the disease. But what are stem cells and how far is scientific research from being able to offer stem cell-based therapy in the clinic to patients suffering from Parkinson's disease, and other chronic diseases? These questions are addressed here together with a critical evaluation of short and long-term clinical perspectives, and a discussion of possible alternatives such as adult stem cells.

Mummery, C. L., et al. (1993). "Fibroblast growth factor-mediated growth regulation and receptor expression in embryonal carcinoma and embryonic stem cells and human germ cell tumours." Biochem Biophys Res Commun **191**(1): 188-195.

FGFs have been implicated in the induction of mesoderm in amphibian development and are present in the mouse embryo at stages that would be appropriate for a similar function in mammals. Primitive ectoderm would then be the target tissue. We have now changes in the expression of receptors for FGFs during the differentiation of embryonal carcinoma (EC) and embryonic stem (ES) cells from the mouse. These cells resemble those of the inner cell mass and later primitive ectoderm. On Northern blots of mRNA from undifferentiated cells, transcripts for FGF R1, R2 and R3 are expressed. All are upregulated during differentiation of ES cells and are upregulated or remain constant as EC cells differentiate. FGF R4 is only expressed after differentiation to derivatives resembling parietal endoderm. By contrast in human EC cells, FGF R2 is downregulated during differentiation, FGF R1 and FGF R3 are unchanged and FGF R4 is expressed before and after differentiation. In both human and mouse EC cells three members of the FGF family (a FGF, b FGF and k FGF, also known as FGFs 1,2 and 4) are mitogenic in serum-free medium and one (KGF or FGF 7) appears to have no effect on growth although cellular morphology is altered. Differences between human

and mouse cells are primarily in the effects of heparin on the FGF-induced response.

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.

Murakami, K., et al. (2011). "Choice of random rather than imprinted X inactivation in female embryonic stem cell-derived extra-embryonic cells." Development **138**(2): 197-202.

In female mammals, one of two X chromosomes is epigenetically inactivated for gene dosage compensation, known as X inactivation (Xi). Inactivation occurs randomly in either the paternal or maternal X chromosome in all embryonic cell lineages, designated as random Xi. By contrast, in extra-embryonic cell lineages, which are segregated from somatic cell lineages in pre-implantation development, the paternal X chromosome is selectively inactivated, known as imprinted Xi. Although it is speculated that erasure of the imprinted mark on either the maternal or paternal X chromosome in somatic cell lineages might change the mode of Xi from imprinted to random, it is not known when this event is completed in development. Here, we tested the mode of Xi during the differentiation of female mouse embryonic stem (ES) cells derived from the inner cell mass (ICM) of blastocyst-stage embryos toward trophectoderm (TE) and primitive endoderm (PrE) lineages induced by artificial activation of transcription factor genes Cdx2 and Gata6, respectively. We found that random Xi occurs in both TE and PrE cells. Moreover, cloned embryos generated by the transfer of nuclei from the female ES cells showed random Xi in TE, suggesting the complete erasure of all X imprints for imprinted Xi in ICM-derived ES cells.

Muramatsu, S., et al. (2009). "Multitracer assessment of dopamine function after transplantation of embryonic stem cell-derived neural stem cells in a primate model of Parkinson's disease." Synapse **63**(7): 541-548.

The ability of primate embryonic stem (ES) cells to differentiate into dopamine (DA)-synthesizing neurons has raised hopes of creating novel cell therapies for Parkinson's disease (PD). As the primary purpose of cell transplantation in PD is restoration of dopaminergic neurotransmission in the striatum, in vivo assessment of DA function after grafting is necessary to achieve better therapeutic effects. A chronic model of PD was produced in two cynomolgus monkeys (M-1 and M-2) by systemic administration of neurotoxin. Neural stem cells (NSCs) derived from cynomolgus ES cells were implanted unilaterally in the putamen. To evaluate DA-specific functions, we used multiple [(11)C]-labeled positron emission tomography (PET) tracers, including [beta-(11)C]L-3,4-dihydroxyphenylalanine (L-[beta-(11)C]DOPA, DA precursor ligand), [(11)C]-2beta-carbomethoxy-3beta-(4-fluorophenyl)tropane ([(11)C]beta-CFT, DA transporter ligand) and [(11)C]raclopride (D (2) receptor ligand). At 12 weeks after grafting NSCs, PET demonstrated significantly increased uptake of L-[beta-(11)C]DOPA (M-1:41%, M-2:61%) and [(11)C]beta-CFT (M-1:31%, M-2:36%) uptake in the grafted putamen. In addition, methamphetamine challenge in M-2 induced reduced [(11)C]raclopride binding (16%) in the transplanted putamen, suggesting release of DA. These results show that transplantation of NSCs derived from cynomolgus monkey ES cells can restore DA function in the putamen of a primate model of PD. PET with multitracers is useful for functional studies in developing cell-based therapies against PD.

Murdoch, A., et al. (2012). "The procurement of cells for the derivation of human embryonic stem cell lines for therapeutic use: recommendations for good practice." Stem Cell Rev **8**(1): 91-99.

The donation of human embryos for the derivation of embryonic stem cell lines that may be used in the development of therapeutic products raises more complex ethical, practical and regulatory problems than the donation of embryos for non-clinical research. This review considers these issues and offers recommendations for good practice.

Murray, P., et al. (2013). "The self-renewal of mouse embryonic stem cells is regulated by cell-substratum adhesion and cell spreading." Int J Biochem Cell Biol **45**(11): 2698-2705.

Mouse embryonic stem cells (mESCs) undergo self-renewal in the presence of the cytokine, leukaemia inhibitory factor (LIF). Following LIF withdrawal, mESCs differentiate, and this is accompanied by an increase in cell-substratum adhesion and cell spreading. The purpose of this study was to investigate the relationship between cell spreading and mESC differentiation. Using E14 and R1 mESC lines, we have restricted cell spreading in the absence of LIF by either culturing mESCs on chemically defined, weakly adhesive biomaterial substrates, or by manipulating the cytoskeleton. We demonstrate that by restricting the degree of spreading by either method, mESCs can be maintained in an undifferentiated and pluripotent state. Under these conditions, self-renewal occurs without the need for LIF and is independent of nuclear translocation of tyrosine-phosphorylated STAT3 or beta-catenin, which have previously been implicated in self-renewal. We also demonstrate that the effect of restricted cell spreading on mESC self-renewal is not mediated by increased intercellular adhesion, as evidenced by the observations that inhibition of mESC adhesion using a function blocking anti E-cadherin antibody or siRNA do not promote differentiation. These results show that mESC spreading and differentiation are regulated both by LIF and by cell-substratum adhesion, consistent with the hypothesis that cell spreading is the common intermediate step in the regulation of mESC differentiation by either LIF or cell-substratum adhesion.

Toya, S. P., et al. (2011). "Interaction of a specific population of human embryonic stem cell-derived progenitor cells with CD11b+ cells ameliorates sepsis-induced lung inflammatory injury." Am J Pathol **178**(1): 313-324.

Human embryonic stem cells differentiated under mesoderm-inducing conditions have important therapeutic properties in sepsis-induced lung injury in mice. Single cell suspensions obtained from day 7 human embryoid bodies (d7EBs) injected i.v. 1 hour after cecal ligation and puncture significantly reduced lung inflammation and edema as well as production of tumor necrosis factor-alpha and interferon-gamma in lungs compared with controls, whereas interleukin-10 production remained elevated. d7EB cell transplantation also reduced mortality to 50% from 90% in the control group. The protection was ascribed to d7EB cell interaction with lung resident CD11b+ cells, and was correlated with the ability of d7EB cells to reduce it also reduced production of proinflammatory cytokines by CD11+ cells, and to endothelial NO synthase-derived NO by d7EB cells, leading to inhibition of inducible macrophage-type NO synthase activation in CD11b+ cells. The protective progenitor cells were positive for the endothelial and hematopoietic lineage marker angiotensin converting enzyme (ACE). Only the ACE+ fraction modulated the proinflammatory profile of CD11b+ cells and reduced mortality in septic mice. In contrast to the nonprotective ACE-cell fraction, the ACE+ cell fraction also produced NO. These findings suggest that an ACE+ subset of human embryonic stem cell-derived progenitor cells has a highly specialized anti-inflammatory function that ameliorates sepsis-induced lung inflammation and reduces mortality.

Zemel'ko, V. I., et al. (2011). "[Multipotent mesenchymal stem cells of desquamated endometrium: isolation, characterization and use as feeder layer for maintenance of human embryonic stem cell lines]." Tsitologiia **53**(12): 919-929.

In this study, we characterize new multipotent human mesenchymal stem cell (MSC) lines derived from desquamated (shedding) endometrium in menstrual blood. The isolated endometrial MSC (eMSC) is an adhesive to plastic heterogeneous population composed mainly of endometrial glandular and stromal cells. The established cell lines meet the criteria of the International Society for Cellular Therapy for defining multipotent human MSC of any origin. The eMSCs have positive expression of CD73, CD90, CD105, CD13, CD29, CD44 markers and the absence of expression of the hematopoietic cell surface antigens CD19, CD34, CD45, CD117, CD130 and HLA-DR (class II). Multipotency of the established eMSC is confirmed by their ability to differentiate into other mesodermal cell types such as osteocytes and adipocytes. Besides, the isolated eMSC lines partially (over 50%) express the pluripotency marker SSEA-4, but do not express Oct-4. Immunofluorescent analysis of the derived cells revealed the expression of the neural precursor markers nestin and beta-III-tubulin. This suggests a neural predisposition of the established eMSC. These cells are characterized by high rate of cell proliferation (doubling time 22-23 h) and high

cloning efficiency (about 60%). In vitro the eMSCs undergo more than 45 population doublings revealing normal karyotype without karyotipic abnormalilies. We demonstrate, that the mititotically inactivated eMSCs are perfect feeder cells for human embryonic stem cell lines (hESC) C612 and C910. The eMSC being a feeder culture maintain the pluripotent status of the hESC, which is revealed by the expression of Oct-4, alkaline phosphatase and SSEA-4. When co-culturing, hESC retain their morphology, proliferative rate for more than 40 passages and capability for spontaneous differentiation into embryoid bodies comprising the three embryonic germ layers. Thus, an easy and non-invasive extraction of the eMSC in menstrual blood, their multipotency and high proliferative activity in vitro without karyotypic abnormalities demonstrate the potential of use of these stem cells in regenerative medicine. Using the derived eMSCs as the feeder culture eliminates the risks associated with animal cells while transferring hESC to clinical setting.

Zweigerdt, R., et al. (2003). "Generation of confluent cardiomyocyte monolayers derived from embryonic stem cells in suspension: a cell source for new therapies and screening strategies." Cytotherapy **5**(5): 399-413.

BACKGROUND: Cellular cardiomyoplasty is evolving as a new strategy to treat cardiac diseases. A prerequisite is a reliable source of pure cardiomyocytes, which could also help in the exploitation of recent advances in genomics and drug screening. Our goal was to establish a robust lab-scale process for the generation of embryonic stem (ES)-cell-derived cardiomyocytes in suspension. METHODS: A 71 ES cell clone carrying a construct consisting of the alpha-cardiac myosin heavy chain (alphaMHC) promoter driving the neomycin resistance gene was used for antibiotic-driven cardiomyocyte enrichment. Rotating suspension culture was established to initiate embryoid body (EB) formation. To track growth and differentiation kinetics, cell count and flow cytometry for SSEA-I, E-cadherin (stem-cell marker)and sarcomeric myosin (cardiomyocytes marker) was performed. Oct4 expression was measured via real time (RT)-PCR. RESULTS: Cultures comprising 2.5-8 x 10(6) differentiating FS cells/mL were obtained after 9 days in rotating suspension. Upon G418 addition,vigorous contracting spheres, termed cardiac bodies (CB), developed. These cultures consisted of about 2.1 x 10(5) enriched cardiomyocytes/mL after 6- 10 days of selection. Suspensions comprising 90- 95%viable single cells were generated using an improved dissociation method. Seeding of cardiomyocytes with 7 x 10(4) cell/cm (2) resulted in a homogeneous monolayer of synchronously contracting cells. Myocyte specific immunohistochemistry indicated purity of > 99%. DISCUSSION: We have established a reliable lab-scale protocol to generate cultures of highly enriched cardiomyocytes in suspension. This will facilitate development of larger-scale processes for stem-cell based cardiomyocyte supply. An improved method is provided to derive vital suspensions of cardiomyocytes, which could be utilized for transplantation as well as for drug screening purposes.

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