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Stem Cell

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Stem Cell Research Literatures (3)

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

Introduction

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

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.

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.

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.

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.

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.

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.

Belzile, J. P., et al. (2014). "Human cytomegalovirus infection of human embryonic stem cell-derived primitive neural stem cells is restricted at several steps but leads to the persistence of viral DNA." J Virol **88**(8): 4021-4039.

UNLABELLED: Congenital human cytomegalovirus (HCMV) infection is a major cause of central nervous system structural anomalies and sensory impairments. It is likely that the stage of fetal development, as well as the state of differentiation of susceptible cells at the time of infection, affects the severity of the disease. We used human embryonic stem (ES) cell-derived primitive prerosette neural stem cells (pNSCs) and neural progenitor cells (NPCs) maintained in chemically defined conditions to study HCMV replication in cells at the early stages of neural development. In contrast to what was observed previously using fetus-derived NPCs, infection of ES cell-derived pNSCs with HCMV was nonprogressive. At a low multiplicity of infection, we observed only a small percentage of cells expressing immediate-early genes (IE) and early genes. IE expression was found to be restricted to cells negative for the anterior marker FORSE-1, and treatment of pNSCs with retinoic acid restored IE expression. Differentiation of pNSCs into NPCs restored IE expression but not the transactivation of early genes. Virions produced in NPCs and pNSCs were exclusively cell associated and were mostly non-

neural tropic. Finally, we found that viral genomes could persist in pNSC cultures for up to a month after infection despite the absence of detectable IE expression by immunofluorescence, and infectious virus could be produced upon differentiation of pNSCs to neurons. In conclusion, our results highlight the complex array of hurdles that HCMV must overcome in order to infect primitive neural stem cells and suggest that these cells might act as a reservoir for the virus. IMPORTANCE: Human cytomegalovirus (HCMV) is a betaherpesvirus that is highly prevalent in the population. HCMV infection is usually asymptomatic but can lead to severe consequences in immunosuppressed individuals. HCMV is also the most important infectious cause of congenital developmental birth defects. Manifestations of fetal HCMV disease range from deafness and learning disabilities to more severe symptoms such as microcephaly. In this study, we have used embryonic stem cells to generate primitive neural stem cells and have used these to model HCMV infection of the fetal central nervous system (CNS) in vitro. Our results reveal that these cells, which are similar to those present in the developing neural tube, do not support viral replication but instead likely constitute a viral reservoir. Future work will define the effect of viral persistence on cellular functions as well as the exogenous signals leading to the reactivation of viral replication in the CNS.

Bencsik, R., et al. (2016). "Improved transgene expression in doxycycline-inducible embryonic stem cells by repeated chemical selection or cell sorting." Stem Cell Res **17**(2): 228-234.

Transgene-mediated programming is a preeminent strategy to direct cellular identity. To facilitate cell fate switching, lineage regulating genes must be efficiently and uniformly induced. However, gene expression is often heterogeneous in transgenic systems. Consistent with this notion, a non-uniform reporter gene expression was detected in our doxycycline (DOX)-regulated, murine embryonic stem (ES) cell clones. Interestingly, a significant fraction of cells within each clone failed to produce any reporter signals upon DOX treatment. We found that the majority of these non-responsive cells neither carry reporter transgene nor geneticin/G418 resistance. This observation suggested that our ES cell clones contained non-recombined cells that survived the G418 selection which was carried out during the establishment of these clones. We successfully eliminated most of these corrupted cells with repeated chemical (G418) selection, however, even after prolonged G418 treatments, a few cells remained non-responsive due to epigenetic silencing. We found that cell sorting has been the most efficient approach to select those cells which can uniformly and stably induce the integrated transgene in this ES cell based platform. Together, our data revealed that post-cloning chemical re-selection or cell sorting strongly facilitate the production of ES cell lines with a uniform transgene induction capacity.

Bendall, S. C., et al. (2008). "Human embryonic stem cells: lessons from stem cell niches in vivo." Regen Med **3**(3): 365-376.

In vivo the stem cell niche is an essential component in controlling and maintaining the stem cells' ability to survive and respond to injury. Human embryonic stem cells (hESCs) appear to be an exception to this rule as they can be removed from their blastocytic microenvironment and maintained indefinitely in vitro. However, recent observations reveal the existence of an autonomously derived in vitro hESC niche. This provides a previously unappreciated mechanism to control hESC expansion and differentiation. Recognizing this, it may now be possible to take aspects of in vivo stem cell niches, namely extracellular matrices, paracrine signals and accessory cell types, and exploit them in order to gain fidelity in directed hESC differentiation. In doing so, routine customization of hESC lines and their application in regenerative therapies may be further enhanced using unique hESC niche-based approaches.

Bentz, K., et al. (2007). "Embryonic stem cells produce neurotrophins in response to cerebral tissue extract: Cell line-dependent differences." J Neurosci Res **85**(5): 1057-1064.

In the present study, we compare the capacity of two different embryonic stem (ES) cell lines to secrete neurotrophins in response to cerebral tissue extract derived from healthy or injured rat brains. The intrinsic capacity of the embryonic cell lines BAC7 (feeder cell-dependent cultivation) to release brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3) exceeded the release of these factors by CGR8 cells (feeder cell-free growth) by factors of 10 and 4, respectively. Nerve growth factor (NGF) was secreted only by BAC7 cells. Conditioning of cell lines with cerebral tissue extract derived from healthy or fluid percussion-injured rat brains resulted in a significant time-dependent increase in BDNF release in both cell lines. The increase in BDNF release by BAC7 cells was more pronounced when cells were incubated with brain extract derived from injured brain. However, differences in neurotrophin release associated with the origin of brain extract were at no time statistically significant. Neutrophin-3 and NGF release was inhibited when cell lines were exposed to cerebral tissue extract. The magnitude of the response to cerebral tissue extract was dependent on the intrinsic capacity of the cell lines to release neurotrophins. Our

results clearly demonstrate significant variations in the intrinsic capability of different stem cell lines to produce neurotrophic factors. Furthermore, a significant modulation of neurotrophic factor release was observed following conditioning of cell lines with tissue extract derived from rat brains. A significant modulation of neurotrophin release dependent on the source of cerebral tissue extract used was not observed.

Ben-Yehudah, A., et al. (2009). "Evaluating protocols for embryonic stem cell differentiation into insulin-secreting beta-cells using insulin II-GFP as a specific and noninvasive reporter." Cloning Stem Cells **11**(2): 245-257.

Stable and full differentiation of pluripotent stem cells into functional beta-cells offers the potential to treat type I diabetes with a theoretically inexhaustible source of replacement cells. In addition to the difficulties in directed differentiation, progress toward an optimized and reliable protocol has been hampered by the complication that cultured cells will concentrate insulin from the media, thus making it difficult to tell which, if any, cells are producing insulin. To address this, we utilized a novel murine embryonic stem cell (mESC) research model, in which the green fluorescent protein (GFP) has been inserted within the C-peptide of the mouse insulinII gene (InsulinII-GFP). Using this method, cells producing insulin are easily identified. We then compared four published protocols for differentiating mESCs into beta-cells to evaluate their relative efficiency by assaying intrinsic insulin production. Cells differentiated using each protocol were easily distinguished based on culture conditions and morphology. This comparison is strengthened because all testing is performed within the same laboratory by the same researchers, thereby removing interlaboratory variability in culture, cells, or analysis. Differentiated cells were analyzed and sorted based on GFP fluorescence as compared to wild type cells. Each differentiation protocol increased GFP fluorescence but only modestly. None of these protocols yielded more than 3% of cells capable of insulin biosynthesis indicating the relative inefficiency of all analyzed protocols. Therefore, improved beta-cells differentiation protocols are needed, and these insulin II GFP cells may prove to be an important tool to accelerate this process.

Berger, C. N., et al. (1995). "The development of haematopoietic cells is biased in embryonic stem cell chimaeras." Dev Biol **170**(2): 651-663.

The haematopoietic development of embryonic stem (ES) cell injection chimaeras was analysed using beta-galactosidase expression from an X-linked transgene as a marker to distinguish the ES-derived cell population from the host cells. The number of cells in the different haematopoietic cell subpopulations was determined by flow cytometry. When the proportions of ES-derived cells in the antigen-positive lineages were compared to the ES cell contribution to all cells in teh organs, we found an unexpected bias in the haematopoietic differentiation of ES-derived cells. ES descendants were overrepresented in the bone marrow B lymphoid cell population and the splenic myeloid cells but were underrepresented in the CD4-positive T lymphoid cells in the spleen. These results were obtained by comparison with control female animals that were X chromosome mosaic for beta-galactosidase expression. These findings of uneven contribution to haematopoietic development by ES cells indicate that the commitment of ES cell descendants may be different from that of the host cells.

Bergmans, B. A., et al. (2010). "Neurons generated from APP/APLP1/APLP2 triple knockout embryonic stem cells behave normally in vitro and in vivo: lack of evidence for a cell autonomous role of the amyloid precursor protein in neuronal differentiation." Stem Cells **28**(3): 399-406.

Alzheimer's disease amyloid precursor protein (APP) has been implicated in many neurobiologic processes, but supporting evidence remains indirect. Studies are confounded by the existence of two partially redundant APP homologues, APLP1 and APLP2. APP/APLP1/APLP2 triple knockout (APP tKO) mice display cobblestone lissencephaly and are perinatally lethal. To circumvent this problem, we generated APP triple knockout embryonic stem (ES) cells and differentiated these to APP triple knockout neurons in vitro and in vivo. In comparison with wild-type (WT) ES cell-derived neurons, APP tKO neurons formed equally pure neuronal cultures, had unaltered in vitro migratory capacities, had a similar acquisition of polarity, and were capable of extending long neurites and forming active excitatory synapses. These data were confirmed in vivo in chimeric mice with APP tKO neurons expressing the enhanced green fluorescent protein (eGFP) present in a WT background brain. The results suggest that the loss of the APP family of proteins has no major effect on these critical neuronal processes and that the apparent multitude of functions in which APP has been implicated might be characterized by molecular redundancy. Our stem cell culture provides an excellent tool to circumvent the problem of lack of viability of APP/APLP triple knockout mice and will help to explore the function of this intriguing protein further in vitro and in vivo.

Bernreuther, C., et al. (2006). "Neural cell adhesion molecule L1-transfected embryonic stem cells promote

functional recovery after excitotoxic lesion of the mouse striatum." J Neurosci **26**(45): 11532-11539.

We have generated a murine embryonic stem cell line constitutively expressing L1 at all stages of neural differentiation to investigate the effects of L1 overexpression on stem cell proliferation, migration, differentiation, cell death, and ability to influence drug-induced rotation behavior in an animal model of Huntington's disease. L1-transfected cells showed decreased cell proliferation in vitro, enhanced neuronal differentiation in vitro and in vivo, and decreased astrocytic differentiation in vivo without influencing cell death compared with nontransfected cells. L1 overexpression also resulted in an increased yield of GABAergic neurons and enhanced migration of embryonic stem cell-derived neural precursor cells into the lesioned striatum. Mice grafted with L1-transfected cells showed recovery in rotation behavior 1 and 4 weeks, but not 8 weeks, after transplantation compared with mice that had received nontransfected cells, thus demonstrating for the first time that a recognition molecule is capable of improving functional recovery during the initial phase in a syngeneic transplantation paradigm.

Berrill, A., et al. (2004). "Assessment of stem cell markers during long-term culture of mouse embryonic stem cells." Cytotechnology **44**(1-2): 77-91.

Embryonic stem (ES) cells have been in the fore front of scientific literature lately as having the potential for regeneration of many tissue types. Two important issues that need to be addressed are the culture conditions for maintaining ES cells and the accuracy of ES cell markers in monitoring the undifferentiated state. Leukaemia inhibitory factor (LIF) is routinely used to sustain mouse ES cells (mES) in a pluripotent fashion. In this paper, we assessed three markers during long-term maintenance of ES cells with various concentrations of LIF to see if decreasing concentration would lead to changes in marker expressions and growth behavior. Common markers of pluripotency such as alkaline phosphatase enzyme activity (ALP), surface staining for stage specific embryonic antigen 1 (SSEA-1), Oct-4 transcription factor, cell doubling time, as well as visual observations of cell morphology were analyzed during long-term maintenance of mES cells with LIF concentrations ranging from 0 to 500 pM. The morphology of the cells at LIF concentrations of 0 25 pM changed from being tight clusters to more flattened shapes while cells in 50-500 pM retained the clustered shape but growth rates remained essentially identical at between 10 and 16 h. ES cells at all concentrations of LIF continued expressing ALP, SSEA-1 and Oct-4 markers over a period of 6 weeks, which indicate that mES cells are capable of either producing autocrine LIF or are able to proliferate at very low levels of LIF. Pluripotency markers such as Oct-4 and SSEA-1 are only moderately reduced after 5-6 weeks. Oct-4 mRNA expression levels were partially diminished in LIF free conditions only at weeks 5 and 6 compared to controls with LIF at 500 pM. Changes in morphology of cells by visual observation seemed to be a faster indication of the onset of differentiation in mES cells, although other reliable means also include decreased levels of Oct-4, SSEA-1 and ALP markers. It is preferable to maintain long-term cultures of mES cells above 50 pM of LIF to have a more homogenous, stable population of pluripotent cells.

Berthier, R., et al. (1997). "The MS-5 murine stromal cell line and hematopoietic growth factors synergize to support the megakaryocytic differentiation of embryonic stem cells." Exp Hematol **25**(6): 481-490.

Murine embryonic stem (ES) cells are able to differentiate into erythroid, mast, and granulomonocytic cells by using appropriate culture conditions. Because we were interested in the regulation of tissue-specific expression of the platelet glycoprotein IIb gene, we studied the culture conditions, aiming at the reproducible production of myeloid cells that included megakaryocytes (MKs) from ES cells. We showed that even a complex cocktail of HGFs (stem cell factor, interleukin 3, IL6, IL11, granulocyte colony-stimulating factor, and erythropoietin) is unable to induce significant myeloid differentiation in day 12 embryoid bodies. Cocultures of MS-5 stromal cells with ES cells were slightly more productive than HGFs. A strong synergistic effect was observed on the growth of myeloid colonies and MKs when we used a combination of MS-5 cells plus the HGF cocktail. Conditioned medium from MS-5 cells also synergized with the HGF cocktail to produce a substantial number of mixed colonies containing MKs. The addition of fibroblast growth factor-2 (FGF-2) to the HGF cocktail plus MS-5 nearly doubled the number of myeloid progenitors, including those with MKs. Thrombopoietin (TPO) alone or in any combination with MS-5 or HGFs, did not increase the number of MK-containing colonies. However, when TPO was added to the HGF cocktail + FGF-2 + MS-5, the number of MKs in liquid cultures and mixed colonies increased, and many exhibited a "hairy" appearance resembling pseudopodial proplatelet formation. Having defined the culture conditions of ES cells that allow the production of all the myeloid lineages including MKs, we conclude that the hematopoietic differentiation model of ES cells is especially useful for studying the regulation of expression of any gene important in early hematopoiesis.

Beyer, T. A., et al. (2013). "Switch enhancers interpret TGF-beta and Hippo signaling to control cell fate in human embryonic stem cells." Cell Rep **5**(6): 1611-1624.

A small toolkit of morphogens is used repeatedly to direct development, raising the question of how context dictates interpretation of the same cue. One example is the transforming growth factor beta (TGF-beta) pathway that in human embryonic stem cells fulfills two opposite functions: pluripotency maintenance and mesendoderm (ME) specification. Using proteomics coupled to analysis of genome occupancy, we uncover a regulatory complex composed of transcriptional effectors of the Hippo pathway (TAZ/YAP/TEAD), the TGF-beta pathway (SMAD2/3), and the pluripotency regulator OCT4 (TSO). TSO collaborates with NuRD repressor complexes to buffer pluripotency gene expression while suppressing ME genes. Importantly, the SMAD DNA binding partner FOXH1, a major specifier of ME, is found near TSO elements, and upon fate specification we show that TSO is disrupted with subsequent SMAD-FOXH1 induction of ME. These studies define switch-enhancer elements and provide a framework to understand how cellular context dictates interpretation of the same morphogen signal in development.

Bhartiya, D., et al. (2012). "Very small embryonic-like stem cells with maximum regenerative potential get discarded during cord blood banking and bone marrow processing for autologous stem cell therapy." Stem Cells Dev **21**(1): 1-6.

Very small embryonic-like stem cells (VSELs) are possibly lost during cord blood banking and bone marrow (BM) processing for autologus stem cell therapy mainly because of their small size. The present study was conducted on human umbilical cord blood (UCB, n=6) and discarded red blood cells (RBC) fraction obtained after separation of mononuclear cells from human BM (n=6), to test this hypothesis. The results show that VSELs, which are pluripotent stem cells with maximum regenerative potential, settle along with the RBCs during Ficoll-Hypaque density separation. These cells are very small in size (3-5 mum), have high nucleo-cytoplasmic ratio, and express nuclear Oct-4, cell surface protein SSEA-4, and other pluripotent markers such as Nanog, Sox-2, Rex-1, and Tert as indicated by immunolocalization and quantitative polymerase chain reaction (Q-PCR) studies. Interestingly, a distinct population of slightly larger, round hematopoietic stem cells (HSCs) with cytoplasmic Oct-4 were detected in the "buffy" coat, which usually gets banked or used during autologus stem cell therapy. Immunohistochemical studies on the umbilical cord tissue (UCT) sections (n=3) showed the presence of nuclear Oct-4-positive VSELs and many fibroblast-like mesenchymal stem cells (MSCs) with cytoplasmic Oct-4. These VSELs with nuclear Oct-4, detected in UCB, UCT, and discarded RBC fraction obtained after BM processing, may persist throughout life, maintain tissue homeostasis, and undergo asymmetric cell division to self-renew as well as produce larger progenitor stem cells, viz. HSCs or MSCs, which follow differentiation trajectories depending on the somatic niche. Hence, it can be concluded that the true stem cells in adult body tissues are the VSELs, whereas the HSCs and MSCs are actually progenitor stem cells that arise by asymmetric cell division of VSELs. The results of the present study may help explain low efficacy reported during adult autologous stem cell trials, wherein unknowingly progenitor stem cells are injected rather than the pluripotent stem cells with maximum regenerative potential.

Bikorimana, E., et al. (2014). "Retroviral infection of murine embryonic stem cell derived embryoid body cells for analysis of hematopoietic differentiation." J Vis Exp(92): e52022.

Embryonic stem cells (ESCs) are an outstanding model for elucidating the molecular mechanisms of cellular differentiation. They are especially useful for investigating the development of early hematopoietic progenitor cells (HPCs). Gene expression in ESCs can be manipulated by several techniques that allow the role for individual molecules in development to be determined. One difficulty is that expression of specific genes often has different phenotypic effects dependent on their temporal expression. This problem can be circumvented by the generation of ESCs that inducibly express a gene of interest using technology such as the doxycycline-inducible transgene system. However, generation of these inducible cell lines is costly and time consuming. Described here is a method for disaggregating ESC-derived embryoid bodies (EBs) into single cell suspensions, retrovirally infecting the cell suspensions, and then reforming the EBs by hanging drop. Downstream differentiation is then evaluated by flow cytometry. Using this protocol, it was demonstrated that exogenous expression of a microRNA gene at the beginning of ESC differentiation blocks HPC generation. However, when expressed in EB derived cells after nascent mesoderm is produced, the microRNA gene enhances hematopoietic differentiation. This method is useful for investigating the role of genes after specific germ layer tissue is derived.

Boast, S. and C. D. Stern (2013). "Simple methods for generating neural, bone and endodermal cell types from

chick embryonic stem cells." Stem Cell Res **10**(1): 20-28.

Most work on embryonic stem cell differentiation uses mammalian cells derived from the blastocyst stage and some of the most widely used protocols to induce differentiation involve growing these cells in monolayer culture. Equivalent stem cells can be obtained from embryos of non-mammalian vertebrates, but to date this has only been successful in birds. These cells can contribute to all somatic lineages in chimaeras and can be induced to differentiate into a variety of cell types in vitro via embryoid body formation. However to date there are no reliable methods for differentiating them into descendants from each of the germ layers in monolayer culture, comparable to the protocols used in mammals. Here we describe three simple and reproducible protocols for differentiation of chick embryonic stem cells into mesoderm (bone), endoderm and neuroectoderm (neurons and glia) in monolayer culture. These methods open the way for more direct comparisons of the properties of mammalian and avian embryonic stem cells that may highlight similarities and differences.

Boheler, K. R., et al. (2011). "Embryonic stem cell-derived cardiomyocyte heterogeneity and the isolation of immature and committed cells for cardiac remodeling and regeneration." Stem Cells Int **2011**: 214203.

Pluripotent stem cells represent one promising source for cell replacement therapy in heart, but differentiating embryonic stem cell-derived cardiomyocytes (ESC-CMs) are highly heterogeneous and show a variety of maturation states. In this study, we employed an ESC clonal line that contains a cardiac-restricted ncx1 promoter-driven puromycin resistance cassette together with a mass culture system to isolate ESC-CMs that display traits characteristic of very immature CMs. The cells display properties of proliferation, CM-restricted markers, reduced mitochondrial mass, and hypoxia-resistance. Following transplantation into rodent hearts, bioluminescence imaging revealed that immature cells, but not more mature CMs, survived for at least one month following injection. These data and comparisons with more mature cells lead us to conclude that immature hypoxia resistant ESC-CMs can be isolated in mass in vitro and, following injection into heart, form grafts that may mediate long-term recovery of global and regional myocardial contractile function following infarction.

Bonde, S., et al. (2010). "Cell fusion of bone marrow cells and somatic cell reprogramming by embryonic stem cells." FASEB J **24**(2): 364-373.

Bone marrow transplantation is a curative treatment for many diseases, including leukemia, autoimmune diseases, and a number of immunodeficiencies. Recently, it was claimed that bone marrow cells transdifferentiate, a much desired property as bone marrow cells are abundant and therefore could be used in regenerative medicine to treat incurable chronic diseases. Using a Cre/loxP system, we studied cell fusion after bone marrow transplantation. Fused cells were chiefly Gr-1(+), a myeloid cell marker, and found predominantly in the bone marrow; in parenchymal tissues. Surprisingly, fused cells were most abundant in the kidney, Peyer's patches, and cardiac tissue. In contrast, after cell fusion with embryonic stem cells, bone marrow cells were reprogrammed into new tetraploid pluripotent stem cells that successfully differentiated into beating cardiomyocytes. Together, these data suggest that cell fusion is ubiquitous after cellular transplants and that the subsequent sharing of genetic material between the fusion partners affects cellular survival and function. Fusion between tumor cells and bone marrow cells could have consequences for tumor malignancy.

Bonig, H., et al. (2008). "Blood types of current embryonic stem cell lines are not conducive to culturing "universal-donor" red blood cells." Transfusion **48**(5): 1039-1040.

Boras-Granic, K., et al. (2014). "Embryonic cells contribute directly to the quiescent stem cell population in the adult mouse mammary gland." Breast Cancer Res **16**(6): 487.

INTRODUCTION: Studies have identified multi-potent stem cells in the adult mammary gland. More recent studies have suggested that the embryonic mammary gland may also contain stem/progenitor cells that contribute to initial ductal development. We were interested in determining whether embryonic cells might also directly contribute to long-lived stem cells that support homeostasis and development in the adult mammary gland. METHODS: We used DNA-label retention to detect long label-retaining cells in the mammary gland. Mouse embryos were labeled with 5-ethynl-2'-deoxyuridine (EdU) between embryonic day 14.5 and embryonic day 18.5 and were subsequently sacrificed and examined for EdU retention at various intervals after birth. EdU retaining cells were co-stained for various lineage markers and identified after fluorescence activated cell sorting analysis of specific epithelial subsets. EdU-labeled mice were subjected to subsequent 5-bromo-2'-deoxyuridine administration to determine whether EdU-labeled cells could re-enter the cell cycle. Finally, EdU-labeled cells were grown under non-adherent conditions to assess their ability to form mammospheres. RESULTS: We demonstrate embryonically-derived, long label-retaining cells (eLLRCs) in the adult mammary gland. eLLRCs stain

for basal markers and are enriched within the mammary stem cell population identified by cell sorting. eLLRCs are restricted to the primary ducts near the nipple region. Interestingly, long label retaining cells (labeled during puberty) are found just in front of the eLLRCs, near where the ends of the ducts had been at the time of DNA labeling in early puberty. A subset of eLLRCs becomes mitotically active during periods of mammary growth and in response to ovarian hormones. Finally, we show that eLLRCs are contained within primary and secondary mammospheres. CONCLUSIONS: Our findings suggest that a subset of proliferating embryonic cells subsequently becomes quiescent and contributes to the pool of long-lived mammary stem cells in the adult. eLLRCs can re-enter the cell cycle, produce both mammary lineages and self-renew. Thus, our studies have identified a putative stem/progenitor cell population of embryonic origin. Further study of these cells will contribute to an understanding of how quiescent stem cells are generated during development and how fetal exposures may alter future breast cancer risk in adults.

Borghese, L., et al. (2010). "Inhibition of notch signaling in human embryonic stem cell-derived neural stem cells delays G1/S phase transition and accelerates neuronal differentiation in vitro and in vivo." Stem Cells **28**(5): 955-964.

The controlled in vitro differentiation of human embryonic stem cells (hESCs) and other pluripotent stem cells provides interesting prospects for generating large numbers of human neurons for a variety of biomedical applications. A major bottleneck associated with this approach is the long time required for hESC-derived neural cells to give rise to mature neuronal progeny. In the developing vertebrate nervous system, Notch signaling represents a key regulator of neural stem cell (NSC) maintenance. Here, we set out to explore whether this signaling pathway can be exploited to modulate the differentiation of hESC-derived NSCs (hESNSCs). We assessed the expression of Notch pathway components in hESNSCs and demonstrate that Notch signaling is active under self-renewing culture conditions. Inhibition of Notch activity by the gamma-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) in hESNSCs affects the expression of human homologues of known targets of Notch and of several cell cycle regulators. Furthermore, DAPT-mediated Notch inhibition delays G1/S-phase transition and commits hESNSCs to neurogenesis. Combined with growth factor withdrawal, inhibition of Notch signaling results in a marked acceleration of differentiation, thereby shortening the time required for the generation of electrophysiologically active hESNSC-derived neurons. This effect can be exploited for neural cell transplantation, where transient Notch inhibition before grafting suffices to promote the onset of neuronal differentiation of hESNSCs in the host tissue. Thus, interference with Notch signaling provides a tool for controlling human NSC differentiation both in vitro and in vivo.

Boroviak, T., et al. (2014). "The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification." Nat Cell Biol **16**(6): 516-528.

The precise relationship of embryonic stem cells (ESCs) to cells in the mouse embryo remains controversial. We present transcriptional and functional data to identify the embryonic counterpart of ESCs. Marker profiling shows that ESCs are distinct from early inner cell mass (ICM) and closely resemble pre-implantation epiblast. A characteristic feature of mouse ESCs is propagation without ERK signalling. Single-cell culture reveals that cell-autonomous capacity to thrive when the ERK pathway is inhibited arises late during blastocyst development and is lost after implantation. The frequency of deriving clonal ESC lines suggests that all E4.5 epiblast cells can become ESCs. We further show that ICM cells from early blastocysts can progress to ERK independence if provided with a specific laminin substrate. These findings suggest that formation of the epiblast coincides with competence for ERK-independent self-renewal in vitro and consequent propagation as ESC lines.

Bose, B., et al. (2012). "Human embryonic stem cell differentiation into insulin secreting beta-cells for diabetes." Cell Biol Int **36**(11): 1013-1020.

hESC (human embryonic stem cells), when differentiated into pancreatic beta ILC (islet-like clusters), have enormous potential for the cell transplantation therapy for Type 1 diabetes. We have developed a five-step protocol in which the EBs (embryoid bodies) were first differentiated into definitive endoderm and subsequently into pancreatic lineage followed by formation of functional endocrine beta islets, which were finally matured efficiently under 3D conditions. The conventional cytokines activin A and RA (retinoic acid) were used initially to obtain definitive endoderm. In the last step, ILC were further matured under 3D conditions using amino acid rich media (CMRL media) supplemented with anti-hyperglycaemic hormone-Glp1 (glucagon-like peptide 1) analogue Liraglutide with prolonged t((1/2)) and Exendin 4. The differentiated islet-like 3D clusters expressed bonafide mature and functional beta-cell markers-PDX1 (pancreatic and duodenal homoeobox-1), C-peptide, insulin and MafA. Insulin synthesis de

novo was confirmed by C-peptide ELISA of culture supernatant in response to varying concentrations of glucose as well as agonist and antagonist of functional 3D beta islet cells in vitro. Our results indicate the presence of almost 65% of insulin producing cells in 3D clusters. The cells were also found to ameliorate hyperglycaemia in STZ (streptozotocin) induced diabetic NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mouse up to 96 days of transplantation. This protocol provides a basis for 3D in vitro generation of long-term in vivo functionally viable islets from hESC.

Bottai, D., et al. (2010). "Embryonic stem cells promote motor recovery and affect inflammatory cell infiltration in spinal cord injured mice." Exp Neurol **223**(2): 452-463.

The purpose of this study was to determine the fate and the effects of undifferentiated embryonic stem cells (ESCs) in mice after contusive lesion of the spinal cord (SCI). Reproducible traumatic lesion to the cord was performed at T8 level by means of the Infinite Horizon Device, and was followed by intravenous injection of one million of undifferentiated ESCs through the tail vein within 2 h from the lesion. The ESCs-treated animals showed a significant improvement of the recovery of motor function 28 days after lesion, with an average score of 4.61+/-0.13 points of the Basso Mouse Scale (n=14), when compared to the average score of vehicle treated mice, 3.58+/-0.23 (n=10). The number of identified ESCs found at the lesion site was 0.6% of the injected cells at 1 week after transplantation, and further reduced to 0.04% at 1 month. It is, thus, apparent that the promoted hind-limb recovery cannot be correlated to a substitution of the lost tissue performed by the exogenous ESC. The extensive evaluation of production of several neuroprotective and inflammatory cytokines did not reveal any effect by ESC-treatment, but unexpectedly the number of invading macrophages and neutrophils was greatly reduced. This may explain the improved preservation of lesion site ventral myelin, at both 1 week (29+/-11%) and 1 month (106+/-14%) after injury. No teratoma formation was observed, although an inappropriate colonization of the sacral cord by differentiated nestin- and beta-tubulin III-positive ESCs was detected.

Boulanger, C. A., et al. (2013). "Embryonic stem cells are redirected to non-tumorigenic epithelial cell fate by interaction with the mammary microenvironment." PLoS One **8**(4): e62019.

Experiments were conducted to redirect mouse Embryonic Stem (ES) cells from a tumorigenic phenotype to a normal mammary epithelial phenotype in vivo. Mixing LacZ-labeled ES cells with normal mouse mammary epithelial cells at ratios of 1:5 and 1:50 in phosphate buffered saline and immediately inoculating them into epithelium-divested mammary fat pads of immune-compromised mice accomplished this. Our results indicate that tumorigenesis occurs only when normal mammary ductal growth is not achieved in the inoculated fat pads. When normal mammary gland growth occurs, we find ES cells (LacZ+) progeny interspersed with normal mammary cell progeny in the mammary epithelial structures. We demonstrate that these progeny, marked by LacZ expression, differentiate into multiple epithelial subtypes including steroid receptor positive luminal cells and myoepithelial cells indicating that the ES cells are capable of epithelial multipotency in this context but do not form teratomas. In addition, in secondary transplants, ES cell progeny proliferate, contribute apparently normal mammary progeny, maintain their multipotency and do not produce teratomas.

Bourne, S., et al. (2004). "Osteogenic differentiation of mouse embryonic stem cells: differential gene expression analysis by cDNA microarray and purification of osteoblasts by cadherin-11 magnetically activated cell sorting." Tissue Eng **10**(5-6): 796-806.

We have previously shown osteogenic differentiation of mouse embryonic stem (ES) cells and temporal enrichment with osteoblastic cells, by stimulation with serum-containing culture medium supplemented with beta-glycerophosphate, ascorbate, and dexamethasone. In our present study we have used similar culture conditions to further investigate osteogenic differentiation of mouse ES cells. Using reverse transcription-polymerase chain reaction (RT-PCR) we demonstrated the expression of genes associated with osteoblast differentiation including the bone matrix protein osteocalcin and the transcription factor Cbfa-1/runx2. Furthermore, results of cDNA microarray analysis, and subsequent RT-PCR analysis of differentiating ES cells after exposure to osteogenic stimuli, revealed a combination of upregulation of genes involved in osteoblast differentiation including osteopontin, HSP-47, and IGF-II coupled with downregulation of genes involved in differentiation of other phenotypes such as the neuroectoderm factor Stra-13. Finally, we have applied magnetically activated cell-sorting methods to ES cell cultures treated with osteogenic stimuli and, using an antibody to cadherin-11, have purified a subpopulation of cells with osteoblastic characteristics.

Boyd, A. S., et al. (2005). "Transplanting stem cells: potential targets for immune attack. Modulating the immune response against embryonic stem cell transplantation." Adv Drug Deliv Rev **57**(13): 1944-1969.

The curative promise of stem cells and their descendants for tissue regeneration and repair is currently the subject of an intense research effort worldwide. If it proves feasible to differentiate stem cells into specific tissues reliably and safely, this approach will be invaluable in the treatment of diseases that lead to organ degeneration or failure, providing an alternative or supplementary source of tissue for transplantation. Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of a pre-implantation blastocyst that can produce all cells and tissues of the foetus. In recent years, several laboratories have described the directed differentiation of ES cells into multiple mature cell types including: cardiomyocytes; haemopoietic cells; hepatocytes; neurones; muscle cells and both endocrine and exocrine cells of the pancreas. How the immune system of the host will respond when these ES cell-derived mature cells are transplanted is ill defined. This review will focus on the potential mechanisms that the immune system could use to target ES cell-derived transplants and how unwanted responses might be prevented.

Boyd, A. S., et al. (2008). "A comparison of protocols used to generate insulin-producing cell clusters from mouse embryonic stem cells." Stem Cells **26**(5): 1128-1137.

Embryonic stem cells (ESCs) have the capacity to generate a panoply of tissue types and may therefore provide an alternative source of tissue in regenerative medicine to treat potentially debilitating conditions like Type 1 diabetes mellitus. However, the ability of mouse ESCs to generate insulin-producing cell clusters (IPCCs) remains highly contentious. In an attempt to clarify this issue, three protocols for the ESC-based generation of IPCCs (referred to as Blyszczuk, Hori, and Lumelsky protocols) were modified and evaluated for their ability to express pancreatic islet genes and proteins and their capacity to function. Herein, we show that the Blyszczuk protocol reproducibly generated IPCCs with gene-expression characteristics that were qualitatively and quantitatively most reminiscent of those found in pancreatic islets. Furthermore, compared to the Hori and Lumelsky protocols, Blyszczuk-derived IPCCs exhibited superior expression of c-peptide, a by-product of de novo insulin synthesis. Functionally, Blyszczuk IPCCs, in contrast to Hori and Lumelsky IPCCs, were able to transiently restore normal blood glucose levels in diabetic mice (<1 week). Longer normoglycemic rescue (>2 weeks) was also achieved in a third of diabetic recipients receiving Blyszczuk IPCCs. Yet Blyszczuk IPCCs were less able to rescue experimental diabetes than isolated syngeneic pancreatic islet tissue. Therefore, depending on the mode of differentiation, ESCs can be driven to generate de novo IPCCs that possess limited functionality. Further modifications to differentiation protocols will be essential to improve the generation of functional IPCCs from mouse ESCs.

Boyd, N. L., et al. (2011). "Microvascular mural cell functionality of human embryonic stem cell-derived mesenchymal cells." Tissue Eng Part A **17**(11-12): 1537-1548.

Microvascular mural or perivascular cells are required for the stabilization and maturation of the remodeling vasculature. However, much less is known about their biology and function compared to large vessel smooth muscle cells. We have developed lines of multipotent mesenchymal cells from human embryonic stem cells (hES-MC); we hypothesize that these can function as perivascular mural cells. Here we show that the derived cells do not form teratomas in SCID mice and independently derived lines show similar patterns of gene expression by microarray analysis. When exposed to platelet-derived growth factor-BB, the platelet-derived growth factor receptor beta is activated and hES-MC migrate in response to a gradient. We also show that in a serum-free medium, transforming growth factor beta1 (TGFbeta1) induces robust expression of multiple contractile proteins (alpha smooth muscle actin, smooth muscle myosin heavy chain, smooth muscle 22alpha, and calponin). TGFbeta1 signaling is mediated through the TGFbetaR1/Alk5 pathway as demonstrated by inhibition of alpha smooth muscle actin expression by treatment of the Alk5-specific inhibitor SB525334 and stable retroviral expression of the Alk5 dominant negative (K232R). Coculture of human umbilical vein endothelial cell (HUVEC) with hES-MC maintains network integrity compared to HUVEC alone in three-dimensional collagen I-fibronectin by paracrine signaling. Using high-resolution laser confocal microscopy, we show that hES-MC also make direct contact with HUVEC. This demonstrates that hESC-derived mesenchymal cells possess the molecular machinery expected in a perivascular progenitor cells and can play a functional role in stabilizing EC networks in in vitro three-dimensional culture.

Boyd, N. L., et al. (2013). "Dissecting the role of human embryonic stem cell-derived mesenchymal cells in human umbilical vein endothelial cell network stabilization in three-dimensional environments." Tissue Eng Part A **19**(1-2): 211-223.

The microvasculature is principally composed of two cell types: endothelium and mural support cells. Multiple sources are available for human endothelial cells (ECs) but sources for human microvascular mural cells (MCs) are limited. We derived multipotent

mesenchymal progenitor cells from human embryonic stem cells (hES-MC) that can function as an MC and stabilize human EC networks in three-dimensional (3D) collagen-fibronectin culture by paracrine mechanisms. Here, we have investigated the basis for hES-MC-mediated stabilization and identified the pleiotropic growth factor hepatocyte growth factor/scatter factor (HGF/SF) as a putative hES-MC-derived regulator of EC network stabilization in 3D in vitro culture. Pharmacological inhibition of the HGF receptor (Met) (1 mum SU11274) inhibits EC network formation in the presence of hES-MC. hES-MC produce and release HGF while human umbilical vein endothelial cells (HUVEC) do not. When HUVEC are cultured alone the networks collapse, but in the presence of recombinant human HGF or conditioned media from human HGF-transduced cells significantly more networks persist. In addition, HUVEC transduced to constitutively express human HGF also form stable networks by autocrine mechanisms. By enzyme-linked immunosorbent assay, the coculture media were enriched in both angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2), but at significantly different levels (Ang1=159+/-15 pg/mL vs. Ang2=30,867+/-2685 pg/mL) contributed by hES-MC and HUVEC, respectively. Although the coculture cells formed stabile network architectures, their morphology suggests the assembly of an immature plexus. When HUVEC and hES-MC were implanted subcutaneously in immune compromised Rag1 mice, hES-MC increased their contact with HUVEC along the axis of the vessel. This data suggests that HUVEC and hES-MC form an immature plexus mediated in part by HGF and angiopoietins that is capable of maturation under the correct environmental conditions (e.g., in vivo). Therefore, hES-MC can function as microvascular MCs and may be a useful cell source for testing EC-MC interactions.

Boyd, N. L., et al. (2009). "Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells." Tissue Eng Part A **15**(8): 1897-1907.

Human embryonic stem cells (hESC) have the potential to produce all of the cells in the body. They are able to self-renew indefinitely, potentially making them a source for large-scale production of therapeutic cell lines. Here, we developed a monolayer differentiation culture that induces hESC (WA09 and BG01) to form epithelial sheets with mesodermal gene expression patterns (BMP4, RUNX1, and GATA4). These E-cadherin+ CD90low cells then undergo apparent epithelial-mesenchymal transition for the derivation of mesenchymal progenitor cells (hESC-derived mesenchymal cells [hES-MC]) that by flow cytometry are negative for hematopoietic (CD34, CD45, and CD133) and endothelial (CD31 and CD146) markers, but positive for markers associated with mesenchymal stem cells (CD73, CD90, CD105, and CD166). To determine their functionality, we tested their capacity to produce the three lineages associated with mesenchymal stem cells and found they could form osteogenic and chondrogenic, but not adipogenic lineages. The derived hES-MC were able to remodel and contract collagen I lattice constructs to an equivalent degree as keloid fibroblasts and were induced to express alpha-smooth muscle actin when exposed to transforming growth factor (TGF)-beta1, but not platelet derived growth factor-B (PDGF-B). These data suggest that the derived hES-MC are multipotent cells with potential uses in tissue engineering and regenerative medicine and for providing a highly reproducible cell source for adult-like progenitor cells.

Brederlau, A., et al. (2006). "Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of in vitro differentiation on graft survival and teratoma formation." Stem Cells **24**(6): 1433-1440.

Human embryonic stem cells (hESCs) have been proposed as a source of dopamine (DA) neurons for transplantation in Parkinson's disease (PD). We have investigated the effect of in vitro predifferentiation on in vivo survival and differentiation of hESCs implanted into the 6-OHDA (6-hydroxydopamine)-lesion rat model of PD. The hESCs were cocultured with PA6 cells for 16, 20, or 23 days, leading to the in vitro differentiation into DA neurons. Grafted hESC-derived cells survived well and expressed neuronal markers. However, very few exhibited a DA neuron phenotype. Reversal of lesion-induced motor deficits was not observed. Rats grafted with hESCs predifferentiated in vitro for 16 days developed severe teratomas, whereas most rats grafted with hESCs predifferentiated for 20 and 23 days remained healthy until the end of the experiment. This indicates that prolonged in vitro differentiation of hESCs is essential for preventing formation of teratomas.

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

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

Brochner, C. B., et al. (2012). "YKL-40 is differentially expressed in human embryonic stem cells and in cell progeny of the three germ layers." J Histochem Cytochem **60**(3): 188-204.

The secreted glycoprotein YKL-40 participates in cell differentiation, inflammation, and cancer progression. High YKL-40 expression is reported during early human development, but its functions are unknown. Six human embryonic stem cell (hESC) lines were cultured in an atmosphere of low or high oxygen tension, in culture medium with or without basic fibroblast growth factor, and on feeder layers comprising mouse embryonic fibroblasts or human foreskin fibroblasts to evaluate whether hESCs and their progeny produced YKL-40 and to characterize YKL-40 expression during differentiation. Secreted YKL-40 protein and YKL-40 mRNA expression were measured by enzyme-linked immunosorbent assay (ELISA) and quantitative RT-PCR. Serial-sectioned colonies were stained for YKL-40 protein and for pluripotent hESC (OCT4, NANOG) and germ layer (HNF-3beta, PDX1, CD34, p63, nestin, PAX6) markers. Double-labeling showed YKL-40 expression in OCT4-positive hESCs, PAX6-positive neuroectodermal cells, and HNF-3beta-positive endodermal cells. The differentiating progeny showed strong YKL-40 expression. Abrupt transition between YKL-40 and OCT4-positive hESCs and YKL-40-positive ecto- and neuroectodermal lineages was observed within the same epithelial-like layer. YKL-40-positive cells within deeper layers lacked contact with OCT4-positive cells. YKL-40 may be important in initial cell differentiation from hESCs toward ectoderm and neuroectoderm, with retained epithelial morphology, whereas later differentiation into endoderm and mesoderm involves a transition into the deeper layers of the colony.

Brolen, G. K., et al. (2005). "Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing beta-cell-like cells." Diabetes **54**(10): 2867-2874.

The recent success in restoring normoglycemia in type 1 diabetes by islet cell transplantation indicates that cell replacement therapy of this severe disease is achievable. However, the severe lack of donor islets has increased the demand for alternative sources of beta-cells, such as adult and embryonic stem cells. Here, we investigate the potential of human embryonic stem cells (hESCs) to differentiate into beta-cells. Spontaneous differentiation of hESCs under two-dimensional growth conditions resulted in differentiation of Pdx1(+)/Foxa2(+) pancreatic progenitors and Pdx1(+)/Isl1(+) endocrine progenitors but no insulin-producing cells. However, cotransplantation of differentiated hESCs with the dorsal pancreas, but not with the liver or telencephalon, from mouse embryos resulted in differentiation of beta-cell-like cell clusters. Comparative analysis of the basic characteristics of hESC-derived insulin(+) cell clusters with human adult islets demonstrated that the insulin(+) cells share important features with normal beta-cells, such as synthesis (proinsulin) and processing (C-peptide) of insulin and nuclear localization of key beta-cell transcription factors, including Foxa2, Pdx1, and Isl1.

Bruin, J. E., et al. (2015). "Treating diet-induced diabetes and obesity with human embryonic stem cell-derived pancreatic progenitor cells and antidiabetic drugs." Stem Cell Reports **4**(4): 605-620.

Human embryonic stem cell (hESC)-derived pancreatic progenitor cells effectively reverse hyperglycemia in rodent models of type 1 diabetes, but their capacity to treat type 2 diabetes has not been reported. An immunodeficient model of type 2 diabetes was generated by high-fat diet (HFD) feeding in SCID-beige mice. Exposure to HFDs did not impact the maturation of macroencapsulated pancreatic progenitor cells into glucose-responsive insulin-secreting cells following transplantation, and the cell therapy improved glucose tolerance in HFD-fed transplant recipients after 24 weeks. However, since diet-induced hyperglycemia and obesity were not fully ameliorated by transplantation alone, a second cohort of HFD-fed mice was treated with pancreatic progenitor cells combined with one of three antidiabetic drugs. All combination therapies rapidly improved body weight and co-treatment with either sitagliptin or metformin improved hyperglycemia after only 12 weeks. Therefore, a stem cell-based therapy may be effective for treating type 2 diabetes, particularly in combination with antidiabetic drugs.

Brzeszczynska, J., et al. (2014). "Differentiation and molecular profiling of human embryonic stem cell-derived corneal epithelial cells." Int J Mol Med **33**(6): 1597-1606.

It has been suggested that the isolation of scalable populations of limbal stem cells may lead to radical changes in ocular therapy. In particular, the derivation and transplantation of corneal stem cells from these populations may result in therapies providing clinical normality of the diseased or damaged cornea. Although feasible in theory, the lack of donor material in sufficient quantity and quality currently limits such a strategy. A potential scalable source of corneal cells could be derived from pluripotent stem cells (PSCs). We developed an in vitro and serum-free corneal differentiation model which displays significant promise. Our stepwise

differentiation model was designed with reference to development and gave rise to cells which displayed similarities to epithelial progenitor cells which can be specified to cells displaying a corneal epithelial phenotype. We believe our approach is novel, provides a robust model of human development and in the future, may facilitate the generation of corneal epithelial cells that are suitable for clinical use. Additionally, we demonstrate that following continued cell culture, stem cell-derived corneal epithelial cells undergo transdifferentiation and exhibit squamous metaplasia and therefore, also offer an in vitro model of disease.

Burdon, T., et al. (2002). "Signalling, cell cycle and pluripotency in embryonic stem cells." Trends Cell Biol **12**(9): 432-438.

Pluripotent mouse embryonic stem (ES) cells can be expanded in large numbers in vitro owing to a process of symmetrical self-renewal. Self-renewal entails proliferation with a concomitant suppression of differentiation. Here we describe how the cytokine leukaemia inhibitory factor (LIF) sustains self-renewal through activation of the transcription factor STAT3, and how two other signals - extracellular-signal-related kinase (ERK) and phosphatidylinositol-3-OH kinase (PI3K) - can influence differentiation and propagation, respectively. We relate these observations to the unusual cell-cycle properties of ES cells and speculate on the role of the cell cycle in maintaining pluripotency.

Busch, C., et al. (2007). "Embryonic stem cells in human sacrococcygeal teratomas: Isolation and characterization of an embryonic stem cell line." J Stem Cells Regen Med **2**(1): 76.

Buschke, D. G., et al. (2012). "Cell death, non-invasively assessed by intrinsic fluorescence intensity of NADH, is a predictive indicator of functional differentiation of embryonic stem cells." Biol Cell **104**(6): 352-364.

BACKGROUND INFORMATION: Continued advances in stem cell biology and stem cell transplantation rely on non-invasive biomarkers to characterise cells and stem cell aggregates. The non-invasive quality of such biomarkers is essential because exogenous labels, probes or reporters can unintentionally and dramatically alter stem cell state as can disruption of cell-cell and cell-matrix interactions. Here, we investigate the utility of the autofluorescent metabolite, nicotinamide adenine dinucleotide (NADH), as a non-invasive, intrinsic biomarker of cell death when detected with multi-photon optical-based approaches. To test this possibility, cell death was induced in murine embryoid bodies (EBs) at an early stage (day 3) of differentiation using staurosporine, an ATP-competitive kinase inhibitor of electron transport. Several hours after staurosporine treatment, EBs were stained with a single-colour, live/dead probe. A single-cross-sectional plane of each EB was imaged to detect the fluorescence intensity of the live/dead probe (extrinsic fluorescence) as well as the fluorescence intensity of NADH (intrinsic fluorescence). EBs were assessed at subsequent time points (days 6-12) for the formation of beating areas as an indicator of functional differentiation. RESULTS: Statistical comparison indicated a strong positive correlation between extrinsic fluorescence intensity of the live/dead stain and intrinsic fluorescence of NADH, suggesting that the intensity of NADH fluorescence could be used to reliably and non-invasively assess death of cells of EBs. Furthermore, EBs that had high levels of cell death soon after aggregate formation had limited ability to give rise to functional cardiomyocytes at later time points. CONCLUSIONS: We demonstrate the utility of NADH fluorescence intensity as a non-invasive indicator of cell death in stem cell aggregates when measured using multi-photon excitation. In addition, we show that the degree of stem cell death at early stages of differentiation is predictive for the formation of functional cardiomyocytes.

Byrne, J. A., et al. (2007). "Producing primate embryonic stem cells by somatic cell nuclear transfer." Nature **450**(7169): 497-502.

Derivation of embryonic stem (ES) cells genetically identical to a patient by somatic cell nuclear transfer (SCNT) holds the potential to cure or alleviate the symptoms of many degenerative diseases while circumventing concerns regarding rejection by the host immune system. However, the concept has only been achieved in the mouse, whereas inefficient reprogramming and poor embryonic development characterizes the results obtained in primates. Here, we used a modified SCNT approach to produce rhesus macaque blastocysts from adult skin fibroblasts, and successfully isolated two ES cell lines from these embryos. DNA analysis confirmed that nuclear DNA was identical to donor somatic cells and that mitochondrial DNA originated from oocytes. Both cell lines exhibited normal ES cell morphology, expressed key stem-cell markers, were transcriptionally similar to control ES cells and differentiated into multiple cell types in vitro and in vivo. Our results represent successful nuclear reprogramming of adult somatic cells into pluripotent ES cells and demonstrate proof-of-concept for therapeutic cloning in primates.

Calderon, D., et al. (2012). "Immune response to human embryonic stem cell-derived cardiac progenitors and adipose-derived stromal cells." J Cell Mol Med **16**(7): 1544-1552.

Transplantation of allogeneic human embryonic stem cell-derived cardiac progenitors triggers an immune response. We assessed whether this response could be modulated by the concomitant use of adipose-derived stromal cells (ADSC). Peripheral blood mononuclear cells were collected from 40 patients with coronary artery disease (CAD) and nine healthy controls. Cardiac progenitors (CD15(+) Mesp1(+)) were generated as already reported from the I6 cell line treated with bone morphogenetic protein (BMP)-2. Adipose-derived stromal cells were obtained from abdominal dermolipectomies. We assessed the proliferative response of peripheral lymphocytes from patients and controls to cardiac progenitors cultured on a monolayer of ADSC, to allogeneic lymphocytes in mixed lymphocyte culture and to the T cell mitogen phytohemaglutin A in presence or absence of ADSC. Cardiac progenitors cultured on a monolayer of ADSC triggered a proliferation of lymphocytes from both patients and controls albeit lower than that induced by allogeneic lymphocytes. When cultured alone, ADSC did not induce any proliferation of allogeneic lymphocytes. When added to cultures of lymphocytes, ADSC significantly inhibited the alloantigen or mitogen-induced proliferative response. Compared to healthy controls, lymphocytes from patients presenting CAD expressed a decreased proliferative capacity, in particular to mitogen-induced stimulation. Adipose-derived stromal cells express an immunomodulatory effect that limits both alloantigen and mitogen-induced lymphocyte responses. Furthermore, lymphocytes from patients with CAD are low responders to conventional stimuli, possibly because of their age and disease-associated treatment regimens. We propose that, in combination, these factors may limit the in vivo immunogenicity of cardiac progenitors co-implanted with ADSC in patients with CAD.

Campbell, C., et al. (2015). "Zebrafish embryonic stromal trunk (ZEST) cells support hematopoietic stem and progenitor cell (HSPC) proliferation, survival, and differentiation." Exp Hematol **43**(12): 1047-1061.

Forward genetic screens in zebrafish have been used to identify genes essential for the generation of primitive blood and the emergence of hematopoietic stem cells (HSCs), but have not elucidated the genes essential for hematopoietic stem and progenitor cell (HSPC) proliferation and differentiation because of the lack of methodologies to functionally assess these processes. We previously described techniques used to test the developmental potential of HSPCs by culturing them on zebrafish kidney stromal (ZKS) cells, derived from the main site of hematopoiesis in the adult teleost. Here we describe an additional primary stromal cell line we refer to as zebrafish embryonic stromal trunk (ZEST) cells, derived from tissue surrounding the embryonic dorsal aorta, the site of HSC emergence in developing fish. ZEST cells encouraged HSPC differentiation toward the myeloid, lymphoid, and erythroid pathways when assessed by morphologic and quantitative reverse transcription polymerase chain reaction analyses. Additionally, ZEST cells significantly expanded the number of cultured HSPCs in vitro, indicating that these stromal cells are supportive of both HSPC proliferation and multilineage differentiation. Examination of ZEST cells indicates that they express numerous cytokines and Notch ligands and possess endothelial characteristics. Further characterization of ZEST cells should prove to be invaluable in understanding the complex signaling cascades instigated by the embryonic hematopoietic niche required to expand and differentiate HSPCs. Elucidating these processes and identifying possibilities for the modulation of these molecular pathways should allow the in vitro expansion of HSPCs for a multitude of therapeutic uses.

Carr, A. J., et al. (2009). "Molecular characterization and functional analysis of phagocytosis by human embryonic stem cell-derived RPE cells using a novel human retinal assay." Mol Vis **15**: 283-295.

PURPOSE: To examine the ability of retinal pigment epithelial (RPE) cells derived from human embryonic stem cells (HESC) to phagocytose photoreceptor outer segments, and to determine whether exposure to human retina induces any morphological changes in these cells. METHODS: HESC-RPE cells were derived from a super-confluent preparation of the Shef1 HESC line. Pigmented colonies were isolated and expanded into pigmented monolayers on Matrigel matrix-coated dishes or filters. Cells were exposed to fluorescently labeled outer segments isolated from the porcine eye and assessed for phagocytic activity at regular intervals. Expression of molecules associated with RPE phagocytosis was analyzed by RT-PCR, immunocytochemistry, and western blot. The role of Mer Tyrosine Kinase (MERTK) in the phagocytosis of outer segments was investigated using antibodies directed against MERTK to block function. In a novel approach, cells were also exposed to fresh human neural retina tissue then examined by electron microscopy for evidence of phagocytosis and changes in cell morphology. RESULTS: HESC-derived RPE cells are capable of phagocytosing isolated porcine outer segments and express molecules associated with RPE-specific phagocytosis, including MERTK. Pre-incubation with antibodies against MERTK blocked phagocytosis of photoreceptor outer segments, but not polystyrene beads. HESC-RPE cells also phagocytosed outer segments in a novel human retinal explant system. Furthermore co-culture adjacent to human retina tissue

in this preparation resulted in the appearance of features in HESC-derived RPE cells normally observed only as the RPE matures. CONCLUSIONS: The ingestion of photoreceptor outer segments from an isolated population and an artificial ex vivo human retina system demonstrates HESC-derived RPE cells are functional. HESC-derived RPE possess the relevant molecules required for phagocytosis, including MERTK, which is essential for the phagocytosis of outer segments but not latex beads. Furthermore, some changes observed in cell morphology after co-culture with human retina may have implications for understanding the full development and differentiation of RPE cells.

Caspi, O. and L. Gepstein (2006). "Regenerating the heart using human embryonic stem cells--from cell to bedside." Isr Med Assoc J **8**(3): 208-214.

The adult human heart has limited regenerative capacity and, therefore, functional restoration of the damaged heart presents a great challenge. Despite the progress achieved in the pharmacological and surgical treatment of degenerative myocardial diseases, they are still considered a major cause of morbidity and mortality in the western world. Repopulation of the damaged heart with cardiomyocytes represents a novel conceptual therapeutic paradigm but is hampered by the lack of sources for human cardiomyocytes. The recent derivation of pluripotent human embryonic stem cell lines may provide a solution for this cell sourcing problem. This review will focus on the derivation of the hESC lines, their mechanism of self-renewal, and their differentiation to cardiomyocytes. The possible signals and cues involved in the commitment and early differentiation of cardiomyocytes in this model will be discussed as well as the molecular, structural and electrophysiologic characteristics of the generated hESC-derived cardiomyocytes. Finally, the hurdles and challenges toward fully harnessing the potential clinical applications of these unique cells will be described.

Cechin, S., et al. (2014). "Influence of in vitro and in vivo oxygen modulation on beta cell differentiation from human embryonic stem cells." Stem Cells Transl Med **3**(3): 277-289.

The possibility of using human embryonic stem (hES) cell-derived beta cells as an alternative to cadaveric islets for the treatment of type 1 diabetes is now widely acknowledged. However, current differentiation methods consistently fail to generate meaningful numbers of mature, functional beta cells. In order to address this issue, we set out to explore the role of oxygen modulation in the maturation of pancreatic progenitor (PP) cells differentiated from hES cells. We have previously determined that oxygenation is a powerful driver of murine PP differentiation along the endocrine lineage of the pancreas. We hypothesized that targeting physiological oxygen partial pressure (pO2) levels seen in mature islets would help the differentiation of PP cells along the beta-cell lineage. This hypothesis was tested both in vivo (by exposing PP-transplanted immunodeficient mice to a daily hyperbaric oxygen regimen) and in vitro (by allowing PP cells to mature in a perfluorocarbon-based culture device designed to carefully adjust pO2 to a desired range). Our results show that oxygen modulation does indeed contribute to enhanced maturation of PP cells, as evidenced by improved engraftment, segregation of alpha and beta cells, body weight maintenance, and rate of diabetes reversal in vivo, and by elevated expression of pancreatic endocrine makers, beta-cell differentiation yield, and insulin production in vitro. Our studies confirm the importance of oxygen modulation as a key variable to consider in the design of beta-cell differentiation protocols and open the door to future strategies for the transplantation of fully mature beta cells.

Cerdan, C., et al. (2006). "Complement targeting of nonhuman sialic acid does not mediate cell death of human embryonic stem cells." Nat Med **12**(10): 1113-1114; author reply 1115.

Chaddah, R., et al. (2012). "Clonal neural stem cells from human embryonic stem cell colonies." J Neurosci **32**(23): 7771-7781.

Clonal cell culture is crucial for experimental protocols that require growth or selection of pure populations of cells. High-density derivation of neural progenitors from human embryonic stem cells (hESCs) can lead to incomplete differentiation, and transplantation of resulting heterogeneous cell mixtures can cause proliferation of tumorigenic clusters in vivo. We have identified the neural precursor that resides among normal hESC colonies as a TRA-1-60(-)/SSEA4(-)/SOX1(+) cell and developed a method that allows for the clonal expansion of these FACS-selected progenitors to neural stem cells (NSCs) in serum-free conditions. Single TRA-1-60(-)/SSEA4(-)/SOX1(+) cells grown in serum-free media give rise to multipotent NSCs with an efficiency of 0.7%. The fate of the TRA-1-60(-)/SSEA4(-)/SOX1(+) neural precursor becomes specified in maintenance conditions by inhibition of BMP signaling. This clonal culture method can be scaled up to produce NSCs for differentiation and use in cell therapies.

Chaerkady, R., et al. (2011). "Quantitative temporal proteomic analysis of human embryonic stem cell

differentiation into oligodendrocyte progenitor cells." Proteomics **11**(20): 4007-4020.

Oligodendrocytes (OLs) are glial cells of the central nervous system, which produce myelin. Cultured OLs provide immense therapeutic opportunities for treating a variety of neurological conditions. One of the most promising sources for such therapies is human embryonic stem cells (ESCs) as well as providing a model to study human OL development. For these purposes, an investigation of proteome level changes is critical for understanding the process of OL differentiation. In this report, an iTRAQ-based quantitative proteomic approach was used to study multiple steps during OL differentiation including neural progenitor cells, glial progenitor cells and oligodendrocyte progenitor cells (OPCs) compared to undifferentiated ESCs. Using a 1% false discovery rate cutoff, approximately 3145 proteins were quantitated and several demonstrated progressive stage-specific expression. Proteins such as transferrin, neural cell adhesion molecule 1, apolipoprotein E and wingless-related MMTV integration site 5A showed increased expression from the neural progenitor cell to the OPC stage. Several proteins that have demonstrated evidence or been suspected in OL maturation were also found upregulated in OPCs including fatty acid-binding protein 4, THBS1, bone morphogenetic protein 1, CRYAB, transferrin, tenascin C, COL3A1, TGFBI and EPB41L3. Thus, by providing the first extensive proteomic profiling of human ESC differentiation into OPCs, this study provides many novel proteins that are potentially involved in OL development.

Chambers, C. A., et al. (1994). "Exogenous Mtv-7 superantigen transgene expression in major histocompatibility complex class II I-E- mice reconstituted with embryonic stem cell-derived hematopoietic stem cells." Proc Natl Acad Sci U S A **91**(3): 1138-1142.

Direct genetic manipulation of hematopoietic cells is limited by the lack of an established hematopoietic stem cell line. It has been demonstrated that embryonic stem (ES) cell<-->tetraploid embryos are completely ES cell-derived and that fetal liver (FL) cells from these embryos support hematopoiesis in lethally irradiated recipients. In this report, we demonstrate that FL cells from ES cell<-->tetraploid embryos support normal lymphopoiesis and T-cell repertoire development. Moreover, the introduction of the Mtv-7 superantigen transgene coding for minor lymphocyte stimulatory antigen 1 into murine hematopoietic cells via reconstitution with ES cell<-->tetraploid FL cells demonstrates that this method can effectively confer stable genetic changes into the hematopoietic tissues without going through the germ line. Long-term and secondary reconstitution with ES cell<-->tetraploid FL cells expressing the Mtv-7 superantigen transgene clonally deleted minor lymphocyte stimulatory antigen 1-reactive T-cell receptor V beta 6+, -8.1+, and -9+ T cells, but not V beta 7+ T cells, in H-2b (I-E-) mice. This model system will be extremely important for analyzing structure-function relationships of molecules involved in proliferation, differentiation, and selection of hematopoietic cells in vivo and for examining hematopoiesis-specific effects of mutations that are lethal during embryogenesis.

Chan, H. Y., et al. (2015). "Morphometric Analysis of Human Embryonic Stem Cell-Derived Ventricular Cardiomyocytes: Determining the Maturation State of a Population by Quantifying Parameters in Individual Cells." Stem Cells Int **2015**: 586908.

Quantitative methods were established to determine the level of maturation of human embryonic stem cell-derived ventricular cardiomyocytes (hESC-vCMs) that were treated with different metabolic stimulants (i.e., isoproterenol and oleic acid) during early differentiation. Cells were double-immunolabeled with alpha-actinin and COX IV antibodies, to label the myofibrils and mitochondria, respectively, after which images were acquired via confocal microscopy. In order to determine the extent of differentiation, image analysis protocols were then used to quantify cell shape and area, as well as the degree of myofibrillar organization and intercalation of mitochondria between the myofibrils within the cells. We demonstrated that oleic acid or isoproterenol alone, or a combination of the two, induced a more elongated hESC-vCM phenotype than the untreated controls. In addition, cells treated with isoproterenol alone exhibited a similar level of myofibrillar organization as the controls, but those treated with oleic acid with/without isoproterenol exhibited a more organized (parallel) orientation of myofibrils. The combined isoproterenol/oleic acid treatment also resulted in enhanced intercalation of mitochondria between the myofibrils. We suggest that these quantitative morphometric methods might serve as simple and effective tools that can be utilized in the determination of the level of structural maturation of hESC-vCMs.

Chan, K. M., et al. (2008). "Hematopoiesis and immunity of HOXB4-transduced embryonic stem cell-derived hematopoietic progenitor cells." Blood **111**(6): 2953-2961.

The ability of embryonic stem (ES) cells to form cells and tissues from all 3 germ layers can be exploited to generate cells that can be used to treat diseases. In particular, successful generation of hematopoietic cells from ES cells could provide safer and less immunogenic cells than bone marrow cells,

which require severe host preconditioning when transplanted across major histocompatibility complex barriers. Here, we exploited the self-renewal properties of ectopically expressed HOXB4, a homeobox transcription factor, to generate hematopoietic progenitor cells (HPCs) that successfully induce high-level mixed chimerism and long-term engraftment in recipient mice. The HPCs partially restored splenic architecture in Rag2(-/-)gamma(c)(-/-)-immunodeficient mice. In addition, HPC-derived newly generated T cells were able to mount a peptide-specific response to lymphocytic choriomeningitis virus and specifically secreted interleukin-2 and interferon-gamma upon CD3 stimulation. In addition, HPC-derived antigen presenting cells in chimeric mice efficiently presented viral antigen to wild-type T cells. These results demonstrate for the first time that leukocytes derived from ES cells ectopically expressing HOXB4 are immunologically functional, opening up new opportunities for the use of ES cell-derived HPCs in the treatment of hematologic and immunologic diseases.

Chan, K. M., et al. (2013). "Hepatic stellate cells promote the differentiation of embryonic stem cell-derived definitive endodermal cells into hepatic progenitor cells." Hepatol Res **43**(6): 648-657.

AIM: Hepatic non-parenchymal cells are well known to be capable of providing an important microenvironment and growth factors for hepatic regeneration, but their capacity for directing embryonic stem cells (ESC) toward hepatocytes remains to be assessed. Thus, this study aims to investigate the role of hepatic stellate cells (HSC), the major type of hepatic non-parenchymal cells, in the differentiation of ESC as well as exploring the potentiality of ESC in regeneration medicine for cell-based therapy. METHODS: A two-step differentiation procedure that utilized the capability of HSC to regulate proliferation and differentiation of hepatocytes was used to develop an approach for directing the differentiation of ESC towards hepatic progenitor cells. Mouse ESC were cultivated in a serum-free medium containing Activin A and fibroblast growth factor to generate definitive endodermal cells characterized by the CXCR4 cell-surface marker. After 6-8 days in culture, approximately 60% of the differentiated cells expressed CXCR4, and more than 90% of the CXCR4 positive cells could be recovered by cell sorting. The purified CXCR4 positive cells were co-cultured with mouse HSC as feeder cells in basal medium without additional hepatocyte growth factors. Differentiation was complete after 10-12 days of co-culture, and hepatic progenitor cell markers such as alpha-fetoprotein (afp) and albumin (alb) were detected in the terminally differentiated ESC. CONCLUSION: These results show that HSC provide an appropriate microenvironment and pivotal growth factors for generation of hepatic progenitor cells from ESC-derived definitive endodermal cells, and suggest that this approach possibly allows for hepatic differentiation of ESC imitating the process of hepatic regeneration.

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

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

Chandrasekar, M. P., et al. (2010). "The role of AMP Kinase on the beta-cell differentiation of mouse embryonic stem cells." J Stem Cells Regen Med **6**(2): 55.

Chang, D. J., et al. (2013). "Contralaterally transplanted human embryonic stem cell-derived neural precursor cells (ENStem-A) migrate and improve brain

functions in stroke-damaged rats." Exp Mol Med **45**: e53.

The transplantation of neural precursor cells (NPCs) is known to be a promising approach to ameliorating behavioral deficits after stroke in a rodent model of middle cerebral artery occlusion (MCAo). Previous studies have shown that transplanted NPCs migrate toward the infarct region, survive and differentiate into mature neurons to some extent. However, the spatiotemporal dynamics of NPC migration following transplantation into stroke animals have yet to be elucidated. In this study, we investigated the fates of human embryonic stem cell (hESC)-derived NPCs (ENStem-A) for 8 weeks following transplantation into the side contralateral to the infarct region using 7.0T animal magnetic resonance imaging (MRI). T2- and T2*-weighted MRI analyses indicated that the migrating cells were clearly detectable at the infarct boundary zone by 1 week, and the intensity of the MRI signals robustly increased within 4 weeks after transplantation. Afterwards, the signals were slightly increased or unchanged. At 8 weeks, we performed Prussian blue staining and immunohistochemical staining using human-specific markers, and found that high percentages of transplanted cells migrated to the infarct boundary. Most of these cells were CXCR4-positive. We also observed that the migrating cells expressed markers for various stages of neural differentiation, including Nestin, Tuj1, NeuN, TH, DARPP-32 and SV38, indicating that the transplanted cells may partially contribute to the reconstruction of the damaged neural tissues after stroke. Interestingly, we found that the extent of gliosis (glial fibrillary acidic protein-positive cells) and apoptosis (TUNEL-positive cells) were significantly decreased in the cell-transplanted group, suggesting that hESC-NPCs have a positive role in reducing glia scar formation and cell death after stroke. No tumors formed in our study. We also performed various behavioral tests, including rotarod, stepping and modified neurological severity score tests, and found that the transplanted animals exhibited significant improvements in sensorimotor functions during the 8 weeks after transplantation. Taken together, these results strongly suggest that hESC-NPCs have the capacity to migrate to the infarct region, form neural tissues efficiently and contribute to behavioral recovery in a rodent model of ischemic stroke.

Chang, J. C., et al. (2006). "Correction of the sickle cell mutation in embryonic stem cells." Proc Natl Acad Sci U S A **103**(4): 1036-1040.

Sickle cell anemia is one of the most common genetic diseases worldwide. Patients often suffer from anemia, painful crises, infections, strokes, and cardiopulmonary complications. Although current management has improved the quality of life and survival of patients, cure can be achieved only with bone marrow transplantation when histocompatible donors are available. The ES cell technology suggests that a therapeutic cloning approach may be feasible for treatment of this disease. Using a transgenic/knockout sickle cell anemia mouse model, which harbors 240 kb of human DNA sequences containing the beta(S)-globin gene, we prepared ES cells from blastocysts that had the sickle cells anemia genotype and carried out homologous recombination with DNA constructs that contained the beta(A)-globin gene. We obtained ES cells in which the beta(S) was corrected to the beta(A) sequence. Hematopoietic cells differentiated from these ES cells produced both hemoglobin A and hemoglobin S. This approach can be applied to human ES cells to correct the sickle mutation as well as beta-thalassemia mutations.

Chang, T. C., et al. (2010). "Rho kinases regulate the renewal and neural differentiation of embryonic stem cells in a cell plating density-dependent manner." PLoS One **5**(2): e9187.

BACKGROUND: Rho kinases (ROCKs) mediate cell contraction, local adhesion, and cell motility, which are considered to be important in cell differentiation. We postulated that ROCKs are involved in controlling embryonic stem (ES) cell renewal and differentiation. METHODOLOGY/PRINCIPAL FINDINGS: CCE, a murine ES cell, was treated with Y-27632 for 48 to 96 hours and colony formation was evaluated. Y-27632 blocked CCE colony formation and induced CCE to grow as individual cells, regardless of the initial seeding cell density either at 10(4)/cm(2) ("high" seeding density) or 2x10(3)/cm(2) ("low" density). However, at high seeding density, Y-27632-treated cells exhibited reduction of alkaline phosphatase (AP) staining and Oct3/4 expression. They expressed SOX-1, nestin, and MAP2c, but not betaIII-tubulin or NG-2. They did not express endoderm or mesoderm lineage markers. After removal of Y-27632, the cells failed to form colonies or regain undifferentiated state. Silencing of ROCK-1 or ROCK-2 with selective small interference RNA induced CCE morphological changes similar to Y-27632. Silencing of ROCK-1 or ROCK-2 individually was sufficient to cause reduction of AP and Oct3/4, and expression of SOX-1, nestin, and MAP2c; and combined silencing of both ROCKs did not augment the effects exerted by individual ROCK siRNA. Y-27632-treated CCE cells seeded at 2x10(3) or 6.6x10(3) cells/cm(2) did not lose renewal factors or express differentiation markers. Furthermore, they were able to form AP-positive colonies after removal of Y-27632 and reseeding. Similar to ROCK inhibition by Y-27632, silencing of ROCK-1 or ROCK-2 in cells

seeded at 2x10(3)/cm(2) did not change renewal factors. CONCLUSIONS/SIGNIFICANCE: We conclude that ROCKs promote ES cell colony formation, maintain them at undifferentiated state, and prevent them from neural differentiation at high seeding density. ROCK inhibition represents a new strategy for preparing large numbers of neural progenitor cells.

Chao, J. R., et al. (2017). "Transplantation of Human Embryonic Stem Cell-Derived Retinal Cells into the Subretinal Space of a Non-Human Primate." Transl Vis Sci Technol **6**(3): 4.

PURPOSE: Previous studies have demonstrated the ability of retinal cells derived from human embryonic stem cells (hESCs) to survive, integrate into the host retina, and mediate light responses in murine mouse models. Our aim is to determine whether these cells can also survive and integrate into the retina of a nonhuman primate, Saimiri sciureus, following transplantation into the subretinal space. METHODS: hESCs were differentiated toward retinal neuronal fates using our previously published technique and cultured for 60 to 70 days. Differentiated cells were further treated with 20 muM N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) for a period of 5 days immediately prior to subretinal transplantation. Differentiated cells were labeled with a lentivirus expressing GFP. One million cells (10,000 cells/muL) were injected into the submacular space into a squirrel monkey eye, using an ab externo technique. RESULTS: RetCam imaging demonstrated the presence and survival of human donor cells 3 months after transplantation in the S. sciureus eye. Injected cells consolidated in the temporal macula. GFP(+) axonal projections were observed to emanate from the central consolidation of cells at 1 month, with some projecting into the optic nerve by 3 months after transplantation. CONCLUSIONS: Human ES cell-derived retinal neurons injected into the submacular space of a squirrel monkey survive at least 3 months postinjection without immunosuppression. Some donor cells appeared to integrate into the host inner retina, and numerous donor axonal projections were noted throughout, with some projecting into the optic nerve. TRANSLATIONAL RELEVANCE: These data illustrate the feasibility of hESC-derived retinal cell replacement in the nonhuman primate eye.

Chen, B., et al. (2009). "Xeno-free culture of human spermatogonial stem cells supported by human embryonic stem cell-derived fibroblast-like cells." Asian J Androl **11**(5): 557-565.

Spermatogonial stem cells (SSCs) divide continuously to support spermatogenesis throughout postnatal life and transmit genetic information to the next generation. Here, we report the successful establishment of the method for the isolation and identification of human SSCs from testicular tissue, and to determine the culture conditions required to expand SSCs on human embryonic stem cell-derived fibroblast-like cells (hdFs). Large-scale cultures of SSCs were maintained on hdF feeder layers and expanded in the presence of a combination of cytokines and glial cell line-derived neurotrophic factor for at least 2 months. Cell surface marker analysis showed that SSCs retained high levels of alkaline phosphatase activity and stained strongly for anti-stage-specific embryonic antigen (SSEA)-1, OCT4 and CD49f. They also expressed the genes OCT4, SOX3 and STRA8 as detected by reverse transcription polymerase chain reaction (RT-PCR) analysis. These data clearly illustrate a novel approach for the growth of human SSCs using hdFs as feeder cells, potentially eliminating xenogeneic contaminants. This system provides a new opportunity for the study of the regulatory mechanism of the 'niche' that governs SSC self-renewal, and will be a valuable source of SSCs for potential clinical applications.

Chen, B. Z., et al. (2011). "Identification of microRNAs expressed highly in pancreatic islet-like cell clusters differentiated from human embryonic stem cells." Cell Biol Int **35**(1): 29-37.

Type 1 diabetes is an autoimmune destruction of pancreatic islet beta cell disease, making it important to find a new alternative source of the islet beta cells to replace the damaged cells. hES (human embryonic stem) cells possess unlimited self-renewal and pluripotency and thus have the potential to provide an unlimited supply of different cell types for tissue replacement. The hES-T3 cells with normal female karyotype were first differentiated into EBs (embryoid bodies) and then induced to generate the T3pi (pancreatic islet-like cell clusters derived from T3 cells), which expressed pancreatic islet cell-specific markers of insulin, glucagon and somatostatin. The expression profiles of microRNAs and mRNAs from the T3pi were analysed and compared with those of undifferentiated hES-T3 cells and differentiated EBs. MicroRNAs negatively regulate the expression of protein-coding mRNAs. The T3pi showed very high expression of microRNAs, miR-186, miR-199a and miR-339, which down-regulated the expression of LIN28, PRDM1, CALB1, GCNT2, RBM47, PLEKHH1, RBPMS2 and PAK6. Therefore, these microRNAs and their target genes are very likely to play important regulatory roles in the development of pancreas and/or differentiation of islet cells, and they may be manipulated to increase the proportion of beta cells and insulin synthesis in the differentiated T3pi for cell therapy of type I diabetics.

Chen, C., et al. (2011). "Characterization of an in vitro differentiation assay for pancreatic-like cell development from murine embryonic stem cells: detailed gene expression analysis." Assay Drug Dev Technol **9**(4): 403-419.

Embryonic stem (ES) cell technology may serve as a platform for the discovery of drugs to treat diseases such as diabetes. However, because of difficulties in establishing reliable ES cell differentiation methods and in creating cost-effective plating conditions for the high-throughput format, screening for molecules that regulate pancreatic beta cells and their immediate progenitors has been limited. A relatively simple and inexpensive differentiation protocol that allows efficient generation of insulin-expressing cells from murine ES cells was previously established in our laboratories. In this report, this system is characterized in greater detail to map developmental cell stages for future screening experiments. Our results show that sequential activation of multiple gene markers for undifferentiated ES cells, epiblast, definitive endoderm, foregut, and pancreatic lineages was found to follow the sequence of events that mimics pancreatic ontogeny. Cells that expressed enhanced green fluorescent protein, driven by pancreatic and duodenal homeobox 1 or insulin 1 promoter, correctly expressed known beta cell lineage markers. Overexpression of Sox17, an endoderm fate-determining transcription factor, at a very early stage of differentiation (days 2-3) enhanced pancreatic gene expression. Overexpression of neurogenin3, an endocrine progenitor cell marker, induced glucagon expression at stages when pancreatic and duodenal homeobox 1 message was present (days 10-16). Forced expression (between days 16 and 25) of MafA, a pancreatic maturation factor, resulted in enhanced expression of insulin genes, glucose transporter 2 and glucokinase, and glucose-responsive insulin secretion. Day 20 cells implanted in vivo resulted in pancreatic-like cells. Together, our differentiation assay recapitulates the proceedings and behaviors of pancreatic development and will be valuable for future screening of beta cell effectors.

Chen, G., et al. (2008). "Trophoblast differentiation defect in human embryonic stem cells lacking PIG-A and GPI-anchored cell-surface proteins." Cell Stem Cell **2**(4): 345-355.

Pluripotent human embryonic stem (hES) cells can differentiate into various cell types derived from the three embryonic germ layers and extraembryonic tissues such as trophoblasts. The mechanisms governing lineage choices of hES cells are largely unknown. Here, we report that we established two independent hES cell clones lacking a group of cell surface molecules, glycosyl-phosphatidyl-inositol-anchored proteins (GPI-APs). The GPI-AP deficiency in these two hES clones is due to the deficiency in the gene expression of PIG-A (phosphatidyl-inositol-glycan class A), which is required for the first step of GPI synthesis. GPI-AP-deficient hES cells were capable of forming embryoid bodies and initiating cell differentiation into the three embryonic germ layers. However, GPI-AP-deficient hES cells failed to form trophoblasts after differentiation induction by embryoid body formation or by adding exogenous BMP4. The defect in trophoblast formation was due to the lack of GPI-anchored BMP coreceptors, resulting in the impairment of full BMP4 signaling activation in the GPI-AP-deficient hES cells. These data reveal that GPI-AP-enhanced full activation of BMP signaling is required for human trophoblast formation.

Chen, H. F., et al. (2007). "Derivation, characterization and differentiation of human embryonic stem cells: comparing serum-containing versus serum-free media and evidence of germ cell differentiation." Hum Reprod **22**(2): 567-577.

BACKGROUND: This study was designed to establish human embryonic stem cell (hESC) lines, to identify the differences when maintained in serum-containing versus serum-free medium and to test their potential of in vitro differentiation. METHODS: Procedures including immunosurgery were performed on 11 donated human blastocysts to establish hESC lines. The cell lines were characterized and maintained using either serum-free or serum-containing media to compare their morphology, Oct-4 expression, apoptosis and growth speed. Differentiation of these lines was evaluated by the morphology and the expression of genes belonging to the three embryonic germ layers and the germ cell lineage. RESULTS: Three hESC lines were established, and they grew at similar speed in both media (serum-containing or serum-free), but hESC cultured in serum-containing medium yielded significantly higher percentages of morphologically good colonies and cells expressing Oct-4. These cell lines differentiated spontaneously in vitro into cells expressing markers belonging to all three embryonic germ layers and germ cell markers, including c-Kit, STELLA, VASA and growth differentiation factor 9 (GDF9), in directly adherent culture. CONCLUSIONS: Three hESC lines with Taiwanese ancestry have been established, and they retain the in vitro differentiation potential with or without embryoid body (EB) formation. The data support that hESC may be capable of differentiation into germ cells although further confirmation is needed. It is also suggested that strategies such as stepwise adaptation will be needed before implementing a serum-free culture condition for hESC lines that have previously been derived in a medium containing serum.

Chen, J., et al. (2005). "Cell adhesion molecule l1-transfected embryonic stem cells with enhanced survival support regrowth of corticospinal tract axons in mice after spinal cord injury." J Neurotrauma **22**(8): 896-906.

Previous studies have indicated that the cell adhesion molecule L1 enhances neuronal survival and neurite outgrowth. L1-mediated promotion of neurite outgrowth has been shown to occur also in an inhibitory environment not only in vitro, but also in vivo. To further investigate the effects of L1 in spinal cord injury, we transfected embryonic stem cells with a plasmid encoding the full-length mouse L1 molecule under the control of PGK promoter. An embryonic stem cell line derived from C57BL/6J transgenic mice that express green fluorescent protein under control of the beta-actin promoter was transfected with L1 and injected into the lesion site of 3-month-old C57BL/6J female mice 7 days after compression injury. Non-transfected embryonic stem cells were detectable at the lesion site 3 days after transplantation, but lost their cellular integrity 7 days after transplantation and were barely detectable 1 month after transplantation. In contrast, L1-transfected embryonic stem cells were detected 1 month after transplantation in numbers comparable to those of the injected cells and demonstrated extended processes. Further, in contrast to the few detectable nontransfected stem cells that remained at the injection site 1 month post-transplantation, the L1-transfected embryonic stem cells had migrated rostrally and caudally from the lesion. Anterogradely labeled corticospinal tract axons showed interdigitation with L1-transfected embryonic stem cells and, in contrast to non-transfected stem cells, extended into the lesion site 1 month after transplantation and, in some cases, extended beyond it. Our observations encourage the use of L1-transfected embryonic stem cells that express L1 not only at the cell surface, but also as a soluble and secreted form. Their use could condition the inhibitory environment for homophilic L1-enhanced axon regrowth not only in spinal cord regeneration, but also in other lesion paradigms.

Chen, T., et al. (2012). "Cell growth arrest and apoptosis induced by Oct4 or Nanog knockdown in mouse embryonic stem cells: a possible role of Trp53." Mol Biol Rep **39**(2): 1855-1861.

It has been clear that both Oct4 and Nanog play essential roles in maintaining embryonic stem cells (ESCs) undifferentiation. However, the roles of Oct4 and Nanog in ESCs growth and apoptosis have been much less explored. In this study, we systematically examined the effects of Oct4 or Nanog knockdown on mouse ESCs (mESCs) growth and apoptosis as well as potential mechanisms. Our results show that Oct4 or Nanog knockdown induces growth arrest and apoptosis in mESCs, indicating that the two genes also play important roles in mESCs survival and growth. Moreover, upregulation in Trp53 and its downstream genes expression was detected in Oct4 or Nanog knockdown mESCs, suggesting a possible role of Trp53 in Oct4 or Nanog knockdown induced mESCs growth arrest and apoptosis.

Chen, W., et al. (2012). "Retinoic acid regulates germ cell differentiation in mouse embryonic stem cells through a Smad-dependent pathway." Biochem Biophys Res Commun **418**(3): 571-577.

Murine embryonic stem cells (ESCs) are pluripotent cells that differentiate into multiple cell lineages. It was recently observed that all-trans retinoic acid (RA) provides instructive signals for the commitment of the germ cell lineage from ESCs. However, little is known about the molecular mechanisms by which RA signals lead to germ cell commitment. In this study, we determined if RA induced ESC differentiation to the germ lineage through modulation of the (bone morphogenetic protein) BMP/Smad pathway activity. In a monolayer culture, RA significantly induced both the expression of the early germ-specific genes, Stra8, Dazl and Mvh, and prolonged activation of Smad1/5 (for at least 24h). Meanwhile, dorsomorphin (a BMP-Smad1/5 specific inhibitor) significantly reduced the RA-induced germ-specific gene expression and completely blocked the RA-induced activation of Smad1/5. Moreover, RA-induced germ-specific gene expression was significantly increased by treatment with the potential activator of Smad1/5, SB431542. Furthermore, the biochemical manipulation of Smad1/5 expression through shRNA knockdown significantly reduced RA-mediated up-regulation of germ-specific gene expression. Our results clearly demonstrate that the Smad1/5 pathway is specifically required at an early stage of germ cell differentiation, corresponding to the RA-dependent commitment of ESCs.

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

Angiogenesis is a limiting factor in regenerating large bone defects. The objective of this study was to investigate angiogenic and osteogenic effects of co-culture on calcium phosphate cement (CPC) scaffold using human umbilical vein endothelial cells (hUVECs) and mesenchymal stem cells (MSCs)

from different origins for the first time. hUVECs were co-cultured with four types of cell: human umbilical cord MSCs (hUCMSCs), human bone marrow MSCs (hBMSCs) and MSCs from induced pluripotent stem cells (hiPSC-MSCs) and embryonic stem cells (hESC-MSCs). Constructs were implanted in 8 mm cranial defects of rats for 12 weeks. CPC without cells served as control 1. CPC with hBMSCs served as control 2. Microcapillary-like structures were successfully formed on CPC in vitro in all four co-cultured groups. Microcapillary lengths increased with time ($p < 0.05$). Osteogenic and angiogenic gene expressions were highly elevated and mineralization by co-cultured cells increased with time ($p < 0.05$). New bone amount and blood vessel density of co-cultured groups were much greater than controls ($p < 0.05$) in an animal study. hUVECs co-cultured with hUCMSCs, hiPSC-MSCs and hESC-MSCs achieved new bone and vessel density similar to hUVECs co-cultured with hBMSCs ($p > 0.1$). Therefore, hUCMSCs, hiPSC-MSCs and hESC-MSCs could serve as alternative cell sources to hBMSCs, which require an invasive procedure to harvest. In conclusion, this study showed for the first time that co-cultures of hUVECs with hUCMSCs, hiPSC-MSCs, hESC-MSCs and hBMSCs delivered via CPC scaffold achieved excellent osteogenic and angiogenic capabilities in vivo. The novel co-culture constructs are promising for bone reconstruction with improved angiogenesis for craniofacial/orthopaedic applications. Copyright (c) 2017 John Wiley & Sons, Ltd.

Chen, X., et al. (2018). "Directed Differentiation of Human Corneal Endothelial Cells From Human Embryonic Stem Cells by Using Cell-Conditioned Culture Media." Invest Ophthalmol Vis Sci **59**(7): 3028-3036.

Purpose: A shortage of human corneal endothelial cells (HCEC) for transplant and current methods of differentiation induction require chemical compounds, which might cast further influences after differentiation induction. Therefore, we developed a simple and straightforward approach to endothelial cell differentiation from human embryonic stem cells (hESC). Methods: HESC are used to differentiate into HCEC by employing a two-stage method, which involves the application of two different types of conditioned culture medium, human corneal stromal cell-conditioned medium (HCSC-CM) and lens epithelial cell (LEC) plus HCSC-CM (LEC-CM+HCEC-CM). In brief, hESCs were treated with different conditioned media to induce directed endothelial differentiation. Results: In the presence of conditioned culture medium, embryonic stem cells differentiate first under the control of periocular mesenchymal precursors (POMPs). Consequently, the expression of several POMP markers was observed. Following this first stage differentiation, POMPs were further directed to differentiate into corneal endothelial cell (CEC)-like cells in the presence of the second-conditioned culture medium. The differentiation of POMPs into CEC-like cells is regulated by a TGFbeta-2/FOXC1 signaling pathway that is activated by the factors present in the conditioned culture medium. Conclusions: HCEC-like cells could be differentiated from hESC by simply using a two-step, preconditioned, medium-mediated approach, which could significantly minimize the workload to generate HCEC for potential clinical use. This research may provide an ideal cell source for corneal regenerative medicine and clinical treatment for corneal diseases in the future.

Chen, X., et al. (2014). "Scleraxis-overexpressed human embryonic stem cell-derived mesenchymal stem cells for tendon tissue engineering with knitted silk-collagen scaffold." Tissue Eng Part A **20**(11-12): 1583-1592.

AIM: Despite our previous study that demonstrates that human embryonic stem cells (hESCs) can be used as seed cells for tendon tissue engineering after stepwise induction, suboptimal tendon regeneration implies that a new strategy needs to be developed for tendon repair. We investigated whether overexpression of the tendon-specific transcription factor scleraxis (SCX) in hESC-derived mesenchymal stem cells (hESC-MSCs) together with knitted silk-collagen sponge scaffold could promote tendon regeneration. METHODS AND RESULTS: hESCs were initially differentiated into MSCs and then engineered with scleraxis (SCX+hESC-MSCs). Engineered tendons were constructed with SCX+hESC-MSCs and a knitted silk-collagen sponge scaffold and then mechanical stress was applied. SCX elevated tendon gene expression in hESC-MSCs and concomitantly attenuated their adipogenic and chondrogenic potential. Mechanical stress further augmented the expression of tendon-specific genes in SCX+hESC-MSC-engineered tendon. Moreover, in vivo mechanical stimulation promoted the alignment of cells and increased the diameter of collagen fibers after ectopic transplantation. In the in vivo tendon repair model, the SCX+hESC-MSC-engineered tendon enhanced the regeneration process as shown by histological scores and superior mechanical performance compared with control cells, especially at early stages. CONCLUSION: Our study offers new evidence concerning the roles of SCX in tendon differentiation and regeneration. We demonstrated a novel strategy of combining hESCs, genetic engineering, and tissue-engineering principles for tendon regeneration, which are important for the future application of hESCs and silk scaffolds for tendon repair.

Chen, Y., et al. (2018). "Long-Term Engraftment Promotes Differentiation of Alveolar Epithelial Cells from Human Embryonic Stem Cell Derived Lung Organoids." Stem Cells Dev **27**(19): 1339-1349.

Human embryonic stem cell (hESC) derived 3D human lung organoids (HLOs) provide a promising model to study human lung development and disease. HLOs containing proximal or/and immature distal airway epithelial cells have been successfully generated in vitro, such as early staged alveolar type 2 (AT2) cells (SPC(+)/SOX9(+)) and immature alveolar type 1 (AT1) cells (HOPX(+)/SOX9(+)). When HLOs were transplanted into immunocompromised mice for further differentiation in vivo, only few distal epithelial cells could be observed. In this study, we transplanted different stages of HLOs into immunocompromised mice to assess whether HLOs could expand and mature in vivo. We found that short-term transplanted HLOs contained lung progenitor cells (NKX2.1(+), SOX9(+), and P63(+)), but not SPC(+) AT2 cells or AQP5(+) AT1 cells. Meanwhile, long-term engrafted HLOs could differentiate into lung distal bipotent progenitor cells (PDPN(+)/SPC(+)/SOX9(+)), AT2 cells (SPC(+), SPB(+)), and immature AT1 cells (PDPN(+), AQP5(-)). However, HLOs at late in vitro stage turned into mature AT1-like cells (AQP5(+)/SPB(-)/SOX9(-)) in vivo. Immunofluorescence staining and transmission electron microscopy (TEM) results revealed that transplanted HLOs contained mesenchymal cells (collagen I(+)), vasculature (ACTA2(+)), neuroendocrine-like cells (PGP9.5(+)), and nerve fiber structures (myelin sheath structure). Together, these data reveal that hESC-derived HLOs would be useful for human lung development modeling, and transplanted HLOs could mimic lung organ-like structures in vivo by possessing vascular network and neuronal network.

Cheung, C. and S. Sinha (2011). "Human embryonic stem cell-derived vascular smooth muscle cells in therapeutic neovascularisation." J Mol Cell Cardiol **51**(5): 651-664.

Ischemic diseases remain one of the major causes of morbidity and mortality throughout the world. In recent clinical trials on cell-based therapies, the use of adult stem and progenitor cells only elicited marginal benefits. Therapeutic neovascularisation is the Holy Grail for ischemic tissue recovery. There is compelling evidence from animal transplantation studies that the inclusion of mural cells in addition to endothelial cells (ECs) can enhance the formation of functional blood vessels. Vascular smooth muscle cells (SMCs) and pericytes are essential for the stabilisation of nascent immature endothelial tubes. Despite the intense interest in the utility of human embryonic stem cells (ESCs) for vascular regenerative medicine, ESC-derived vascular SMCs have received much less attention than ECs. This review begins with developmental insights into a range of smooth muscle progenitors from studies on embryos and ESC differentiation systems. We then summarise the methods of derivation of smooth muscle progenitors and cells from human ESCs. The primary emphasis is on the inherent heterogeneity of smooth muscle progenitors and cells and the limitations of current in vitro characterisation. Essential transplantation issues such as the type and source of therapeutic cells, mode of cell delivery, measures to enhance cell viability, putative mechanisms of benefit and long-term tracking of cell fate are also discussed. Finally, we highlight the challenges of clinical compatibility and scaling up for medical use in order to eventually realise the goal of human ESC-based vascular regenerative medicine.

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

BACKGROUND: Spontaneous in vitro transition of undifferentiated spermatogonia into the pluripotent cell state has been achieved using neonatal and adult mouse testis tissue. In an effort to establish an analogous source of human patient-specific pluripotent stem cells, several research groups have described the derivation of embryonic stem cell-like cells from primary cultures of human testis. These cells are characterized in all studies as growing in compact colonies, expressing pluripotency-associated markers and possessing multilineage differentiation capabilities in vitro, but only one study claimed their ability to induce teratomas. This controversy initiated a debate about the pluripotent state and origin of human testis-derived ES-like cells (htES-like cells). METHODS: htES-like cell colonies were obtained from primary testicular cultures of three individuals and selectively expanded using culture conditions known to support the propagation of blastocyst-derived human embryonic stem cells (ESCs), mouse epiblast stem cells and 'naive' human ESCs. The stem cell properties of htES-like cells were subsequently assessed by testing the expression of ESC-specific markers, differentiation abilities in vitro and in vivo, and microarray profiling. RESULTS: The expression of pluripotency-associated markers in htES-like cells and their differentiation abilities differed significantly from those of ESCs. Gene expression microarray analysis revealed that htES-like cells possess a transcriptome distinct from human ESCs and fibroblasts, but closely resembling the transcriptome of mesenchymal stem cells (MSCs). The similarity to MSCs was confirmed by detection of SSEA4/CD146 expressing cells within htES-like

colonies and efficient in vitro differentiation toward three mesodermal lineages (adipogenic, osteogenic, chondrogenic). CONCLUSIONS: Taken together, these results indicate that htES-like cells, in contrast to pluripotent stem cells derived from adult mouse testis, are not pluripotent and most likely not of germ cell but of mesenchymal origin.

Chinzei, R., et al. (2002). "Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes." Hepatology **36**(1): 22-29.

Embryonic stem (ES) cells have a potential to differentiate into various progenitor cells. Here we investigated the differentiation capacity of mouse ES cells into hepatocytes both in vitro and in vivo. During the culture of embryoid bodies (EBs) derived from ES cells, albumin (ALB) messenger RNA (mRNA) was expressed within 12 days after removal of leukemia inhibitory factor, and alpha-fetoprotein (AFP) mRNA was observed within 9 days without additional exogenous growth factors. In ES cells and early EBs, by contrast, neither ALB mRNA nor AFP mRNA was observed. ALB protein was first detected at day 15 and the level increased with the culture period. The differentiation of EBs facilitated the synthesis of urea with the culture period, whereas early EBs and ES cells produced no urea. These results suggest that cultured EBs contain hepatocytes capable of producing ALB and urea. ES cells and the isolated cells from EBs were transplanted through portal vein to the liver after 30% partial hepatectomy of female mice pretreated with 2-acetylaminofluorene. Four weeks after transplantation with isolated cells from day-9 EBs, ES-derived cells containing Y-chromosome in the liver were positive for ALB (0.2% of total liver cells), whereas teratoma was found in mice transplanted with ES cells or EBs up to day 6. The incidence of teratoma was decreased with the culture duration and no teratoma was observed in the liver transplanted with isolated cells from day-9 EBs. In conclusion, our in vitro and in vivo experiments revealed that cultured EBs contain functional hepatocytes or hepatocyte-like cells.

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

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

Cho, M., et al. (2006). "An alternative method of deriving embryonic stem cell-like clones by aggregation of diploid cells with tetraploid embryos." Fertil Steril **85 Suppl 1**: 1103-1110.

OBJECTIVE: To assess whether embryonic stem (ES) cells could be derived from the aggregation of diploid cells with tetraploid embryos. DESIGN: Randomized, prospective study. SETTING: University embryology and gamete biotechnology laboratory. ANIMAL(S): F1 (C57BL6/DBA2) mice. INTERVENTION(S): Four- to eight-cell F1 tetraploid embryos were aggregated with 10 to 15 donor E14 ES cells. MAIN OUTCOME MEASURE(S): Embryogenesis and ES cell establishment. RESULT(S): No difference (78% to 89%) in blastocyst formation was detected between the aggregated tetraploid and the control diploid embryos. In a total of 27 transfers, pregnancy was detected in three tetraploid (23.1%) and five diploid (35.7%) cases, and three live births developed from the aggregated tetraploid embryos. The tetraploid blastocysts without aggregation were plated, but no ES cell-like colony was formed. Six of eight aggregated blastocysts derived well-proliferated colonies, which were positive for anti-stage-specific embryonic antigen (SSEA)-1 antibody, Oct-4, and alkaline phosphatase. The microsatellite assay confirmed the homogenous makeup among the donor E14 cells and live-birth and ES-like cells derived from the E14-aggregated, tetraploid embryo. CONCLUSION(S): The aggregation of pluripotent

diploid cells with tetraploid embryos yielded live births and ES-like cells that were homogenous to the donor diploid cells.

Cho, M. S., et al. (2012). "Generation of retinal pigment epithelial cells from human embryonic stem cell-derived spherical neural masses." Stem Cell Res **9**(2): 101-109.

Dysfunction and loss of retinal pigment epithelium (RPE) are major pathologic changes observed in various retinal degenerative diseases such as aged-related macular degeneration. RPE generated from human pluripotent stem cells can be a good candidate for RPE replacement therapy. Here, we show the differentiation of human embryonic stem cells (hESCs) toward RPE with the generation of spherical neural masses (SNMs), which are pure masses of hESCs-derived neural precursors. During the early passaging of SNMs, cystic structures arising from opened neural tube-like structures showed pigmented epithelial morphology. These pigmented cells were differentiated into functional RPE by neuroectodermal induction and mechanical purification. Most of the differentiated cells showed typical RPE morphologies, such as a polygonal-shaped epithelial monolayer, and transmission electron microscopy revealed apical microvilli, pigment granules, and tight junctions. These cells also expressed molecular markers of RPE, including Mitf, ZO-1, RPE65, CRALBP, and bestrophin. The generated RPE also showed phagocytosis of isolated bovine photoreceptor outer segment and secreting pigment epithelium-derived factor and vascular endothelial growth factor. Functional RPE could be generated from SNM in our method. Because SNMs have several advantages, including the capability of expansion for long periods without loss of differentiation capability, easy storage and thawing, and no need for feeder cells, our method for RPE differentiation may be used as an efficient strategy for generating functional RPE cells for retinal regeneration therapy.

Cho, Y. M., et al. (2008). "Betacellulin and nicotinamide sustain PDX1 expression and induce pancreatic beta-cell differentiation in human embryonic stem cells." Biochem Biophys Res Commun **366**(1): 129-134.

The major obstacle in cell therapy of diabetes mellitus is the limited source of insulin-producing beta cells. Very recently, it was shown that a five-stage protocol recapitulating in vivo pancreatic organogenesis induced pancreatic beta cells in vitro; however, this protocol is specific to certain cell lines and shows much line-to-line variation in differentiation efficacy. Here, we modified the five-stage protocol for the human embryonic stem cell line SNUhES3 by the addition of betacellulin and nicotinamide. We reproduced in vivo pancreatic islet differentiation by directing the cells through stages that resembled in vivo pancreatic organogenesis. The addition of betacellulin and nicotinamide sustained PDX1 expression and induced beta-cell differentiation. C-peptide-a genuine marker of de novo insulin production-was identified in the differentiated cells, although the insulin mRNA content was very low. Further studies are necessary to develop more efficient and universal protocols for beta-cell differentiation.

Choi, H. S., et al. (2008). "Development of a decoy immunization strategy to identify cell-surface molecules expressed on undifferentiated human embryonic stem cells." Cell Tissue Res **333**(2): 197-206.

Little is known about the cell-surface molecules that are related to the undifferentiated and pluripotent state of human embryonic stem cells (hESCs). Here, we generated a panel of murine monoclonal antibodies (MAb) against undifferentiated hESCs by a modification of a previously described decoy immunization strategy. H9 hESCs were differentiated in the presence of retinoic acid and used as a decoy immunogen. Twelve Balb/c mice were immunized in the right hind footpads with differentiated H9 cells and in the left hind footpads with undifferentiated H9 cells. After immunization, the left popliteal lymph node cells were collected and were fused with mouse myeloma cells. The fusion resulted in 79 hybridomas secreting MAbs that bound to the undifferentiated H9 cells as shown by flow cytometric analysis. Of these, 70 MAbs bound to the undifferentiated H9 cells, but only weakly or not at all to the differentiated H9 cells. We characterized 37 MAbs (32 IgGs, 5 IgMs) recognizing surface molecules that were down-regulated during embryoid body cell formation. One of the MAbs, L125-C2, was confirmed to immunoprecipitate CD9, previously known as a surface molecule on the undifferentiated hESCs. To investigate the relationship between the MAbs and hESC-specific antibodies, two representative MAbs, viz., L125-C2 and 291-D4, were selected and studied by multi-color flow cytometric analysis. This showed that more than 60% of L125-C2- and 291-D4-positive cells were also positive for the expression of hESC-specific surface molecules such as SSEA3, SSEA4, TRA-1-60, and TRA-1-81, indicating the close relationship between the two MAbs and the hESC-specific surface molecules. Our results suggest that the decoy immunization strategy is an efficient method for isolating a panel of MAbs against undifferentiated hESCs, and that the generated MAbs should be useful for studying the surface molecules on hESCs in the pluripotent and undifferentiated state.

Choi, H. S., et al. (2014). "Antibody approaches to prepare clinically transplantable cells from human embryonic stem cells: identification of human embryonic stem cell surface markers by monoclonal antibodies." Biotechnol J **9**(7): 915-920.

Human embryonic stem cells (hESCs) are unique cell populations, possessing both unlimited self-renewal capacity and pluripotency, i.e. the potential to give rise to all kinds of specialized cells in the human body. Marker molecules expressed on the surface of hESCs are important for the identification, characterization, and clinical application of hESCs. Compared with conventional genomics- or proteomics-based approaches, generating monoclonal antibody (mAb) libraries against hESCs using alternative methodologies expands the repertoire of mAbs raised against non-protein markers, for example, glycolipid antigens. Additional information about the conformation and post-translational modification of surface molecules can also be obtained. In this article, we review how mAb libraries against hESC surface markers have been developed using whole-cell and decoy immunization strategies.

Choi, J. H., et al. (2011). "Generation of viable embryos and embryonic stem cell-like cells from cultured primary follicles in mice." Biol Reprod **85**(4): 744-754.

Primary follicles retrieved from B6CBAF1 prepubertal mice were cultured in a stepwise manner in an alpha-minimum essential medium-based medium to generate viable embryos and embryonic stem cell (ESC)-like cells. A significant increase in follicle growth and oocyte maturation accompanied by increased secretion of 17beta-estradiol and progesterone was achieved by exposing primary follicles to 100 or 200 mIU of follicle-stimulating hormone (FSH) during culture. More oocytes developed into blastocysts following in vitro fertilization (IVF) or parthenogenetic activation after culture with 200 mIU of FSH during the entire culture period than with 100 mIU. Eleven ESC-like cell lines, consisting of four heterozygotic and seven homozygotic phenotypes, were established from 25 trials of primary follicle culture combined with IVF or parthenogenetic activation. In conclusion, primary follicles can potentially yield developmentally competent oocytes, which produce viable embryos and ESC-like cell lines following in vitro manipulation. We suggest a method to utilize immature follicles, which are most abundant in ovaries, to improve reproductive efficiency and for use in regenerative medicine.

Choi, S., et al. (2013). "Regulation of Pluripotency-related Genes and Differentiation in Mouse Embryonic Stem Cells by Direct Delivery of Cell-penetrating Peptide-conjugated CARM1 Recombinant Protein." Dev Reprod **17**(1): 9-16.

Coactivator-associated arginine methyltransferase 1 (CARM1) is included in the protein arginine methyltransferase (PRMT) family, which methylates histone arginine residues through posttranslational modification. It has been proposed that CARM1 may up-regulate the expression of pluripotency-related genes through the alteration of the chromatin structure. Mouse embryonic stem cells (mESCs) are pluripotent and have the ability to self-renew. The cells are mainly used to study the genetic function of novel genes, because the cells facilitate the transmission of the manipulated genes into target mice. Since the up-regulated methylation levels of histone arginine residue lead to the maintenance of pluripotency in embryos and stem cells, it may be suggested that CARM1 overexpressing mESCs elevate the expression of pluripotency-related genes in reconstituted embryos for transgenic mice and may resist the differentiation into trophectoderm (TE). We constructed a fusion protein by connecting CARM1 and 7X-arginine (R7). As a cell-penetrating peptide (CPP), can translocate CARM1 protein into mESCs. CPP-CARM1 protein was detected in the nuclei of the mESCs after a treatment of 24 hours. Accordingly, the expression of pluripotency-related genes was up-regulated in CPP-CARM1-treated mESCs. In addition, CPP-CARM1-treated mESC-derived embryoid bodies (EBs) showed an elevated expression of pluripotency-related genes and delayed spontaneous differentiation. This result suggests that the treatment of recombinant CPP-CARM1 protein elevates the expression of pluripotency-related genes of mESCs by epigenetic modification, and this protein-delivery system could be used to modify embryonic fate in reconstituted embryos with mESCs.

Choi, Y. J., et al. (2018). "Phthalazinone Pyrazole Enhances the Hepatic Functions of Human Embryonic Stem Cell-Derived Hepatocyte-Like Cells via Suppression of the Epithelial-Mesenchymal Transition." Stem Cell Rev **14**(3): 438-450.

During liver development, nonpolarized hepatic progenitor cells differentiate into mature hepatocytes with distinct polarity. This polarity is essential for maintaining the intrinsic properties of hepatocytes. The balance between the epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) plays a decisive role in differentiation of polarized hepatocytes. In this study, we found that phthalazinone pyrazole (PP), a selective inhibitor of Aurora-A kinase (Aurora-A), suppressed the EMT during the differentiation of hepatocyte-like cells (HLCs) from human embryonic stem cells. The

differentiated HLCs treated with PP at the hepatoblast stage showed enhanced hepatic morphology and functions, particularly with regard to the expression of drug metabolizing enzymes. Moreover, we found that these effects were mediated though suppression of the AKT pathway, which is involved in induction of the EMT, and upregulation of hepatocyte nuclear factor 4alpha expression rather than Aurora-A inhibition. In conclusion, these findings provided insights into the regulatory role of the EMT on in vitro hepatic maturation, suggesting that inhibition of the EMT may drive transformation of hepatoblast cells into mature and polarized HLCs.

Chowdhury, F., et al. (2010). "Soft substrates promote homogeneous self-renewal of embryonic stem cells via downregulating cell-matrix tractions." PLoS One **5**(12): e15655.

Maintaining undifferentiated mouse embryonic stem cell (mESC) culture has been a major challenge as mESCs cultured in Leukemia Inhibitory Factor (LIF) conditions exhibit spontaneous differentiation, fluctuating expression of pluripotency genes, and genes of specialized cells. Here we show that, in sharp contrast to the mESCs seeded on the conventional rigid substrates, the mESCs cultured on the soft substrates that match the intrinsic stiffness of the mESCs and in the absence of exogenous LIF for 5 days, surprisingly still generated homogeneous undifferentiated colonies, maintained high levels of Oct3/4, Nanog, and Alkaline Phosphatase (AP) activities, and formed embryoid bodies and teratomas efficiently. A different line of mESCs, cultured on the soft substrates without exogenous LIF, maintained the capacity of generating homogeneous undifferentiated colonies with relatively high levels of Oct3/4 and AP activities, up to at least 15 passages, suggesting that this soft substrate approach applies to long term culture of different mESC lines. mESC colonies on these soft substrates without LIF generated low cell-matrix tractions and low stiffness. Both tractions and stiffness of the colonies increased with substrate stiffness, accompanied by downregulation of Oct3/4 expression. Our findings demonstrate that mESC self-renewal and pluripotency can be maintained homogeneously on soft substrates via the biophysical mechanism of facilitating generation of low cell-matrix tractions.

Chowdhury, F., et al. (2010). "Material properties of the cell dictate stress-induced spreading and differentiation in embryonic stem cells." Nat Mater **9**(1): 82-88.

Growing evidence suggests that physical microenvironments and mechanical stresses, in addition to soluble factors, help direct mesenchymal-stem-cell fate. However, biological responses to a local force in embryonic stem cells remain elusive. Here we show that a local cyclic stress through focal adhesions induced spreading in mouse embryonic stem cells but not in mouse embryonic stem-cell-differentiated cells, which were ten times stiffer. This response was dictated by the cell material property (cell softness), suggesting that a threshold cell deformation is the key setpoint for triggering spreading responses. Traction quantification and pharmacological or shRNA intervention revealed that myosin II contractility, F-actin, Src or cdc42 were essential in the spreading response. The applied stress led to oct3/4 gene downregulation in mES cells. Our findings demonstrate that cell softness dictates cellular sensitivity to force, suggesting that local small forces might have far more important roles in early development of soft embryos than previously appreciated.

Christoforou, N., et al. (2010). "Implantation of mouse embryonic stem cell-derived cardiac progenitor cells preserves function of infarcted murine hearts." PLoS One **5**(7): e11536.

Stem cell transplantation holds great promise for the treatment of myocardial infarction injury. We recently described the embryonic stem cell-derived cardiac progenitor cells (CPCs) capable of differentiating into cardiomyocytes, vascular endothelium, and smooth muscle. In this study, we hypothesized that transplanted CPCs will preserve function of the infarcted heart by participating in both muscle replacement and neovascularization. Differentiated CPCs formed functional electromechanical junctions with cardiomyocytes in vitro and conducted action potentials over cm-scale distances. When transplanted into infarcted mouse hearts, CPCs engrafted long-term in the infarct zone and surrounding myocardium without causing teratomas or arrhythmias. The grafted cells differentiated into cross-striated cardiomyocytes forming gap junctions with the host cells, while also contributing to neovascularization. Serial echocardiography and pressure-volume catheterization demonstrated attenuated ventricular dilatation and preserved left ventricular fractional shortening, systolic and diastolic function. Our results demonstrate that CPCs can engraft, differentiate, and preserve the functional output of the infarcted heart.

Chuang, C. Y., et al. (2012). "Meiotic competent human germ cell-like cells derived from human embryonic stem cells induced by BMP4/WNT3A signaling and OCT4/EpCAM (epithelial cell adhesion molecule) selection." J Biol Chem **287**(18): 14389-14401.

The establishment of an effective germ cell selection/enrichment platform from in vitro

differentiating human embryonic stem cells (hESCs) is crucial for studying the molecular and signaling processes governing human germ cell specification and development. In this study, we developed a germ cell-enriching system that enables us to identify signaling factors involved in germ cell-fate induction from differentiating hESCs in vitro. First, we demonstrated that selection through an OCT4-EGFP reporter system can successfully increase the percentage of meiotic-competent, germ cell-like cells from spontaneously differentiating hESCs. Furthermore, we showed that the pluripotency associated surface marker, epithelial cell adhesion molecule (EpCAM), is also expressed in human fetal gonads and can be used as an effective selection marker for germ cell enrichment from differentiating hESCs. Combining OCT4 and EpCAM selection can further enrich the meiotic-competent germ cell-like cell population. Also, with the percentage of OCT4(+)/EpCAM(+) cells as readout, we demonstrated the synergistic effect of BMP4/pSMAD1/5/8 and WNT3A/beta-CATENIN in promoting hESCs toward the germline fate. Combining BMP4/WNT3A induction and OCT4/EpCAM selection can significantly increase the putative germ cell population with meiotic competency. Co-transplantation of these cells with dissociated mouse neonatal ovary cells into SCID mice resulted in a homogenous germ cell cluster formation in vivo. The stepwise platform established in this study provides a useful tool to elucidate the molecular mechanisms of human germ cell development, which has implications not only for human fertility research but regenerative medicine in general.

Clark, A. T. (2007). "Establishment and differentiation of human embryonic stem cell derived germ cells." Soc Reprod Fertil Suppl **63**: 77-86.

Germ cells are absolutely essential for fertility. Aberrant germ cell development can result in abnormal gonadal function, incomplete embryogenesis and infertility, or germ cell tumors. Our understanding of the molecular regulation of normal germ cell development in mammals has progressed significantly due to the utility of the mouse as a genetic model system. However, the molecular regulation of human germ cell development is almost completely unknown due to the historical lack of a malleable model. The purpose of this review is to compare the cell-based events leading up to the specification of the germ cell lineage in both mice and humans and to discuss some of the key signaling pathways that have recently been identified, which regulate germ cell specification. In addition, the new cell-based models for differentiating germ cells from both mouse and human embryonic stem cells (ESCs) will be summarized.

Clark, A. T. and R. A. Reijo Pera (2006). "Modeling human germ cell development with embryonic stem cells." Regen Med **1**(1): 85-93.

There has previously been no robust cell-based model for examining the genetic and epigenetic mechanisms of human germ cell formation. Human embryonic stem cells (hESCs) could potentially fill this need, as all cell types analyzed to date (including mature germ cells) can be identified by marker analysis during hESC differentiation. Furthermore, hESCs could also be used to differentiate mature female germ cells (oocytes) in culture as an alternate reprogramming cell for somatic cell nuclear transfer. However, to differentiate and isolate a functional germ cell from hESCs, the mechanisms that regulate germ cell formation need to be understood. The purpose of this review is to summarize the current understanding of the earliest events in human germ cell formation and to describe some of the known genetic pathways that regulate germ cell specification and development in the mouse. Finally, the current literature on the formation of germ cells from ESCs will be described.

Cobo, F., et al. (2008). "Electron microscopy reveals the presence of viruses in mouse embryonic fibroblasts but neither in human embryonic fibroblasts nor in human mesenchymal cells used for hESC maintenance: toward an implementation of microbiological quality assurance program in stem cell banks." Cloning Stem Cells **10**(1): 65-74.

Human embryonic stem cells (hESCs) are expected to open up new avenues in regenerative medicine by allowing the generation of transplantable cells to be used in future cell replacement therapies. Maintenance of hESCs in the presence of xenogenic compounds is likely to prevent their use in future therapeutic applications in humans. Recently, it has been claimed that human foreskin-derived human embryonic fibroblast (HEFs) and human adult marrow cells have the ability to support prolonged expansion of hESCs in culture similar to murine feeders. Here, to minimize the use of xenogenic components for hESC maintenance, we performed transmission electron microscopy-based microbiological studies in an attempt to implement a microbiological Quality Assurance Program in Stem Cell Banks by determining the potential presence of viral particles in MEFs compared with human HEFs and bone marrow-derived mesenchymal cells. We observed in three out of nine MEF samples (33.3%) viruses belonging to the Retroviridae family. Within the Retroviridae family, these viruses have a C morphology, which indicates they belong to the subfamily Orthoretroviridae. In contrast, no viral particles could be observed in either the HEF samples (n = 5) or the human BM-derived mesenchymal cells (n = 9) analyzed. Based on these

experimental microbiological data, we recommend the implementation of microbiological Quality Assurance Programs by means of transmission electron microscopy as a routine technique to assess the potential presence of viral particles in any feeder cell used in stem cell banks and support the use of human cells rather than murine cells as feeders to maintain hESC cultures in an undifferentiated state.

Coll, J. L., et al. (1995). "Targeted disruption of vinculin genes in F9 and embryonic stem cells changes cell morphology, adhesion, and locomotion." Proc Natl Acad Sci U S A **92**(20): 9161-9165.

Vinculin, a major constituent of focal adhesions and zonula adherens junctions, is thought to be involved in linking the microfilaments to areas of cell-substrate and cell-cell contacts. To test the role of vinculin in cell adhesion and motility, we used homologous recombination to generate F9 embryonal carcinoma and embryonic stem cell clones homozygous for a disrupted vinculin gene. When compared to wild-type cells, vinculin-mutant cells displayed a rounder morphology and a reduced ability to adhere and spread on plastic or fibronectin. Decreased adhesion of the mutant cells was associated with a reduction in lamellipodial extensions, as observed by time-lapse video microscopy. The locomotive activities of control F9 and the vinculin-null cells were compared in two assays. Loss of vinculin resulted in a 2.4-fold increase in cell motility. These results demonstrate an important role for vinculin in determining cell shape, adhesion, surface protrusive activity, and cell locomotion.

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

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

Cong, S., et al. (2014). "Effects of different feeder layers on culture of bovine embryonic stem cell-like cells in vitro." Cytotechnology **66**(6): 995-1005.

To find a suitable feeder layer is important for successful culture conditions of bovine embryonic stem cell-like cells. In this study, expression of pluripotency-related genes OCT4, SOX2 and NANOG in bovine embryonic stem cell-like cells on mouse embryonic fibroblast feeder layers at 1-5 passages were monitored in order to identify the possible reason that bovine embryonic stem cell-like cells could not continue growth and passage. Here, we developed two novel feeder layers, mixed embryonic fibroblast feeder layers of mouse and bovine embryonic fibroblast at different ratios and sources including mouse fibroblast cell lines. The bovine embryonic stem cell-like cells generated in our study displayed typical stem cell morphology and expressed specific markers such as OCT4, stage-specific embryonic antigen 1 and 4, alkaline phosphatase, SOX2, and NANOG mRNA levels. When feeder layers and cell growth factors were removed, the bovine embryonic stem cell-like cells formed embryoid bodies in a suspension culture. Furthermore, we compared the expression of the pluripotent markers during bovine embryonic stem cell-like cell in culture on mixed embryonic fibroblast feeder layers, including mouse fibroblast cell lines feeder layers and mouse embryonic fibroblast feeder layers by real-time quantitative polymerase chain reaction. Results suggested that mixed embryonic fibroblast and sources including mouse fibroblast cell lines feeder layers were more suitable for long-term culture and growth of bovine embryonic stem cell-like cells than mouse embryonic fibroblast feeder layers. The findings may provide useful experimental data for the establishment of an appropriate culture system for bovine embryonic stem cell lines.

Corrales, C. E., et al. (2006). "Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti." J Neurobiol **66**(13): 1489-1500.

Hearing loss in mammals is irreversible because cochlear neurons and hair cells do not regenerate. To determine whether we could replace neurons lost to primary neuronal degeneration, we injected EYFP-expressing embryonic stem cell-derived mouse neural progenitor cells into the cochlear nerve trunk in immunosuppressed animals 1 week after destroying the cochlear nerve (spiral ganglion) cells while leaving hair cells intact by ouabain application to the round window at the base of the cochlea in gerbils. At 3 days post transplantation, small grafts were seen that expressed endogenous EYFP and could be immunolabeled for neuron-specific markers. Twelve days after transplantation, the grafts had neurons that extended processes from the nerve core toward the denervated organ of Corti. By 64-98 days, the grafts had sent out abundant processes that occupied a significant portion of the space formerly occupied by the cochlear nerve. The neurites grew in fasciculating bundles projecting through Rosenthal's canal, the former site of spiral ganglion cells, into the osseous spiral lamina and ultimately into the organ of Corti, where they contacted hair cells. Neuronal counts showed a significant increase in neuronal processes near the sensory epithelium, compared to animals that were denervated without subsequent stem cell transplantation. The regeneration of these neurons shows that neurons differentiated from stem cells have the capacity to grow to a specific target in an animal model of neuronal degeneration.

Corti, S., et al. (2010). "Embryonic stem cell-derived neural stem cells improve spinal muscular atrophy phenotype in mice." Brain **133**(Pt 2): 465-481.

Spinal muscular atrophy, characterized by selective loss of lower motor neurons, is an incurable genetic neurological disease leading to infant mortality. We previously showed that primary neural stem cells derived from spinal cord can ameliorate the spinal muscular atrophy phenotype in mice, but this primary source has limited translational value. Here, we illustrate that pluripotent stem cells from embryonic stem cells show the same potential therapeutic effects as those derived from spinal cord and offer great promise as an unlimited source of neural stem cells for transplantation. We found that embryonic stem cell-derived neural stem cells can differentiate into motor neurons in vitro and in vivo. In addition, following their intrathecal transplantation into spinal muscular atrophy mice, the neural stem cells, like those derived from spinal cord, survived and migrated to appropriate areas, ameliorated behavioural endpoints and lifespan, and exhibited neuroprotective capability. Neural stem cells obtained using a drug-selectable embryonic stem cell line yielded the greatest improvements. As with cells originating from primary tissue, the embryonic stem cell-derived neural stem cells integrated appropriately into the parenchyma, expressing neuron- and motor neuron-specific markers. Our results suggest translational potential for the use of pluripotent cells in neural stem cell-mediated therapies and highlight potential safety improvements and benefits of drug selection for neuroepithelial cells.

Costa, A. and D. Henrique (2015). "Transcriptome profiling of induced hair cells (iHCs) generated by combined expression of Gfi1, Pou4f3 and Atoh1 during embryonic stem cell differentiation." Genom Data **6**: 77-80.

To gain new insights about the genetic networks controlling hair cell (HC) development, we previously developed a direct genetic programming strategy to generate an inexhaustible supply of HC-like cells (induced HCs, iHCs) in vitro, starting from mouse embryonic stem cells (ESC). We found that combined activity of three transcription factors, Gfi1, Pou4f3, and Atoh1, can program ESC-derived progenitors towards HC fate with efficiencies of 55%-80%. These iHCs express several HC markers and exhibit polarized structures that are highly reminiscent of the mechanosensitive hair bundles, with many microvilli-like stereocilia. Here, we describe the experimental design, methodology, and data validation for the microarray analysis used to characterize the transcriptome profile of iHCs at different stages of their differentiation. This approach based on FACS sorting and microarray analysis revealed a highly similar iHC transcriptome to that of endogenous HCs in vivo. The data obtained in this study is available in the Gene Expression Omnibus (GEO) database (accession number GSE60352).

Coulombel, L. (2010). "[A big step forward in the identification of therapeutic human embryonic stem cells-derived progenitors for cardiac cell therapy]." Med Sci (Paris) **26**(4): 439-441.

Couteaudier, M., et al. (2016). "Keratinocytes derived from chicken embryonic stem cells support Marek's disease virus infection: a highly differentiated cell model to study viral replication and morphogenesis." Virol J **13**: 7.

BACKGROUND: Marek's disease is a virus disease with worldwide distribution that causes major losses to poultry production. Vaccines against Marek's disease virus, an oncogenic alphaherpesvirus, reduce

tumour formation but have no effect on virus shedding. Successful horizontal virus transmission is linked to the active viral replication in feather follicle epithelial cells of infected chickens, from which infectious viral particles are shed into the environment. The feather follicle epithelium is the sole tissue in which those infectious particles are produced and no in vitro cell-systems can support this highly efficient morphogenesis. We previously characterized embryonic stem-cell-derived keratinocytes, showing they display a marker-gene profile similar to skin keratinocytes, and therefore we tested their susceptibility to Marek's disease virus infection. FINDINGS: We show herein that keratinocytes derived from chicken embryonic stem-cells are fully permissive to the replication of either non-pathogenic or pathogenic Marek's disease viruses. All viruses replicated on all three keratinocyte lines and kinetics of viral production as well as viral loads were similar to those obtained on primary cells. Morphogenesis studies were conducted on infected keratinocytes and on corneocytes, showing that all types of capsids/virions were present inside the cells, but extracellular viruses were absent. CONCLUSIONS: The keratinocyte lines are the first epithelial cell-line showing ectodermal specific markers supporting Marek's disease virus replication. In this in vitro model the replication lead to the production of cell-associated viral progeny. Further work will be devoted to the study of relationship between 3D differentiation of keratinocytes and Marek's disease virus replication.

Couteaudier, M., et al. (2015). "Derivation of keratinocytes from chicken embryonic stem cells: establishment and characterization of differentiated proliferative cell populations." Stem Cell Res **14**(2): 224-237.

A common challenge in avian cell biology is the generation of differentiated cell-lines, especially in the keratinocyte lineage. Only a few avian cell-lines are available and very few of them show an interesting differentiation profile. During the last decade, mammalian embryonic stem cell-lines were shown to differentiate into almost all lineages, including keratinocytes. Although chicken embryonic stem cells had been obtained in the 1990s, few differentiation studies toward the ectodermal lineage were reported. Consequently, we explored the differentiation of chicken embryonic stem cells toward the keratinocyte lineage by using a combination of stromal induction, ascorbic acid, BMP4 and chicken serum. During the induction period, we observed a downregulation of pluripotency markers and an upregulation of epidermal markers. Three homogenous cell populations were derived, which were morphologically similar to chicken primary keratinocytes, displaying intracellular lipid droplets in almost every pavimentous cell. These cells could be serially passaged without alteration of their morphology and showed gene and protein expression profiles of epidermal markers similar to chicken primary keratinocytes. These cells represent an alternative to the isolation of chicken primary keratinocytes, being less cumbersome to handle and reducing the number of experimental animals used for the preparation of primary cells.

Crocker, S. J., et al. (2011). "Intravenous administration of human embryonic stem cell-derived neural precursor cells attenuates cuprizone-induced central nervous system (CNS) demyelination." Neuropathol Appl Neurobiol **37**(6): 643-653.

AIMS: Previous studies have demonstrated the therapeutic potential for human embryonic stem cell-derived neural precursor cells (hES-NPCs) in autoimmune and genetic animal models of demyelinating diseases. Herein, we tested whether intravenous (i.v.) administration of hES-NPCs would impact central nervous system (CNS) demyelination in a cuprizone model of demyelination. METHODS: C57Bl/6 mice were fed cuprizone (0.2%) for 2 weeks and then separated into two groups that either received an i.v. injection of hES-NPCs or i.v. administration of media without these cells. After an additional 2 weeks of dietary cuprizone treatment, CNS tissues were analysed for detection of transplanted cells and differences in myelination in the region of the corpus callosum (CC). RESULTS: Cuprizone-induced demyelination in the CC was significantly reduced in mice treated with hES-NPCs compared with cuprizone-treated controls that did not receive stem cells. hES-NPCs were identified within the brain tissues of treated mice and revealed migration of transplanted cells into the CNS. A limited number of human cells were found to express the mature oligodendrocyte marker, O1, or the astrocyte marker, glial fibrillary acidic protein. Reduced apoptosis and attenuated microglial and astrocytic responses were also observed in the CC of hES-NPC-treated mice. CONCLUSIONS: These findings indicated that systemically administered hES-NPCs migrated from circulation into a demyelinated lesion within the CNS and effectively reduced demyelination. Observed reductions in astrocyte and microglial responses, and the benefit of hES-NPC treatment in this model of myelin injury was not obviously accountable to tissue replacement by exogenously administered cells.

Cruz, A. C., et al. (2004). "Tumor necrosis factor-alpha-converting enzyme controls surface expression of c-Kit and survival of embryonic stem cell-derived mast cells." J Biol Chem **279**(7): 5612-5620.

Transmembrane metalloproteinases of the disintegrin and metalloproteinase (ADAM) family control cell signaling interactions via hydrolysis of protein extracellular domains. Prior work has shown that the receptor tyrosine kinase, c-Kit (CD117), is essential for mast cell survival and that serum levels of c-Kit increase in proliferative mast cell disorders, suggesting the existence of c-Kit shedding pathways in mast cells. In the present work, we report that tumor necrosis factor alpha-converting enzyme (TACE; ADAM-17) mediates shedding of c-Kit. Stimulation of transfected cells with phorbol 12-myristate 13-acetate (PMA) induced metalloproteinase-mediated release of c-Kit ectodomain, which increased further upon TACE overexpression. By contrast, TACE-deficient fibroblasts did not demonstrate inducible release, thus identifying TACE as the metalloproteinase primarily responsible for PMA-induced c-Kit shedding. Surface expression of c-Kit by the human mast cell-1 line decreased upon phorbol-induced shedding, which involved metalloproteinase activity susceptible to inhibition by tissue inhibitor of metalloproteinase (TIMP)-3. To further explore the role of TACE in shedding of c-Kit from mast cells, we compared the behavior of mast cells derived from murine embryonic stem cells. In these studies, PMA decreased surface c-Kit levels on mast cells expressing wild-type (+/+) TACE but not on those expressing an inactive mutant (DeltaZn/DeltaZn), confirming the role of TACE in PMA-induced c-Kit shedding. Compared with TACE(+/+) cells, TACE(DeltaZn/DeltaZn) mast cells also demonstrated decreased constitutive shedding and increased basal surface expression of c-Kit, with diminished apoptosis in response to c-Kit ligand deprivation. These data suggest that TACE controls mast cell survival by regulating shedding and surface expression of c-Kit.

Cui, L., et al. (2004). "Spatial distribution and initial changes of SSEA-1 and other cell adhesion-related molecules on mouse embryonic stem cells before and during differentiation." J Histochem Cytochem **52**(11): 1447-1457.

We examined the distribution of cell adhesion-related molecules (CAMs) among mouse embryonic stem (ES) cells and the spatial distribution on cell surfaces before and during differentiation. The cell-cell heterogeneity of SSEA-1, PECAM-1, and ICAM-1 among the undifferentiated cells in the ES cell colonies was evident by immunohistochemistry and immuno-SEM, supporting the flow cytometry findings. In contrast, most undifferentiated ES cells strongly expressed CD9. SSEA-1 was located preferentially on the edge of low protuberances and microvilli and formed clusters or linear arrays of 3-20 particles. PECAM-1 and ICAM-1 were randomly localized on the free cell surfaces, whereas CD9 was preferentially localized on the microvilli or protuberances, especially in the cell periphery. Both the SSEA-1(+) fraction and the SSEA-1(-) fraction of magnetic cell sorting (MACS) formed undifferentiated colonies after plating. Flow cytometry showed that these populations reverted separately again to a culture with a mixed phenotype. Differentiation induced by retinoic acid downregulated the expression of all CAMs. Immuno-SEM showed decreases of SSEA-1 in the differentiated ES cells, although some clustering still remained. Our findings help to elucidate the significance of these molecules in ES cell maintenance and differentiation and suggest that cell surface antigens may be useful for defining the phenotype of undifferentiated and differentiated ES cells.

Cusulin, C., et al. (2012). "Embryonic stem cell-derived neural stem cells fuse with microglia and mature neurons." Stem Cells **30**(12): 2657-2671.

Transplantation of neural stem cells (NSCs) is a novel strategy to restore function in the diseased brain, acting through multiple mechanisms, for example, neuronal replacement, neuroprotection, and modulation of inflammation. Whether transplanted NSCs can operate by fusing with microglial cells or mature neurons is largely unknown. Here, we have studied the interaction of a mouse embryonic stem cell-derived neural stem (NS) cell line with rat and mouse microglia and neurons in vitro and in vivo. We show that NS cells spontaneously fuse with cocultured cortical neurons, and that this process requires the presence of microglia. Our in vitro data indicate that the NS cells can first fuse with microglia and then with neurons. The fused NS/microglial cells express markers and retain genetic and functional characteristics of both parental cell types, being able to respond to microglia-specific stimuli (LPS and IL-4/IL-13) and to differentiate to neurons and astrocytes. The NS cells fuse with microglia, at least partly, through interaction between phosphatidylserine exposed on the surface of NS cells and CD36 receptor on microglia. Transplantation of NS cells into rodent cortex results in fusion with mature pyramidal neurons, which often carry two nuclei, a process probably mediated by microglia. The fusogenic role of microglia could be even more important after NSC transplantation into brains affected by neurodegenerative diseases associated with microglia activation. It remains to be elucidated how the occurrence of the fused cells will influence the functional outcome after NSC transplantation in the diseased brain.

Czerwinska, A. M., et al. (2016). "Cell cycle regulation of embryonic stem cells and mouse embryonic

fibroblasts lacking functional Pax7." Cell Cycle **15**(21): 2931-2942.

The transcription factor Pax7 plays a key role during embryonic myogenesis and in adult organisms in that it sustains the proper function of satellite cells, which serve as adult skeletal muscle stem cells. Recently we have shown that lack of Pax7 does not prevent the myogenic differentiation of pluripotent stem cells. In the current work we show that the absence of functional Pax7 in differentiating embryonic stem cells modulates cell cycle facilitating their proliferation. Surprisingly, deregulation of Pax7 function also positively impacts at the proliferation of mouse embryonic fibroblasts. Such phenotypes seem to be executed by modulating the expression of positive cell cycle regulators, such as cyclin E.

Daadi, M. M., et al. (2012). "Dopaminergic neurons from midbrain-specified human embryonic stem cell-derived neural stem cells engrafted in a monkey model of Parkinson's disease." PLoS One **7**(7): e41120.

The use of human embryonic stem cells (hESCs) to repair diseased or injured brain is promising technology with significant humanitarian, societal and economic impact. Parkinson's disease (PD) is a neurological disorder characterized by the loss of midbrain dopaminergic (DA) neurons. The generation of this cell type will fulfill a currently unmet therapeutic need. We report on the isolation and perpetuation of a midbrain-specified self-renewable human neural stem cell line (hNSCs) from hESCs. These hNSCs grew as a monolayer and uniformly expressed the neural precursor markers nestin, vimentin and a radial glial phenotype. We describe a process to direct the differentiation of these hNSCs towards the DA lineage. Glial conditioned media acted synergistically with fibroblastic growth factor and leukemia inhibitory factor to induce the expression of the DA marker, tyrosine hydroxylase (TH), in the hNSC progeny. The glial-derived neurotrophic factor did not fully mimic the effects of conditioned media. The hNSCs expressed the midbrain-specific transcription factors Nurr1 and Pitx3. The inductive effects did not modify the level of the glutamic acid decarboxylase (GAD) transcript, a marker for GABAergic neurons, while the TH transcript increased 10-fold. Immunocytochemical analysis demonstrated that the TH-expressing cells did not co-localize with GAD. The transplantation of these DA-induced hNSCs into the non-human primate MPTP model of PD demonstrated that the cells maintain their DA-induced phenotype, extend neurite outgrowths and express synaptic markers.

Daadi, M. M. and G. K. Steinberg (2009). "Manufacturing neurons from human embryonic stem cells: biological and regulatory aspects to develop a safe cellular product for stroke cell therapy." Regen Med **4**(2): 251-263.

Demographic trends, particularly those related to longer life expectancy, suggest that the demand for tissue and organ transplants will further increase since many disorders result from degeneration, injury or organ failure. The most urgent problem in transplantation medicine is the shortage or lack of suitable donor organs and tissue, leading to ethical and societal problems such as organ trafficking. The discovery of stem cells in the inner cell mass of developing embryos and in adult tissue has revolutionized the medical field by introducing new therapeutic dimensions to consider for previously untreatable diseases and injuries. The unlimited self-renewal ability and pluripotent capacity to become any cell type of the organism make human embryonic stem cells (hESCs) a compelling source of cells to study tissue histogenesis and to apply in a wide array of tissue engineering, cell transplantation therapy and drug discovery applications. In this article, we will focus on hESCs and address the derivation of therapeutic neural stem cell lines from hESCs, as well as the biological and regulatory aspects to developing a safe cellular product for stroke cell therapy.

Dabelsteen, S., et al. (2009). "Epithelial cells derived from human embryonic stem cells display p16INK4A senescence, hypermotility, and differentiation properties shared by many P63+ somatic cell types." Stem Cells **27**(6): 1388-1399.

Human embryonic stem (hES) cells can generate cells expressing p63, K14, and involucrin, which have been proposed to be keratinocytes. Although these hES-derived, keratinocyte-like (hESderK) cells form epithelioid colonies when cultured in a fibroblast feeder system optimal for normal tissue-derived keratinocytes, they have a very short replicative lifespan unless engineered to express HPV16 E6E7. We report here that hESderK cells undergo senescence associated with p16(INK4A) expression, unrelated to telomere status. Transduction to express bmi1, a repressor of the p16(INK4A)/p14(ARF) locus, conferred upon hESderK cells and keratinocytes a substantially extended lifespan. When exposed to transforming growth factor beta or to an incompletely processed form of Laminin-332, three lifespan-extended or immortalized hESderK lines that we studied became directionally hypermotile, a wound healing and invasion response previously characterized in keratinocytes. In organotypic culture, hESderK cells stratified and expressed involucrin and K10, as do epidermal keratinocytes in vivo. However, their growth requirements were less stringent than keratinocytes.

We then extended the comparison to endoderm-derived, p63(+)/K14(+) urothelial and tracheobronchial epithelial cells. Primary and immortalized lines of these cell types had growth requirements and hypermotility responses similar to keratinocytes and bmi1 expression facilitated their immortalization by engineering to express the catalytic subunit of telomerase (TERT). In organotypic culture, they stratified and exhibited squamous metaplasia, expressing involucrin and K10. Thus, hESderK cells proved to be distinct from all three normal p63(+) cell types tested. These results indicate that hESderK cells cannot be identified conclusively as keratinocytes or even as ectodermal cells, but may represent an incomplete form of, or deviation from, normal p63(+) lineage development.

Dado-Rosenfeld, D., et al. (2015). "Tensile forces applied on a cell-embedded three-dimensional scaffold can direct early differentiation of embryonic stem cells toward the mesoderm germ layer." Tissue Eng Part A **21**(1-2): 124-133.

Mechanical forces play an important role in the initial stages of embryo development; yet, the influence of forces, particularly of tensile forces, on embryonic stem cell differentiation is still unknown. The effects of tensile forces on mouse embryonic stem cell (mESC) differentiation within a three-dimensional (3D) environment were examined using an advanced bioreactor system. Uniaxial static or dynamic stretch was applied on cell-embedded collagen constructs. Six-day-long cyclic stretching of the seeded constructs led to a fourfold increase in Brachyury (BRACH-T) expression, associated with the primitive streak phase in gastrulation, confirmed also by immunofluorescence staining. Further examination of gene expression characteristic of mESC differentiation and pluripotency, under the same conditions, revealed changes mostly related to mesodermal processes. Additionally, downregulation of genes related to pluripotency and stemness was observed. Cyclic stretching of the 3D constructs resulted in actin fiber alignment parallel to the stretching direction. BRACH-T expression decreased under cyclic stretching with addition of myosin II inhibitor. No significant changes in gene expression were observed when mESCs were first differentiated in the form of embryoid bodies and then exposed to cyclic stretching, suggesting that forces primarily influence nondifferentiated cells. Understanding the effects of forces on stem cell differentiation provides a means of controlling their differentiation for later use in regenerative medicine applications and sheds light on their involvement in embryogenesis.

Dang, L. T., et al. (2012). "Zfhx1b induces a definitive neural stem cell fate in mouse embryonic stem cells." Stem Cells Dev **21**(15): 2838-2851.

Inducing a stable and predictable program of neural cell fate in pluripotent cells in vitro is an important goal for utilizing these cells for modeling human disease mechanisms. However, the extent to which in vitro neural specification recapitulates in vivo neural specification remains to be fully established. We previously demonstrated that in the mouse embryo, activation of fibroblast growth factor (FGF) signalling promotes definitive neural stem cell (NSC) development through the upregulation of the transcription factor Zfhx1b. Here, we asked whether Zfhx1b is similarly required during neural lineage development of embryonic stem (ES) cells. Zfhx1b gene expression is rapidly upregulated in mouse ES cells cultured in a permissive neural-inducing environment, compared to ES cells in a standard pluripotency maintenance environment, and is potentiated by FGF signalling. However, overexpression of Zfhx1b in ES cells in maintenance conditions, containing serum and leukemia inhibitory factor (LIF), is sufficient to induce Sox1 expression, a marker found in neural precursors and to promote definitive NSC colony formation. Knockdown of Zfhx1b in ES cells using siRNA did not affect the initial transition of ES cells to a neural cell fate, but did diminish the ability of these neural cells to develop further into definitive NSCs. Thus, our findings using ES cells are congruent with evidence from mouse embryos and support a model, whereby intercellular FGF signaling induces Zfhx1b, which promotes the development of definitive NSCs subsequent to an initial neural specification event that is independent of this pathway.

Dang, S. M. and P. W. Zandstra (2005). "Scalable production of embryonic stem cell-derived cells." Methods Mol Biol **290**: 353-364.

Embryonic stem (ES) cells have the ability to self-renew as well as differentiate into any cell type in the body. These traits make ES cells an attractive "raw material" for a variety of cell-based technologies. However, uncontrolled cell aggregation in ES cell differentiation culture inhibits cell proliferation and differentiation and thwarts the use of stirred suspension bioreactors. Encapsulation of ES cells in agarose microdrops prevents physical interaction between developing embryoid bodies (EBs) that, in turn, prevents EB agglomeration. This enables use of stirred suspension bioreactors that can generate large numbers of ES-derived cells under controlled conditions.

Danova-Alt, R., et al. (2012). "Very small embryonic-like stem cells purified from umbilical cord blood lack stem cell characteristics." PLoS One **7**(4): e34899.

Very small embryonic-like (VSEL) cells have been described as putatively pluripotent stem cells present in murine bone marrow and human umbilical cord blood (hUCB) and as such are of high potential interest for regenerative medicine. However, there remain some questions concerning the precise identity and properties of VSEL cells, particularly those derived from hUCB. For this reason, we have carried out an extensive characterisation of purified populations of VSEL cells from a large number of UCB samples. Consistent with a previous report, we find that VSEL cells are CXCR4(+), have a high density, are indeed significantly smaller than HSC and have an extremely high nuclear/cytoplasmic ratio. Their nucleoplasm is unstructured and stains strongly with Hoechst 33342. A comprehensive FACS screen for surface markers characteristic of embryonic, mesenchymal, neuronal or hematopoietic stem cells revealed negligible expression on VSEL cells. These cells failed to expand in vitro under a wide range of culture conditions known to support embryonic or adult stem cell types and a microarray analysis revealed the transcriptional profile of VSEL cells to be clearly distinct both from well-defined populations of pluripotent and adult stem cells and from the mature hematopoietic lineages. Finally, we detected an aneuploid karyotype in the majority of purified VSEL cells by fluorescence in situ hybridisation. These data support neither an embryonic nor an adult stem cell like phenotype, suggesting rather that hUCB VSEL cells are an aberrant and inactive population that is not comparable to murine VSEL cells.

Das, S., et al. (2008). "Generation of embryonic stem cells: limitations of and alternatives to inner cell mass harvest." Neurosurg Focus **24**(3-4): E4.

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the early mammalian embryo. Because of their plasticity and potentially unlimited capacity for self-renewal, ES cells have generated tremendous interest both as models for developmental biology and as possible tools for regenerative medicine. This excitement has been attenuated, however, by scientific, political, and ethical considerations. In this article the authors describe somatic cell nuclear transfer and transcription-induced pluripotency, 2 techniques that have been used in attempts to circumvent the need to derive ES cells by the harvest of embryonic tissue.

David, R., et al. (2005). "Magnetic cell sorting purification of differentiated embryonic stem cells stably expressing truncated human CD4 as surface marker." Stem Cells **23**(4): 477-482.

Embryonic stem (ES) cells offer great potential in regenerative medicine and tissue engineering. Clinical applications are still hampered by the lack of protocols for gentle, high-yield isolation of specific cell types for transplantation expressing no immunogenic markers. We describe labeling of stably transfected ES cells expressing a human CD4 molecule lacking its intracellular domain (DeltaCD4) under control of the phosphoglycerate kinase promoter for magnetic cell sorting (MACS). To track the labeled ES cells, we fused DeltaCD4 to an intracellular enhanced green fluorescent protein domain (DeltaCD4EGFP). We showed functionality of the membrane-bound fluorescent fusion protein and its suitability for MACS leading to purities greater than 97%. Likewise, expression of DeltaCD4 yielded up to 98.5% positive cells independently of their differentiation state. Purities were not limited by the initial percentage of DeltaCD4(+) cells, ranging from 0.6%-16%. The viability of MACS-selected cells was demonstrated by reaggregation and de novo formation of embryoid bodies developing all three germ layers. Thus, expression of DeltaCD4 in differentiated ES cells may enable rapid, high-yield purification of a desired cell type for tissue engineering and transplantation studies.

David, R., et al. (2008). "Connexin 40 promoter-based enrichment of embryonic stem cell-derived cardiovascular progenitor cells." Cells Tissues Organs **188**(1-2): 62-69.

BACKGROUND: Pluripotent embryonic stem (ES) cells that can differentiate into functional cardiomyocytes as well as vascular cells in cell culture may open the door to cardiovascular cell transplantation. However, the percentage of ES cells in embryoid bodies (EBs) which spontaneously undergo cardiovascular differentiation is low (<10%), making strategies for their specific labeling and purification indispensable. METHODS: The human connexin 40 (Cx40) promoter was isolated and cloned in the vector pEGFP. The specificity of the construct was initially assessed in Xenopus embryos injected with Cx40-EGFP plasmid DNA. Stable Cx40-EGFP ES cell clones were differentiated and fluorescent cells were enriched manually as well as via fluorescence-activated cell sorting. Characterization of these cells was performed with respect to spontaneous beating as well as via RT-PCRs and immunofluorescent stainings. RESULTS: Cx40-EGFP reporter plasmid injection led to EGFP fluorescence specifically in the abdominal aorta of frog tadpoles. After crude manual enrichment of highly Cx40-EGFP-positive EBs, the appearance of cardiac and vascular structures was increased approximately 3-fold. Immunofluorescent stainings showed EGFP expression exclusively in vascular-like structures simultaneously expressing von Willebrand

factor and in formerly beating areas expressing alpha-actinin. Cx40-EGFP-expressing EBs revealed significantly higher numbers of beating cardiomyocytes and vascular-like structures. Semiquantitative RT-PCRs confirmed an enhanced cardiovascular differentiation as shown for the cardiac markers Nkx2.5 and MLC2v, as well as the endothelial marker vascular endothelial cadherin. CONCLUSIONS: Our work shows the feasibility of specific labeling and purification of cardiovascular progenitor cells from differentiating EBs based on the Cx40 promoter. We provide proof of principle that the deleted CD4 (DeltaCD4) surface marker-based method for magnetic cell sorting developed by our group will be ideally suitable for transference to this promoter.

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

Stem cells divide by asymmetric division and display different degrees of potency, or ability to differentiate into various specialized cell types. Owing to their unique regenerative capacity, stem cells have generated great enthusiasm worldwide and represent an invaluable tool with unprecedented potential for biomedical research and therapeutic applications. Stem cells play a central role in the understanding of molecular mechanisms regulating tissue development and regeneration in normal and pathological conditions and open large possibilities for the discovery of innovative pharmaceuticals to treat the most devastating diseases of our time. Not least, their intrinsic characteristics allow the engineering of functional tissues for replacement therapies that promise to revolutionize the medical practice in the near future. In this paper, the authors present the characteristics of pluripotent stem cells and new developments of transdifferentiation technologies and explore some of the biomedical applications that this emerging technology is expected to empower.

de Peppo, G. M., et al. (2010). "Osteogenic potential of human mesenchymal stem cells and human embryonic stem cell-derived mesodermal progenitors: a tissue engineering perspective." Tissue Eng Part A **16**(11): 3413-3426.

INTRODUCTION: Human mesenchymal stem cells (hMSCs) are promising candidates for bone engineering and regeneration with a considerable number of experimental successes reported over the last years. However, hMSCs show several limitations for tissue engineering applications, which can be overcome by using human embryonic stem cell-derived mesodermal progenitors (hES-MPs). The aim of this study was to investigate and compare the osteogenic differentiation potential of hMSCs and hES-MPs. MATERIALS AND METHODS: The osteogenic differentiation and mineralization behavior of both cell types were evaluated at passage 5, 10, 15, and 20. Expression of COL1A1, RUNX2, OPN, and OC was evaluated by reverse transcription (RT)-polymerase chain reaction, whereas mineralization was examined by photospectrometry, von Kossa staining, and time-of-flight secondary ion mass spectrometry. The immunoprofile of both cell types was investigated by flow cytometry. RESULTS: We demonstrated that, under proper stimulation, hES-MPs undergo osteogenic differentiation and exhibit significantly increased mineralization ability compared to hMSCs after protracted expansion. hES-MPs were also found to express lower amount of human leukocyte antigens class II proteins. CONCLUSIONS: The high osteogenic ability of hES-MPs, together with low expression of human leukocyte antigens class II, makes these cells an attractive alternative for bulk production of cells for bone engineering applications.

de Peppo, G. M., et al. (2013). "Human embryonic stem cell-derived mesodermal progenitors display substantially increased tissue formation compared to human mesenchymal stem cells under dynamic culture conditions in a packed bed/column bioreactor." Tissue Eng Part A **19**(1-2): 175-187.

Bone tissue engineering represents a promising strategy to obviate bone deficiencies, allowing the ex vivo construction of bone substitutes with unprecedented potential in the clinical practice. Considering that in the human body cells are constantly stimulated by chemical and mechanical stimuli, the use of bioreactor is emerging as an essential factor for providing the proper environment for the reproducible and large-scale production of the engineered substitutes. Human mesenchymal stem cells (hMSCs) are experimentally relevant cells but, regardless the encouraging results reported after culture under dynamic conditions in bioreactors, show important limitations for tissue engineering applications, especially considering their limited proliferative potential, loss of functionality following protracted expansion, and decline in cellular fitness associated with aging. On the other hand, we previously demonstrated that human embryonic stem cell-derived mesodermal progenitors (hES-MPs) hold great potential to provide a homogenous and unlimited source of cells for bone engineering applications. Based on prior scientific evidence using different types of stem cells, in the present study we hypothesized that dynamic culture of hES-MPs in a packed bed/column bioreactor had the potential to affect proliferation, expression of genes involved in osteogenic differentiation, and matrix mineralization, therefore

resulting in increased bone-like tissue formation. The reported findings suggest that hES-MPs constitute a suitable alternative cell source to hMSCs and hold great potential for the construction of bone substitutes for tissue engineering applications in clinical settings.

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.

Devereaux, M. W. (2007). "Alternative sources of adult stem cells: a possible solution to the embryonic stem cell debate." Gend Med **4**(1): 85; author reply 86.

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.

Dihne, M., et al. (2006). "Embryonic stem cell-derived neuronally committed precursor cells with reduced teratoma formation after transplantation into the lesioned adult mouse brain." Stem Cells **24**(6): 1458-1466.

The therapeutic potential of embryonic stem (ES) cells in neurodegenerative disorders has been

widely recognized, and methods are being developed to optimize culture conditions for enriching the cells of interest and to improve graft stability and safety after transplantation. Whereas teratoma formation rarely occurs in xenogeneic transplantation paradigms of ES cell-derived neural progeny, more than 70% of mice that received murine ES cell-derived neural precursor cells develop teratomas, thus posing a major safety problem for allogeneic and syngeneic transplantation paradigms. Here we introduce a new differentiation protocol based on the generation of substrate-adherent ES cell-derived neural aggregates (SENAs) that consist predominantly of neuronally committed precursor cells. Purified SENAs that were differentiated into immature but postmitotic neurons did not form tumors up to four months after syngeneic transplantation into the acutely degenerated striatum and showed robust survival.

Ding, J., et al. (2016). "Induction of differentiation of human embryonic stem cells into functional hair-cell-like cells in the absence of stromal cells." Int J Biochem Cell Biol **81**(Pt A): 208-222.

Sensorineural hearing loss and vestibular dysfunction have become the most common forms of sensory defects. Stem cell-based therapeutic strategies for curing hearing loss are being developed. Several attempts to develop hair cells by using chicken utricle stromal cells as feeder cells have resulted in phenotypic conversion of stem cells into inner ear hair-cell-like cells. Here, we induced the differentiation of human embryonic stem cells (hESCs) into otic epithelial progenitors (OEPs), and further induced the differentiation of OEPs into hair-cell-like cells using different substrates. Our results showed that OEPs cultured on the chicken utricle stromal cells with the induction medium could differentiate into hair-cell-like cells with stereociliary bundles. Co-culture with stromal cells, however, may be problematic for subsequent examination of the induced hair-cell-like cells. In order to avoid the interference from stromal cells, we cultured OEPs on laminin with different induction media and examined the effects of the induction medium on the differentiation potentials of OEPs into hair-cell-like cells.

Ding, X., et al. (2012). "Polycomb group protein Bmi1 promotes hematopoietic cell development from embryonic stem cells." Stem Cells Dev **21**(1): 121-132.

Bmi1 is a component of the Polycomb repressive complexes and essential for maintaining the pool of adult stem cells. Polycomb repressive complexes are key regulators for embryonic development by modifying chromatin architecture and maintaining gene repression. To assess the role of Bmi1 in pluripotent stem cells and on exit from pluripotency during differentiation, we studied forced Bmi1 expression in mouse embryonic stem cells (ESC). We found that ESC do not express detectable levels of Bmi1 RNA and protein and that forced Bmi1 expression had no obvious influence on ESC self-renewal. However, upon ESC differentiation, Bmi1 effectively enhanced development of hematopoietic cells. Global transcriptional profiling identified a large array of genes that were differentially regulated during ESC differentiation by Bmi1. Importantly, we found that Bmi1 induced a prominent up-regulation of Gata2, a zinc finger transcription factor, which is essential for primitive hematopoietic cell generation from mesoderm. In addition, Bmi1 caused sustained growth and a >100-fold expansion of ESC-derived hematopoietic stem/progenitor cells within 2-3 weeks of culture.

Do, E. K., et al. (2014). "Reptin regulates pluripotency of embryonic stem cells and somatic cell reprogramming through Oct4-dependent mechanism." Stem Cells **32**(12): 3126-3136.

Oct4 has been implicated in regulation of pluripotency in embryonic stem cells (ESCs) and reprogramming of somatic cells into induced pluripotent stem cells. However, the molecular mechanisms involved in Oct4-dependent regulation of pluripotency and reprogramming have not been clear. To gain insight into the mechanism of regulation of Oct4-mediated self-renewal of ESCs and reprogramming of somatic cells, we attempted to identify Oct4-binding proteins using affinity purification and mass spectrometry. We identified Reptin, a key component of ATP-dependent chromatin remodeling complexes, as an Oct4-binding protein. Depletion of endogenous Reptin using lentiviral short hairpin RNA (shRNA) led to a decrease in the number and size of alkaline phosphatase-positive colonies of mouse ESCs. In addition, shRNA-mediated silencing of Reptin resulted in decreased expression of pluripotency-specific marker genes, including Oct4, Sox2, Nanog, and SSEA-1. Results of the Oct4 reporter assay showed synergism between Oct4 and Reptin, and depletion of endogenous Reptin abolished Oct4 transcriptional activity. Results of a chromatin immunoprecipitation assay showed the overlapping interaction of Reptin and Oct4 to CR4 in the Oct4 enhancer in ESCs. Knockdown of Reptin using shRNA suppressed the reprogramming of mouse embryonic fibroblasts to induced pluripotent stem cells, whereas overexpression of Reptin resulted in enhanced efficiency of induced pluripotent stem cell generation. These results strongly suggest that Reptin plays a key role in maintaining the pluripotency of ESCs and in establishing the pluripotency during reprogramming of somatic cells by regulation of Oct4-mediated gene regulation.

Dodla, M. C., et al. (2010). "Role of astrocytes, soluble factors, cells adhesion molecules and neurotrophins in functional synapse formation: implications for human embryonic stem cell derived neurons." Curr Stem Cell Res Ther **5**(3): 251-260.

Availability of human embryonic stem cells (hESCs) and its neural derivatives has opened up wide possibilities of using these cells as tools for developmental studies, drug screening and cell therapies for treating neurodegenerative diseases. However, for hESC-derived neurons to fulfill their potential they need to form functional synapses and spontaneously active neural networks. Until recently very few studies have reported hESC-derived neurons capable of forming such networks, suggesting lack of certain components in culture media to promote mature synaptogenesis. In this review we discuss the various factors that enhance functional synapse formation in primary and stem cell-derived neuronal cultures. These factors include astrocytes, astrocyte-derived factors, cell adhesion molecules and neurotrophins. We discuss the current literature on studies that have used these factors for functional differentiation of primary neural cultures, and discuss its implications for stem cell - derived neural cultures.

Domev, H., et al. (2012). "Efficient engineering of vascularized ectopic bone from human embryonic stem cell-derived mesenchymal stem cells." Tissue Eng Part A **18**(21-22): 2290-2302.

Human mesenchymal stem cells (hMSCs) can be derived from various adult and fetal tissues. However, the quality of tissues for the isolation of adult and fetal hMSCs is donor dependent with a nonreproducible yield. In addition, tissue engineering and cell therapy require large-scale production of a pure population of lineage-restricted stem cells that can be easily induced to differentiate into a specific cell type. Therefore, human embryonic stem cells (hESCs) can provide an alternative, plentiful source for generation of reproducible hMSCs. We have developed efficient differentiation protocols for derivation of hMSCs from hESCs, including coculture with murine OP9 stromal cells and feeder layer-free system. Our protocols have resulted in the generation of up to 49% of hMSCs, which expressed CD105, CD90, CD29, and CD44. The hMSCs exhibited high adipogenic, chondrocytic, and osteogenic differentiation in vitro. The latter correlated with osteocalcin secretion and vascular endothelial growth factor (VEGF) production by the differentiating hMSCs. hMSC-derived osteoblasts further differentiated and formed ectopic bone in vivo, and induced the formation of blood vessels in Matrigel implants. Our protocol enables generation of a purified population of hESC-derived MSCs, with the potential of differentiating into several mesodermal lineages, and particularly into vasculogenesis-inducing osteoblasts, which can contribute to the development of bone repair protocols.

Dong, W., et al. (2013). "Antitumor effect of embryonic stem cells in a non-small cell lung cancer model: antitumor factors and immune responses." Int J Med Sci **10**(10): 1314-1320.

Research in recent years has revealed that embryonic stem cells (ESCs) could generate obvious antitumor effects in both vitro and vivo. In vitro, ESCs could secrete soluble factors that are capable of blocking cancer cells proliferation, moreover, embryonic microenvironments could effectively inhibit tumorigenesis and metastasis; while in vivo, administration of ESCs in tumor-bearing mice could generate significant antitumor effects by indirectly activating the antitumor immune system. In this study, non-small cell lung cancer cells (Lewis Lung Carcinoma cells, LLCs) and ESCs were co-injected together into mice, after that subcutaneous tumor growth was monitored, cellular and humoral immune responses were detected, and different control groups were set to compare the results in different conditions.

Doss, M. X., et al. (2007). "Transcriptomic and phenotypic analysis of murine embryonic stem cell derived BMP2+ lineage cells: an insight into mesodermal patterning." Genome Biol **8**(9): R184.

BACKGROUND: Bone morphogenetic protein (BMP)2 is a late mesodermal marker expressed during vertebrate development and plays a crucial role in early embryonic development. The nature of the BMP2-expressing cells during the early stages of embryonic development, their transcriptome and cell phenotypes developed from these cells have not yet been characterized. RESULTS: We generated a transgenic BMP2 embryonic stem (ES) cell lineage expressing both puromycin acetyltransferase and enhanced green fluorescent protein (EGFP) driven by the BMP2 promoter. Puromycin resistant and EGFP positive BMP2+ cells with a purity of over 93% were isolated. Complete transcriptome analysis of BMP2+ cells in comparison to the undifferentiated ES cells and the control population from seven-day-old embryoid bodies (EBs; intersection of genes differentially expressed between undifferentiated ES cells and BMP2+ EBs as well as differentially expressed between seven-day-old control EBs and BMP2+ EBs by t-test, $p < 0.01$, fold change >2) by microarray analysis led to identification of 479 specifically upregulated and 193 downregulated transcripts.

Doss, M. X., et al. (2004). "Embryonic stem cells: a promising tool for cell replacement therapy." J Cell Mol Med **8**(4): 465-473.

Embryonic stem (ES) cells are revolutionizing the field of developmental biology as a potential tool to understand the molecular mechanisms occurring during the process of differentiation from the embryonic stage to the adult phenotype. ES cells harvested from the inner cell mass (ICM) of the early embryo can proliferate indefinitely in vitro while retaining the ability to differentiate into all somatic cells. Emerging results from mice models with ES cells are promising and raising tremendous hope among the scientific community for the ES-cell based cell replacement therapy (CRT) of various severe diseases. ES cells could potentially revolutionize medicine by providing an unlimited renewable source of cells capable of replacing or repairing tissues that have been damaged in almost all degenerative diseases such as diabetes, myocardial infarction and Parkinson's disease. This review updates the progress of ES cell research in CRT, discusses about the problems encountered in the practical utility of ES cells in CRT and evaluates how far this approach is successful experimentally.

Doss, M. X., et al. (2010). "Global transcriptomic analysis of murine embryonic stem cell-derived brachyury(+) (T) cells." Genes Cells **15**(3): 209-228.

Brachyury(+) mesodermal cell population with purity over 79% was obtained from differentiating brachyury embryonic stem cells (ESC) generated with brachyury promoter driven enhanced green fluorescent protein and puromycin-N-acetyltransferase. A comprehensive transcriptomic analysis of brachyury(+) cells enriched with puromycin application from 6-day-old embryoid bodies (EBs), 6-day-old control EBs and undifferentiated ESCs led to identification of 1573 uniquely up-regulated and 1549 uniquely down-regulated transcripts in brachyury(+) cells. Furthermore, transcripts up-regulated in brachyury(+) cells have overrepresented the Gene Ontology annotations (cell differentiation, blood vessel morphogenesis, striated muscle development, placenta development and cell motility) and Kyoto Encyclopedia of Genes and Genomes pathway annotations (mitogen-activated protein kinase signaling and transforming growth factor beta signaling). Transcripts representing Larp2 and Ankrd34b are notably up-regulated in brachyury(+) cells. Knockdown of Larp2 resulted in a significantly down-regulation BMP-2 expression, and knockdown of Ankrd34b resulted in alteration of NF-H, PPARgamma and PECAM1 expression. The elucidation of transcriptomic signatures of ESCs-derived brachyury(+) cells will contribute toward defining the genetic and cellular identities of presumptive mesodermal cells. Furthermore, there is a possible involvement of Larp2 in the regulation of the late mesodermal marker BMP-2. Ankrd34b might be a positive regulator of neurogenesis and a negative regulator of adipogenesis.

Du, K. L., et al. (2004). "Megakaryoblastic leukemia factor-1 transduces cytoskeletal signals and induces smooth muscle cell differentiation from undifferentiated embryonic stem cells." J Biol Chem **279**(17): 17578-17586.

The SAP domain transcription factor myocardin plays a critical role in the transcriptional program regulating smooth muscle cell differentiation. In this report, we describe the capacity of myocardin to physically associate with megakaryoblastic leukemia factor-1 (MKL1) and characterize the function of MKL1 in smooth muscle cells (SMCs). The MKL1 gene is expressed in most human tissues and myocardin and MKL are co-expressed in SMCs. MKL1 and myocardin physically associate via conserved leucine zipper domains. Overexpression of MKL1 transactivates serum response factor (SRF)-dependent SMC-restricted transcriptional regulatory elements including the SM22alpha promoter, smooth muscle myosin heavy chain promoter/enhancer, and SM-alpha-actin promoter/enhancer in non-SMCs. Moreover, forced expression of MKL1 and SRF in undifferentiated SRF(-/-) embryonic stem cells activates multiple endogenous SMC-restricted genes at levels equivalent to, or exceeding, myocardin. Forced expression of a dominant-negative MKL1 mutant reduces myocardin-induced activation of the SMC-specific SM22alpha promoter. In NIH3T3 fibroblasts MKL1 localizes to the cytoplasm and translocates to the nucleus in response to serum stimulation, actin treadmilling, and RhoA signaling.

Duggal, G., et al. (2015). "Exogenous supplementation of Activin A enhances germ cell differentiation of human embryonic stem cells." Mol Hum Reprod **21**(5): 410-423.

Human embryonic stem cells (hESCs) derived in the presence of Activin A (ActA) demonstrate an increased differentiation propensity toward the germ cell lineage. In addition, mouse epiblast stem cells and mouse epiblast-like cells are poised toward germ cell differentiation and are derived in the presence of ActA. We therefore investigated whether supplementation with ActA enhances in vitro hESC differentiation toward germ cell lineage. ActA up-regulated early primordial germ cell (PGC) genes STELLA/DPPA3 (developmental pluripotency associated 3) and tyrosine kinase receptor cKIT in both ActA-derived and standard-derived hESCs indicating its role in priming hESCs toward the PGC lineage. Indeed, ActA plus bone morphogenic protein 4 (BMP4) strongly increased germ cell differentiation potential of hESCs

based on the high expression of late PGC markers DAZL (deleted in azoospermia-like) and VASA/DDX4 (DEAD-box polypeptide 4) at mRNA and protein level. Hence, the combination of ActA with BMP4 provides an additional boost for hESCs to develop into postmigratory germ cells. Together with increased VASA expression in the presence of ActA and BMP4, we also observed up-regulation of endoderm-specific genes GATA4 (GATA binding protein 4) and GATA6. Finally, we were able to further mature these in vitro-derived PGC-like cells (PGCLCs) by culturing them in in vitro maturation (IVM) medium, resulting in the formation of germ cell-like clusters and induction of meiotic gene expression. In conclusion, we demonstrate for the first time a synergism between ActA and BMP4 in facilitating germ cell-directed differentiation of hESCs, which is enhanced by extended culture in IVM medium, as shown by cytoplasmic VASA-expressing PGCLCs. We propose a novel relationship between the endoderm and germ cell lineage during hESC differentiation.

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.

Egozi, D., et al. (2007). "Regulation of the cell cycle inhibitor p27 and its ubiquitin ligase Skp2 in differentiation of human embryonic stem cells." FASEB J **21**(11): 2807-2817.

Embryonic stem cells combine the features of robust proliferation with precise differentiation capacity. p27 is a cell cycle inhibitor that is involved in the regulation of proliferation and differentiation in many developing tissues. Recent studies in murine embryonic stem cells have suggested that p27 is involved in the progression of normal differentiation programs in these cells. However, the expression and regulation of p27 and its role in the differentiation of human embryonic stem cells (hESc) has not been previously explored. Herein we show that p27 expression was low in undifferentiated hESc, but increased markedly in differentiated cells. The expression of Skp2, the ubiquitin ligase that targets p27 for degradation, was inversely related to p27 expression. Moreover, embryoid bodies (EBs) with low p27 expression and high Skp2/p27 ratio showed poorer

differentiation than those with high p27 expression. Modulation of Skp2 expression is mainly regulated by its rate of degradation. In contrast to somatic cells, which have high levels of Skp2 mainly in S and G2/M, in undifferentiated hESc Skp2 levels were also high in G1. These results point to a potentially important role for p27 regulation in hESc.

Eiges, R., et al. (2001). "Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells." Curr Biol **11**(7): 514-518.

Human embryonic stem (ES) cells are pluripotent cell lines that have been derived from the inner cell mass (ICM) of blastocyst stage embryos [1--3]. They are characterized by their ability to be propagated indefinitely in culture as undifferentiated cells with a normal karyotype and can be induced to differentiate in vitro into various cell types [1, 2, 4-- 6]. Thus, human ES cells promise to serve as an unlimited cell source for transplantation. However, these unique cell lines tend to spontaneously differentiate in culture and therefore are difficult to maintain. Furthermore, colonies may contain several cell types and may be composed of cells other than pluripotent cells [1, 2, 6]. In order to overcome these difficulties and establish lines of cells with an undifferentiated phenotype, we have introduced a reporter gene that is regulated by a promoter of an ES cell-enriched gene into the cells. For the introduction of DNA into human ES cells, we have established a specific transfection protocol that is different from the one used for murine ES cells. Human ES cells were transfected with enhanced green fluorescence protein (EGFP), under the control of murine Rex1 promoter. The transfected cells show high levels of GFP expression when in an undifferentiated state. As the cells differentiate, this expression is dramatically reduced in monolayer cultures as well as in the primitive endoderm of early stage (simple) embryoid bodies (EBs) and in mature EBs. The undifferentiated cells expressing GFP can be analyzed and sorted by using a Fluorescence Activated Cell Sorter (FACS). Thus, we have established lines of human ES cells in which only undifferentiated cells are fluorescent, and these cells can be followed and selected for in culture. We also propose that the pluripotent nature of the culture is made evident by the ability of the homogeneous cell population to form EBs. The ability to efficiently transfect human ES cells will provide the means to study and manipulate these cells for the purpose of basic and applied research.

El-Badawy, A. and N. El-Badri (2016). "The cell cycle as a brake for beta-cell regeneration from embryonic stem cells." Stem Cell Res Ther **7**: 9.

The generation of insulin-producing beta cells from stem cells in vitro provides a promising source of cells for cell transplantation therapy in diabetes. However, insulin-producing cells generated from human stem cells show deficiency in many functional characteristics compared with pancreatic beta cells. Recent reports have shown molecular ties between the cell cycle and the differentiation mechanism of embryonic stem (ES) cells, assuming that cell fate decisions are controlled by the cell cycle machinery. Both beta cells and ES cells possess unique cell cycle machinery yet with significant contrasts. In this review, we compare the cell cycle control mechanisms in both ES cells and beta cells, and highlight the fundamental differences between pluripotent cells of embryonic origin and differentiated beta cells. Through critical analysis of the differences of the cell cycle between these two cell types, we propose that the cell cycle of ES cells may act as a brake for beta-cell regeneration. Based on these differences, we discuss the potential of modulating the cell cycle of ES cells for the large-scale generation of functionally mature beta cells in vitro. Further understanding of the factors that modulate the ES cell cycle will lead to new approaches to enhance the production of functional mature insulin-producing cells, and yield a reliable system to generate bona fide beta cells in vitro.

Ellerstrom, C., et al. (2010). "Single cell enzymatic dissociation of human embryonic stem cells: a straightforward, robust, and standardized culture method." Methods Mol Biol **584**: 121-134.

The routine culture and expansion of human embryonic stem (hES) cells has been and is still posing a challenge to researchers wishing to take advantage of the cells' unique potential. In contrast to mouse embryonic stem cells, hES cells usually have to be expanded by tedious mechanical microdissection or by enzymatic dissociation to cell clusters of a very narrow size range.It is essential to use a culture system that allows the robust and reproducible enzymatic dissociation of viable hES cell cultures to single cells to allow the scale-up of hES cell cultures as well as the application of hES cells in various experiments, such as FACS, electroporation, and clonal selection.By the development of enzyme-based protocols, which are less labor intensive and less time consuming, much progress has been made over the recent years with regard to improved culture systems for hES cell. We have developed a culture system that is based on single cell enzymatic dissociation (SCED) in combination with a highly supportive feeder cell layer of human foreskin fibroblasts (hFFs). The culture system allows defined enzymatic propagation while maintaining the hES cell lines in an undifferentiated, pluripotent, and normal state.In this chapter, we will show how hES

cells, which have been derived and passaged by traditional mechanical dissection, can be rapidly adjusted to propagation by enzymatic dissociation to single cells. The protocols we describe are widely applicable and should therefore be of general use for the reliable mass cultivation of hES cells for various experiments.

Ellerstrom, C., et al. (2007). "Facilitated expansion of human embryonic stem cells by single-cell enzymatic dissociation." Stem Cells **25**(7): 1690-1696.

Traditionally, human embryonic stem cells (hESCs) are propagated by mechanical dissection or enzymatic dissociation into clusters of cells. To facilitate up-scaling and the use of hESC in various experimental manipulations, such as fluorescence-activated cell sorting, electroporation, and clonal selection, it is important to develop new, stable culture systems based on single-cell enzymatic propagation. Here, we show that hESCs, which were derived and passaged by mechanical dissection, can be rapidly adjusted to propagation by enzymatic dissociation to single cells. As an indication of the stability of this culture system, we demonstrate that hESCs can be maintained in an undifferentiated, pluripotent, and genetically normal state for up to 40 enzymatic passages. We also demonstrate that a recombinant trypsin preparation increases clonal survival compared with porcine trypsin. Finally, we show that human foreskin fibroblast feeders are superior to the commonly used mouse embryonic fibroblast feeders in terms of their ability to prevent spontaneous differentiation after single-cell passaging. Importantly, the culture system is widely applicable and should therefore be of general use to facilitate reliable large-scale cultivation of hESCs, as well as their use in various experimental manipulations. Disclosure of potential conflicts of interest is found at the end of this article.

Emre, N., et al. (2010). "The ROCK inhibitor Y-27632 improves recovery of human embryonic stem cells after fluorescence-activated cell sorting with multiple cell surface markers." PLoS One **5**(8): e12148.

BACKGROUND: Due to the inherent sensitivity of human embryonic stem cells (hESCs) to manipulations, the recovery and survival of hESCs after fluorescence-activated cell sorting (FACS) can be low. Additionally, a well characterized and robust methodology for performing FACS on hESCs using multiple-cell surface markers has not been described. The p160-Rho-associated coiled kinase (ROCK) inhibitor, Y-27632, previously has been identified as enhancing survival of hESCs upon single-cell dissociation, as well as enhancing recovery from cryopreservation. Here we examined the application of Y-27632 to hESCs after FACS to improve survival in both feeder-dependent and feeder-independent growth conditions. METHODOLOGY/PRINCIPAL FINDINGS: HESCs were sorted using markers for SSEA-3, TRA-1-81, and SSEA-1. Cells were plated after sorting for 24 hours in either the presence or the absence of Y-27632. In both feeder-dependent and feeder-independent conditions, cell survival was greater when Y-27632 was applied to the hESCs after sort. Specifically, treatment of cells with Y-27632 improved post-sort recovery up to four fold. To determine the long-term effects of sorting with and without the application of Y-27632, hESCs were further analyzed. Specifically, hESCs sorted with and without the addition of Y-27632 retained normal morphology, expressed hESC-specific markers as measured by immunocytochemistry and flow cytometry, and maintained a stable karyotype. In addition, the hESCs could differentiate into three germ layers in vitro and in vivo in both feeder-dependent and feeder-independent growth conditions. CONCLUSIONS/SIGNIFICANCE: The application of Y-27632 to hESCs after cell sorting improves cell recovery with no observed effect on pluripotency, and enables the consistent recovery of hESCs by FACS using multiple surface markers. This improved methodology for cell sorting of hESCs will aid many applications such as removal of hESCs from secondary cell types, identification and isolation of stem cell subpopulations, and generation of single cell clones. Finally, these results demonstrate an additional application of ROCK inhibition to hESC research.

Endoh, M., et al. (2017). "PCGF6-PRC1 suppresses premature differentiation of mouse embryonic stem cells by regulating germ cell-related genes." Elife **6**.

The ring finger protein PCGF6 (polycomb group ring finger 6) interacts with RING1A/B and E2F6 associated factors to form a non-canonical PRC1 (polycomb repressive complex 1) known as PCGF6-PRC1. Here, we demonstrate that PCGF6-PRC1 plays a role in repressing a subset of PRC1 target genes by recruiting RING1B and mediating downstream mono-ubiquitination of histone H2A. PCGF6-PRC1 bound loci are highly enriched for promoters of germ cell-related genes in mouse embryonic stem cells (ESCs). Conditional ablation of Pcgf6 in ESCs leads to robust de-repression of such germ cell-related genes, in turn affecting cell growth and viability. We also find a role for PCGF6 in pre- and peri-implantation mouse embryonic development. We further show that a heterodimer of the transcription factors MAX and MGA recruits PCGF6 to target loci. PCGF6 thus links sequence specific target recognition by the MAX/MGA complex to PRC1-dependent transcriptional silencing of germ cell-specific genes in pluripotent stem cells.

Farifteh, F., et al. (2014). "Histone modification of embryonic stem cells produced by somatic cell nuclear transfer and fertilized blastocysts." Cell J **15**(4): 316-323.

OBJECTIVE: Nuclear transfer-embryonic stem cells (NT-ESCs) are genetically identical to the donor's cells; provide a renewable source of tissue for replacement, and therefore, decrease the risk of immune rejection. Trichostatin A (TSA) as a histone deacetylase in- hibitor (HDACi) plays an important role in the reorganization of the genome and epigenetic changes. In this study, we examined whether TSA treatment after somatic cell nuclear transfer (SCNT) can improve the developmental rate of embryos and establishment rate of NT-ESCs line, as well as whether TSA treatment can improve histone modification in NT-ESCs lines. MATERIALS AND METHODS: In this experimental study, mature oocytes were recovered from BDF1 [C57BL/6xDBA/2) F 1 mice] mice and enucleated by micromanipulator. Cumulus cells were injected into enucleated oocytes as donor. Reconstructed embryos were ac- tivated in the presence or absence of TSA and cultured for 5 days. Blastocysts were transferred on inactive mouse embryonic fibroblasts (MEF), so ESCs lines were estab- lished. ESCs markers were evaluated by reverse transcription-polymerase chain reaction (RT-PCR). Histone modifications were analyzed by enzyme linked immunosorbent assay (ELISA). RESULTS: Result of this study showed that TSA treatment after SCNT can improve devel- opmental rate of embryos (21.12 +/- 3.56 vs. 8.08 +/- 7.92), as well as establishment rate of NT-ESCs line (25 vs. 12.5). We established 6 NT-ESCs in two experimental groups, and three embryonic stem cells (ESCs) lines as control group. TSA treatment has no effect in H3K4 acetylation and H3K9 tri-methylation in ESCs. CONCLUSION: TSA plays a key role in the developmental rate of embryos, establishment rate of ESC lines after SCNT, and regulation of histone modification in NT-ESCs, in a man- ner similar to that of ESCs established from normal blastocysts.

Faro-Trindade, I. and P. R. Cook (2006). "A conserved organization of transcription during embryonic stem cell differentiation and in cells with high C value." Mol Biol Cell **17**(7): 2910-2920.

Although we have detailed information on the alterations occurring in steady-state levels of all cellular mRNAs during differentiation, we still know little about more global changes. Therefore, we investigated the numbers of molecules of RNA polymerase II that are active--and the way those molecules are organized--as two mouse cells (aneuploid F9 teratocarcinoma, and euploid and totipotent embryonic stem cells) differentiate into parietal endoderm. Quantitative immunoblotting shows the number of active molecules roughly halves. Transcription sites (detected by light and electron microscopy after allowing engaged polymerases to extend nascent transcripts in bromouridine-triphosphate) are uniformly distributed throughout the nucleoplasm. The numbers of such sites fall during differentiation as nuclei become smaller, but site density and diameter remain roughly constant. Similar site densities and diameters are found in salamander (amphibian) cells with 11-fold larger genomes, and in aneuploid HeLa cells. We conclude that active polymerases and their nascent transcripts are concentrated in a limited number of discrete nucleoplasmic sites or factories, and we speculate that the organization of transcription is conserved during both differentiation and evolution to a high C value.

Farzaneh, Z., et al. (2010). "Enhanced functions of human embryonic stem cell-derived hepatocyte-like cells on three-dimensional nanofibrillar surfaces." Stem Cell Rev **6**(4): 601-610.

Human embryonic stem cell (hESC)-derived hepatocytes provide a promising unlimited resource for the treatment of liver disease. However, current protocols for the generation of mature and functional hepatocytes are inefficient. Therefore, in order to better differentiate and maintain the function of differentiating hESCs, we have hypothesized that hESCs undergo better differentiation into hepatocyte-like cells (HLCs) when induced on three-dimensional nanofibrillar surfaces. We have demonstrated that, during stepwise differentiation of induction, the markers of hepatic lineage expressed and finally lead to the generation of functional mature cells. In the presence of an ultraweb nanofiber, HLCs produced lower AFP, greater urea, glycogen storage, metabolic PROD activity, uptake of LDL and organic anion ICG, all of which are indicative of the differentiation of HLCs. These results show that topographically treated hESCs at the nano level have a distinct hepatic functionality profile which has implications for cell therapies.

Fassler, R., et al. (1995). "Lack of beta 1 integrin gene in embryonic stem cells affects morphology, adhesion, and migration but not integration into the inner cell mass of blastocysts." J Cell Biol **128**(5): 979-988.

A gene trap-type targeting vector was designed to inactivate the beta 1 integrin gene in embryonic stem (ES) cells. Using this vector more than 50% of the ES cell clones acquired a disruption in the beta 1 integrin gene and a single clone was mutated in both alleles. The homozygous mutant did not produce beta 1 integrin mRNA or protein, while alpha 3, alpha

5, and alpha 6 integrin subunits were transcribed but not detectable on the cell surface. Heterozygous mutants showed reduced beta 1 expression and surface localization of alpha/beta 1 heterodimers. The alpha V subunit expression was not impaired on any of the mutants. Homozygous ES cell mutants lacked adhesiveness for laminin and fibronectin but not for vitronectin and showed a reduced association with a fibroblast feeder layer. Furthermore, they did not migrate towards chemoattractants in fibroblast medium. None of these functions were impaired in heterozygous mutants. Scanning electron microscopy revealed that homozygous cells showed fewer cell-cell junctions and had many microvilli not usually found on wild type and heterozygous cells. This profound change in cell shape is not associated with gross alterations in the expression and distribution of cytoskeletal components. Unexpectedly, microinjection into blastocysts demonstrated full integration of homozygous and heterozygous mutants into the inner cell mass. This will allow studies of the consequences of beta 1 integrin deficiency in several in vivo situations.

Fathi, A., et al. (2014). "Quantitative proteomics analysis highlights the role of redox hemostasis and energy metabolism in human embryonic stem cell differentiation to neural cells." J Proteomics **101**: 1-16.

UNLABELLED: Neural differentiation of human embryonic stem cells (hESCs) is a unique opportunity for in vitro analyses of neurogenesis in humans. Extrinsic cues through neural plate formation are well described in the hESCs although intracellular mechanisms underlying neural development are largely unknown. Proteome analysis of hESC differentiation to neural cells will help to further define molecular mechanisms involved in neurogenesis in humans. Using a two-dimensional differential gel electrophoresis (2D-DIGE) system, we analyzed the proteome of hESC differentiation to neurons at three stages, early neural differentiation, neural ectoderm and mature neurons. Out of 137 differentially accumulated protein spots, 118 spots were identified using MALDI-TOF/TOF and LC MS/MS. We observed that proteins involved in redox hemostasis, vitamin and energy metabolism and ubiquitin dependent proteolysis were more abundant in differentiated cells, whereas the abundance of proteins associated with RNA processing and protein folding was higher in hESCs. Higher abundance of proteins involved in maintaining cellular redox state suggests the importance of redox hemostasis in neural differentiation. Furthermore, our results support the concept of a coupling mechanism between neuronal activity and glucose utilization. The protein network analysis showed that the majority of the interacting proteins were associated with the cell cycle and cellular proliferation. These results enhanced our understanding of the molecular dynamics that underlie neural commitment and differentiation. BIOLOGICAL SIGNIFICANCE: In highlighting the role of redox and unique metabolic properties of neuronal cells, the present findings add insight to our understanding of hESC differentiation to neurons. The abundance of fourteen proteins involved in maintaining cellular redox state, including 10 members of peroxiredoxin (Prdx) family, mainly increased during differentiation, thus highlighting a link of neural differentiation to redox. Our results revealed markedly higher expression of genes encoding enzymes involved in the glycolysis and amino acid synthesis during differentiation. Protein network analysis predicted a number of critical mediators in hESC differentiation. These proteins included TP53, CTNNB1, SMARCA4, TNF, TERT, E2F1, MYC, RB1, and AR.

Fathi, F., et al. (2008). "Characterizing endothelial cells derived from the murine embryonic stem cell line CCE." Rejuvenation Res **11**(2): 371-378.

Embryonic stem cells (ESC) are defined by two main properties of self-renewal and their multipotency to differentiate into virtually all cell types of the body, including endothelial cells. ESCs have been widely regarded as an unlimited source of cells in regeneration medicine and also an ideal in vitro model to investigate complex developmental processes. Here, we report a simple and efficient in vitro model to derive a nearly pure population of endothelial cells from a murine ESC line. CCE ES cells are exposed to alpha-MEM medium containing 10% FBS for 4 days and then cultured in endothelial basal-2 medium containing vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and 2% FBS for 42 days. The cells acquired a relatively uniform endothelial cell morphology and were able to propagate and expand in culture. When murine ES cell-derived endothelial cells (MESDECs) were cultured on Matrigel and incubated for 48 h, vessel-like tube structures consisting of CD31 (PECAM-1) or BS-1 immunoreactive cells were developed. Immunocytochemistry and RT-PCR analyses revealed that MESDECs express endothelial cell-specific marker proteins such as Flk-1, PECAM-1, Tie-1, and Tie-2, in which the expressions persist for long periods of time after differentiation. The cells were also capable of taking up acetylated low-density lipoprotein (LDL) in culture. Our data suggest that MESDECs could provide a suitable in vitro model to study molecular events involved in vascular development and open up a new therapeutic strategy in regeneration medicine of cardiovascular disorders.

Feigelman, J., et al. (2016). "Analysis of Cell Lineage Trees by Exact Bayesian Inference Identifies Negative Autoregulation of Nanog in Mouse Embryonic Stem Cells." Cell Syst **3**(5): 480-490 e413.

Many cellular effectors of pluripotency are dynamically regulated. In principle, regulatory mechanisms can be inferred from single-cell observations of effector activity across time. However, rigorous inference techniques suitable for noisy, incomplete, and heterogeneous data are lacking. Here, we introduce stochastic inference on lineage trees (STILT), an algorithm capable of identifying stochastic models that accurately describe the quantitative behavior of cell fate markers observed using time-lapse microscopy data collected from proliferating cell populations. STILT performs exact Bayesian parameter inference and stochastic model selection using a particle-filter-based algorithm. We use STILT to investigate the autoregulation of Nanog, a heterogeneously expressed core pluripotency factor, in mouse embryonic stem cells. STILT rejects the possibility of positive Nanog autoregulation with high confidence; instead, model predictions indicate weak negative feedback. We use STILT for rational experimental design and validate model predictions using novel experimental data. STILT is available for download as an open source framework from http://www.imsb.ethz.ch/research/claassen/Software/stilt---stochastic-inference-o n-lineage-trees.html.

Filippi, M. D., et al. (2002). "Requirement for mitogen-activated protein kinase activation in the response of embryonic stem cell-derived hematopoietic cells to thrombopoietin in vitro." Blood **99**(4): 1174-1182.

Enforced expression of c-mpl in embryonic stem (ES) cells inactivated for this gene results in protein expression in all the ES cell progeny, producing cells that do not belong to the megakaryocytic lineage and are responsive to PEG-rhuMGDF, a truncated form of human thrombopoietin (TPO) conjugated to polyethylene glycol. These include a primitive cell called BL-CFC, thought to represent the equivalent of the hemangioblast, and all myeloid progenitor cells. In this model, PEG-rhuMGDF was able to potentiate the stimulating effects of other growth factors, including vascular endothelial growth factor, on BL-CFC and a combination of cytokines on the growth of granulocyte macrophage-colony-forming units. The importance of the C-terminal domain of Mpl and of mitogen-activated protein kinase (MAPK) activation in TPO-dependent megakaryocytic differentiation has been well studied in vitro. Here, the role of this domain and the involvement of MAPK in upstream and nonmegakaryocytic cells are examined by using 2 truncated mutants of Mpl (Delta34, deletion of residues 71 to 121 in the C-terminal domain; and Delta3, deletion of residues 71-94) and specific inhibitors of the MAPK pathway. The 2 deleted regions support different functions, mediated by different signals. Residues 71 to 121 were required for PEG-rhuMGDF-dependent growth of BL-CFC, for megakaryocytic and other myeloid progenitors, and for megakaryocyte polyploidization. These responses were mediated by the ERK1-ERK2 MAPK pathway. In contrast, the only function of the sequence comprising residues 71 to 94 was to mediate the synergistic effects of PEG-rhuMGDF with other hematopoietic growth factors. This function is not mediated by MAPK activation.

Fogel, J. L., et al. (2012). "Use of LysoTracker to detect programmed cell death in embryos and differentiating embryonic stem cells." J Vis Exp(68).

Programmed cell death (PCD) occurs in adults to maintain normal tissue homeostasis and during embryological development to shape tissues and organs(1,2,6,7). During development, toxic chemicals or genetic alterations can cause an increase in PCD or change PCD patterns resulting in developmental abnormalities and birth defects(3-5). To understand the etiology of these defects, the study of embryos can be complemented with in vitro assays that use differentiating embryonic stem (ES) cells. Apoptosis is a well-studied form of PCD that involves both intrinsic and extrinsic signaling to activate the caspase enzyme cascade. Characteristic cell changes include membrane blebbing, nuclear shrinking, and DNA fragmentation. Other forms of PCD do not involve caspase activation and may be the end-result of prolonged autophagy. Regardless of the PCD pathway, dying cells need to be removed. In adults, the immune cells perform this function, while in embryos, where the immune system has not yet developed, removal occurs by an alternative mechanism. This mechanism involves neighboring cells (called "non-professional phagocytes") taking on a phagocytic role-they recognize the 'eat me' signal on the surface of the dying cell and engulf it(8-10). After engulfment, the debris is brought to the lysosome for degradation. Thus regardless of PCD mechanism, an increase in lysosomal activity can be correlated with increased cell death. To study PCD, a simple assay to visualize lysosomes in thick tissues and multilayer differentiating cultures can be useful. LysoTracker dye is a highly soluble small molecule that is retained in acidic subcellular compartments such as the lysosome(11-13). The dye is taken up by diffusion and through the circulation. Since penetration is not a hindrance, visualization of PCD in thick tissues and multi-layer cultures is possible(12,13). In contrast, TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) analysis(14), is limited to small samples, histological sections, and monolayer cultures because the procedure requires the entry/permeability

of a terminal transferase. In contrast to Aniline blue, which diffuses and is dissolved by solvents, LysoTracker Red DND-99 is fixable, bright, and stable. Staining can be visualized with standard fluorescent or confocal microscopy in whole-mount or section using aqueous or solvent-based mounting media(12,13). Here we describe protocols using this dye to look at PCD in normal and sonic hedgehog null mouse embryos. In addition, we demonstrate analysis of PCD in differentiating ES cell cultures and present a simple quantification method. In summary, LysoTracker staining can be a great complement to other methods of detecting PCD.

Foja, S., et al. (2013). "Hypoxia supports reprogramming of mesenchymal stromal cells via induction of embryonic stem cell-specific microRNA-302 cluster and pluripotency-associated genes." Cell Reprogram **15**(1): 68-79.

Pluripotency is characterized by specific transcription factors such as OCT4, NANOG, and SOX2, but also by pluripotency-associated microRNAs (miRs). Somatic cells can be reprogrammed by forced expression of these factors leading to induced pluripotent stem cells (iPSCs) with characteristics similar to embryonic stem cells (ESCs). However, current reprogramming strategies are commonly based on viral delivery of the pluripotency-associated factors, which affects the integrity of the genome and impedes the use of such cells in any clinical application. In an effort to establish nonviral, nonintegrating reprogramming strategies, we examined the influence of hypoxia on the expression of pluripotency-associated factors and the ESC-specific miR-302 cluster in primary and immortalized mesenchymal stromal cells (MSCs). The combination of hypoxia and fibroblast growth factor 2 (FGF2) treatments led to the induction of OCT4 and NANOG in an immortalized cell line L87 and primary MSCs, accompanied with increased doubling rates and decreased senescence. Most importantly, the endogenous ECS-specific cluster miR-302 was induced upon hypoxic culture and FGF2 supplementation. Hypoxia also improved reprogramming of MSCs via episomal expression of pluripotency factors. Thus, our data illustrate that hypoxia in combination with FGF2 supplementation efficiently facilitates reprogramming of MSCs.

Fong, C. Y., et al. (2009). "Separation of SSEA-4 and TRA-1-60 labelled undifferentiated human embryonic stem cells from a heterogeneous cell population using magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS)." Stem Cell Rev **5**(1): 72-80.

A major concern in human embryonic stem cell (hESC)-derived cell replacement therapy is the risk of tumorigenesis from undifferentiated hESCs residing in the population of hESC-derived cells. Separation of these undifferentiated hESCs from the differentiated derivatives using cell sorting methods may be a plausible approach in overcoming this problem. We therefore explored magnetic activated cell sorting (MACS) and fluorescence activated cell sorting (FACS) to separate labelled undifferentiated hESCs from a heterogeneous population of hESCs and hepatocellular carcinoma cells (HepG2) deliberately mixed respectively at different ratios (10:90, 20:80, 30:70, 40:60 and 50:50) to mimic a standard in vitro differentiation protocol, instead of using a hESC-differentiated cell population, so that we could be sure of the actual number of cells separated. HES-3 and HES-4 cells were labelled in separate experiments for the stem cell markers SSEA-4 and TRA-1-60 using primary antibodies. Anti-PE magnetic microbeads that recognize the PE-conjugated SSEA-4 labelled hESCs was added to the heterogeneous cell mixture and passed through the MACS column. The cells that passed through the column ('flow-through' fraction) and those retained ('labelled' fraction') were subsequently analysed using FACS. The maximum efficacy of hESCs retention using MACS was 81.0 +/- 2.9% (HES-3) and 83.6 +/- 4.2% (HES-4). Using FACS, all the undifferentiated hESCs labelled with the two cell-surface markers could be removed by selective gating. Both hESCs and HepG2 cells in the 'flow-through' fraction following MACS separation were viable in culture whereas by FACS separation only the HepG2 cells were viable. FACS efficiently helps to eliminate the undifferentiated hESCs based on their cell-surface antigens expressed.

Fujimori, H., et al. (2012). "Induction of cancerous stem cells during embryonic stem cell differentiation." J Biol Chem **287**(44): 36777-36791.

Stem cell maintenance depends on their surrounding microenvironment, and aberrancies in the environment have been associated with tumorigenesis. However, it remains to be elucidated whether an environmental aberrancy can act as a carcinogenic stress for cellular transformation of differentiating stem cells into cancer stem cells. Here, utilizing mouse embryonic stem cells as a model, it was illustrated that environmental aberrancy during differentiation leads to the emergence of pluripotent cells showing cancerous characteristics. Analogous to precancerous stages, DNA lesions were spontaneously accumulated during embryonic stem cell differentiation under aberrational environments, which activates barrier responses such as senescence and apoptosis. However, overwhelming such barrier responses, piled-up spheres were subsequently induced from the previously senescent cells. The sphere cells exhibit aneuploidy and

dysfunction of the Arf-p53 module as well as enhanced tumorigenicity and a strong self-renewal capacity, suggesting development of cancerous stem cells. Our current study suggests that stem cells differentiating in an aberrational environment are at risk of cellular transformation into malignant counterparts.

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

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

Fukuda, H. and J. Takahashi (2005). "Embryonic stem cells as a cell source for treating Parkinson's disease." Expert Opin Biol Ther **5**(10): 1273-1280.

Parkinson's disease (PD) is a neurodegenerative disease characterised by a loss of midbrain dopaminergic (DA) neurons. Transplantation of DA neurons represents a promising treatment for PD, and embryonic stem (ES) cells are a good candidate source for DA neurons. However, although recent reports have demonstrated that DA neurons can be efficiently induced from ES cells and function therapeutically in an animal model of PD, many problems remain to be solved in order for ES cells to be used for clinical applications. This review will describe the current status of this field and the obstacles yet to be overcome, and will outline future research approaches from the clinical perspective.

Fukumitsu, K., et al. (2009). "Establishment of a cell line derived from a mouse fetal liver that has the characteristic to promote the hepatic maturation of mouse embryonic stem cells by a coculture method." Tissue Eng Part A **15**(12): 3847-3856.

Stromal cells residing in murine fetal livers have the ability to promote the hepatic maturation of murine embryonic stem cells (ESCs) and hepatic progenitor cells (HPCs) 3848 in vitro. These stromal cells were isolated as the CD49f(+/-)CD45(-)Thy1(+)gp38(+) cell fraction. The present study established a murine fetal liver stromal cell line that induced hepatic maturation in mouse ESCs and HPCs. A transgene containing a temperature-sensitive SV40 large T antigen was transfected into the primary fetal liver stromal cells. These immortalized cells, which were named as the gp38-positive and Thy1-positive murine liver stromal (MLSgt) cells, induced both mouse ESCs and HPCs to differentiate into mature hepatocyte-like cells using a coculture method. Since MLSgt is not a cloned cell line, one clone, MLSgt20, was selected as a line with the characteristic to induce hepatic differentiation, which was comparable to its parental stromal cells. The ESC-derived endoderm cells cocultured with the MLSgt20 cells expressed mature hepatocyte-specific gene markers, including glucose-6-phosphatase, tyrosine aminotransferase, tryptophan 2,3-dioxgenase, and cytochrome P450 (CYP1a1, Cyp1b1, Cyp1a2, and Cyp3a11). In addition, these cells also exhibited hepatic functions, such as glycogen storage and ammonia metabolism. Transmission electron microscopy showed that the cocultured ESCs expressed the morphologic features of mature hepatocytes. In conclusion, a cell line was established that has the characteristic to promote the hepatic maturation of mouse ESCs and HPCs by a coculture method.

Fukunaga, N., et al. (2010). "Leukemia inhibitory factor (LIF) enhances germ cell differentiation from primate embryonic stem cells." Cell Reprogram **12**(4): 369-376.

Recently, several research groups have shown that germ cells can be produced in vitro from pluripotent embryonic stem cells (ESCs). In the mouse, live births of offspring using germ cells induced from ESCs in vitro have been reported. Furthermore, some efficient methods for inducing the useful number of germ cells from ESCs have also been developed. On

the other hand, in primates, despite the appearances of germ cell-like cells including meiotic cells were observed by spontaneous differentiation or introducing transgenes, it has not been determined whether fully functional germ cells can be derived from ESCs. To elucidate the property for the germ cells induced from primate ESCs, specification of the promoting factors for the germ cell development and improving the efficiency of germ cell derivation are essential. Leukemia inhibitory factor (LIF) has been reported as one of the important factors for mouse primordial germ cell (PGC) survival in vitro. However, the effects of LIF on germ cell formation from pluripotent cells of primates have not been examined. The aim of this study is to determine whether LIF addition can improve in vitro germ cell production from cynomolgus monkey ESCs (cyESCs). After 8 days of differentiation, LIF added culture induced dome-shaped germ cell colonies as indicated by the intense expression of alkaline phosphatase activity (ALP).

Funakoshi, N., et al. (2011). "Comparison of hepatic-like cell production from human embryonic stem cells and adult liver progenitor cells: CAR transduction activates a battery of detoxification genes." Stem Cell Rev **7**(3): 518-531.

In vitro production of human hepatocytes is of primary importance in basic research, pharmacotoxicology and biotherapy of liver diseases. We have developed a protocol of differentiation of human embryonic stem cells (ES) towards hepatocyte-like cells (ES-Hep). Using a set of human adult markers including CAAT/enhancer binding protein (C/EBPalpha), hepatocyte nuclear factor 4/7 ratio (HNF4alpha1/HNF4alpha7), cytochrome P450 7A1 (CYP7A1), CYP3A4 and constitutive androstane receptor (CAR), and fetal markers including alpha-fetoprotein, CYP3A7 and glutathione S-transferase P1, we analyzed the expression of a panel of 41 genes in ES-Hep comparatively with human adult primary hepatocytes, adult and fetal liver. The data revealed that after 21 days of differentiation, ES-Hep are representative of fetal hepatocytes at less than 20 weeks of gestation. The glucocorticoid receptor pathway was functional in ES-Hep. Extending protocols of differentiation to 4 weeks did not improve cell maturation. When compared with hepatocyte-like cells derived from adult liver non parenchymal epithelial (NPE) cells (NPE-Hep), ES-Hep expressed several adult and fetal liver makers at much greater levels (at least one order of magnitude), consistent with greater expression of liver-enriched transcription factors Forkhead box A2, C/EBPalpha, HNF4alpha and HNF6. It therefore seems that ES-Hep reach a better level of differentiation than NPE-Hep and that these cells use different lineage pathways towards the hepatic phenotype.

Furusawa, T., et al. (2004). "Embryonic stem cells expressing both platelet endothelial cell adhesion molecule-1 and stage-specific embryonic antigen-1 differentiate predominantly into epiblast cells in a chimeric embryo." Biol Reprod **70**(5): 1452-1457.

We examined the expression of cell-surface markers on subpopulations of mouse embryonic stem (ES) cells to identify those that were associated with cells that had the highest pluripotency. Flow cytometry analysis revealed a wide variation in the expression of platelet endothelial cell adhesion molecule 1 (PECAM-1) and stage-specific embryonic antigen (SSEA)-1 in ES cells. Almost all SSEA-1+ cells expressed a high level of PECAM- 1, and reversible repopulation was observed between PECAM- 1+SSEA-1+ and PECAM-1+SSEA-1- cells. The ES cells carrying the lacZ gene were sorted into three subpopulations: PECAM- 1-SSEA-1-, PECAM-1+SSEA-1-, and PECAM-1+SSEA-1+. Quantitative reverse transcription-polymerase chain reaction revealed a low level of Oct3/4 mRNA expression and an elevation in differentiation maker gene expression in PECAM-1- cells. To compare the pluripotency of these three subpopulations, a single cell from each was injected into eight-cell embryo and ES cells identified at later stages by X-gal staining.

Gadkari, R., et al. (2014). "Human embryonic stem cell derived-mesenchymal stem cells: an alternative mesenchymal stem cell source for regenerative medicine therapy." Regen Med **9**(4): 453-465.

AIM: To enumerate and characterize mesenchymal stem cells (MSC) derived from human embryonic stem cells (hESC) for clinical application. MATERIALS & METHODS: hESC were differentiated into hESC-MSC and characterized by the expression of surface markers using flow cytometry. hESC-MSC were evaluated with respect to growth kinetics, colony-forming potential, as well as osteogenic and adipogenic differentiation capacity. Immunosuppressive effects were assessed using peripheral blood mononuclear cell (PBMC) proliferation and cytotoxicity assays. RESULTS: hESC-MSC showed similar morphology, and cell surface markers as adipose (AMSC) and bone marrow-derived MSC (BMSC). hESC-MSC exhibited a higher growth rate during early in vitro expansion and equivalent adipogenic and osteogenic differentiation and colony-forming potential as AMSC and BMSC. hESC-MSC demonstrated similar immunosuppressive effects as AMSC and BMSC.

Gage, B. K., et al. (2013). "Initial cell seeding density influences pancreatic endocrine development during in vitro differentiation of human embryonic stem cells." PLoS One **8**(12): e82076.

Human embryonic stem cells (hESCs) have the ability to form cells derived from all three germ layers, and as such have received significant attention as a possible source for insulin-secreting pancreatic beta-cells for diabetes treatment. While considerable advances have been made in generating hESC-derived insulin-producing cells, to date in vitro-derived glucose-responsive beta-cells have remained an elusive goal. With the objective of increasing the in vitro formation of pancreatic endocrine cells, we examined the effect of varying initial cell seeding density from 1.3 x 10(4) cells/cm(2) to 5.3 x 10(4) cells/cm(2) followed by a 21-day pancreatic endocrine differentiation protocol.

Galat, V., et al. (2010). "[Cell engineering and genetic approaches to the development of models of human embryonic stem cells for studying genetic disorders]." Biofizika **55**(3): 481-485.

A novel approach to the establishment of genetically modified human embryonic stem cell (hESC) lines has been developed, and it has been shown that mutant hESC may be derived from affected embryos after preimplantation genetic diagnosis screening for a particular single gene disorder. Here we provide the description of embryo and cell manipulation procedures, diagnostic lay out, analysis of the efficiency of embryo development and hESC establishment, as well as developments for hESC derivation in animal free conditions. The high efficiency of the approach (50%) is especially crucial in the work with rare and unique resources, such as genetically screened embryos necessary for the derivation of hESC lines representative of specific genetic diseases.

Gan, Q., et al. (2007). "Concise review: epigenetic mechanisms contribute to pluripotency and cell lineage determination of embryonic stem cells." Stem Cells **25**(1): 2-9.

Epigenetic mechanisms, such as histone modifications and DNA methylation, have been shown to play a key role in the regulation of gene transcription. Results of recent studies indicate that a novel "bivalent" chromatin structure marks key developmental genes in embryonic stem cells (ESCs), wherein a number of untranscribed lineage-control genes, such as Sox1, Nkx2-2, Msx1, Irx3, and Pax3, are epigenetically modified with a unique combination of activating and repressive histone modifications that prime them for potential activation (or repression) upon cell lineage induction and differentiation. However, results of these studies also showed that a subset of lineage-control genes, such as Myf5 and Mash1, were not marked by these histone modifications, suggesting that distinct epigenetic mechanisms might exist for lineage-control genes in ESCs. In this review article, we summarize evidence regarding possible mechanisms that control these unique histone modifications at lineage-control gene loci in ESCs and consider their possible contribution to ESC pluripotency. In addition, we propose a novel "histone modification pulsing" model wherein individual pluripotent stem cells within the inner cell mass of blastocysts undergo transient asynchronous histone modifications at these developmental gene loci, thereby conferring differential responsiveness to environmental cues and morphogenic gradients important for cell lineage determination. Finally, we consider how these rapid histone modification exchanges become progressively more stable as ESCs undergo differentiation and maturation into specialized cell lineages.

Gangemi, R. M., et al. (2004). "Regulatory genes controlling cell fate choice in embryonic and adult neural stem cells." J Neurochem **89**(2): 286-306.

Neural stem cells are the most immature progenitor cells in the nervous system and are defined by their ability to self-renew by symmetric division as well as to give rise to more mature progenitors of all neural lineages by asymmetric division (multipotentiality). The interest in neural stem cells has been growing in the past few years following the demonstration of their presence also in the adult nervous system of several mammals, including humans. This observation implies that the brain, once thought to be entirely post-mitotic, must have at least a limited capacity for self-renewal. This raises the possibility that the adult nervous system may still have the necessary plasticity to undergo repair of inborn defects and acquired injuries, if ways can be found to exploit the potential of neural stem cells (either endogenous or derived from other sources) to replace damaged or defective cells.

Gao, Q., et al. (2012). "Expression pattern of embryonic stem cell markers in DFAT cells and ADSCs." Mol Biol Rep **39**(5): 5791-5804.

Mature adipocytes can revert to a more primitive phenotype and gain cell proliferative ability under the condition of ceiling method, named dedifferentiated fat cells (DFAT cells). These cells exhibit multilineage potential as adipose tissue-derived stromal cells (ADSCs). However, the stem molecular signature of DFAT cells and the difference distinct from ADSCs are still not sure. To study the molecular signature of DFAT cells better, highly purified mature

adipocytes were obtained from rats and the purity was more than 98%, and about 98.6% were monocytes. These mature adipocytes dedifferentiated into fibroblast-like cells spontaneously by the ceiling culture method, these cells proliferated rapidly in vitro, grew in the same direction and formed vertex, and expressed extensively embryonic stem cell markers such as Oct4, Sox2, c-Myc, and Nanog, surface antigen SSEA-1, CD105, and CD31, moreover, these cells possessed ALP and telomerase activity.

Garcia-Lavandeira, M., et al. (2012). "Craniopharyngiomas express embryonic stem cell markers (SOX2, OCT4, KLF4, and SOX9) as pituitary stem cells but do not coexpress RET/GFRA3 receptors." J Clin Endocrinol Metab **97**(1): E80-87.

CONTEXT: Adult stem cells maintain some markers expressed by embryonic stem cells and express other specific markers depending on the organ where they reside. Recently, stem/progenitor cells in the rodent and human pituitary have been characterized as expressing GFRA2/RET, PROP1, and stem cell markers such as SOX2 and OCT4 (GPS cells). OBJECTIVE: Our objective was to detect other specific markers of the pituitary stem cells and to investigate whether craniopharyngiomas (CRF), a tumor potentially derived from Rathke's pouch remnants, express similar markers as normal pituitary stem cells. DESIGN: We conducted mRNA and Western blot studies in pituitary extracts, and immunohistochemistry and immunofluorescence on sections from normal rat and human pituitaries and 20 CRF (18 adamantinomatous and two papillary). RESULTS: Normal pituitary GPS stem cells localized in the marginal zone (MZ) express three key embryonic stem cell markers, SOX2, OCT4, and KLF4, in addition to SOX9 and PROP1 and beta-catenin overexpression. They express the RET receptor and its GFRA2 coreceptor but also express the coreceptor GFRA3 that could be detected in the MZ of paraffin pituitary sections. CRF maintain the expression of SOX2, OCT4, KLF4, SOX9, and beta-catenin. However, RET and GFRA3 expression was altered in CRF. In 25% (five of 20), both RET and GFRA3 were detected but not colocalized in the same cells. The other 75% (15 of 20) lose the expression of RET, GFRA3, or both proteins simultaneously. CONCLUSIONS: Human pituitary adult stem/progenitor cells (GPS) located in the MZ are characterized by expression of embryonic stem cell markers SOX2, OCT4, and KLF4 plus the specific pituitary embryonic factor PROP1 and the RET system. Redundancy in RET coreceptor expression (GFRA2 and GFRA3) suggest an important systematic function in their physiological behavior. CRF share the stem cell markers suggesting a common origin with GPS. However, the lack of expression of the RET/GFRA system could be related to the cell mislocation and deregulated growth of CRF.

Gavrilov, S., et al. (2009). "Non-viable human embryos as a source of viable cells for embryonic stem cell derivation." Reprod Biomed Online **18**(2): 301-308.

Human embryonic stem cells (hESC) hold great promise for use in regenerative medicine. However, the extraordinary potential of hESC as therapeutic tools is tempered by ethical, moral and political issues surrounding their derivation from human embryos. It has previously been proposed that ethical criteria applied to essential organ donation could be employed for derivation of hESC from irreversibly arrested, and thus organismically dead, human embryos produced during routine IVF procedures. Here, it is shown that arrested embryos do not resume normal development during extended culture, yet most of them contain a substantial number of living cells on embryonic day 6 (72% have <1 viable cell, 47% have <5 viable cells), suggesting that this class of non-viable embryos could be a rich source of viable cells for derivation of hESC lines.

Geens, M., et al. (2011). "Sertoli cell-conditioned medium induces germ cell differentiation in human embryonic stem cells." J Assist Reprod Genet **28**(5): 471-480.

PURPOSE: To investigate the spontaneous germ cell differentiation capacity of VUB hESC lines, develop a protocol for the induction of germ cell differentiation using conditioned medium from Sertoli cells (SCCM) and compare it to existing protocols. METHODS: hESC were allowed to differentiate spontaneously or after the addition of bone morphogenetic proteins (BMPs) and/or SCCM. VASA transcripts were measured by relative quantification real-time RT-PCR to determine the efficiency of germ cell differentiation. RESULTS: VUB hESC lines can differentiate spontaneously towards the germ cell lineage, however, more consistently in an embryoid body approach than in monolayer cultures. BMPs and SCCM significantly improve VASA expression, but do not have a synergistic effect. Direct contact of differentiating hESC with Sertoli cells does not improve VASA expression. CONCLUSIONS: SCCM contains inductive factors for germ cell differentiation and could represent an element for in-vitro differentiation to germ cells.

George, A., et al. (2011). "Production of cloned and transgenic embryos using buffalo (Bubalus bubalis) embryonic stem cell-like cells isolated from in vitro fertilized and cloned blastocysts." Cell Reprogram **13**(3): 263-272.

Here, we report the isolation and characterization of embryonic stem (ES) cell-like cells from cloned blastocysts, generated using fibroblasts derived from an adult buffalo (BAF). These nuclear transfer embryonic stem cell-like cells (NT-ES) grew in well-defined and dome-shaped colonies. The expression pattern of pluripotency marker genes was similar in both NT-ES and in vitro fertilization (IVF) embryo-derived embryonic stem cell-like cells (F-ES). Upon spontaneous differentiation via embryoid body formation, cells of different morphology were observed, among which predominant were endodermal-like and epithelial-like cell types. The ES cell-like cells could be passaged only mechanically and did not form colonies when plated as single cell suspension at different concentrations. When F-ES cell-like, NT-ES cell-like, and BAF cells of same genotype were used for hand-made cloning (HMC), no significant difference ($p > 0.05$) was observed in cleavage and blastocyst rate. Following transfer of HMC embryos to synchronized recipients, pregnancies were established only with F-ES cell-like and BAF cell-derived embryos, and one live calf was born from F-ES cell-like cells. Further, when transfected NT-ES cell-like cells and BAF were used for HMC, no significant difference ($p > 0.05$) was observed between cleavage and blastocyst rate. In conclusion, here we report for the first time the derivation of ES cell-like cells from an adult buffalo, and its genetic modification. We also report the birth of a live cloned calf from buffalo ES cell-like cells.

Gerecht-Nir, S. and J. Itskovitz-Eldor (2004). "Cell therapy using human embryonic stem cells." Transpl Immunol **12**(3-4): 203-209.

Cell therapy refers to the transplantation of healthy, functional and propagating cells to restore the viability or function of deficient tissues. Stem cells are characterized by self-renewal and the potential to form differentiated cells. In early mammalian embryos, at the blastocyst stage, the inner cell mass is pluripotent. Thus, it has been recognized that human embryonic stem cells (hESCs), which are derived from such cells of blastocysts, may serve as a source of numerous types of differentiated cells. The first part of this review summarizes different techniques for the derivation and maintenance of undifferentiated hESCs. In the second part, issues concerning the safety and bulk production, which may enable hESCs use in future clinical applications, are presented. The last part of this review details accumulated data regarding the in vitro differentiation potential of hESCs.

Germano, I. M., et al. (2006). "Apoptosis in human glioblastoma cells produced using embryonic stem cell-derived astrocytes expressing tumor necrosis factor-related apoptosis-inducing ligand." J Neurosurg **105**(1): 88-95.

OBJECT: Embryonic stem (ES) cell-derived astrocytes have several theoretical and practical advantages as gene therapy vectors in the treatment of malignant gliomas. The aim of this study was to test the proapoptotic effects of ES cell-derived astrocytes expressing transgenic tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in human malignant glioma cells. METHODS: Mouse ES cells containing a doxycycline-inducible transgene were engineered with human TRAIL (hTRAIL) and then directed to differentiate into astrocytes. The ES cell-derived-TRAIL-expressing astrocytes were cocultured with human malignant glioma cells. Reverse transcriptase polymerase chain reaction, immunocytochemistry, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling, and flow cytometry were used to quantify results. In vitro coculture of ES cell-derived astrocytes expressing hTRAIL with A172 human malignant glioma cells after doxycycline induction caused a significant decrease in cell viability from 85 +/- 2% at baseline to 8 +/- 2% posttreatment ($p < 0.001$). Labeling with apoptotic markers showed that cell death occurred by means of apoptosis. A significant increase in apoptotic rate (88 +/- 3%) from baseline (4 +/- 2%) was found in A172 cells after doxycycline induction ($p < 0.005$). This effect was superior to the apoptotic rate seen after treatment with recombinant TRAIL (57 +/- 2%).

Ghahrizjani, F. A., et al. (2015). "Enhanced expression of FNDC5 in human embryonic stem cell-derived neural cells along with relevant embryonic neural tissues." Gene **557**(2): 123-129.

Availability of human embryonic stem cells (hESCs) has enhanced the capability of basic and clinical research in the context of human neural differentiation. Derivation of neural progenitor (NP) cells from hESCs facilitates the process of human embryonic development through the generation of neuronal subtypes. We have recently indicated that fibronectin type III domain containing 5 protein (FNDC5) expression is required for appropriate neural differentiation of mouse embryonic stem cells (mESCs). Bioinformatics analyses have shown the presence of three isoforms for human FNDC5 mRNA. To differentiate which isoform of FNDC5 is involved in the process of human neural differentiation, we have used hESCs as an in vitro model for neural differentiation by retinoic acid (RA) induction. The hESC line, Royan H5, was differentiated into a neural lineage in defined adherent culture treated by RA and basic fibroblast growth factor (bFGF). We collected all cell types that included hESCs, rosette structures, and

neural cells in an attempt to assess the expression of FNDC5 isoforms. There was a contiguous increase in all three FNDC5 isoforms during the neural differentiation process. Furthermore, the highest level of expression of the isoforms was significantly observed in neural cells compared to hESCs and the rosette structures known as neural precursor cells (NPCs). High expression levels of FNDC5 in human fetal brain and spinal cord tissues have suggested the involvement of this gene in neural tube development. Additional research is necessary to determine the major function of FDNC5 in this process.

Ghodsizadeh, A., et al. (2014). "Galactosylated collagen matrix enhanced in vitro maturation of human embryonic stem cell-derived hepatocyte-like cells." Biotechnol Lett **36**(5): 1095-1106.

Due to their important biomedical applications, functional human embryonic stem cell-derived hepatocyte-like cells (hESC-HLCs) are an attractive topic in the field of stem cell differentiation. Here, we have initially differentiated hESCs into functional hepatic endoderm (HE) and continued the differentiation by replating them onto galactosylated collagen (GC) and collagen matrices. The differentiation of hESC-HE cells into HLCs on GC substrate showed significant up-regulation of hepatic-specific genes such as ALB, HNF4alpha, CYP3A4, G6P, and ASGR1. There was more albumin secretion and urea synthesis, as well as more cytochrome p450 activity, in differentiated HLCs on GC compared to the collagen-coated substrate. These results suggested that GC substrate has the potential to be used for in vitro maturation of hESC-HLCs.

Gholamitabar Tabari, M., et al. (2018). "Evaluation of Novel Mouse-Specific Germ Cell Gene Expression in Embryonic Stem Cell-Derived Germ Cell-Like Cells In Vitro with Retinoic Acid Treatment." Cell Reprogram **20**(4): 245-255.

We designed a study to induce differentiation of Oct4-GFP (expression of Green Fluorescent Protein of oct4) embryonic stem cells (ESCs) by embryoid body (EB) culture system into germ cells (GCs) using retinoic acid (RA) and evaluated the expression level of (Fkbp6, Mov10l1, 4930432K21Rik, and Tex13) in differentiated cells. The expression levels of four GC-related genes, Oct4, Mvh, Scp3, and Stra8, was determined by quantitative real-time polymerase chain reaction (q-RT-PCR). Immunostaining and flow cytometry were used as additional tests to confirm q-RT-PCR findings. A significant increase occurred in the expression of meiotic markers and specific genes, Fkbp6 ($p = 0.00$), Mov10l1 ($p = 0.01$), and Tex13 ($p = 0.00$) in ESCs treated with RA (+RA) compared with the controls (-RA). Oct4 expression was decreased in all studied groups. The expression levels of 4930432K21Rik, Mvh, Stra8, and Scp3 in the +RA group was higher than that of the -RA group. Flow cytometry analysis showed that mean number of Mvh-positive cells in the +RA group was greater as compared with ESCs, -RA and EB7 groups ($p = 0.00$). Downregulation of Oct4 as a pluripotency factor as well as the expression of meiosis markers, this hypothesis is raised that ESCs are differentiated by RA, and have been introduced into the zygote/pachytene of first meiosis as GC-like cells.

Gholamitabar Tabari, M., et al. (2018). "Evaluation of specific germ cell genes expression in mouse embryonic stem cell-derived germ cell like cells treated with bone morphogenetic protein 4 in vitro." Int J Reprod Biomed (Yazd) **16**(8): 507-518.

Background: Bone morphogenetic protein 4 (BMP4) is a significant signaling molecule that involves in initiating of differentiation and performs multifunctional effects on embryonic stem cells (ESCs) and embryos. Objective: The goal of the present study was to evaluate an in vitro differentiation model of mouse embryonic stem cells into germ cells, using BMP4. Materials and Methods: in this experimental study, we used Oct4-GFP mouse ESCs to form embryoid body (EB) aggregations for two days. Then, single cells from EB were cultured for four days with BMP4. Using MTT assay and gene expression levels for evaluation of Mvh and Riken by real-time RT-PCR of six concentrations, 12.5 ng/ ml BMP4 was determined as an optimized dose.

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

Human induced pluripotent stem cells (hiPSCs) generated by de-differentiation of adult somatic cells offer potential solutions for the ethical issues surrounding human embryonic stem cells (hESCs), as well as their immunologic rejection after cellular transplantation. However, although hiPSCs have been described as "embryonic stem cell-like", these cells have a distinct gene expression pattern compared to hESCs, making incomplete reprogramming a potential pitfall. It is unclear to what degree the difference in tissue of origin may contribute to these gene expression differences. To answer these important questions, a careful transcriptional profiling analysis is necessary to investigate the exact reprogramming state of hiPSCs, as well as analysis of the impression, if any, of the tissue of origin on the resulting hiPSCs. In this study, we compare the gene profiles of hiPSCs derived from fetal fibroblasts, neonatal fibroblasts, adipose stem cells, and

keratinocytes to their corresponding donor cells and hESCs.

Ghule, P. N., et al. (2007). "Cell cycle dependent phosphorylation and subnuclear organization of the histone gene regulator p220(NPAT) in human embryonic stem cells." J Cell Physiol **213**(1): 9-17.

Human embryonic stem (ES) cells have an expedited cell cycle (approximately 15 h) due to an abbreviated G1 phase (approximately 2.5 h) relative to somatic cells. One principal regulatory event during cell cycle progression is the G1/S phase induction of histone biosynthesis to package newly replicated DNA. In somatic cells, histone H4 gene expression is controlled by CDK2 phosphorylation of p220(NPAT) and localization of HiNF-P/p220(NPAT) complexes with histone genes at Cajal body related subnuclear foci. Here we show that this 'S point' pathway is operative in situ in human ES cells (H9 cells; NIH-designated WA09). Immunofluorescence microscopy shows an increase in p220(NPAT) foci in G1 reflecting the assembly of histone gene regulatory complexes in situ. In contrast to somatic cells where duplication of p220(NPAT) foci is evident in S phase, the increase in the number of p220(NPAT) foci in ES cells appears to precede the onset of DNA synthesis as measured by BrdU incorporation. Phosphorylation of p220(NPAT) at CDK dependent epitopes is most pronounced in S phase when cells exhibit elevated levels of cyclins E and A. Our data indicate that subnuclear organization of the HiNF-P/p220(NPAT) pathway is rapidly established as ES cells emerge from mitosis and that p220(NPAT) is subsequently phosphorylated in situ. Our findings establish that the HiNF-P/p220(NPAT) gene regulatory pathway operates in a cell cycle dependent microenvironment that supports expression of DNA replication-linked histone genes and chromatin assembly to accommodate human stem cell self-renewal.

Ghule, P. N., et al. (2009). "The subnuclear organization of histone gene regulatory proteins and 3' end processing factors of normal somatic and embryonic stem cells is compromised in selected human cancer cell types." J Cell Physiol **220**(1): 129-135.

Human histone gene expression is controlled at the level of transcription initiation and subsequent 3'end processing to generate non-polyadenylated stem-loop containing histone mRNAs. Transcription is controlled at the G1/S phase transition by the Cyclin E/CDK2 mediated induction of p220(NPAT)/HiNF-P complexes at subnuclear domains designated Histone Locus Bodies (HLBs) that associate with histone gene clusters. Histone mRNA maturation is mediated by Lsm10 containing U7snRNP complexes. In normal human somatic and embryonic stem cells, the 6p histone locus, the transcription marker p220(NPAT) and the 3'end processing marker Lsm10 (but not the Cajal Body marker coilin) co-localize, reflecting the assembly of an integrated factory for histone gene expression. Using in situ immuno-fluorescence microscopy and fluorescence in situ hybridization (FISH), we show that this subnuclear organization is compromised in some cancer cell lines. In aneuploid cells, the presence of HLBs correlates with the number of histone gene loci. More importantly, the in situ co-localization of p220(NPAT) and Lsm10 is disrupted in HeLa S3 cervical carcinoma cells and MCF7 breast adenocarcinoma cells, with most Lsm10 residing in Cajal Bodies. The finding that the subnuclear integration of transcriptional initiation and 3'end processing of histone gene transcripts is deregulated may be causally linked to tumor-related modifications in molecular pathways controlling histone gene expression during the cell cycle.

Ghule, P. N., et al. (2008). "Staged assembly of histone gene expression machinery at subnuclear foci in the abbreviated cell cycle of human embryonic stem cells." Proc Natl Acad Sci U S A **105**(44): 16964-16969.

Human embryonic stem (hES) cells have an abbreviated G(1) phase of the cell cycle. How cells expedite G(1) events that are required for the initiation of S phase has not been resolved. One key regulatory pathway that controls G(1)/S-phase transition is the cyclin E/CDK2-dependent activation of the coactivator protein nuclear protein, ataxia-telangiectasia locus/histone nuclear factor-P (p220(NPAT)/HiNF-P) complex that induces histone gene transcription. In this study, we use the subnuclear organization of factors controlling histone gene expression to define mechanistic differences in the G(1) phase of hES and somatic cells using in situ immunofluorescence microscopy and fluorescence in situ hybridization (FISH).

Gibson, J. D., et al. (2009). "Single-cell transcript analysis of human embryonic stem cells." Integr Biol (Camb) **1**(8-9): 540-551.

We demonstrate the qualitative and quantitative power of single-cell transcript analysis to characterize transcriptome dynamics in human embryonic stem cells (hESC's). Single-cell analysis can systematically determine unique cellular profiles for use in cell sorting and identification, show the potential to augment standing models of cellular differentiation, and elucidate the behavior of stem cells exiting pluripotency. Using single-cell analysis of H9 hESC's differentiating under three culture conditions, we revealed transient expression of mesendodermal markers in all three protocols, followed by increasingly

stable expression of embryonic endoderm and extra-embryonic endoderm markers. Our single-cell profiles reveal mixed populations of cell types, with both transcriptional and temporal heterogeneity marking differentiation under all conditions. Interestingly, we also observe extensive and prolonged co-expression of markers regulating both pluripotency and lineage differentiation in all culture conditions, and we find that pluripotency marker transcripts remain detectable in the majority of cells for many days. Finally, we show that cells derived from undifferentiated hESC colonies display consistent gene expression profiles characterized by three cohorts of transcripts: uniform, absent and sporadically detected messages, and that a striking correlation exists between genes' membership in these cohorts and their hESC promoter chromatin state, with bivalent promoters dominating the sporadic transcripts.

Gioviale, M. C., et al. (2013). "Beyond islet transplantation in diabetes cell therapy: from embryonic stem cells to transdifferentiation of adult cells." Transplant Proc **45**(5): 2019-2024.

Exogenous insulin is, at the moment, the therapy of choice of diabetes, but does not allow tight regulation of glucose leading to long-term complications. Recently, pancreatic islet transplantation to reconstitute insulin-producing beta cells, has emerged as an alternative promising therapeutic approach. Unfortunately, the number of donor islets is too low compared with the high number of patients needing a transplantation leading to a search for renewable sources of high-quality beta-cells. This review, summarizes more recent promising approaches to the generation of new beta-cells from embryonic stem cells for transdifferentiation of adult cells, particularly a critical examination of the seminal work by Lumelsky et al.

Giuffrida, D., et al. (2009). "Human embryonic stem cells secrete soluble factors that inhibit cancer cell growth." Cell Prolif **42**(6): 788-798.

OBJECTIVES: The aim of this study was to determine whether normal human embryonic stem cells (hESC) would secrete factors that arrest growth of human epithelial cancer cell lines. MATERIALS AND METHODS: Cell proliferation was examined using the MTT assay then haemocytometer cell counts. Staining with propidium iodide followed by flow cytometry was used to detect cell cycle stages. Heat denaturation and molecular fractionation experiments were also performed. RESULTS: We found that hESC conditioned medium (hESC CM) inhibited SKOV-3 and HEY cell proliferation. Similar results were also obtained when we used breast and prostate cancer cell lines, whereas little or no inhibitory effect was observed when human fibroblasts were tested. Moreover, a co-culture model confirmed that inhibition of cancer cell proliferation is mediated by soluble factors produced by hESCs. We also determined that the proportion of cancer cells in G(1) phase was increased by hESC CM treatment, accompanied by decrease in cells in S and G(2)/M phases, suggesting that the factors slow progression of cancer cells by cell cycle inhibition. Heat denaturation and molecular fractionation experiments indicated a low molecular weight thermostable factor was responsible for these properties.

Glaser, D. E., et al. (2011). "Functional characterization of embryonic stem cell-derived endothelial cells." J Vasc Res **48**(5): 415-428.

Endothelial cells (EC) derived from embryonic stem cells (ESC) require additional functional characterization before they are used as a cell therapy in order to enhance their potential for engraftment and proliferation. We explore several physiologically relevant functions of ESC-derived EC (ESC-EC), such as its capacity to produce nitric oxide (NO), regulate permeability, activate and express surface molecules for the recruitment of leukocytes in response to inflammatory stimuli, migrate and grow new blood vessels, lay down extracellular matrix, and take up low-density lipoproteins. We also examined the ESC-EC ability to upregulate NO in response to shear stress and downregulate NO in response to pro-inflammatory TNF-alpha activation. Functional responses of ESC-EC were compared with those of cultured mouse aortic ECs. The ESC-EC exhibit most aspects of functional endothelium, but interesting differences remain. The ESC-EC produced less NO on a per cell basis, but the same amount of NO if quantified based on the area of endothelial tissue. They also exhibit increased angiogenic sprouting and are more resistant to inflammatory signals. We further characterized the subphenotype of our ESC-EC and observed both venous and arterial markers on individual cells with a larger percentage of the cells exhibiting a venous phenotype. These data support the hypothesis that the developmental default pathway is toward a venous EC, and that refinement of methods for differentiation towards arterial EC is required to maintain a homogeneous population.

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

Type 1 diabetes mellitus--characterized by the permanent destruction of insulin-secreting beta-cells--is responsive to cell-based treatments that replace lost beta-cell populations. The current gold standard of

pancreas transplantation provides only temporary independence from exogenous insulin and is fraught with complications, including increased mortality. Stem cells offer a number of theoretical advantages over current therapies. Our review will focus on the development of treatments involving tissue stem cells from bone marrow, liver and pancreatic cells, as well as the potential use of embryonic and induced pluripotent stem cells for Type 1 diabetes therapy. While the body of research involving stem cells is at once promising and inconsistent, bone marrow-derived mesenchymal stem cell transplantation seems to offer the most compelling evidence of efficacy.

Gomi, M., et al. (2011). "Single and local blockade of interleukin-6 signaling promotes neuronal differentiation from transplanted embryonic stem cell-derived neural precursor cells." J Neurosci Res **89**(9): 1388-1399.

Safe and efficient transplantation of embryonic stem (ES) cells to the brain requires that local inflammatory and immune responses to allogeneic grafts are inhibited. To investigate cytokines that affect graft cell survival and differentiation, we used stromal cell-derived inducing activity to induce the differentiation of neural progenitor cells (NPCs) from mouse ES cells and transplanted the NPCs into mouse brain. Examination of surrounding brain tissue revealed elevated expression levels of interleukin (IL)-1beta, IL-4, and IL-6 in response to NPC transplantation. Among these, only IL-6 reduced neuronal differentiation and promoted glial differentiation in vitro. When we added anti-IL-6 receptor antibodies to NPCs during transplantation, this single and local blockade of IL-6 signaling reduced the accumulation of host-derived leukocytes, including microglia. Furthermore, it also promoted neuronal differentiation and reduced glial differentiation from the grafted NPCs to an extent similar to that with systemic and continuous administration of cyclosporine A. These results suggest that local administration of anti-IL-6 receptor antibodies with NPCs may promote neuronal differentiation during the treatment of neurological diseases with cell replacement therapy.

Gong, S. P., et al. (2014). "The co-injection of somatic cells with embryonic stem cells affects teratoma formation and the properties of teratoma-derived stem cell-like cells." PLoS One **9**(9): e105975.

The aim of this study was to assess the biological reactions triggered by stem cell transplantation related to phenotypic alteration, host-to-cell response, chromosomal stability, transcriptional alteration, and stem cell-like cell re-expansion. B6CBAF1 mouse embryonic stem cells (ESCs) were injected subcutaneously into homologous or heterologous (B6D2F1) recipients, and heterologous injections were performed with or without co-injection of B6D2F1 fetal fibroblasts. All homologous injections resulted in teratoma formation, whereas a sharp decrease in formation was detected after heterologous injection (100 vs. 14%; $p<0.05$). The co-injection of somatic cells in heterologous injections enhanced teratoma formation significantly (14 vs. 75%; $p<0.05$). Next, ESC-like cell colonies with the same genotype as parental ESCs were formed by culturing teratoma-dissociated cells. Compared with parental ESCs, teratoma-derived ESC-like cells exhibited significantly increased aneuploidy, regardless of homologous or heterologous injections. Repopulation of the parental ESCs was the main factor that induced chromosomal instability, whereas the co-injection of somatic cells did not restore chromosomal normality. Different genes were expressed in the parental ESCs and teratoma-derived ESC-like cells; the difference was larger with parental vs. heterologous than parental vs. homologous co-injections. The co-injection of somatic cells decreased this difference further. In conclusion, the host-to-cell interactions triggered by ESC transplantation could be modulated by co-injection with somatic cells. A mouse model using homologous or heterologous transplantation of stem cells could help monitor cell adaptability and gene expression after injection.

Gong, S. P., et al. (2010). "Embryonic stem cell-like cells established by culture of adult ovarian cells in mice." Fertil Steril **93**(8): 2594-2601, 2601 e2591-2599.

OBJECTIVE: To suggest an alternative strategy for deriving histocompatible stems cells without undertaking genetic manipulation. DESIGN: Prospective approach using an animal model. SETTING: Stem cell and bioevaluation laboratory, Seoul National University. ANIMAL(S): F1 (C57BL6 X DBA2) and outbred (ICR) mice. INTERVENTION(S): Ovarian stroma cells of less than 40 mum in diameter were subcultured with fibroblast monolayer, and colony-forming cells were characterized. MAIN OUTCOME MEASURE(S): Stemness, genotype, and imprinted gene methylation. RESULT(S): Two-lines of colony-forming cells were established, which expressed markers specific for embryonic stem cells (ESC) and formed embryoid bodies and teratomas. Complete matching of microsatellite markers with the cell donor strain confirmed their establishment from ovarian tissue, and identification of both homozygotic and heterozygotic chromosomes raised the possibility of their derivation from parthenogenetic oocytes. However, the use of cells smaller than mature oocytes for primary culture, the difference in imprinted gene methylation compared with parthenogenetic ESCs, and failure to establish the

ESC-like cells by primary follicle culture collectively suggested the irrelevancy to gametes. CONCLUSION(S): Coculture of adult ovarian cells with somatic fibroblasts can yield colony-forming cells having ESC-like activity, which may provide an alternative for establishing autologous stem cells from adults that can be obtained without genetic manipulation.

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

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

Gonzalez, S., et al. (2011). "Influence of E-cadherin-mediated cell adhesion on mouse embryonic stem cells derivation from isolated blastomeres." Stem Cell Rev **7**(3): 494-505.

Efforts to efficiently derive embryonic stem cells (ESC) from isolated blastomeres have been done to minimize ethical concerns about human embryo destruction. Previous studies in our laboratory indicated a poor derivation efficiency of mouse ESC lines from isolated blastomeres at the 8-cell stage (1/8 blastomeres) due, in part, to a low division rate of the single blastomeres in comparison to their counterparts with a higher number of blastomeres (2/8, 3/8 and 4/8 blastomeres). Communication and adhesion between blastomeres from which the derivation process begins could be important aspects to efficiently derive ESC lines. In the present study, an approach consisting in the adhesion of a chimeric E-cadherin (E-cad-Fc) to the blastomere surface was devised to recreate the signaling produced by native E-cadherin between neighboring blastomeres inside the embryo. By this approach, the division rate of 1/8 blastomeres increased from 44.6% to 88.8% and a short exposure of 24 h to the E-cad-Fc produced an ESC derivation efficiency of 33.6%, significantly higher than the 2.2% obtained from the control group without E-cad-Fc. By contrast, a longer exposure to the same chimeric protein resulted in higher proportions of trophoblastic vesicles. Thus, we establish an important role of E-cadherin-mediated adherens junctions in promoting both the division of single 1/8 blastomeres and the efficiency of the ESC derivation process.

Gonzalo-Gil, E., et al. (2016). "Human embryonic stem cell-derived mesenchymal stromal cells ameliorate collagen-induced arthritis by inducing host-derived indoleamine 2,3 dioxygenase." Arthritis Res Ther **18**: 77.

BACKGROUND: The immunosuppressive and anti-inflammatory properties of mesenchymal stromal cells (MSC) have prompted their therapeutic application in several autoimmune diseases, including rheumatoid arthritis. Adult MSC are finite and their clinical use is restricted by the need for long-term expansion protocols that can lead to genomic instability. Inhibition of Smad2/3 signaling in human pluripotent stem cells (hPSC) provides an infinite source of MSC that match the phenotype and functional properties of adult MSC. Here, we test the therapeutic potential of hPSC-MSC of embryonic origin (embryonic stem cell-derived mesenchymal stromal cells, hESC-MSC) in the experimental model of collagen-induced arthritis (CIA). METHODS: CIA was induced in DBA/1 mice by immunization with type II collagen (CII) in Complete Freund's Adjuvant (CFA). Mice were treated with either a single dose (10(6) cells/mouse) of hESC-MSC on the day of immunization (prophylaxis) or with three doses of hESC-MSC every other day starting on the day of arthritis onset (therapy). Arthritis severity was evaluated daily for six weeks and ten days, respectively. Frequency of Treg (FoxP3(+)), Th1 (IFNgamma(+)) and Th17 (IL17(+)) CD4(+) T cells in inguinal lymph nodes (ILN) was quantified by flow cytometry. Serum levels of anti-CII antibodies were determined by ELISA. Detection of hESC-MSC and quantification of murine and human indoleamine 2,3 dioxygenase (IDO1) expression was performed by quantitative real-time PCR. Statistical differences were analyzed by ANOVA and the Mann-Whitney U test. RESULTS: Administration of hESC-MSC to mice with established arthritis reduced disease severity compared to control-treated mice. Analysis of CD4 T cell populations in treated mice showed an increase in FoxP3(+) Treg and IFNgamma(+) Th1 cells but not in Th17 cells in the ILN. Anti-CII antibody levels were not affected by treatment. Migration of hESC-MSC to the ILN in

treated mice was associated with the induction of murine IDO1. CONCLUSION: Treatment with hESC-MSC ameliorates CIA by inducing IFNgamma(+) Th1 cells and IDO1 in the host. Thus, hESC-MSC can provide an infinite cellular source for treatment of rheumatoid arthritis.

Goodrich, A. D., et al. (2010). "In vivo generation of beta-cell-like cells from CD34(+) cells differentiated from human embryonic stem cells." Exp Hematol **38**(6): 516-525 e514.

OBJECTIVE: CD34(+) cells, present within the bone marrow, have previously been shown to possess pancreatic endocrine potential. Based on this observation, we explored the capacity of CD34(+) cells derived in culture from the differentiation of human embryonic stem cells (hESC), for their in vivo pancreatic endocrine capacity. MATERIALS AND METHODS: Sheep were transplanted with hESC-derived CD34(+) cells, as well as nonsorted differentiated cultures. Transplantations were carried out with in utero intraperitoneal injections prior to development of the immune system in the fetus so that tolerance toward foreign antigens was acquired during gestation and persisted in the adult. RESULTS: All cell populations that were tested demonstrated human cellular activity and long-term presence up to 5 years. However, the in vivo beta-cell-like activity achieved from the transplantation of the sorted CD34(+) cell population was not augmented by transplanting the entire cell population from which the CD34(+) cells were isolated. Human DNA and insulin messenger RNA were detected in sheep pancreases. An average of 1.51 ng/mL human C-peptide was detected in serum from eight animals transplanted with differentiated cell populations and assayed up to 55 months posttransplantation. Transplantation of as few as 23,500 cells resulted in long-term sustainable beta-cell-like activity. Teratomas were absent in the transplanted animals. CONCLUSION: Our data suggest that hESC-derived CD34(+) cells have a potential for long-term in vivo endocrine cellular activity that could prove useful in regenerative medicine. Because the same cell population has previously been shown to contain hematopoietic potential, it could be used for the induction of immunological tolerance and bone marrow chimerism prior to cellular therapy for diabetes.

Hajizadeh-Saffar, E., et al. (2015). "Inducible VEGF expression by human embryonic stem cell-derived mesenchymal stromal cells reduces the minimal islet mass required to reverse diabetes." Sci Rep **5**: 9322.

UNLABELLED: Islet transplantation has been hampered by loss of function due to poor revascularization. We hypothesize that co-transplantation of islets with human embryonic stem cell-derived mesenchymal stromal cells that conditionally overexpress VEGF (hESC-MSC:VEGF) may augment islet revascularization and reduce the minimal islet mass required to reverse diabetes in mice. HESC-MSCs were transduced by recombinant lentiviruses that allowed conditional (Dox-regulated) overexpression of VEGF. HESC-MSC: VEGF were characterized by tube formation assay. After co-transplantation of hESC-MSC:VEGF with murine islets in collagen-fibrin hydrogel in the omental pouch of diabetic nude mice, we measured blood glucose, body weight, glucose tolerance and serum C-peptide. As control, islets were transplanted alone or with non-transduced hESC-MSCs. Next, we compared functional parameters of 400 islets alone versus 200 islets co-transplanted with hESC-MSC:VEGF. As control, 200 islets were transplanted alone. Metabolic function of islets transplanted with hESC-MSC:VEGF significantly improved, accompanied by superior graft revascularization, compared with control groups. Transplantation of 200 islets with hESC-MSC:VEGF showed superior function over 400 islets alone. We conclude that co-transplantation of islets with VEGF-expressing hESC-MSCs allowed for at least a 50% reduction in minimal islet mass required to reverse diabetes in mice. This approach may contribute to alleviate the need for multiple donor organs per patient.

Hall, V. (2008). "Porcine embryonic stem cells: a possible source for cell replacement therapy." Stem Cell Rev **4**(4): 275-282.

The development of porcine embryonic stem cell lines (pESC) has received renewed interest given the advances being made in the production of immunocompatible transgenic pigs. However, difficulties are evident in the production of pESCs in-vitro. This may largely be attributable to differences in porcine pre-implantation development compared to the mouse and human. Expression of oct4, nanog and sox2 differs in the zona-enclosed porcine blastocyst compared to its mouse and human counterparts, which may suggest that other factors may be responsible for maintaining porcine pluripotency in the early blastocyst. In addition, the epiblast forms considerably later, at days 7 to 8 when the porcine blastocyst begins to hatch and is maintained for 4 days before completely differentiating. This review covers an outline of the known molecular profile during porcine pre-implantation development and provides a history in the development of putative pESCs to date. Greater knowledge on the molecular mechanisms that underlie porcine pluripotency and pre-implantation development may aid in improving the development of pESCs.

Hall, V. J. (2008). "Embryonic stem cells and Parkinson's disease: cell transplantation to cell therapy." Ann Acad Med Singapore **37**(3): 163-162.

Han, L., et al. (2012). "A chemical small molecule induces mouse embryonic stem cell differentiation into functional vascular endothelial cells via Hmbox1." Stem Cells Dev **21**(15): 2762-2769.

Embryonic stem cells (ESCs) can differentiate to endothelial progenitor cells and vascular endothelial cells (VECs), but the mechanism is largely unknown. In this study, we synthesized 2 chiral compounds (R-ABO and S-ABO) and identified R-ABO as an effective inducer of ESC differentiation into VECs. Furthermore, we found that R-ABO induced ESC differentiation into VECs via homeobox containing 1 (Hmbox1) that acted upstream of fibroblast growth factor 2 (FGF-2). The data suggest that R-ABO is a novel tool for ESC differentiation into VECs, and Hmbox1 is a key regulator in this differentiation process. These findings provide information on a novel target and a new platform for further investigating the gene control of ESC differentiation to VECs.

Han, S., et al. (2018). "Endothelial cells instruct liver specification of embryonic stem cell-derived endoderm through endothelial VEGFR2 signaling and endoderm epigenetic modifications." Stem Cell Res **30**: 163-170.

Liver organogenesis requires complex cell-cell interactions between hepatic endoderm cells and adjacent cell niches. Endothelial cells are key players for endoderm hepatic fate decision. We previously demonstrated that the endothelial cell niche promotes hepatic specification of mouse embryonic stem cell(ESC)-derived endoderm through dual repression of Wnt and Notch pathways in endoderm cells. In the present study, we dissected further the mechanisms by which endothelial cells trigger endoderm hepatic specification. Using our previously established in vitro mouse ESC system mimicking the early hepatic specification process, endoderm cells were purified and co-cultured with endothelial cells to induce hepatic specification. The comparison of transcriptome profiles between hepatic endoderm cells isolated from co-cultures and endoderm cells cultured alone revealed that VEGF signaling instructs hepatic specification of endoderm cells through endothelial VEGFR2 activation. Additionally, epigenetic mark inhibition assays upon co-cultures uncovered that histone acetylation and DNA methylation promote hepatic specification while histone methylation inhibits it. This study provides an efficient 2D platform modelling the endothelial cell niche crosstalk with endoderm, and reveals mechanisms by which endothelial cells promote hepatic specification of mouse ESC-derived endoderm cells through endothelial VEGFR2 activation and endoderm epigenetic modifications.

Han, X., et al. (2017). "Efficient and Fast Differentiation of Human Neural Stem Cells from Human Embryonic Stem Cells for Cell Therapy." Stem Cells Int **2017**: 9405204.

Stem cell-based therapies have been used for repairing damaged brain tissue and helping functional recovery after brain injury. Aberrance neurogenesis is related with brain injury, and multipotential neural stem cells from human embryonic stem (hES) cells provide a great promise for cell replacement therapies. Optimized protocols for neural differentiation are necessary to produce functional human neural stem cells (hNSCs) for cell therapy. However, the qualified procedure is scarce and detailed features of hNSCs originated from hES cells are still unclear. In this study, we developed a method to obtain hNSCs from hES cells, by which we could harvest abundant hNSCs in a relatively short time. Then, we examined the expression of pluripotent and multipotent marker genes through immunostaining and confirmed differentiation potential of the differentiated hNSCs. Furthermore, we analyzed the mitotic activity of these hNSCs. In this report, we provided comprehensive features of hNSCs and delivered the knowledge about how to obtain more high-quality hNSCs from hES cells which may help to accelerate the NSC-based therapies in brain injury treatment.

Handschel, J., et al. (2011). "Embryonic stem cells in scaffold-free three-dimensional cell culture: osteogenic differentiation and bone generation." Head Face Med **7**: 12.

Extracorporeal formation of mineralized bone-like tissue is still an unsolved challenge in tissue engineering. Embryonic stem cells may open up new therapeutic options for the future and should be an interesting model for the analysis of fetal organogenesis. Here we describe a technique for culturing embryonic stem cells (ESCs) in the absence of artificial scaffolds which generated mineralized miromasses. Embryonic stem cells were harvested and osteogenic differentiation was stimulated by the addition of dexamethasone, ascorbic acid, and ss-glycerolphosphate (DAG). After three days of cultivation microspheres were formed. These spherical three-dimensional cell units showed a peripheral zone consisting of densely packed cell layers surrounded by minerals that were embedded in the extracellular matrix. Alizarine red staining confirmed evidence of mineralization after 10 days of DAG stimulation in the stimulated but not in the control group. Transmission electron microscopy demonstrated scorching crystallites and collagenous fibrils as early indication

of bone formation. These extracellular structures resembled hydroxyl apatite-like crystals as demonstrated by distinct diffraction patterns using electron diffraction analysis. The micromass culture technique is an appropriate model to form three-dimensional bone-like micro-units without the need for an underlying scaffold. Further studies will have to show whether the technique is applicable also to pluripotent stem cells of different origin.

Hansson, M. L., et al. (2015). "Efficient delivery and functional expression of transfected modified mRNA in human embryonic stem cell-derived retinal pigmented epithelial cells." J Biol Chem **290**(9): 5661-5672.

Gene- and cell-based therapies are promising strategies for the treatment of degenerative retinal diseases such as age-related macular degeneration, Stargardt disease, and retinitis pigmentosa. Cellular engineering before transplantation may allow the delivery of cellular factors that can promote functional improvements, such as increased engraftment or survival of transplanted cells. A current challenge in traditional DNA-based vector transfection is to find a delivery system that is both safe and efficient, but using mRNA as an alternative to DNA can circumvent these major roadblocks. In this study, we show that both unmodified and modified mRNA can be delivered to retinal pigmented epithelial (RPE) cells with a high efficiency compared with conventional plasmid delivery systems. On the other hand, administration of unmodified mRNA induced a strong innate immune response that was almost absent when using modified mRNA. Importantly, transfection of mRNA encoding a key regulator of RPE gene expression, microphthalmia-associated transcription factor (MITF), confirmed the functionality of the delivered mRNA. Immunostaining showed that transfection with either type of mRNA led to the expression of roughly equal levels of MITF, primarily localized in the nucleus. Despite these findings, quantitative RT-PCR analyses showed that the activation of the expression of MITF target genes was higher following transfection with modified mRNA compared with unmodified mRNA. Our findings, therefore, show that modified mRNA transfection can be applied to human embryonic stem cell-derived RPE cells and that the method is safe, efficient, and functional.

Hao, J., et al. (2009). "Human parthenogenetic embryonic stem cells: one potential resource for cell therapy." Sci China C Life Sci **52**(7): 599-602.

Pluripotent stem cells derived from somatic cells through such processes as nuclear transfer or induced pluripotent stem (iPS) cells present an important model for biomedical research and provide potential resources for cell replacement therapies. However, the overall efficiency of the conversional nuclear transfer is very low and the safety issue remains a major concern for iPS cells. Embryonic stem cells (ESCs) generated from parthenogenetic embryos are one attractive alternative as a source of histocompatible cells and tissues for cell therapy. Recent studies on human parthenogenetic embryonic stem cells (hPG ESCs) have revealed that these ESCs are very similar to the hESCs derived from IVF or in vivo produced blastocysts in gene expression and other characteristics, but full differentiation and development potential of these hPG ESCs have to be further investigated before clinical research and therapeutic interventions. To generate various pluripotent stem cells, diverse reprogramming techniques and approaches will be developed and integrated. This may help elucidate the fundamental mechanisms underlying reprogramming and stem cell biology, and ultimately benefit cell therapy and regenerative medicine.

Hao, Q., et al. (2015). "Study of Bone Marrow and Embryonic Stem Cell-Derived Human Mesenchymal Stem Cells for Treatment of Escherichia coli Endotoxin-Induced Acute Lung Injury in Mice." Stem Cells Transl Med **4**(7): 832-840.

UNLABELLED: : Mesenchymal stem cells (MSCs) can be derived from multiple tissue sources. However, the optimal source of MSCs for cell-based therapy for acute lung injury (ALI) is unclear. In the present experiments, we studied bone marrow (BM)-derived and embryonic stem cell-derived human MSC (ES-MSCs) as a therapeutic agent in Escherichia coli endotoxin-induced ALI in mice. We hypothesized that ES-MSCs would be more potent than BM-MSCs owing to its more primitive source of origin. ALI was induced by the intratracheal instillation of endotoxin at 4 mg/kg into 10-12-week-old C57BL/6 mice with or without BM-MSCs, ES-MSCs, or normal human lung fibroblasts as a cellular control. Compared with the endotoxin-injured mice at 48 hours, the administration of ES-MSCs provided results similar to those of BM-MSCs, significantly reducing the influx of white blood cells and neutrophils and decreasing the secretion of the inflammatory cytokines, macrophage inflammatory protein-2 and tumor necrosis factor-alpha, in the injured alveolus. BM-MSCs also reduced extravascular lung water, a measure of pulmonary edema, by 60% and the total protein levels, a measure of lung permeability, by 66%. However, surprisingly, ES-MSCs did not have these protective effects, which was partially explained by the increased secretion of matrix metallopeptidase 9 by ES-MSCs, an enzyme known to increase lung protein permeability. In conclusion, both BM-MSCs and ES-MSCs markedly decreased endotoxin-induced inflammation. However, ES-MSCs did not show any beneficial effect on reducing

pulmonary edema and lung protein permeability compared with BM-MSCs, suggesting that not all MSCs behave in a similar fashion. Our results highlight the need perhaps for a disease-specific potency assay for MSCs. SIGNIFICANCE: To determine the optimal source of mesenchymal stem cells (MSCs) for cell-based therapy for acute lung injury, bone marrow (BM)- and embryonic stem cell-derived human MSC (ES-MSCs) were compared as therapeutic agents for Escherichia coli endotoxin-induced lung injury in mice. ES-MSCs behaved similarly to BM-MSCs by markedly decreasing the inflammatory response induced by endotoxin. However, unlike BM-MSCs, ES-MSCs provided no protective effects against increasing lung water and protein permeability, in part because of an increase in expression of matrix metallopeptidase 9 by ES-MSCs. In patients with acute respiratory distress syndrome, impaired alveolar fluid clearance (i.e., no resolution of pulmonary edema fluid) has been associated with higher mortality rates. Although ES-MSCs might ultimately be found to have properties superior to those of BM-MSCs, such as for immunomodulation, these results highlight the need for a disease-specific potency assay for stem cell-based therapy.

Haraguchi, S., et al. (2012). "Establishment of self-renewing porcine embryonic stem cell-like cells by signal inhibition." J Reprod Dev **58**(6): 707-716.

Although the establishment of putative porcine embryonic stem cells (ESCs) has been reported, such cell lines quickly lose their self-renewal ability, as they easily differentiate or become extinct after only a limited number of passages in culture. ESC-like cells exhibiting self-renewal rather than pluripotency are considered to be a valuable resource in applications such as drug screening and toxicology testing in humans, livestock and veterinary medicine. Here, we report the generation of unique cell lines established from the inner cell mass (ICM) of porcine embryos by using inhibitors of glycogen synthase kinase 3beta and mitogen-activated protein kinase kinase 1. These ICM-derived cell lines were initially cultured and passaged in conventional ES medium for human ESCs and showed porcine ESC-like morphology with alkaline phosphatase (AP) activity. After transfer to culture in ES medium containing inhibitors, the morphology of the colonies was dramatically changed, i.e., they were closely packed smooth-edged colonies with close cell-cell boundaries and showed the expression of undifferentiated markers including OCT4 (POU5F1) and NANOG. Notably, the self-renewal capacity and morphology of the cells were LIF-dependent, consistent with the expression of LIF receptors and phosphorylation of signal transducer and activator of transcription 3. To date, our established cell lines have been cultured continuously for over 100 passages without any overt morphological changes. Thus, the established cell lines reported here provide a new ESC-like cell culture system for use not only in the fields of veterinary medicine and livestock but also human medical research, since porcine physiology closely resembles that of humans.

Haridass, D., et al. (2009). "Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice." Am J Pathol **175**(4): 1483-1492.

Fetal liver progenitor cell suspensions (FLPC) and hepatic precursor cells derived from embryonic stem cells (ES-HPC) represent a potential source for liver cell therapy. However, the relative capacity of these cell types to engraft and repopulate a recipient liver compared with adult hepatocytes (HC) has not been comprehensively assessed. We transplanted mouse and human HC, FLPC, and ES-HPC into a new immunodeficient mouse strain (Alb-uPA(tg(+/-))Rag2(-/-)gamma(c)(-/-) mice) and estimated the percentages of HC after 3 months. Adult mouse HC repopulated approximately half of the liver mass (46.6 +/- 8.0%, 1 x 10(6) transplanted cells), whereas mouse FLPC derived from day 13.5 and 11.5 post conception embryos generated only 12.1 +/- 3.0% and 5.1 +/- 1.1%, respectively, of the recipient liver and smaller cell clusters. Adult human HC and FLPC generated overall less liver tissue than mouse cells and repopulated 10.0 +/- 3.9% and 2.7 +/- 1.1% of the recipient livers, respectively. Mouse and human ES-HPC did not generate HC clusters in our animal model. We conclude that, in contrast to expectations, adult HC of human and mouse origin generate liver tissue more efficiently than cells derived from fetal tissue or embryonic stem cells in a highly immunodeficient Alb-uPA transgenic mouse model system. These results have important implications in the context of selecting the optimal strategy for human liver cell therapies.

Harrill, J. A., et al. (2010). "Quantitative assessment of neurite outgrowth in human embryonic stem cell-derived hN2 cells using automated high-content image analysis." Neurotoxicology **31**(3): 277-290.

Throughout development neurons undergo a number of morphological changes including neurite outgrowth from the cell body. Exposure to neurotoxic chemicals that interfere with this process may result in permanent deficits in nervous system function. Traditionally, rodent primary neural cultures and immortalized human and non-human clonal cell lines have been used to investigate the molecular mechanisms controlling neurite outgrowth and examine chemical effects on this process. The present study

characterizes the molecular phenotype of hN2 human embryonic stem cell (hESC)-derived neural cells and uses automated high-content image analysis to measure neurite outgrowth in vitro. At 24h post-plating hN2 cells express a number of protein markers indicative of a neuronal phenotype, including: nestin, beta(III)-tubulin, microtubule-associated protein 2 (MAP2) and phosphorylated neurofilaments. Neurite outgrowth in hN2 cells proceeded rapidly, with a majority of cells extending one to three neurites by 48h in culture. In addition, concentration-dependent decreases in neurite outgrowth and ATP-content were observed following treatment of hN2 cells with either bisindolylmaleimide I, U0126, lithium chloride, sodium orthovanadate and brefeldin A, all of which have previously been shown to inhibit neurite outgrowth in primary rodent neural cultures. Overall, the molecular phenotype, rate of neurite outgrowth and sensitivity of hN2 cells to neurite outgrowth inhibitors were comparable to other in vitro models previously characterized in the literature. hN2 cells provide a model in which to investigate chemical effects on neurite outgrowth in a non-transformed human-derived cells and provide an alternative to the use of primary rodent neural cultures or immortalized clonal cell lines.

Harrison, N. J., et al. (2007). "Culture adaptation of embryonic stem cells echoes germ cell malignancy." Int J Androl **30**(4): 275-281; discussion 281.

Teratocarcinomas are a subset of tumours that result from the neoplastic transformation of primordial germ cells. Such germ cell tumours (GCT) are histologically heterogeneous, reflecting a capacity for differentiation (pluripotency) of their embryonal carcinoma (EC) stem cells. However, malignant evolution of these tumours may ultimately correlate with a decrease in pluripotency, because this would tend to increase the propensity of EC cells for self-renewal. Human embryonic stem (ES) cells, derived from early blastocysts, closely resemble EC cells and, on prolonged culture in vitro, acquire progressive genetic changes that show striking similarity to those seen in GCT (e.g. gain of material from chromosome 12). In parallel, these abnormal ES cells show enhanced population growth rates and plating efficiencies, indicative of their adaptation to culture conditions. Understanding the mechanisms that drive such culture adaptation of ES cells may also provide insights into the development and progression of GCT.

Hartman, B. H., et al. (2018). "Fbxo2(VHC) mouse and embryonic stem cell reporter lines delineate in vitro-generated inner ear sensory epithelia cells and enable otic lineage selection and Cre-recombination." Dev Biol.

While the mouse has been a productive model for inner ear studies, a lack of highly specific genes and tools has presented challenges. The absence of definitive otic lineage markers and tools is limiting in vitro studies of otic development, where innate cellular heterogeneity and disorganization increase the reliance on lineage-specific markers. To address this challenge in mice and embryonic stem (ES) cells, we targeted the lineage-specific otic gene Fbxo2 with a multicistronic reporter cassette (Venus/Hygro/CreER = VHC). In otic organoids derived from ES cells, Fbxo2(VHC) specifically delineates otic progenitors and inner ear sensory epithelia. In mice, Venus expression and CreER activity reveal a cochlear developmental gradient, label the prosensory lineage, show enrichment in a subset of type I vestibular hair cells, and expose strong expression in adult cerebellar granule cells. We provide a toolbox of multiple spectrally distinct reporter combinations for studies that require use of fluorescent reporters, hygromycin selection, and conditional Cre-mediated recombination.

Hayakawa-Yano, Y., et al. (2017). "An RNA-binding protein, Qki5, regulates embryonic neural stem cells through pre-mRNA processing in cell adhesion signaling." Genes Dev **31**(18): 1910-1925.

Cell type-specific transcriptomes are enabled by the action of multiple regulators, which are frequently expressed within restricted tissue regions. In the present study, we identify one such regulator, Quaking 5 (Qki5), as an RNA-binding protein (RNABP) that is expressed in early embryonic neural stem cells and subsequently down-regulated during neurogenesis. mRNA sequencing analysis in neural stem cell culture indicates that Qki proteins play supporting roles in the neural stem cell transcriptome and various forms of mRNA processing that may result from regionally restricted expression and subcellular localization. Also, our in utero electroporation gain-of-function study suggests that the nuclear-type Qki isoform Qki5 supports the neural stem cell state. We next performed in vivo transcriptome-wide protein-RNA interaction mapping to search for direct targets of Qki5 and elucidate how Qki5 regulates neural stem cell function. Combined with our transcriptome analysis, this mapping analysis yielded a bona fide map of Qki5-RNA interaction at single-nucleotide resolution, the identification of 892 Qki5 direct target genes, and an accurate Qki5-dependent alternative splicing rule in the developing brain. Last, our target gene list provides the first compelling evidence that Qki5 is associated with specific biological events; namely, cell-cell adhesion. This prediction was confirmed by histological analysis of mice in which Qki proteins were genetically ablated, which revealed disruption of the apical surface of the lateral wall in the developing brain. These data

collectively indicate that Qki5 regulates communication between neural stem cells by mediating numerous RNA processing events and suggest new links between splicing regulation and neural stem cell states.

He, H., et al. (2016). "p53 and p73 Regulate Apoptosis but Not Cell-Cycle Progression in Mouse Embryonic Stem Cells upon DNA Damage and Differentiation." Stem Cell Reports **7**(6): 1087-1098.

Embryonic stem cells (ESCs) are fast proliferating cells capable of differentiating into all somatic cell types. In somatic cells, it is well documented that p53 is rapidly activated upon DNA damage to arrest the cell cycle and induce apoptosis. In mouse ESCs, p53 can also be functionally activated, but the precise biological consequences are not well characterized. Here, we demonstrated that doxorubicin treatment initially led to cell-cycle arrest at G2/M in ESCs, followed by the occurrence of massive apoptosis. Neither p53 nor its target gene p73 was required for G2/M arrest. Instead, p53 and p73 were fully responsible for apoptosis. p53 and p73 were also required for differentiation-induced apoptosis in mouse ESCs. In addition, doxorubicin treatment induced the expression of retinoblastoma protein in a p53-dependent manner. Therefore, both p53 and p73 are critical in apoptosis induced by DNA damage and differentiation.

He, K., et al. (2016). "Epigenetics changes caused by the fusion of human embryonic stem cell and ovarian cancer cells." Biosci Rep **36**(5).

To observe the effect of gene expression and tumorigenicity in hybrid cells of human embryonic stem cells (hESCs) and ovarian cancer cells in vitro and in vivo using a mouse model, and to determine its feasibility in reprogramming tumour cells growth and apoptosis, for a potential exploration of the role of hESCs and tumour cells fusion in the management of ovarian cancer. Stable transgenic hESCs (H1) and ovarian cancer cell line OVCAR-3 were established before fusion, and cell fusion system was established to analyse the related indicators. PTEN expression in HO-H1 cells was higher than those in the parental stem cells and lower than those in parental tumour cells; the growth of OV-H1 (RFP+GFP) hybrid cells with double fluorescence expressions were obviously slower than that of human embryonic stem cells and OVCAR-3 ovarian cancer cells. The apoptosis signal of the OV-H1 hybrid cells was significantly higher than that of the hESCs and OVCAR-3 ovarian cancer cells. In vivo results showed that compared with 7 days, 28 days and 35 days after inoculation of OV-H1 hybrid cells; also, apoptotic cell detection indicated that much stronger apoptotic signal was found in OV-H1 hybrid cells inoculated mouse. The hESCs can inhibit the growth of OVCAR-3 cells in vitro by suppressing p53 and PTEN expression to suppress the growth of tumour that may be achieved by inducing apoptosis of OVCAR-3 cells. The change of epigenetics after fusion of ovarian cancer cells and hESCs may become a novel direction for treatment of ovarian cancer.

Hirata, S., et al. (2005). "Prevention of experimental autoimmune encephalomyelitis by transfer of embryonic stem cell-derived dendritic cells expressing myelin oligodendrocyte glycoprotein peptide along with TRAIL or programmed death-1 ligand." J Immunol **174**(4): 1888-1897.

Experimental autoimmune encephalomyelitis (EAE) is caused by activation of myelin Ag-reactive CD4+ T cells. In the current study, we tested a strategy to prevent EAE by pretreatment of mice with genetically modified dendritic cells (DC) presenting myelin oligodendrocyte glycoprotein (MOG) peptide in the context of MHC class II molecules and simultaneously expressing TRAIL or Programmed Death-1 ligand (PD-L1). For genetic modification of DC, we used a recently established method to generate DC from mouse embryonic stem cells (ES cells) in vitro (ES-DC). ES cells were sequentially transfected with an expression vector for TRAIL or PD-L1 and an MHC class II-associated invariant chain-based MOG epitope-presenting vector. Subsequently, double-transfectant ES cell clones were induced to differentiate to ES-DC, which expressed the products of introduced genes. Treatment of mice with either of the double-transfectant ES-DC significantly reduced T cell response to MOG, cell infiltration into spinal cord, and the severity of MOG peptide-induced EAE. In contrast, treatment with ES-DC expressing MOG alone, irrelevant Ag (OVA) plus TRAIL, or OVA plus PD-L1, or coinjection with ES-DC expressing MOG plus ES-DC-expressing TRAIL or PD-L1 had no effect in reducing the disease severity. In contrast, immune response to irrelevant exogenous Ag (keyhole limpet hemocyanin) was not impaired by treatment with any of the genetically modified ES-DC. The double-transfectant ES-DC presenting Ag and simultaneously expressing immune-suppressive molecules may well prove to be an effective therapy for autoimmune diseases without inhibition of the immune response to irrelevant Ag.

Hirata, Y., et al. (2010). "Transactivation of the dopamine receptor 3 gene by a single provirus integration results in development of B-cell lymphoma in transgenic mice generated from retrovirally transduced embryonic stem cells." Blood **115**(19): 3930-3938.

Gene transfer vectors based on retroviruses are commonly used in gene therapy applications because of their unique ability to integrate efficiently into host genomes. This ability also forms the basis of a transformation event that can be induced in transduced cells by transactivation of proto-oncogenes near the vector integration sites. Here, we report on the development of lymphoma in mice generated from embryonic stem cells transduced with an enhanced green fluorescent protein. The cells expressed B220, CD5, Mac1, and IgM on their surfaces and expressed transcription factors characteristic of B-cell lymphoma. Importantly, each mouse had a single copy of the provirus in its genome; the copy was integrated into the second intron of the dopamine receptor 3 (D3) gene, and high-level expression of D3 was detected only in the lymphoma cells. Ectopic expression of D3 in murine marrow cells resulted in preferential proliferation of cells at the pre-B-cell stage in response to a D3-specific agonist, but this proliferation was not observed in vivo. Cells cotransduced with D3 and Bcl-x(L) genes had a phenotype similar to that of lymphoma in vivo, suggesting that the leukemogenesis induced by retroviral integration required "second hit" mutations of additional genes.

Hiroyama, T., et al. (2008). "Establishment of mouse embryonic stem cell-derived erythroid progenitor cell lines able to produce functional red blood cells." PLoS One **3**(2): e1544.

BACKGROUND: The supply of transfusable red blood cells (RBCs) is not sufficient in many countries. If erythroid cell lines able to produce transfusable RBCs in vitro were established, they would be valuable resources. However, such cell lines have not been established. To evaluate the feasibility of establishing useful erythroid cell lines, we attempted to establish such cell lines from mouse embryonic stem (ES) cells. METHODOLOGY/PRINCIPAL FINDINGS: We developed a robust method to obtain differentiated cell lines following the induction of hematopoietic differentiation of mouse ES cells and established five independent hematopoietic cell lines using the method. Three of these lines exhibited characteristics of erythroid cells. Although their precise characteristics varied, each of these lines could differentiate in vitro into more mature erythroid cells, including enucleated RBCs. Following transplantation of these erythroid cells into mice suffering from acute anemia, the cells proliferated transiently, subsequently differentiated into functional RBCs, and significantly ameliorated the acute anemia. In addition, we did not observe formation of any tumors following transplantation of these cells. CONCLUSION/SIGNIFICANCE: To the best of our knowledge, this is the first report to show the feasibility of establishing erythroid cell lines able to produce mature RBCs. Considering the number of human ES cell lines that have been established so far, the intensive testing of a number of these lines for erythroid potential may allow the establishment of human erythroid cell lines similar to the mouse erythroid cell lines described here. In addition, our results strongly suggest the possibility of establishing useful cell lines committed to specific lineages other than hematopoietic progenitors from human ES cells.

Hisamatsu-Sakamoto, M., et al. (2008). "Embryonic stem cells cultured in serum-free medium acquire bovine apolipoprotein B-100 from feeder cell layers and serum replacement medium." Stem Cells **26**(1): 72-78.

Previous studies have demonstrated that cell populations that are cultured with heterologous animal products can acquire xenoantigens, potentially limiting their clinical utility because of immune responses. Embryonic stem cells (ESCs) are an attractive source of multiple potential cellular therapies and are typically derived and routinely cultured on murine embryonic fibroblast (MEF) feeder cell layers in commercially available serum replacement (SR) medium or fetal calf serum (FCS)-containing medium. Recently, we found that a strong antibody response was generated in human subjects after the second infusion of therapeutic cells cultured in FCS-containing medium. This response was specific for bovine apolipoprotein B-100 (apoB-100), which is the major protein component of low-density lipoproteins (LDL) and which targets its binding to abundant low-density lipoprotein receptors on the cell surface, from which it is internalized. Here, we have shown that ESCs cultured on MEFs in SR medium acquired bovine apoB-100 from MEFs and from the SR medium as well. Our findings also suggest that bovine LDL are used as critical nutrients for ESC propagation.

Ho, H. Y. and M. Li (2006). "Potential application of embryonic stem cells in Parkinson's disease: drug screening and cell therapy." Regen Med **1**(2): 175-182.

Embryonic stem (ES) cells are genetically normal, continuous cell lines that can give rise to a variety of somatic cells in culture. These include the midbrain dopaminergic neurons, a major cell type lost in Parkinson's disease. With the promising outcome of mesencephalic fetal transplantation in some Parkinson's disease patients, the establishment of human ES cells has sparked much attention in both the scientific and general community regarding their potential as an alternative to aborted fetal tissue for cell replacement therapies. There is also great interest in developing the ES cell system as a platform for pharmaceutical and toxicological screening. Progress has been made in

developing protocols for dopaminergic neuronal specification in ES cell development. Research to define the criteria for the 'right' category of therapeutic dopaminergic cells is underway. However, the promise of human ES cells rests largely on our ability to expand stem cells without genetic and epigenetic compromise, and to direct stem cell differentiation with absolute phenotypic fidelity. The delivery of these goals will require a much better understanding of the control of ES cell self-renewal, proliferation and the commitment of differentiation.

Ho, J. C., et al. (2012). "Reversal of endothelial progenitor cell dysfunction in patients with type 2 diabetes using a conditioned medium of human embryonic stem cell-derived endothelial cells." Diabetes Metab Res Rev **28**(5): 462-473.

BACKGROUND: The potential clinical application of bone marrow or peripheral blood-derived progenitor cells for cardiovascular regeneration in patients with diabetes mellitus (DM) is limited by their functional impairment. We sought to determine the mechanisms of impaired therapeutic efficacy of peripheral blood-derived progenitor cells in type 2 DM patients and evaluated the use of cell-free conditioned medium obtained from human embryonic stem cell-derived endothelial-like cells (ESC-ECs) to reverse their functional impairment. METHODS: The angiogenic potential of late outgrowth endothelial cells (OECs) and cytokine profile of the conditional medium of proangiogenic cells (PACs) derived from peripheral blood-mononuclear cells of healthy control and DM patients and ESC-ECs was compared by in vitro tube formation assay and a multiplex bead-based immunoassay kit, respectively. The in vivo angiogenic potential of ESC-ECs derived conditioned medium in rescuing the functional impairment of PB-PACs in DM patients was investigated using a hindlimb ischemia model. RESULTS: Human ESC-ECs had similar functional and phenotypic characteristics as OECs in healthy controls. Cytokine profiling showed that vascular endothelial growth factor, stromal cell-derived factor 1 and placental growth factor were down-regulated in PACs from DM patients. Tube formation assay that revealed functional impairment of OECs from DM patients could be rescued by ESC-ECs conditioned medium. Administration of ESC-ECs conditioned medium restored the therapeutic efficacy of PB-PACs from DM patients in a mouse model of hindlimb ischemia. CONCLUSIONS: Our results showed that peripheral blood-derived progenitor cells from DM patients have impaired function because of defective secretion of angiogenic cytokines, which could be restored by supplementation of ESC-ECs conditioned medium.

Husseini, L., et al. (2008). "Functional analysis of embryonic stem cell-derived glial cells after integration into hippocampal slice cultures." Stem Cells Dev **17**(6): 1141-1152.

Embryonic stem (ES) cell-derived neural progenitor cells (ESNPs) generated in vitro are multipotent progenitors which can differentiate into oligodendrocytes, astrocytes, and neurons. Given the exciting prospects for ES cell-based treatments of neurological disorders, several studies investigated the migration, integration, and differentiation of grafted ESNPs into neurons and glial cells. However, little is known about the functional properties of transplanted ESNPs on the single cell level. In this study, we combined electrophysiology, single cell reverse transcription polymerase chain reaction (RT-PCR) and immunochemistry to determine the developmental time course of molecular and functional properties of ES cell-derived glial precursors (ESGPs) after deposition onto hippocampal slice cultures. Based on functional criteria, donor cells possessed three different phenotypes. During an observation period of 3 weeks after engraftment, the proportion of donor cells with a passive current pattern (type 3) continuously increased. The majority of these cells expressed astroglial markers. Type 3 host cells underwent similar developmental changes. In contrast, donor and host cells expressing time- and voltage-dependent currents (types 1, 2) displayed different developmental profiles. Importantly, type 2 donor and host cells also differed in the expression of inwardly rectifying K(+) channels. This suggests that despite several similarities in overall current phenotypes and timing of maturation, many donor cells integrated into host tissue but did not acquire the full set of ion channels present in their native counterparts. These findings emphasize the need to carefully characterize ES cell-derived progeny aimed for neural repair and cell-mediated gene transfer strategies.

Hwang, I., et al. (2016). "Intrathecal Transplantation of Embryonic Stem Cell-Derived Spinal GABAergic Neural Precursor Cells Attenuates Neuropathic Pain in a Spinal Cord Injury Rat Model." Cell Transplant **25**(3): 593-607.

Neuropathic pain following spinal cord injury (SCI) is a devastating disease characterized by spontaneous pain such as hyperalgesia and allodynia. In this study, we investigated the therapeutic potential of ESC-derived spinal GABAergic neurons to treat neuropathic pain in a SCI rat model. Mouse embryonic stem cell-derived neural precursor cells (mESC-NPCs) were cultured in media supplemented with sonic hedgehog (SHH) and retinoic acid (RA) and efficiently differentiated into GABAergic neurons. Interestingly, low doses of SHH and RA induced MGE-like

progenitors, which expressed low levels of DARPP32 and Nkx2.1 and high levels of Irx3 and Pax6. These cells subsequently generated the majority of the DARPP32(-) GABAergic neurons after in vitro differentiation. The spinal mESC-NPCs were intrathecally transplanted into the lesion area of the spinal cord around T10-T11 at 21 days after SCI. The engrafted spinal GABAergic neurons remarkably increased both the paw withdrawal threshold (PWT) below the level of the lesion and the vocalization threshold (VT) to the level of the lesion (T12, T11, and T10 vertebrae), which indicates attenuation of chronic neuropathic pain by the spinal GABAergic neurons. The transplanted cells were positive for GABA antibody staining in the injured region, and cells migrated to the injured spinal site and survived for more than 7 weeks in L4-L5. The mESC-NPC-derived spinal GABAergic neurons dramatically attenuated the chronic neuropathic pain following SCI, suggesting that the spinal GABAergic mESC-NPCs cultured with low doses of SHH and RA could be alternative cell sources for treatment of SCI neuropathic pain by stem cell-based therapies.

Hwang, N. S., et al. (2008). "In vivo commitment and functional tissue regeneration using human embryonic stem cell-derived mesenchymal cells." Proc Natl Acad Sci U S A **105**(52): 20641-20646.

Development of clinically relevant regenerative medicine therapies using human embryonic stem cells (hESCs) requires production of a simple and readily expandable cell population that can be directed to form functional 3D tissue in an in vivo environment. We describe an efficient derivation method and characterization of mesenchymal stem cells (MSCs) from hESCs (hESCd-MSCs) that have multilineage differentiation potential and are capable of producing fat, cartilage, and bone in vitro. Furthermore, we highlight their in vivo survival and commitment to the chondrogenic lineage in a microenvironment comprising chondrocyte-secreted morphogenetic factors and hydrogels. Normal cartilage architecture was established in rat osteochondral defects after treatment with chondrogenically-committed hESCd-MSCs. In view of the limited available cell sources for tissue engineering applications, these embryonic-derived cells show significant potential in musculoskeletal tissue regeneration applications.

Hwang, N. S., et al. (2006). "Chondrogenic differentiation of human embryonic stem cell-derived cells in arginine-glycine-aspartate-modified hydrogels." Tissue Eng **12**(9): 2695-2706.

Human embryonic stem cells (hESCs) have the potential to self-renew and generate multiple cell types, producing critical building blocks for tissue engineering and regenerative medicine applications. Here, we describe the efficient derivation and chondrogenic differentiation of mesenchymal-like cells from hESCs. These cells exhibit mesenchymal stem cell (MSC) surface markers, including CD29, CD44, CD105, and platelet-derived growth factor receptor-alpha. Under appropriate growth conditions, the hESC-derived cells proliferated without phenotypic changes and maintained MSC surface markers. The chondrogenic capacity of the cells was studied in pellet culture and after encapsulation in poly(ethylene glycol)-diacrylate (PEGDA) hydrogels with exogenous extracellular proteins or argininеglycine- aspartate (RGD)-modified PEGDA hydrogels. The hESC-derived cells exhibited growth factor- dependent matrix production in pellet culture but did not produce tissue characteristic of cartilage morphology. In PEGDA hydrogels containing exogenous hyaluronic acid or type I collagen, no significant cell growth or matrix production was observed. In contrast, when these cells were encapsulated in RGDmodified poly(ethylene glycol)hydrogels, neocartilage with basophilic extracellular matrix deposition was observed within 3 weeks of culture, producing cartilage-specific gene up-regulation and extracellular matrix production. Our results indicate that precursor cells characteristic of a MSC population can be cultured from differentiating hESCs through embryoid bodies, thus holding great promise for a potentially unlimited source of cells for cartilage tissue engineering.

Hyka-Nouspikel, N., et al. (2012). "Deficient DNA damage response and cell cycle checkpoints lead to accumulation of point mutations in human embryonic stem cells." Stem Cells **30**(9): 1901-1910.

Human embryonic stem cells (hESCs) tend to lose genomic integrity during long periods of culture in vitro and to acquire a cancer-like phenotype. In this study, we aim at understanding the contribution of point mutations to the adaptation process and at providing a mechanistic explanation for their accumulation. We observed that, due to the absence of p21/Waf1/Cip1, cultured hESCs lack proper cell cycle checkpoints and are vulnerable to the kind of DNA damage usually repaired by the highly versatile nucleotide excision repair (NER) pathway. In response to UV-induced DNA damage, the majority of hESCs succumb to apoptosis; however, a subpopulation continues to proliferate, carrying damaged DNA and accumulating point mutations with a typical UV-induced signature. The UV-resistant cells retain their proliferative capacity and potential for pluripotent differentiation and are markedly less apoptotic to subsequent UV exposure. These findings demonstrate that, due to deficient DNA damage response, the modest NER activity in hESCs is insufficient to

prevent increased mutagenesis. This provides for the appearance of genetically aberrant hESCs, paving the way for further major genetic changes.

Inzunza, J., et al. (2005). "Derivation of human embryonic stem cell lines in serum replacement medium using postnatal human fibroblasts as feeder cells." Stem Cells **23**(4): 544-549.

Derivation and culture of human embryonic stem cells (hESCs) without animal-derived material would be optimal for cell transplantation. We derived two new hES (HS293 and HS306) and 10 early cell lines using serum replacement (SR) medium instead of conventional fetal calf serum and human foreskin fibroblasts as feeder cells. Line HS293 has been in continuous culture, with a passage time of 5-8 days, since October 2003 and is at passage level 56. Line HS306 has been cultured since February 2004, now at passage 41. The lines express markers of pluripotent hESCs (Oct-4, SSEA-4, TRA-1-60, TRA-1-81, GCTM-2, and alkaline phosphatase). The pluripotency has been shown in embryoid bodies in vitro, and the pluripotency of line 293 has also been shown in vivo by teratoma formation in severe combined immunodeficiency/beige mice. The karyotype of HS293 is 46,XY, and that of HS306 is 46,XX. Ten more early lines have been derived under similar conditions since September 2004. We conclude that hESC lines can be successfully derived using SR medium and postnatal human fibroblasts as feeder cells. This is a step toward xeno-free conditions and facilitates the use of these cells in transplantation.

Ishii, S., et al. (2010). "Stromal cell-secreted factors promote the survival of embryonic stem cell-derived early neural stem/progenitor cells via the activation of MAPK and PI3K-Akt pathways." J Neurosci Res **88**(4): 722-734.

Neural stem/progenitor cells (NS/PCs) have been studied extensively with the hope of using them clinically to repair the damaged central nervous system. However, little is known about the signals that regulate the proliferation, survival, and differentiation of NS/PCs in early development. To clarify the underlying mechanisms, we took advantage of an in vitro ES cell differentiation system from which we can obtain neurospheres containing NS/PCs with characteristics of the early caudal neural tube, by treating embryoid bodies (EBs) with a low concentration of retinoic acid (RA). We found that conditioned medium from the PA6 stromal cell line (PA6CM) increased the efficiency of neurosphere formation by suppressing apoptosis and promoting the survival of the NS/PCs. PA6CM also induced the phosphorylation of Erk1/2 and Akt1 in cells derived from the EBs. Furthermore, inhibitors of the MAPK and PI3K-Akt signaling pathways, U0126 and LY294002, attenuated the effects of PA6CM, significantly increasing the number of apoptotic cells and decreasing the number of viable cells among the ES cell-derived NS/PCs. Thus, PA6CM appears to contain soluble factors that promote the survival of ES cell-derived early NS/PCs through the activation of the MAPK and PI3K-Akt pathways.

Ishii, T., et al. (2010). "In vitro hepatic maturation of human embryonic stem cells by using a mesenchymal cell line derived from murine fetal livers." Cell Tissue Res **339**(3): 505-512.

Hepatocytes derived from human embryonic stem cells (hESCs) are an attractive cell source for regenerative medicine. We previously reported the differentiation of hESCs into alpha-fetoprotein (AFP)-producing endodermal cells by using extracellular matrix and growth factors. We also reported the establishment of the MLSgt20 cell line, which was derived from mesenchymal cells residing in murine fetal livers and accelerated the hepatic maturation of both murine hepatic progenitor cells and murine ESCs. In this study, hESC-derived AFP-producing cells were isolated by using a flow cytometer and co-cultured with MLSgt20 cells. The co-cultured hESC-derived AFP-producing cells had the immunocytological characteristics of hepatocytes, expressed mature hepatocyte markers (as indicated by reverse transcription and the polymerase chain reaction), and displayed higher hepatocyte functions including ammonia removal, cytochrome P450 3A4/7 activity, and the ability to produce and store glycogen. However, the MLSgt20 cells did not directly cause undifferentiated hESCs to mature into hepatocyte-like cells. The co-culture method was thus successfully shown to induce the differentiation of hESC-derived endodermal cells into functional hepatocyte-like cells.

Ishii, T., et al. (2007). "Transplantation of embryonic stem cell-derived endodermal cells into mice with induced lethal liver damage." Stem Cells **25**(12): 3252-3260.

ESCs are a potential cell source for cell therapy. However, there is no evidence that cell transplantation using ESC-derived hepatocytes is therapeutically effective. The main objective of this study was to assess the therapeutic efficacy of the transplantation of ESC-derived endodermal cells into a liver injury model. The beta-galactosidase-labeled mouse ESCs were differentiated into alpha-fetoprotein (AFP)-producing endodermal cells. AFP-producing cells or ESCs were transplanted into transgenic mice that expressed diphtheria toxin (DT) receptors under the control of an albumin enhancer/promoter. Selective damage was induced in the recipient hepatocytes by the

administration of DT. Although the transplanted AFP-producing cells had repopulated only 3.4% of the total liver mass 7 days after cell transplantation, they replaced 32.8% of the liver by day 35. However, these engrafted cells decreased (18.3% at day 40 and 7.9% at day 50) after the cessation of DT administration, and few donor cells were observed by days 60-90. The survival rate of the AFP-producing cell-transplanted group (66.7%) was significantly higher in comparison with that of the sham-operated group (17.6%). No tumors were detected by day 50 in the AFP-producing cell-transplanted group; however, splenic teratomas did form 60 days or more after transplantation. ESC transplantation had no effect on survival rates; furthermore, there was a high frequency of tumors in the ESC-transplanted group 35 days after transplantation. In conclusion, this study demonstrates, for the first time, that ESC-derived endodermal cells improve the survival rates after transplantation into mice with induced hepatocellular injury. Disclosure of potential conflicts of interest is found at the end of this article.

Ishiwata, I., et al. (2003). "Organogenesis of heart-vascular system derived from mouse 2 cell stage embryos and from early embryonic stem cells in vitro." Hum Cell **16**(1): 15-22.

Regenerative medical treatment with embryonic stem cells (an ES cell) is a goal for organ transplantation. Structures that are tubular in nature (i.e. blood capillaries) were induced from early embryonic stem (EES) cells in vitro using embryotrophic factor (ETFs). In addition, cardiac muscle cells could be identified as well. However, differentiation of EES cells into a complete cardiovascular system was difficult because 3 germ layer primordial organs are directed embryologically in various ways and it is not possible to guide only cardiovascular organs. Thus, we introduced ETFs after the formation of an embryoid body and were successful in cloning cell clusters that beat, thus deriving only cardiovascular organs. The application of this to the treatment of various cardiovascular diseases is promising.

Islam, M. S., et al. (2010). "Use of human embryonic stem cells to understand hematopoiesis and hematopoietic stem cell niche." Curr Stem Cell Res Ther **5**(3): 245-250.

Intensive research of hematopoiesis using human embryonic stem cells (hESC) as a unique starting cell population has enabled differentiation and isolation of diverse hematopoietic cell lineages. However, there has been only limited success in derivation of hematopoietic stem cells (HSCs) capable of long-term, multi-lineage engraftment when transplanted into xenogeneic models. Better understanding of the HSC developmental niche, the home for hematopoietic stem and progenitor cells, will aid to advance strategies to derive and assay putative HSCs from hESCs. This review discusses recent status of hematopoietic development from the hESCs and highlights the possibility of developing HSC niche using hESC-derived niche components.

Israely, E., et al. (2014). "Akt suppression of TGFbeta signaling contributes to the maintenance of vascular identity in embryonic stem cell-derived endothelial cells." Stem Cells **32**(1): 177-190.

The ability to generate and maintain stable in vitro cultures of mouse endothelial cells (ECs) has great potential for genetic dissection of the numerous pathologies involving vascular dysfunction as well as therapeutic applications. However, previous efforts at achieving sustained cultures of primary stable murine vascular cells have fallen short, and the cellular requirements for EC maintenance in vitro remain undefined. In this study, we have generated vascular ECs from mouse embryonic stem (ES) cells and show that active Akt is essential to their survival and propagation as homogeneous monolayers in vitro. These cells harbor the phenotypical, biochemical, and functional characteristics of ECs and expand throughout long-term cultures, while maintaining their angiogenic capacity. Moreover, Akt-transduced embryonic ECs form functional perfused vessels in vivo that anastomose with host blood vessels. We provide evidence for a novel function of Akt in stabilizing EC identity, whereby the activated form of the protein protects mouse ES cell-derived ECs from TGFbeta-mediated transdifferentiation by downregulating SMAD3. These findings identify a role for Akt in regulating the developmental potential of ES cell-derived ECs and demonstrate that active Akt maintains endothelial identity in embryonic ECs by interfering with active TGFbeta-mediated processes that would ordinarily usher these cells to alternate fates.

Iuchi, S., et al. (2006). "An immortalized drug-resistant cell line established from 12-13-day mouse embryos for the propagation of human embryonic stem cells." Differentiation **74**(4): 160-166.

Human embryonic stem (ES) cells are usually co-cultivated with supporting cells consisting of short-term cultures of fibroblasts (not an immortalized line) in a medium lacking serum. This method has promoted important progress in the field, but suffers from certain disadvantages. By serial cultivation for 27 consecutive transfers and about 63 cell generations, we have evolved an immortalized line from fibroblastic cells of 12-13-day mouse embryos. This line (MMM) supports the multiplication of H9 cells better than the 3T3 line. It supports the growth of H9 cells as well as do

available short-term fibroblast cultures, but maintains more effectively the stem cell character of the H9 cells, judging by their better retention of Oct4. We have made MMM cells resistant to blasticidin and zeocin, the most efficient antibiotics for selection of stable transformants. In the presence of zeocin, the resistant MMM were able to support multiplication and selection of ES cells transfected with an exogenous gene encoding zeocin resistance.

Ivey, K. N., et al. (2008). "MicroRNA regulation of cell lineages in mouse and human embryonic stem cells." Cell Stem Cell **2**(3): 219-229.

Cell fate decisions of pluripotent embryonic stem (ES) cells are dictated by activation and repression of lineage-specific genes. Numerous signaling and transcriptional networks progressively narrow and specify the potential of ES cells. Whether specific microRNAs help refine and limit gene expression and, thereby, could be used to manipulate ES cell differentiation has largely been unexplored. Here, we show that two serum response factor (SRF)-dependent muscle-specific microRNAs, miR-1 and miR-133, promote mesoderm formation from ES cells but have opposing functions during further differentiation into cardiac muscle progenitors. Furthermore, miR-1 and miR-133 were potent repressors of nonmuscle gene expression and cell fate during mouse and human ES cell differentiation. miR-1's effects were in part mediated by translational repression of the Notch ligand Delta-like 1 (Dll-1). Our findings indicate that muscle-specific miRNAs reinforce the silencing of nonmuscle genes during cell lineage commitment and suggest that miRNAs may have general utility in regulating cell-fate decisions from pluripotent ES cells.

Jacobs, V. R., et al. (2005). "[The STEMMAT-project as part of health initiative BayernAktiv: adult stem cells from umbilical cord and cord blood as alternative to embryonic stem cell research]." Zentralbl Gynakol **127**(6): 368-372.

Adult stem cells from umbilical cord and cord blood are an interesting alternative to embryonic stem cells because such research is commonly recognized as ethical undisputed and many aspects are still insufficiently investigated. In the context of the STEMMAT research project (STEM = Stem Cell and MAT = Material) different aspects of stem cells from umbilical cord and cord blood are investigated, to improve basic science understanding and potentially leading someday to a clinical application.

Jafary, H., et al. (2008). "Differential effect of activin on mouse embryonic stem cell differentiation in insulin-secreting cells under nestin-positive selection and spontaneous differentiation protocols." Cell Biol Int **32**(2): 278-286.

Parallel to the importance of the development of cell therapies to treat diabetes is the production of sufficient numbers of pancreatic endocrine cells that function like primary islets. To increase the efficiency of endocrine pancreatic-like cell differentiation from mouse embryonic stem cells (ESCs), we applied activin-B to nestin-positive selection (protocol 1) and spontaneous differentiation (protocol 2) in different groups including: [A] activin-B, or [B] basic fibroblast growth factor (bFGF), and/or [C] activin-B+bFGF. The differentiated cells expressed most pancreatic-related genes. The number of insulin- and C peptide-positive cells, as well as dithizone-positive clusters in group A of protocol 1 was higher than in the other groups. Significant insulin concentrations in protocol 1 were produced when glucose was added to the medium, in comparison with protocol 2. Moreover, insulin release was increased significantly in group A of protocol 1 even with lower glucose. In conclusion, Addition of activin-B in a nestin-positive selection protocol increased the insulin-secreting cells in comparison with the same protocol with bFGF and/or spontaneous differentiation in presence of bFGF and/or activin-B alone. However, improvements of the current method are required to generate a sufficient source of true beta-cells for the treatment of diabetes mellitus.

Jagatha, B., et al. (2009). "In vitro differentiation of retinal ganglion-like cells from embryonic stem cell derived neural progenitors." Biochem Biophys Res Commun **380**(2): 230-235.

ES cells have been reported to serve as an excellent source for obtaining various specialized cell types and could be used in cell replacement therapy. Here, we demonstrate the potential of ES cells to differentiate along retinal ganglion cell (RGC) lineage. FGF2-induced ES cell derived neural progenitors (ES-NPs) were able to generate RGC-like cells in vitro upon differentiation. These cells expressed RGC regulators and markers such as, Ath5, Brn3b, RPF-1, Thy-1 and Islet-1, confirming their potential to differentiate into RGCs. The generation of RGCs from ES-NPs was enhanced with the exposure of FGF2 and Sonic hedgehog (Shh), although Shh treatment alone did not affect RGC differentiation significantly. ES-NPs, after exposure to FGF2, were capable of integrating and differentiating into RGCs in vivo upon transplantation. Thus, our study suggests that ES cells can serve an excellent renewable source for generating RGCs that can be used to treat neurodegenerative diseases like glaucoma.

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.

Ji, Y., et al. (2017). "Microvesicles released from human embryonic stem cell derived-mesenchymal stem cells inhibit proliferation of leukemia cells." Oncol Rep **38**(2): 1013-1020.

Human embryonic stem cell derived-mesenchymal stem cells (hESCMSCs) are able to inhibit proliferation of leukemia cells. Microvesicles released from human embryonic stem cell derived-mesenchymal stem cells (hESCMSCMVs) might play an important part in antitumor activity. Microvesicles were isolated by ultracentrifugation and identified under a scanning electron microscopy and transmission electron microscope separately. After 48-h cocultured with hESCMSCs and hESCMSCMVs, the number of K562 and HL60 was counted and tumor cell viability was measured by CCK8 assay. The expression of proteins Bcl-2 and Bax were estimated by western blotting. Transmission electron microscope and western blot analysis were adopted to evaluate the autophagy level. Results showed that both hESCMSCs and hESCMSCMVs inhibited proliferation of leukemia cells in a concentration-dependent manner. hESCMSCMVs reduced the ratio of Bcl/Bax, enhanced the protein level of Beclin-1 and LC3-II conversion, thus upregulating autophagy and apoptosis. In conclusion, microvesicles released from human embryonic stem cell derived-mesenchymal stem cells inhibited tumor growth and stimulated autophagy and excessive autophagy might induce apoptosis.

Jiang, S., et al. (2010). "Reconstitution of mammary epithelial morphogenesis by murine embryonic stem cells undergoing hematopoietic stem cell differentiation." PLoS One **5**(3): e9707.

BACKGROUND: Mammary stem cells are maintained within specific microenvironments and recruited throughout lifetime to reconstitute de novo the mammary gland. Mammary stem cells have been isolated through the identification of specific cell surface markers and in vivo transplantation into cleared mammary fat pads. Accumulating evidence showed that during the reformation of mammary stem cell niches by dispersed epithelial cells in the context of the intact epithelium-free mammary stroma, non-mammary epithelial cells may be sequestered and reprogrammed to perform mammary epithelial cell functions and to adopt mammary epithelial characteristics during reconstruction of mammary epithelium in regenerating mammary tissue in vivo. METHODOLOGY/PRINCIPAL FINDINGS: To examine whether other types of progenitor cells are able to contribute to mammary branching morphogenesis, we examined the potential of murine embryonic stem (mES) cells, undergoing hematopoietic differentiation, to support mammary reconstitution in

vivo. We observed that cells from day 14 embryoid bodies (EBs) under hematopoietic differentiation condition, but not supernatants derived from these cells, when transplanted into denuded mammary fat pads, were able to contribute to both the luminal and myoepithelial lineages in branching ductal structures resembling the ductal-alveolar architecture of the mammary tree. No teratomas were observed when these cells were transplanted in vivo. CONCLUSIONS/SIGNIFICANCE: Our data provide evidence for the dominance of the tissue-specific mammary stem cell niche and its role in directing mES cells, undergoing hematopoietic differentiation, to reprogram into mammary epithelial cells and to promote mammary epithelial morphogenesis. These studies should also provide insights into regeneration of damaged mammary gland and the role of the mammary microenvironment in reprogramming cell fate.

Kaitsuka, T., et al. (2014). "Generation of functional insulin-producing cells from mouse embryonic stem cells through 804G cell-derived extracellular matrix and protein transduction of transcription factors." Stem Cells Transl Med **3**(1): 114-127.

Embryonic stem (ES) and induced pluripotent stem (iPS) cells have potential applications to regenerative medicine for diabetes; however, a useful and safe way to generate pancreatic beta cells has not been developed. In this study, we tried to establish an effective method of differentiation through the protein transduction of three transcription factors (Pdx1, NeuroD, and MafA) important to pancreatic beta cell development. The method poses no risk of unexpected genetic modifications in target cells. Transduction of the three proteins induced the differentiation of mouse ES and mouse iPS cells into insulin-producing cells. Furthermore, a laminin-5-rich extracellular matrix efficiently induced differentiation under feeder-free conditions. Cell differentiation was confirmed with the expression of the insulin 1 gene in addition to marker genes in pancreatic beta cells, the differentiated cells secreted glucose-responsive C-peptide, and their transplantation restored normoglycemia in diabetic mice. Moreover, Pdx1 protein transduction had facilitative effects on differentiation into pancreatic endocrine progenitors from human iPS cells. These results suggest the direct delivery of recombinant proteins and treatment with laminin-5-rich extracellular matrix to be useful for the generation of insulin-producing cells.

Karamali, F., et al. (2018). "Hepatocyte growth factor promotes the proliferation of human embryonic stem cell derived retinal pigment epithelial cells." J Cell Physiol.

Research that pertains to the molecular mechanisms involved in retinal pigment epithelial (RPE) development can significantly contribute to cell therapy studies. The effects of periocular mesenchymal cells on the expansion of RPE cells remain elusive. We have examined the possible proliferative role of hepatocyte growth factor (HGF) as a mesenchymal cell secretory factor against human embryonic stem cell derived RPE (hESC-RPE). We found that the conditioned medium of human mesenchymal stem cells from apical papilla and/or exogenous HGF promoted proliferation of the hESC-RPE cells as single cells and cell sheets, in addition to rabbit RPE sheets in vitro. Blockage of HGF signaling by HGF receptor inhibitor, PHA-665752, inhibited proliferation of hESC-RPE cells. However, differentiation of hESCs and human-induced pluripotent stem cells to a rostral fate and eye-field specification was unaffected by HGF. Our in vivo analysis showed HGF expression in periocular mesenchymal cells after optic cup formation in chicken embryos. Administration of HGF receptor inhibitor at this developmental stage in chicken embryos led to reduced eye size and disorganization of the RPE sheet. These findings suggested that HGF administration could be beneficial for obtaining higher numbers of hESC-RPE cells in human preclinical and clinical trials.

Kasuda, S., et al. (2008). "Establishment of embryonic stem cells secreting human factor VIII for cell-based treatment of hemophilia A." J Thromb Haemost **6**(8): 1352-1359.

BACKGROUND: Hemophilia A is an X-chromosome-linked recessive bleeding disorder resulting from an F8 gene abnormality. Although various gene therapies have been attempted with the aim of eliminating the need for factor VIII replacement therapy, obstacles to their clinical application remain. OBJECTIVES: We evaluated whether embryonic stem (ES) cells with a tetracycline-inducible system could secrete human FVIII. METHODS AND RESULTS: We found that embryoid bodies (EBs) developed under conditions promoting liver differentiation efficiently secreted human FVIII after doxycycline induction. Moreover, use of a B-domain variant F8 cDNA (226aa/N6) dramatically enhanced FVIII secretion. Sorting based on green fluorescent protein (GFP)-brachyury (Bry) and c-kit revealed that GFP-Bry(+)/c-kit(+) cells during EB differentiation with serum contain an endoderm progenitor population. When GFP-Bry(+)/c-kit(+) cells were cultured under the liver cell-promoting conditions, these cells secreted FVIII more efficiently than other populations tested. CONCLUSION: Our findings suggest the potential for future development of an effective ES cell-based approach to treating hemophilia A.

Kato, K., et al. (2010). "Identification of stem cell transcriptional programs normally expressed in embryonic and neural stem cells in alloreactive CD8+ T cells mediating graft-versus-host disease." Biol Blood Marrow Transplant **16**(6): 751-771.

A hallmark of graft-versus-host-disease (GVHD), a life-threatening complication after allogeneic hematopoietic stem cell transplantation, is the cytopathic injury of host tissues mediated by persistent alloreactive effector T cells (T(E)). However, the mechanisms that regulate the persistence of alloreactive T(E) during GVHD remain largely unknown. Using mouse GVHD models, we demonstrate that alloreactive CD8(+) T(E) rapidly diminished in vivo when adoptively transferred into irradiated secondary congenic recipient mice. In contrast, although alloreactive CD8(+) T(E) underwent massive apoptosis upon chronic exposure to alloantigens, they proliferated in vivo in secondary allogeneic recipients, persisted, and caused severe GVHD. Thus, the continuous proliferation of alloreactive CD8(+) T(E), which is mediated by alloantigenic stimuli rather than homeostatic factors, is critical to maintaining their persistence. Gene expression profile analysis revealed that although alloreactive CD8(+) T(E) increased the expression of genes associated with cell death, they activated a group of stem cell genes normally expressed in embryonic and neural stem cells. Most of these stem cell genes are associated with cell cycle regulation, DNA replication, chromatin modification, and transcription. One of these genes, Ezh2, which encodes a chromatin modifying enzyme, was abundantly expressed in CD8(+) T(E). Silencing Ezh2 significantly reduced the proliferation of alloantigen-activated CD8(+) T cells. Thus, these findings identify that a group of stem cell genes could play important roles in sustaining terminally differentiated alloreactive CD8(+) T(E) and may be therapeutic targets for controlling GVHD.

Katsman, D., et al. (2012). "Embryonic stem cell-derived microvesicles induce gene expression changes in Muller cells of the retina." PLoS One **7**(11): e50417.

Cell-derived microvesicles (MVs), recognized as important components of cell-cell communication, contain mRNAs, miRNAs, proteins and lipids and transfer their bioactive contents from parent cells to cells of other origins. We have studied the effect that MVs released from embryonic stem cells (ESMVs) have on retinal progenitor Muller cells. Cultured human Muller cells were exposed to mouse ESMVs every 48 hours for a total of 9 treatments. Morphological changes were observed by light microscopy in the treated cells, which grew as individual heterogeneous cells, compared to the uniform, spindle-like adherent cellular sheets of untreated cells. ESMVs transferred to Muller cells embryonic stem cell (ESC) mRNAs involved in the maintenance of pluripotency, including Oct4 and Sox2, and the miRNAs of the 290 cluster, important regulators of the ESC-specific cell cycle. Moreover, ESMV exposure induced up-regulation of the basal levels of endogenous human Oct4 mRNA in Muller cells. mRNA and miRNA microarrays of ESMV-treated vs. untreated Muller cells revealed the up-regulation of genes and miRNAs involved in the induction of pluripotency, cellular proliferation, early ocular genes and genes important for retinal protection and remodeling, as well as the down-regulation of inhibitory and scar-related genes and miRNAs involved in differentiation and cell cycle arrest. To further characterize the heterogeneous cell population of ESMV-treated Muller cells, their expression of retinal cell markers was compared to that in untreated control cells by immunocytochemistry. Markers for amacrine, ganglion and rod photoreceptors were present in treated but not in control Muller cells. Together, our findings indicate that ESMs induce de-differentiation and pluripotency in their target Muller cells, which may turn on an early retinogenic program of differentiation.

Kattman, S. J., et al. (2007). "Specification of multipotential cardiovascular progenitor cells during embryonic stem cell differentiation and embryonic development." Trends Cardiovasc Med **17**(7): 240-246.

The fully formed heart is composed of diverse cell lineages including myocytes, endothelial cells, vascular smooth muscle cells, and fibroblasts that derive from distinct subsets of mesoderm during embryonic development. Findings from lineage tracing studies indicate that cardiomyocytes develop from cells that express fetal liver kinase-1, suggesting that the cardiac lineages may arise from a progenitor cell with vascular cardiomyocyte potential. Recent studies using the embryonic stem cell model have led to the identification of a fetal liver kinase-1(+) progenitor cell that displays both vascular and cardiomyocyte potential. A comparable progenitor was also isolated from the early mouse embryo. Identification and isolation of these cardiovascular progenitor cells establishes a new model of heart development that will provide insights into the mechanisms regulating cardiovascular lineage diversification. These progenitor cells may also represent a novel cell population for models of congenital heart disease and cell replacement therapy.

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

Developmental exposure to endocrine disruptors has resulted in the increased incidence of

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

Kauts, M. L., et al. (2018). "In Vitro Differentiation of Gata2 and Ly6a Reporter Embryonic Stem Cells Corresponds to In Vivo Waves of Hematopoietic Cell Generation." Stem Cell Reports **10**(1): 151-165.

In vivo hematopoietic generation occurs in waves of primitive and definitive cell emergence. Differentiation cultures of pluripotent embryonic stem cells (ESCs) offer an accessible source of hematopoietic cells for blood-related research and therapeutic strategies. However, despite many approaches, it remains a goal to robustly generate hematopoietic progenitor and stem cells (HP/SCs) in vitro from ESCs. This is partly due to the inability to efficiently promote, enrich, and/or molecularly direct hematopoietic emergence. Here, we use Gata2Venus (G2V) and Ly6a(SCA1)GFP (LG) reporter ESCs, derived from well-characterized mouse models of HP/SC emergence, to show that during in vitro differentiation they report emergent waves of primitive hematopoietic progenitor cells (HPCs), definitive HPCs, and B-lymphoid cell potential. These results, facilitated by enrichment of single and double reporter cells with HPC properties, demonstrate that in vitro ESC differentiation approximates the waves of hematopoietic cell generation found in vivo, thus raising possibilities for enrichment of rare ESC-derived HP/SCs.

Kawazoe, S., et al. (2009). "Extrinsic factors derived from mouse embryonal carcinoma cell lines maintain pluripotency of mouse embryonic stem cells through a novel signal pathway." Dev Growth Differ **51**(2): 81-93.

Embryonic carcinoma (EC) cells, which are malignant stem cells of teratocarcinoma, have numerous morphological and biochemical properties in common with pluripotent stem cells such as embryonic stem (ES) cells. However, three EC cell lines (F9, P19 and PCC3) show different developmental potential and self-renewal capacity from those of ES cells. All three EC cell lines maintain self-renewal capacity in serum containing medium without Leukemia Inhibitory factor (LIF) or feeder layer, and show limited differentiation capacity into restricted lineage and cell types. To reveal the underlying mechanism of these characteristics, we took the approach of characterizing extrinsic factors derived from EC cells on the self-renewal capacity and pluripotency of mouse ES cells. Here we demonstrate that EC cell lines F9 and P19 produce factor(s) maintaining the undifferentiated state of mouse ES cells via an unidentified signal pathway, while P19 and PCC3 cells produce self-renewal factors of ES cells other than LIF that were able to activate the STAT3 signal; however, inhibition of STAT3 activation with Janus kinase inhibitor shows only partial impairment on the maintenance of the undifferentiated state of ES cells. Thus, these factors present in EC cells-derived conditioned medium may be responsible for the self-renewal capacity of EC and ES cells independently of LIF signaling.

Kayama, M., et al. (2010). "Transfection with pax6 gene of mouse embryonic stem cells and subsequent cell cloning induced retinal neuron progenitors, including retinal ganglion cell-like cells, in vitro." Ophthalmic Res **43**(2): 79-91.

OBJECTIVE: It is theoretically possible to induce various cell types, including retinal neurons, from embryonic stem cells (ESCs). pax6 regulates early events in eye development, including the generation of retinal ganglion cells (RGCs). We previously reported the successful induction of corneal epithelial cells from ESCs transfected with the pax6 gene. Here, we attempted to establish cloned RGC-like cells from ESCs transfected with the pax6 gene. METHODS: Undifferentiated mouse ESCs were

transfected with pax6 cDNA by electroporation, followed by selection with G418. We conducted limiting-dilution culture of pax6-transfected cells. We expanded the cloned pax6-transfected cells, which expressed nestin and musashi-1, for further characterization in culture media containing fibronectin. The cells were characterized using RT-PCR, immunostaining, electron microscopy, renal subcapsular transplantation assay and Ca imaging. RESULTS: We obtained clonally expanding pax6-transfected cells, all of which were positive for six3, sonic hedgehog (shh), math5, brn3, thy1 and melanopsin, by using several ESCs. When transplanted into a mouse renal capsule, they differentiated into neurons with elongated axons, expressing betaIII tubulin and neurofilament middle chain, and were free from teratoma development. Electron-microscopic examination showed neurotubules and neurofilaments in the axon-like processes of the cloned pax6-transfected cells. High KCl stimulation increased free Ca influx on Ca2+ imaging. CONCLUSIONS: ESCs were applicable for the induction of retinal progenitor cells, including RGC-like cells, by transfection with the pax6 gene and subsequent limiting-dilution culture. Cloned cell lines may be useful to analyze the requirements for retinal progenitor cell differentiation, and our study suggests the clinical application of this cell type.

Kayama, M., et al. (2007). "Recent advances in corneal regeneration and possible application of embryonic stem cell-derived corneal epithelial cells." Clin Ophthalmol **1**(4): 373-382.

The depletion of limbal stem cells due to various diseases leads to corneal opacification and visual loss. The unequivocal identification and isolation of limbal stem cells may be a considerable advantage because long-term, functional recovery of corneal epithelium is linked to graft constructs that retain viable stem cell populations. As specific markers of limbal stem cells, the ATP-binding cassette, sub-family G, member2 (ABCG2), a member of the multiple drug-resistance (MDR) family of membrane transporters which leads to a side population phenotype, and transcription factor p63 were proposed recently. Conventional corneal transplantation is not applicable for patients with limbal stem cells deficiency, because the conventional allograft lacks limbal stem cells. The introduction of limbal epithelial cell transplantation was a major advance in the therapeutic techniques for reconstruction of the corneal surface. Limbal epithelial cell transplantation is clinically conducted when cultured allografts as well as autografts are available; however, allografts have a risk of immunologic rejection and autografts are hardly available for patients with bilateral ocular surface disorders. Embryonic stem (ES) cells are characterized by their capacity to proliferate indefinitely and to differentiate into any cell type. We induced corneal epithelial cells from ES cells by culturing them on type IV collagen or alternatively, by introduction of the pax6 gene into ES cells. Recent advances in our study supports the possibility of their clinical use as a cell source for reconstruction of the damaged corneal surface. This review summarizes the recent advances in corneal regeneration therapies and the possible application of ES cell-derived corneal epithelial cells.

Kee, K., et al. (2006). "Bone morphogenetic proteins induce germ cell differentiation from human embryonic stem cells." Stem Cells Dev **15**(6): 831-837.

The growth factors bone morphogenetic protein-4 (BMP4), BMP7, and BMP8b are required for specification of primordial germ cells (PGCs) in mice. Disruption of the genes that encode these factors leads to a severe reduction in number, or the complete absence, of PGCs. In addition, several studies have demonstrated that human BMP4 can promote PGC differentiation from mouse embryonic stem (ES) cells and in organ cultures. Here, we sought to determine whether recombinant human BMPs could induce differentiation of germ cells from human (h) ES cells. We found that addition of recombinant human BMP4 increased the expression of the germ cell-specific markers VASA and SYCP3 during differentiation of hES cells to embryoid bodies (EBs). In addition, BMP7 and BMP8b showed additive effects on germ cell induction when added together with BMP4. Finally, we observed that addition of BMPs to differentiating ES cells also increased the percentage of cells that stained positively for VASA. We note that the effects of recombinant BMPs were modest but reproducible and suggest that addition of BMPs to differentiation media increases differentiation of human germ cells from hES cells.

Kee, K. and R. A. Reijo Pera (2008). "Human germ cell lineage differentiation from embryonic stem cells." CSH Protoc **2008**: pdb prot5048.

INTRODUCTIONBiological and ethical constraints hinder studies of human germ cell development despite its importance to reproductive health, including fertility and tumorigenesis. Thus, most of what we know of human germ cell development has been extrapolated from studies in model organisms. Human embryonic stem cells (hESCs) may provide an ideal system for probing the developmental genetics of germ cell formation and differentiation in vitro. The growth factors BMP (bone morphogenetic protein) 4, BMP7, and BMP8b are required for development of primordial germ cells (PGCs) in mice. It has been shown that these BMPs

significantly increase germ cell differentiation from hESCs in vitro. This protocol describes a method to induce germ cell differentiation from hESCs by the addition of BMPs to hESC differentiation medium. The protocol can be used to study the basic mechanism of germ cell development in human cells.

Kelly, O. G., et al. (2011). "Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells." Nat Biotechnol **29**(8): 750-756.

Using a flow cytometry-based screen of commercial antibodies, we have identified cell-surface markers for the separation of pancreatic cell types derived from human embryonic stem (hES) cells. We show enrichment of pancreatic endoderm cells using CD142 and of endocrine cells using CD200 and CD318. After transplantation into mice, enriched pancreatic endoderm cells give rise to all the pancreatic lineages, including functional insulin-producing cells, demonstrating that they are pancreatic progenitors. In contrast, implanted, enriched polyhormonal endocrine cells principally give rise to glucagon cells. These antibodies will aid investigations that use pancreatic cells generated from pluripotent stem cells to study diabetes and pancreas biology.

Kerkis, I., et al. (2006). "Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers." Cells Tissues Organs **184**(3-4): 105-116.

We report the isolation of a population of immature dental pulp stem cells (IDPSC), which express embryonic stem cell markers Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81 as well as several other mesenchymal stem cell markers during at least 25 passages while maintaining the normal karyotype and the rate of expansion characteristic of stem cells. The expression of these markers was maintained in subclones obtained from these cells. Moreover, in vitrothese cells can be induced to undergo uniform differentiation into smooth and skeletal muscles, neurons, cartilage, and bone under chemically defined culture conditions. After in vivo transplantation of these cells into immunocompromised mice, they showed dense engraftment in various tissues. The relative ease of recovery and the expression profiles of various markers justify further exploration of IDPSC for clinical therapy.

Kern, I., et al. (2013). "Embryonic stem cell-based screen for small molecules: cluster analysis reveals four response patterns in developing neural cells." Curr Med Chem **20**(5): 710-723.

Neural differentiation of embryonic stem cells (ESC) is considered a promising model to perform in vitro testing for neuroactive and neurotoxic compounds. We studied the potential of a dual reporter murine ESC line to identify bioactive and/or toxic compounds. This line expressed firefly luciferase under the control of the neural cell-specific tubulin alpha promoter (TUBA1A), and renilla luciferase under the control of the ubiquitous translation elongation factor 1-alpha-1 (EEF1A1) promoter. During neural differentiation, TUBA1A activity increased, while EEF1A1 activity decreased. We first validated our test system using the known neurotoxin methyl mercury. This compound altered expression of both reporter genes, with ESC-derived neural precursors being affected at markedly lower concentrations than undifferentiated ESCs. Analysis of a library of 1040 bioactive compounds picked up 127 compounds with altered EEF1A1 and/or TUBA1A promoter activity, which were classified in 4 clusters. Cluster 1 (low EEF1A1 and TUBA1A) was the largest cluster, containing many cytostatic drugs, as well as known neurodevelopmental toxicants, psychotropic drugs and endocrine disruptors. Cluster 2 (high EEF1A1, stable TUBA1A) was limited to three sulfonamides. Cluster 3 (high EEF1A1 and TUBA1A) was small, but markedly enriched in neuroactive and neurotoxic compounds. Cluster 4 (stable EEF1A1, high TUBA1A) was heterogeneous, containing endocrine disruptors, neurotoxic and cytostatic drugs. The dual reporter gene assay described here might be a useful addition to in vitro drug testing panels. Our two-dimensional testing strategy provides information on complex response patterns, which could not be achieved by a single marker approach.

Keskintepe, L., et al. (2007). "Derivation and comparison of C57BL/6 embryonic stem cells to a widely used 129 embryonic stem cell line." Transgenic Res **16**(6): 751-758.

Typically, embryonic stem (ES) cells derived from 129 mouse substrains are used to generate genetically altered mouse models. Resulting chimeric mice were then usually converted to a C57BL/6 background, which takes at least a year, even in the case of speed congenics. In recent years, embryonic stem cells have been derived from various mouse strains. However, 129 ES cells are still widely used partially due to poor germline transmission of ES cells derived from other strains. Availability of highly germline-competent C57BL/6 ES cells would enormously facilitate generation of genetically altered mice in a pure C57BL/6 genetic background by eliminating backcrossing time, and thus significantly reducing associated costs and efforts. Here, we describe establishment of a C57BL/6 ES cell line (LK1) and compare its efficacy to a widely used 129SvJ ES cell line (GSI-1) in generating germline chimeras. In contrast to earlier studies, our data shows that highly

germline-competent C57BL/6 ES cell lines can be derived using a simple approach, and thus support broader use of C57BL/6 ES cell lines for genetically engineered mouse models.

Kibschull, M., et al. (2011). "Human embryonic fibroblasts support single cell enzymatic expansion of human embryonic stem cells in xeno-free cultures." Stem Cell Res **6**(1): 70-82.

The future application of human embryonic stem cells (hESC) for therapeutic approaches requires the development of xeno-free culture conditions to prevent the potential transmission of animal pathogens or xenobiotic substances to hESC. An important component of the majority of hESC culture systems developed is the requirement for fibroblasts to serve as feeders. For this purpose, several studies have used human foreskin fibroblasts established under xeno-free conditions. In this study we report xeno-free establishment and maintenance of human embryonic fibroblasts (XHEF) and demonstrate their ability to support long-term self-renewal of hESC under xeno-free culture conditions, using a commercially available complete medium. Importantly, our culture conditions allow enzymatic passaging of hESC. In contrast, hESC cultured on human foreskin fibroblasts (XHFF) under the same conditions were poorly maintained and rapidly subject to differentiation. Our study clearly shows that the source of human fibroblasts is essential for long-term xeno-free hESC maintenance.

Kidder, B. L., et al. (2008). "Embryonic stem cells contribute to mouse chimeras in the absence of detectable cell fusion." Cloning Stem Cells **10**(2): 231-248.

Embryonic stem (ES) cells are capable of differentiating into all embryonic and adult cell types following mouse chimera production. Although injection of diploid ES cells into tetraploid blastocysts suggests that tetraploid cells have a selective disadvantage in the developing embryo, tetraploid hybrid cells, formed by cell fusion between ES cells and somatic cells, have been reported to contribute to mouse chimeras. In addition, other examples of apparent stem cell plasticity have recently been shown to be the result of cell fusion. Here we investigate whether ES cells contribute to mouse chimeras through a cell fusion mechanism. Fluorescence in situ hybridization (FISH) analysis for X and Y chromosomes was performed on dissociated tissues from embryonic, neonatal, and adult wild-type, and chimeric mice to follow the ploidy distributions of cells from various tissues. FISH analysis showed that the ploidy distributions in dissociated tissues, notably the tetraploid cell number, did not differ between chimeric and wild-type tissues. To address the possibility that early cell fusion events are hidden by subsequent reductive divisions or other changes in cell ploidy, we injected Z/EG (lacZ/EGFP) ES cells into ACTB-cre blastocysts. Recombination can only occur as the result of cell fusion, and the recombined allele should persist through any subsequent changes in cell ploidy. We did not detect evidence of fusion in embryonic chimeras either by direct fluorescence microscopy for GFP or by PCR amplification of the recombined Z/EG locus on genomic DNA from ACTB-cre::Z/EG chimeric embryos. Our results argue strongly against cell fusion as a mechanism by which ES cells contribute to chimeras.

Kiechle, M. (2008). "[Human embryonic stem cell research in Germany. The scientific reviewing of applications for the import and use of human embryonic stem cells]." Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz **51**(9): 961-964.

From July 2002 to May 2008, 36 applications for the import and use of human embryonic stem cells (hES) were reviewed by the German Central Ethics Committee for Stem Cell Research (ZES). A flood of applications anticipated by opponents to human embryonic stem cell research has not occurred since the enactment of the German Stem Cell Act in 2002. On the contrary, German hES cell research is below international average in terms of project numbers. The current restrictions for using hES cells in Germany might be causative for the opinion that this type of research is not considered to be very promising. This could hold true especially for research aiming at clinical applications. Consequently, potential research goals of premium importance, especially those of potential clinical relevance, could be seriously jeopardized.

Kim, B. M., et al. (2012). "MicroRNAs are indispensable for reprogramming mouse embryonic fibroblasts into induced stem cell-like cells." PLoS One **7**(6): e39239.

MicroRNAs play a pivotal role in cellular maintenance, proliferation, and differentiation. They have also been implicated to play a key role in disease pathogenesis, and more recently, cellular reprogramming. Certain microRNA clusters can enhance or even directly induce reprogramming, while repressing key proteins involved in microRNA processing decreases reprogramming efficiency. Although microRNAs clearly play important roles in cellular reprogramming, it remains unknown whether microRNAs are absolutely necessary. We endeavored to answer this fundamental question by attempting to reprogram Dicer-null mouse embryonic fibroblasts (MEFs) that lack almost all functional microRNAs

using a defined set of transcription factors. Transduction of reprogramming factors using either lentiviral or piggyBac transposon vector into two, independently derived lines of Dicer-null MEFs failed to produce cells resembling embryonic stem cells (ESCs). However, expression of human Dicer in the Dicer-null MEFs restored their reprogramming potential. Our study demonstrates for the first time that microRNAs are indispensable for dedifferentiation reprogramming.

Kim, D. W., et al. (2006). "Stromal cell-derived inducing activity, Nurr1, and signaling molecules synergistically induce dopaminergic neurons from mouse embryonic stem cells." Stem Cells **24**(3): 557-567.

To induce differentiation of embryonic stem cells (ESCs) into specialized cell types for therapeutic purposes, it may be desirable to combine genetic manipulation and appropriate differentiation signals. We studied the induction of dopaminergic (DA) neurons from mouse ESCs by overexpressing the transcription factor Nurr1 and coculturing with PA6 stromal cells. Nurr1-expressing ESCs (N2 and N5) differentiated into a higher number of neurons (approximately twofold) than the naive ESCs (D3). In addition, N2/N5-derived cells contained a significantly higher proportion (>50%) of tyrosine hydroxylase (TH)+ neurons than D3 (<30%) and an even greater proportion of TH+ neurons (approximately 90%) when treated with the signaling molecules sonic hedgehog, fibroblast growth factor 8, and ascorbic acid. N2/N5-derived cells express much higher levels of DA markers (e.g., TH, dopamine transporter, aromatic amino acid decarboxylase, and G protein-regulated inwardly rectifying K+ channel 2) and produce and release a higher level of dopamine, compared with D3-derived cells. Furthermore, the majority of generated neurons exhibited electrophysiological properties characteristic of midbrain DA neurons. Finally, transplantation experiments showed efficient in vivo integration/generation of TH+ neurons after implantation into mouse striatum. Taken together, our results show that the combination of genetic manipulation(s) and in vitro cell differentiation conditions offers a reliable and effective induction of DA neurons from ESCs and may pave the way for future cell transplantation therapy in Parkinson's disease.

Kim, E. M., et al. (2014). "Embryonic stem cell-derived haematopoietic progenitor cells down-regulate the CD3 xi chain on T cells, abrogating alloreactive T cells." Immunology **142**(3): 421-430.

Murine embryonic stem (ES) cell-derived haematopoietic progenitor cells (HPCs) engraft and populate lymphoid organs. In vivo, HPCs engraft across MHC barriers protecting donor-type allografts from rejection. However, the underlying phenomenon remains elusive. Here, we sought to determine the mechanism by which ES cell-derived HPCs regulate alloreactive T cells. We used the 2C mouse, which expresses a transgenic T-cell receptor against H2-L(d) to determine whether HPCs are deleted by cytotoxic T lymphocytes (CTLs). Previously, we reported that HPCs express MHC class I antigens poorly and do not express class II antigens. In vitro stimulated 2C CTLs failed to lyse H2-L(d) HPCs in a standard 4-hr (51) chromium release assay. Similarly, when the HPCs were tested in an ELISPOT assay measuring the release of interferon-gamma by CTLs, HPCs failed to induce CTL degranulation. In addition, mice that were injected with HPCs showed a marked decrease in T-cell responses to alloantigen and CD3 stimulation, but showed a normal response to PMA/ionomycin, suggesting that HPCs impaired T-cell signalling through the T-cell receptor/CD3 complex. Here, we show that HPCs secrete arginase, an enzyme that scavenges l-arginine, leading to metabolites that down-regulate CD3 zeta chain. Indeed an arginase inhibitor partially restored expression of the CD3 zeta chain, implicating arginase 1 in the down-regulation of T cells. This previously unrecognized property of ES cell-derived HPCs could positively enhance the engraftment of ES cell-derived HPCs across MHC barriers by preventing rejection.

Kim, E. M., et al. (2012). "Embryonic stem cell-derived T cells induce lethal graft-versus-host disease and reject allogenic skin grafts upon thymic selection." Am J Transplant **12**(3): 600-609.

Efficient differentiation of embryonic stem cells (ESC) into hematopoietic progenitor cells (HPCs) is crucial for the establishment of stem cell-based therapies targeting the treatment of immunological and hematological disorders. However, so far, it has not been possible to induce long-term survival of murine ESC-derived HPCs without the overexpression of HoxB4, a homeobox transcription factor that confers self-renewal properties to hematopoietic cells. Yet it has not been feasible to generate T cells from HoxB4-expressing HPCs, a problem that has been attributed to HoxB4. Here, we show that Notch1 signaling in HoxB4-transduced ESCs leads to efficient derivation of T cells that survive long term. These T cells display a normal T-cell Vbeta repertoire, respond to mitogen stimulation and induce lethal graft-versus-host disease. Thymic selection in fetal thymic organ cultures (FTOCs) allowed negative selection and generation of T cells tolerant to 'self' and capable of rejecting MHC-mismatched skin allografts. Our data show that ESC-

derived T cells, despite high expression of HoxB4, are fully immunocompetent.

Kim, G. D., et al. (2012). "Honokiol inhibits vascular vessel formation of mouse embryonic stem cell-derived endothelial cells via the suppression of PECAM and MAPK/mTOR signaling pathway." Cell Physiol Biochem **30**(3): 758-770.

Embryonic stem cells, which are characterized by pluripotency and self-renewal, have recently been highlighted in drug discovery. In particular, the potential of ES cells to differentiate into specific-cell types make them an extremely useful tool in the evaluation of the biological activity of test compounds. Honokiol, a major neolignan derived from the bark of Magnolia obovata, has been shown an anti-tumor activity. However, the precise mechanism of action in the anti-tumor activity of honokiol is still poorly understood. Here, we evaluated the antiangiogenic activity of honokiol using mouse ES cell-derived embryoid bodies. mES-derived EBs were formed using hanging drop cultures and vascular formation was induced on gelatincoated plates in EGM-2 medium. The growth inhibition of honokiol was found to be more sensitive in the differentiated EB-derived endothelial cells compared to the undifferentiated EB-derived cells. Honokiol also inhibited the vascular formation of mES cells on 3-D collagen gel and decreased the expression of endothelial biomarkers VEGFR2 and PECAM in the differentiated EB-derived endothelial cells. In addition, honokiol suppressed the MAPK and mTOR signaling pathways in the EB-derived endothelial cells. Therefore, the anti-angiogenic activity of honokiol is associated in part with the suppression of PECAM and MAPK/mTOR pathways in EB-derived endothelial cells.

Kim, G. D., et al. (2009). "Cytotoxicity of 5-fluorouracil: Effect on endothelial differentiation via cell cycle inhibition in mouse embryonic stem cells." Toxicol In Vitro **23**(4): 719-727.

Embryonic stem cells (ESCs) are known to characteristics for pluripotency and self-renewal, but the precise mechanisms of ES-derived cells to specific toxicants have not been determined. Here, we evaluated the cytotoxicity of 5-fluorouracil (5-FU) and see its effect on cell viability, proliferation, and differentiation in mouse ESC-derived endothelial differentiation. Mouse ESCs were exposed to 5-FU (10 microM) and combined with probucol (50 microM) for 24h, which is an antagonist of 5-FU. Changes in gene expression as a result of 5-FU exposure in mouse ESC-derived endothelial precursor cells (ES-EPCs) were assessed using an oligonucleotide microarray (AB1700). The expression of Oct-4 was decreased during the differentiation of mouse ESCs into endothelial cells; otherwise, the expression of PECAM was increased. Mouse ES-EPCs were shown to have a decrease in viability (49.8%) and PECAM expression, and induce G1/S phase (31.1%/60.6%) when compared with/without treatment of 5-FU. Expression of cell cycle-related proteins was increased in endothelial precursor cells exposed to 5-FU without probucol treatment. From theses results suggest that 5-FU inhibit endothelial differentiation as well as inducing the G1/S phase arrest. We propose that mouse ES-EPCs might be a useful tool for screening the cytotoxicity of compounds in endothelial cells.

Koch, K. S., et al. (2006). "Immune-privileged embryonic Swiss mouse STO and STO cell-derived progenitor cells: major histocompatibility complex and cell differentiation antigen expression patterns resemble those of human embryonic stem cell lines." Immunology **119**(1): 98-115.

Embryonic mouse STO (S, SIM; T, 6-thioguanine resistant; O, ouabain resistant) and 3(8)21-enhanced green fluorescent protein (EGFP) cell lines exhibit long-term survival and hepatic progenitor cell behaviour after xenogeneic engraftment in non-immunosuppressed inbred rats, and were previously designated major histocompatibility complex (MHC) class I- and class II-negative lines. To determine the molecular basis for undetectable MHC determinants, the expression and haplotype of H-2K, H-2D, H-2L and I-A proteins were reassessed by reverse transcriptase-polymerase chain reaction (RT-PCR), cDNA sequencing, RNA hybridization, immunoblotting, quantitative RT-PCR (QPCR), immunocytochemistry and flow cytometry. To detect cell differentiation (CD) surface antigens characteristic of stem cells, apoptotic regulation or adaptive immunity that might facilitate progenitor cell status or immune privilege, flow cytometry was also used to screen untreated and cytokine [interferon (IFN)-gamma]-treated cultures. Despite prior PCR genotyping analyses suggestive of H-2q haplotypes in STO, 3(8)21-EGFP and parental 3(8)21 cells, all three lines expressed H-2K cDNA sequences identical to those of d-haplotype BALB/c mice, as well as constitutive and cytokine-inducible H-2K(d) determinants. In contrast, apart from H-2L(d[LOW]) display in 3(8)21 cells, H-2Dd, H-2Ld and I-Ad determinants were undetectable. All three lines expressed constitutive and cytokine-inducible CD34; however, except for inducible CD117([LOW]) expression in 3(8)21 cells, no expression of CD45, CD117, CD62L, CD80, CD86, CD90.1 or CD95L/CD178 was observed. Constitutive and cytokine-inducible CD95([LOW]) expression was detected in STO and 3(8)21 cells, but not in 3(8)21-EGFP cells. MHC (class I(+[LOW])/class II-) and CD

(CD34+/CD80-/CD86-/CD95L-) expression patterns in STO and STO cell-derived progenitor cells resemble patterns reported for human embryonic stem cell lines. Whether these patterns reflect associations with mechanisms that are regulatory of immune privilege or functional tissue-specific plasticity is unknown.

Kodama, M., et al. (2008). "Pancreatic endocrine and exocrine cell ontogeny from renal capsule transplanted embryonic stem cells in streptozocin-injured mice." J Histochem Cytochem **56**(1): 33-44.

In this study, we describe pancreatic cell ontogeny in renal capsule-transplanted embryonic stem cells (ES) after injury by streptozocin (STZ), showing pancreatogenesis in situ. Seven-week-old female BALB/c nude mice were treated with either a single 175- or 200-mg/kg STZ dose, a regimen that induces substantial beta-cell damage without overt hyperglycemia, and transplanted 24 hr later with 1 x 10(5) ES. Immunohistochemistry was performed on ES tissue at 15, 21, and 28 days after transplantation using antibodies against stage- and lineage-specific pancreatic markers. After 21 days, PDX-1+ pancreatic foci first appeared in the renal capsule and expressed both amylase and endocrine hormones (insulin, glucagon, and somatostatin). These foci increased in size by day 28 because of acinar and duct cell proliferation, whereas endocrine cells remained non-dividing, and made up 2-4% of ES tumor volume. PDX-1, Nkx6.1, Ngn3, and ISL-1 protein localization patterns in pancreatic foci were comparable with embryonic pancreatogenesis. A prevalence of multihormonal endocrine cells, a characteristic of adult beta-cell regeneration, indicated a possible divergence from embryonic islet cell development. The results indicate that beta-cell damage, without overt hyperglycemia, induces a process of fetal-like pancreatogenesis in renal capsule-transplanted ES, leading to beta-cell neogenesis.

Koel, M., et al. (2017). "Optimizing bone morphogenic protein 4-mediated human embryonic stem cell differentiation into trophoblast-like cells using fibroblast growth factor 2 and transforming growth factor-beta/activin/nodal signalling inhibition." Reprod Biomed Online **35**(3): 253-263.

Several studies have demonstrated that human embryonic stem cells (hESC) can be differentiated into trophoblast-like cells if exposed to bone morphogenic protein 4 (BMP4) and/or inhibitors of fibroblast growth factor 2 (FGF2) and the transforming growth factor beta (TGF-beta)/activin/nodal signalling pathways. The goal of this study was to investigate how the inhibitors of these pathways improve the efficiency of hESC differentiation when compared with basic BMP4 treatment. RNA sequencing was used to analyse the effects of all possible inhibitor combinations on the differentiation of hESC into trophoblast-like cells over 12 days. Genes differentially expressed compared with untreated cells were identified at seven time points. Additionally, expression of total human chorionic gonadotrophin (HCG) and its hyperglycosylated form (HCG-H) were determined by immunoassay from cell culture media. We showed that FGF2 inhibition with BMP4 activation up-regulates syncytiotrophoblast-specific genes (CGA, CGB and LGALS16), induces several molecular pathways involved in embryo implantation and triggers HCG-H production. In contrast, inhibition of the TGF-beta/activin/nodal pathway decreases the ability of hESC to form trophoblast-like cells. Information about the conditions needed for hESC differentiation toward trophoblast-like cells helps us to find an optimal model for studying the early development of human trophoblasts in normal and in complicated pregnancy.

Kofidis, T., et al. (2005). "They are not stealthy in the heart: embryonic stem cells trigger cell infiltration, humoral and T-lymphocyte-based host immune response." Eur J Cardiothorac Surg **28**(3): 461-466.

OBJECTIVE: The in vivo immunogenicity of Embryonic Stem Cells is controversial. At present, there is only in vitro evidence of MHC I expression by this cell population but vivid speculation about their immune-privileged state. The immunology aspect of ESC transplantation deserves thorough investigation. METHODS: We injected mouse ESC (expressing Green Fluorescent Protein, GFP) into injured myocardium of syngeneic, allogeneic and SCID recipients. Furthermore, we monitored host response for up to 4 weeks post cell transfer. We determined local response (CD 3, CD 11c expression by host cells), MHC I expression by donor cells, MHC-II expression within and around the graft, humoral response of allogeneic hosts using Flow Cytometry and evaluated the hosts' cytokine response using stimulated spleenocytes by means of ELISPOT. Cell survival was estimated by morphometry, by calculating the area of the GFP+ graft over the area of infarction at multiple sections of the harvested heart. RESULTS: There was significant cellular infiltration into and around the graft consisting of T-lymphocytes (CD3+) and dendritic cells (CD 11c). Infiltration was detectable at 1 week and progressed through 4 weeks following cell transplantation. The humoral Ab response was moderate at 2 weeks but frank at 4 weeks. ELISPOT demonstrated a Th1 pathway of donor specific T-lymphocyte response with strong IFN-gamma and Il-2 production (figure A). MHC I expression was significant within the graft and maximal in the allogeneic groups. CONCLUSIONS: An immune response against transplanted ESC was demonstrated

and the future use of ESC will likely require the use of systemic immunosuppression.

Koh, K. P., et al. (2011). "Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells." Cell Stem Cell **8**(2): 200-213.

TET family enzymes convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in DNA. Here, we show that Tet1 and Tet2 are Oct4-regulated enzymes that together sustain 5hmC in mouse embryonic stem cells (ESCs) and are induced concomitantly with 5hmC during reprogramming of fibroblasts to induced pluripotent stem cells. ESCs depleted of Tet1 by RNAi show diminished expression of the Nodal antagonist Lefty1 and display hyperactive Nodal signaling and skewed differentiation into the endoderm-mesoderm lineage in embryoid bodies in vitro. In Fgf4- and heparin-supplemented culture conditions, Tet1-depleted ESCs activate the trophoblast stem cell lineage determinant Elf5 and can colonize the placenta in midgestation embryo chimeras. Consistent with these findings, Tet1-depleted ESCs form aggressive hemorrhagic teratomas with increased endoderm, reduced neuroectoderm, and ectopic appearance of trophoblastic giant cells. Thus, 5hmC is an epigenetic modification associated with the pluripotent state, and Tet1 functions to regulate the lineage differentiation potential of ESCs.

Koifman, G., et al. (2018). "A mutant p53-dependent embryonic stem cell gene signature is associated with augmented tumorigenesis of stem cells." Cancer Res.

Mutations in the tumor suppressor p53 are the most frequent alterations in human cancer. These mutations include p53-inactivating mutations as well as oncogenic gain-of-function (GOF) mutations that endow p53 with capabilities to promote tumor progression. A primary challenge in cancer therapy is targeting stemness features and cancer stem cells (CSC) that account for tumor initiation, metastasis, and cancer relapse. Here we show that in vitro cultivation of tumors derived from mutant p53 murine bone marrow (BM) mesenchymal stem cells (MSC) gives rise to aggressive tumor lines (TL). These MSC-TL exhibited CSC features as displayed by their augmented oncogenicity and high expression of CSC markers. Comparative analyses between MSC-TL with their parental mutant p53 MSC allowed for identification of the molecular events underlying their tumorigenic properties, including an embryonic stem cell (ESC) gene signature specifically expressed in MSC-TL. Knockout of mutant p53 led to a reduction in tumor development and tumorigenic cell frequency, which was accompanied by reduced expression of CSC markers and the ESC MSC-TL signature. In human cancer, MSC-TL ESC signature-derived genes correlated with poor patient survival and were highly expressed in human tumors harboring p53 hotspot mutations. These data indicate that the ESC gene signature-derived genes may serve as new stemness-based prognostic biomarkers as well as novel cancer therapeutic targets.

Kokudo, T., et al. (2008). "Snail is required for TGFbeta-induced endothelial-mesenchymal transition of embryonic stem cell-derived endothelial cells." J Cell Sci **121**(Pt 20): 3317-3324.

Epithelial-mesenchymal transition (EMT) plays important roles in various physiological and pathological processes, and is regulated by signaling pathways mediated by cytokines, including transforming growth factor beta (TGFbeta). Embryonic endothelial cells also undergo differentiation into mesenchymal cells during heart valve formation and aortic maturation. However, the molecular mechanisms that regulate such endothelial-mesenchymal transition (EndMT) remain to be elucidated. Here we show that TGFbeta plays important roles during mural differentiation of mouse embryonic stem cell-derived endothelial cells (MESECs). TGFbeta2 induced the differentiation of MESECs into mural cells, with a decrease in the expression of the endothelial marker claudin 5, and an increase in expression of the mural markers smooth muscle alpha-actin, SM22alpha and calponin, whereas a TGFbeta type I receptor kinase inhibitor inhibited EndMT. Among the transcription factors involved in EMT, Snail was induced by TGFbeta2 in MESECs. Tetracycline-regulated expression of Snail induced the differentiation of MESECs into mural cells, whereas knockdown of Snail expression abrogated TGFbeta2-induced mural differentiation of MESECs. These results indicate that Snail mediates the actions of endogenous TGFbeta signals that induce EndMT.

Koledova, Z., et al. (2010). "Cell-cycle regulation in embryonic stem cells: centrosomal decisions on self-renewal." Stem Cells Dev **19**(11): 1663-1678.

Embryonic stem cells seem to have the intriguing capacity to divide indefinitely while retaining their pluripotency. This self-renewal is accomplished by specialized mechanisms of cell-cycle control. In the last few years, several studies have provided evidence for a direct link between cell-cycle regulation and cell-fate decisions in stem cells. In this review, we discuss the peculiarities of embryonic stem cell-cycle control mechanisms, implicate their involvement in cell-fate decisions, and distinguish centrosomes as important players in the self-renewal versus differentiation roulette.

Koltsova, A. M., et al. (2015). "[Characteristics of New Mesenchymal Stem Cell Line Derived from Human Embryonic Stem Cells]." Tsitologiia **57**(11): 761-770.

New nonimmortalized fibroblast-like cell line SC6-MSC has been obtained from a line of human embryonic stem cells (ESC)--SC6. Numerical and structural karyotypic analysis has shown hypodiploidy karyotypic: 45, X0 in this line. The average cell population doublings time, for SC6-MSC is 26.0 +/- 0.4 h at the 8th passage and 82.0 +/- 9.2 h at the 18th passage. The growth curves showed active proliferation for 8-10 passages with a consequent gradual decrease of proliferative activity, which ended to 20th passage. To determine the line's status, the analysis of the surface markers by flow cytometry was carried out. We have revealed the expression of surface antigens CD44, CD73, CD90, CD105 and HLA-ABC characteristic for human MSC, and the absence of CD34 and HLA-DR expression. However, the level of expression of surface markers CD90 and CD105 was significantly lower in comparison with other MSC lines including the line SC5-MSC derived from the line human ESC-SC5. Immunofluorescence analysis of the expression of the surface markers and transcription factor Oct-4 characteristic for human embryonic stem cells showed the absence of Oct-4 expression and the presence of SSEA-4 and TRA-1-60 expression, which is characteristic for a number of MSC lines with normal karyotype. Immunofluorescence analysis has shown the presence of the markers of early differentiation in the derivates of three germ layers, characteristic for human ESC, which in corresponding microenvironments may allow MSC to be useful for reparation of tissue injures. The directed osteogenic and chondrogenic differentiation of line SC6-MSC has shown. However, no directed adipogenic differentiation of this line has been found. The obtained results with high probability may indicate what alteration of chromosomal and, accordingly, gene balance, in line SC6-MSC with karyotype 45, X0 resulted in decrease in differential potential, in expression CD90, associated in particular with the processes of differentiation and aging of cells.

Konig, N., et al. (2017). "Murine neural crest stem cells and embryonic stem cell-derived neuron precursors survive and differentiate after transplantation in a model of dorsal root avulsion." J Tissue Eng Regen Med **11**(1): 129-137.

Spinal root avulsion results in paralysis and sensory loss, and is commonly associated with chronic pain. In addition to the failure of avulsed dorsal root axons to regenerate into the spinal cord, avulsion injury leads to extensive neuroinflammation and degeneration of second-order neurons in the dorsal horn. The ultimate objective in the treatment of this condition is to counteract degeneration of spinal cord neurons and to achieve functionally useful regeneration/reconnection of sensory neurons with spinal cord neurons. Here we compare survival and migration of murine boundary cap neural crest stem cells (bNCSCs) and embryonic stem cells (ESCs)-derived, predifferentiated neuron precursors after their implantation acutely at the junction between avulsed dorsal roots L3-L6 and the spinal cord. Both types of cells survived transplantation, but showed distinctly different modes of migration. Thus, bNCSCs migrated into the spinal cord, expressed glial markers and formed elongated tubes in the peripheral nervous system (PNS) compartment of the avulsed dorsal root transitional zone (DRTZ) area. In contrast, the ESC transplants remained at the site of implantation and differentiated to motor neurons and interneurons. These data show that both stem cell types successfully survived implantation to the acutely injured spinal cord and maintained their differentiation and migration potential. These data suggest that, depending on the source of neural stem cells, they can play different beneficial roles for recovery after dorsal root avulsion.

Konorov, S. O., et al. (2013). "Label-free determination of the cell cycle phase in human embryonic stem cells by Raman microspectroscopy." Anal Chem **85**(19): 8996-9002.

The cell cycle is a series of integrated and coordinated physiological events that results in cell growth and replication. Besides observing the event of cell division it is not feasible to determine the cell cycle phase without fatal and/or perturbing invasive procedures such as cell staining, fixing, and/or dissociation. Raman microspectroscopy (RMS) is a chemical imaging technique that exploits molecular vibrations as a contrast mechanism; it can be applied to single living cells noninvasively to allow unperturbed analysis over time. We used RMS to determine the cell cycle phase based on integrating the composite 783 cm(-1) nucleic acid band intensities across individual cell nuclei. After correcting for RNA contributions using the RNA 811 cm(-1) band, the measured intensities essentially reflected DNA content. When quantifying Raman images from single cells in a population of methanol-fixed human embryonic stem cells, the histogram of corrected 783 cm(-1) band intensities exhibited a profile analogous to that obtained using flow-cytometry with nuclear stains. The two population peaks in the histogram occur at Raman intensities corresponding to a 1-fold and 2-fold diploid DNA complement per cell, consistent with a distribution of cells with a population peak due to cells at the end of G1 phase (1-fold) and a peak due to cells entering M phase (2-fold). When treated with EdU to label the replicating DNA and block cell division, cells

with higher EdU-related fluorescence generally had higher integrated Raman intensities. This provides proof-of-principle of an analytical method for label-free RMS determination in situ of cell cycle phase in adherent monolayers or even single adherent cells.

Kurtovic, S., et al. (2015). "Leptin enhances endothelial cell differentiation and angiogenesis in murine embryonic stem cells." Microvasc Res **97**: 65-74.

The metabolic regulation of leptin and its angiogenic effects have been well characterized in adult mammals. However, the role of leptin in the differentiation of embryonic stem cells (ESCs) to endothelial cells (ECs) has not been characterized. We hypothesized that leptin enhances the generation of ECs derived from ESCs and, in this way, promotes angiogenesis in embryonic vessels. To address this hypothesis, we utilized an in vitro model consisting of murine ESCs-derived embryoid bodies (EBs). Vascular density, EC and angiogenesis markers as well as phosphorylation levels of signal transducer and activator of transcription 3 (pSTAT3) were investigated in leptin-treated EBs and in untreated EBs as controls. ESC-derived ECs were isolated by magnetic sorting based on the expression of platelet endothelial cell adhesion molecule (PECAM-1/CD31). Significant upregulation of EC and angiogenic markers as well as higher vessel density were found in leptin-treated EBs compared to controls. CD31 positive enriched cells derived from leptin-treated EBs had improved proliferation and survival rate and showed higher levels of pSTAT3. These results suggested that leptin promotes EC differentiation and angiogenesis in mouse EBs and that janus tyrosine kinase (JAK)/STAT pathway can play a role in this biological process. Leptin-mediated EC differentiation and angiogenesis in ESCs can be a useful application towards regenerative medicine and tissue engineering.

Lau, Y. T., et al. (2011). "Effects of hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blockers on the proliferation and cell cycle progression of embryonic stem cells." Pflugers Arch **461**(1): 191-202.

Embryonic stem cells (ESCs) can uniquely proliferate indefinitely and differentiate into all cell lineages. ESCs may therefore provide an unlimited supply of cells for cell-based therapies. Previous study reported the presence of hyperpolarization-activated inward currents in undifferentiated mouse (m) ESCs, but the functional role of this hyperpolarization-activated current in mESCs is unknown. In this study, the role of this current in maintaining the proliferative capacity and the cell cycle progression of ESCs was investigated. In D3 mESCs, this hyperpolarization-activated inward current can be blocked by HCN channel blocker ZD7288. Application of the HCN channel blockers, cesium (1-10 mM) or ZD7288 (0.1-30 muM), attenuated cell proliferation in a concentration-dependent manner. Both HCN blockers were found to be non-cytotoxic to mESCs as determined by cell viability test. Interestingly, ZD7288 at 10 and 30 muM was found to decrease the proportion of cells in G(0)/G(1) phase and increase the proportion of cells in S phase. This suggests that this hyperpolarization-activated current can affect the cell cycle progression in mESCs. In summary, the present investigation suggests that ESC proliferation and cell cycle progression can be regulated by this hyperpolarization-activated current.

Laundos, T. L., et al. (2017). "Rotary orbital suspension culture of embryonic stem cell-derived neural stem/progenitor cells: impact of hydrodynamic culture on aggregate yield, morphology and cell phenotype." J Tissue Eng Regen Med **11**(8): 2227-2240.

Embryonic stem (ES)-derived neural stem/progenitor cells (ES-NSPCs) constitute a promising cell source for application in cell therapies for the treatment of central nervous system disorders. In this study, a rotary orbital hydrodynamic culture system was applied to single-cell suspensions of ES-NSPCs, to obtain homogeneously-sized ES-NSPC cellular aggregates (neurospheres). Hydrodynamic culture allowed the formation of ES-NSPC neurospheres with a narrower size distribution than statically cultured neurospheres, increasing orbital speeds leading to smaller-sized neurospheres and higher neurosphere yield. Neurospheres formed under hydrodynamic conditions (72 h at 55 rpm) showed higher cell compaction and comparable percentages of viable, dead, apoptotic and proliferative cells. Further characterization of cellular aggregates provided new insights into the effect of hydrodynamic shear on ES-NSPC behaviour. Rotary neurospheres exhibited reduced protein levels of N-cadherin and beta-catenin, and higher deposition of laminin (without impacting fibronectin deposition), matrix metalloproteinase-2 (MMP-2) activity and percentage of neuronal cells. In line with the increased MMP-2 activity levels found, hydrodynamically-cultured neurospheres showed higher outward migration on laminin. Moreover, when cultured in a 3D fibrin hydrogel, rotary neurospheres generated an increased percentage of neuronal cells. In conclusion, the application of a constant orbital speed to single-cell suspensions of ES-NSPCs, besides allowing the formation of homogeneously-sized neurospheres, promoted ES-NSPC differentiation and outward migration, possibly by influencing the expression of cell-cell adhesion molecules and the

secretion of proteases/extracellular matrix proteins. These findings are important when establishing the culture conditions needed to obtain uniformly-sized ES-NSPC aggregates, either for use in regenerative therapies or in in vitro platforms for biomaterial development or pharmacological screening. Copyright (c) 2016 John Wiley & Sons, Ltd.

Lavial, F., et al. (2009). "Ectopic expression of Cvh (Chicken Vasa homologue) mediates the reprogramming of chicken embryonic stem cells to a germ cell fate." Dev Biol **330**(1): 73-82.

When they are derived from blastodermal cells of the pre-primitive streak in vitro, the pluripotency of Chicken Embryonic Stem Cells (cESC) can be controlled by the cPouV and Nanog genes. These cESC can differentiate into derivatives of the three germ layers both in vitro and in vivo, but they only weakly colonize the gonads of host embryos. By contrast, non-cultured blastodermal cells and long-term cultured chicken primordial germ cells maintain full germline competence. This restriction in the germline potential of the cESC may result from either early germline determination in the donor embryos or it may occur as a result of in vitro culture. We are interested in understanding the genetic determinants of germline programming. The RNA binding protein Cvh (Chicken Vasa Homologue) is considered as one such determinant, although its role in germ cell physiology is still unclear. Here we show that the exogenous expression of Cvh, combined with appropriate culture conditions, induces cESC reprogramming towards a germ cell fate. Indeed, these cells express the Dazl, Tudor and Sycp3 germline markers, and they display improved germline colonization and adopt a germ cell fate when injected into recipient embryos. Thus, our results demonstrate that Vasa can drive ES cell differentiation towards the germ cell lineage, both in vitro and in vivo.

Lavial, F. and B. Pain (2010). "Chicken embryonic stem cells as a non-mammalian embryonic stem cell model." Dev Growth Differ **52**(1): 101-114.

Embryonic stem cells (ESCs) were isolated in the early 1980s from mouse and in the late 1990s from primate and human. These cells present the unique property of self-renewal and the ability to generate differentiated progeny in all embryonic lineages both in vitro and in vivo. The mESCs (mouse embryonic stem cells) can contribute to both somatic and germinal lineages once re-injected into a recipient embryo at the blastocyst stage. In avian species, chicken embryonic stem cells (cESCs) have been isolated from the in vitro culture of early chicken blastodermal cells (cBCs) taken from stage X embryo (EG&K) These cESCs can be maintained under specific culture conditions and have been characterized on the basis of their morphology, biochemical features, in vitro differentiation potentialities and in vivo morphogenetic properties. The relationship between these cESCs and some of the chicken germ cells identified and grown under specific culture conditions are still under debate, in particular with the identification of the Cvh gene as a key factor for germ cell determination. Moreover, by cloning the avian homologue of the Oct4 mammalian gene, we have demonstrated that this gene, as well as the chicken Nanog gene, was involved in the characterization and maintenance of the chicken pluripotency. These first steps toward the understanding of pluripotency control in a non-mammalian species opens the way for the development and characterization of putative new cell types such as chicken EpiSC and raises the question of the existence of reprogramming in avian species. These different points are discussed.

Le Coz, F., et al. (2015). "Hand1-Luc embryonic stem cell test (Hand1-Luc EST): a novel rapid and highly reproducible in vitro test for embryotoxicity by measuring cytotoxicity and differentiation toxicity using engineered mouse ES cells." J Toxicol Sci **40**(2): 251-261.

The embryonic stem cell test (EST) is a promising alternative method for evaluating embryotoxicity of test chemicals by measuring cytotoxicity and differentiation toxicity using mouse ES cells. Differentiation toxicity is analyzed by microscopically counting the beating of embryonic bodies after 10 days of culture. However, improvements are necessary to reduce the laborious manipulations involved and the time required to obtain results. We have previously reported the successful stable transfection of ES cells (ES-D3) with the heart and neural crest derivatives expressed transcript 1 (Hand1) gene and the establishment of a 96-well multi-plate-based new EST with luciferase reporter assay 6 days after treatment with test chemicals. Now, we propose an even more rapid and easier EST, named Hand1-Luc EST. We established another cell line to monitor the Hand1 gene expression via a luciferase reporter gene. By mRNA analysis and luciferase assay, we examined in detail the luciferase activity during cell differentiation, which allowed us to reduce the time of measurement from day 6 to day 5 (120 hr). Furthermore, the protocol was improved, with, among others, the measurement of cytotoxicity and differentiation toxicity taking place in the same 96-well round bottom plate instead of two different plates. With the positive control, 5-fluorouracil (5-FU), and 9 test chemicals, data with high reproducibility and very low variation (CV < 50%) in the relevant endpoints were obtained. This study shows that the Hand1-Luc EST

could provide an accurate and sensitive short-term test for prediction of embryotoxicants by measuring cytotoxicity and differentiation toxicity from the same sample.

Leavitt, A. D. and I. Hamlett (2011). "Homologous recombination in human embryonic stem cells: a tool for advancing cell therapy and understanding and treating human disease." Clin Transl Sci **4**(4): 298-305.

Human embryonic stem cells (hESCs) hold great promise for ushering in an era of novel cell therapies to treat a wide range of rare and common diseases, yet they also provide an unprecedented opportunity for basic research to yield clinical benefit. HESCs can be used to better understand human development, to model human diseases, to understand the contribution of specific mutations to the pathogenesis of disease, and to develop human cell-based screening systems to identify novel therapeutic agents and evaluate potential toxicity of therapeutic agents under development. Such basic research will benefit greatly from efficient methods to perform targeted gene modification, an area of hESC investigation that is currently in its infancy. Moreover, the reality of hESC-based cellular therapies will require improved methods for generating the specific cells of interest, and reporter cell lines generated through targeted gene modifications are expected to play an important role in developing optimal cell-specific differentiation protocols. Herein, we review the current status of homologous recombination in hESCs, a gene targeting technique that is sure to continue to improve, and to play an important role in realizing the maximal human benefit from hESCs.

Lee, A. S., et al. (2009). "Effects of cell number on teratoma formation by human embryonic stem cells." Cell Cycle **8**(16): 2608-2612.

Teratoma formation is a critical obstacle to safe clinical translation of human embryonic stem (ES) cell-based therapies in the future. As current methods of isolation are unable to yield 100% pure population of differentiated cells from a pluripotent donor source, potential development of these tumors is a significant concern. Here we used non-invasive reporter gene imaging to investigate the relationship between human ES cell number and teratoma formation in a xenogenic model of ES cell transplantation. Human ES cells (H9 line) were stably transduced with a double fusion (DF) reporter construct containing firefly luciferase and enhanced green fluorescent protein (Fluc-eGFP) driven by a human ubiquitin promoter. Immunodeficient mice received intramyocardial (n = 35) or skeletal muscle (n = 35) injection of 1 x 10(2), 1 x 10(3), 1 x 10(4), 1 x 10(5) or 1 x 10(6) DF positive ES cells suspended in saline for myocardium and Matrigel for skeletal muscle. Cell survival and proliferation were monitored via bioluminescence imaging (BLI) for an 8 week period following transplantation. Mice negative for Fluc signal after 8 weeks were followed out to day 365 to confirm tumor absence. Significantly, in this study, a minimum of 1 x 10(5) ES cells in the myocardium and 1 x 10(4) cells in the skeletal muscle was observed to be requisite for teratoma development, suggesting that human ES cell number may be a critical factor in teratoma formation. Engraftment and tumor occurrence were also observed to be highly dependent on ES cell number. We anticipate these results should yield useful insights to the safe and reliable application of human ES cell derivatives in the clinic.

Lee, E. J., et al. (2012). "New culture system for human embryonic stem cells: autologous mesenchymal stem cell feeder without exogenous fibroblast growth factor 2." Differentiation **83**(1): 92-100.

Human embryonic stem (hES) cells have been successfully maintained using human-cell feeder systems or feeder-free systems. However, despite advances in culture techniques, hES cells require supplementation with fibroblast growth factor 2 (FGF-2), an exogenous stemness factor, which is needed to sustain the authentic undifferentiated status. We developed a new culture system for hES cells; this system does not require supplementation with FGF-2 to obtain hES cells that are suitable for tissue engineering and regenerative medicine. This culture system employed mesenchymal stem cells derived from hES cells (hESC-MSCs) as autologous human feeder cells in the absence of FGF-2. The hES cell line SNUhES3 cultured in this new autologous feeder culture system maintained the typical morphology of hES cells and expression of pluripotency-related proteins, SSEA-4, TRA-1-60, OCT4, and alkaline phosphatase, without development of abnormal karyotypes after more than 30 passages. RNA expression of the pluripotency-related genes OCT4 and NANOG was similar to the expression in SNUhES3 cells maintained on xenofeeder STO cells. To identify the mechanism that enables the cells to be maintained without exogenous FGF-2, we checked the secretion of FGF-2 from the mitomycin-C treated autofeeder hESC-MSCs versus xenofeeder STO cells, and confirmed that hESC-MSCs secreted FGF-2 whereas STO cells did not. The level of FGF-2 in the media from the autofeeder system without exogenous FGF-2 was comparable to that from the xenofeeder system with addition of FGF-2. In conclusion, our new culture system for hES cells, which employs a feeder layer of autologous hESC-MSCs, supplies sufficient amounts of secreted FGF-2 to eliminate the requirement for exogenous FGF-2.

Levy, Y. S., et al. (2004). "Embryonic and adult stem cells as a source for cell therapy in Parkinson's disease." J Mol Neurosci **24**(3): 353-386.

The rationale behind the use of cells as therapeutic modalities for neurodegenerative diseases in general, and in Parkinson's disease (PD) in particular, is that they will improve patient's functioning by replacing the damaged cell population. It is reasoned that these cells will survive, grow neurites, establish functional synapses, integrate best and durably with the host tissue mainly in the striatum, renew the impaired wiring, and lead to meaningful clinical improvement. To increase the generation of dopamine, researchers have already transplanted non-neuronal cells, without any genetic manipulation or after introduction of genes such as tyrosine hydroxylase, in animal models of PD. Because these cells were not of neuronal origin, they developed without control, did not integrate well into the brain parenchyma, and their survival rates were low. Clinical experiments using cell transplantation as a therapy for PD have been conducted since the 1980s. Most of these experiments used fetal dopaminergic cells originating in the ventral mesencephalic tissue obtained from fetuses. Although it was shown that the transplanted cells survived and some patients benefited from this treatment, others suffered from severe dyskinesia, probably caused by the graft's excessive and uncontrolled production and release of dopamine. It is now recognized that cell-replacement strategy will be effective in PD only if the transplanted cells have the same abilities, such as dopamine synthesis and control release, reuptake, and metabolizing dopamine, as the original dopaminergic neurons. Recent studies on embryonic and adult stem cells have demonstrated that cells are able to both self-renew and produce differentiated tissues, including dopaminergic neurons. These new methods offer real hope for tissue replacement in a wide range of diseases, especially PD. In this review we summarize the evidence of dopaminergic neuron generation from embryonic and adult stem cells, and discuss their application for cell therapy in PD.

Leydon, C., et al. (2013). "Human embryonic stem cell-derived epithelial cells in a novel in vitro model of vocal mucosa." Tissue Eng Part A **19**(19-20): 2233-2241.

A satisfactory in vitro model of vocal fold mucosa does not exist, thus precluding a systematic, controlled study of vocal fold biology and biomechanics. We sought to create a valid, reproducible three-dimensional (3D) in vitro model of human origin of vocal fold mucosa of human origin. We hypothesized that coculture of human embryonic stem cell (hESC)-derived simple epithelial cells with primary vocal fold fibroblasts under appropriate conditions would elicit morphogenesis of progenitor cells into vocal fold epithelial-like cells and creation of a basement membrane. Using an in vitro prospective study design, hESCs were differentiated into cells that coexpressed the simple epithelial cell marker, keratin 18 (K18), and the transcription factor, p63. These simple epithelial cells were cocultured with primary vocal fold fibroblasts seeded in a collagen gel scaffold. The cells were cultured for 3 weeks in a keratinocyte medium at an air-liquid interface. After that time, the engineered mucosa demonstrated a stratified, squamous epithelium and a continuous basement membrane recapitulating the key morphologic and phenotypic characteristics of native vocal fold mucosa. hESC-derived epithelial cells exhibited positive staining for vocal fold stratified, squamous epithelial markers, keratin 13 (K13) and 14 (K14), as well as tight junctions, adherens junctions, gap junctions, and desmosomes. Despite the presence of components critical for epithelial structural integrity, the epithelium demonstrated greater permeability than native tissue indicating compromised functional integrity. While further work is warranted to improve functional barrier integrity, this study demonstrates that hESC-derived epithelial progenitor cells can be engineered to create a replicable 3D in vitro model of vocal fold mucosa featuring a multilayered, terminally differentiated epithelium.

Li, D., et al. (2013). "Cell-based screening of traditional Chinese medicines for proliferation enhancers of mouse embryonic stem cells." Biotechnol Prog **29**(3): 738-744.

A high-throughput cell-based method was developed for screening traditional Chinese herbal medicines (TCHMs) for potential stem cell growth promoters. Mouse embryonic stem (mES) cells expressing enhanced green fluorescent protein (EGFP) were cultured in growth media supplemented with various TCHM extracts. The dosage-dependent effects of TCHM extracts on cell growth, including proliferation and cytotoxicity, were assessed via EGFP fluorescence measurement. Seven TCHMs were investigated, and among them Panax notoginseng (PN), Rhizoma Atractylodis macrocephalae, Rhizoma chuanxiong, and Ganoderma lucidum spores (GLS) showed potential to improve mES cell proliferation. Eleven mixtures of these four TCHMs were then studied, and the results showed that the mixture of PN and GLS had the strongest growth promoting effect, increasing the specific growth rate of mES cells by 29.5% at a low dosage of 0.01% (wt/vol) PN/GLS ($P<0.01$) and 34.2% at 0.1% (wt/vol) PN/GLS ($P<0.05$) compared to the control. The growth promoting effect of PN/GLS was further confirmed with ES cells cultured in spinner flasks. A 29.3-fold increase in the

total cell number was achieved in the medium supplemented with 0.01% PN/GLS after 5 days, while the control culture only gave a 16.8-fold increase. This cell-based screening method thus can provide an efficient and high-throughput way to explore potential stem cell growth promoters from TCHMs.

Liew, C. G., et al. (2008). "PAX4 enhances beta-cell differentiation of human embryonic stem cells." PLoS One **3**(3): e1783.

BACKGROUND: Human embryonic stem cells (HESC) readily differentiate into an apparently haphazard array of cell types, corresponding to all three germ layers, when their culture conditions are altered, for example by growth in suspension as aggregates known as embryoid bodies (EBs). However, this diversity of differentiation means that the efficiency of producing any one particular cell type is inevitably low. Although pancreatic differentiation has been reported from HESC, practicable applications for the use of beta-cells derived from HESC to treat diabetes will only be possible once techniques are developed to promote efficient differentiation along the pancreatic lineages. METHODS AND FINDINGS: Here, we have tested whether the transcription factor, Pax4 can be used to drive the differentiation of HESC to a beta-cell fate in vitro. We constitutively over-expressed Pax4 in HESCs by stable transfection, and used Q-PCR analysis, immunocytochemistry, ELISA, Ca(2+) microfluorimetry and cell imaging to assess the role of Pax4 in the differentiation and intracellular Ca(2+) homeostasis of beta-cells developing in embryoid bodies produced from such HESC. Cells expressing key beta-cell markers were isolated by fluorescence-activated cell sorting after staining for high zinc content using the vital dye, Newport Green. CONCLUSION: Constitutive expression of Pax4 in HESC substantially enhances their propensity to form putative beta-cells. Our findings provide a novel foundation to study the mechanism of pancreatic beta-cells differentiation during early human development and to help evaluate strategies for the generation of purified beta-cells for future clinical applications.

Lifantseva, N., et al. (2011). "Expression patterns of cancer-testis antigens in human embryonic stem cells and their cell derivatives indicate lineage tracks." Stem Cells Int **2011**: 795239.

Pluripotent stem cells can differentiate into various lineages but undergo genetic and epigenetic changes during long-term cultivation and, therefore, require regular monitoring. The expression patterns of cancer-testis antigens (CTAs) MAGE-A2, -A3, -A4, -A6, -A8, -B2, and GAGE were examined in undifferentiated human embryonic stem (hES) cells, their differentiated derivatives, teratocarcinoma (hEC) cells, and cancer cell lines of neuroectodermal and mesodermal origin. Undifferentiated hES cells and embryoid body cells expressed MAGE-A3, -A6, -A4, -A8, and GAGEs while later differentiated derivatives expressed only MAGE-A8 or MAGE-A4. Likewise, mouse pluripotent stem cells also express CTAs of Magea but not Mageb family. Despite similarity of the hES and hEC cell expression patterns, MAGE-A2 and MAGE-B2 were detected only in hEC cells but not in hES cells. Moreover, our analysis has shown that CTAs are aberrantly expressed in cancer cell lines and display low tissue specificity. The identification of CTA expression patterns in pluripotent stem cells and their derivatives may be useful for isolation of abnormally CTA-expressing cells to improve the safety of stem-cell based therapy.

Lim, J. J., et al. (2014). "Three-step method for proliferation and differentiation of human embryonic stem cell (hESC)-derived male germ cells." PLoS One **9**(4): e90454.

The low efficiency of differentiation into male germ cell (GC)-like cells and haploid germ cells from human embryonic stem cells (hESCs) reflects the culture method employed in the two-dimensional (2D)-microenvironment. In this study, we applied a three-step media and calcium alginate-based 3D-culture system for enhancing the differentiation of hESCs into male germ stem cell (GSC)-like cells and haploid germ cells. In the first step, embryoid bodies (EBs) were derived from hESCs cultured in EB medium for 3 days and re-cultured for 4 additional days in EB medium with BMP4 and RA to specify GSC-like cells. In the second step, the resultant cells were cultured in GC-proliferation medium for 7 days. The GSC-like cells were then propagated after selection using GFR-alpha1 and were further cultured in GC-proliferation medium for 3 weeks. In the final step, a 3D-co-culture system using calcium alginate encapsulation and testicular somatic cells was applied to induce differentiation into haploid germ cells, and a culture containing approximately 3% male haploid germ cells was obtained after 2 weeks of culture. These results demonstrated that this culture system could be used to efficiently induce GSC-like cells in an EB population and to promote the differentiation of ESCs into haploid male germ cells.

Lim, M. N., et al. (2012). "Ex vivo expanded SSEA-4+ human limbal stromal cells are multipotent and do not express other embryonic stem cell markers." Mol Vis **18**: 1289-1300.

PURPOSE: The presence of multipotent human limbal stromal cells resembling mesenchymal stromal cells (MSC) provides new insights to the characteristic of these cells and its therapeutic potential.

However, little is known about the expression of stage-specific embryonic antigen 4 (SSEA-4) and the embryonic stem cell (ESC)-like properties of these cells. We studied the expression of SSEA-4 surface protein and the various ESC and MSC markers in the ex vivo cultured limbal stromal cells. The phenotypes and multipotent differentiation potential of these cells were also evaluated. METHODS: Limbal stromal cells were derived from corneoscleral rims. The SSEA-4(+) and SSEA-4(-) limbal stromal cells were sorted by fluorescence-activated cells sorting (FACS). Isolated cells were expanded and reanalyzed for their expression of SSEA-4. Expression of MSC and ESC markers on these cells were also analyzed by FACS. In addition, expression of limbal epithelial and corneal stromal proteins such as ATP-binding cassette sub-family G member 2 (ABCG2), tumour protein p63 (p63), paired box 6 (Pax6), cytokeratin 3 (AE5), cytokeratin 10, and keratocan sulfate were evaluated either by immunofluorecence staining or reverse transcription polymerase chain reaction. Appropriate induction medium was used to differentiate these cells into adipocytes, osteocytes, and chondrocytes. RESULTS: Expanded limbal stromal cells expressed the majority of mesenchymal markers. These cells were negative for ABCG2, p63, Pax6, AE-5, and keratocan sulfate. After passaged, a subpopulation of these cells showed low expression of SSEA-4 but were negative for other important ESC surface markers such as Tra-1-60, Tra-1-81, and transcription factors like octamer-binding transcription factor 4 (Oct4), SRY(sex determining region Y)-box 2 (Sox2), and Nanog. Early passaged cells when induced were able to differentiate into adipocytes, osteocytes and chondrocytes. CONCLUSIONS: The expanded limbal stromal cells showed features of multipotent MSC. Our study confirmed the expression of SSEA-4 by a subpopulation of cultured limbal stromal cells. However, despite the expression of SSEA-4, these cells did not express any other markers of ESC. Therefore, we conclude that the cells did not show properties of ESC.

Lim, W. F., et al. (2013). "Hematopoietic cell differentiation from embryonic and induced pluripotent stem cells." Stem Cell Res Ther **4**(3): 71.

Pluripotent stem cells, both embryonic stem cells and induced pluripotent stem cells, are undifferentiated cells that can self-renew and potentially differentiate into all hematopoietic lineages, such as hematopoietic stem cells (HSCs), hematopoietic progenitor cells and mature hematopoietic cells in the presence of a suitable culture system. Establishment of pluripotent stem cells provides a comprehensive model to study early hematopoietic development and has emerged as a powerful research tool to explore regenerative medicine. Nowadays, HSC transplantation and hematopoietic cell transfusion have successfully cured some patients, especially in malignant hematological diseases. Owing to a shortage of donors and a limited number of the cells, hematopoietic cell induction from pluripotent stem cells has been regarded as an alternative source of HSCs and mature hematopoietic cells for intended therapeutic purposes. Pluripotent stem cells are therefore extensively utilized to facilitate better understanding in hematopoietic development by recapitulating embryonic development in vivo, in which efficient strategies can be easily designed and deployed for the generation of hematopoietic lineages in vitro. We hereby review the current progress of hematopoietic cell induction from embryonic stem/induced pluripotent stem cells.

Lin, H. T., et al. (2007). "Enhancement of insulin-producing cell differentiation from embryonic stem cells using pax4-nucleofection method." World J Gastroenterol **13**(11): 1672-1679.

AIM: To enhance the differentiation of insulin producing cell (IPC) ability from embryonic stem (ES) cells in vitro. METHODS: Four-day embryoid body (EB)-formatted ES cells were dissociated as single cells for the followed plasmid DNA delivery. The use of Nucleofector electroporator (Amaxa biosystems, Germany) in combination with medium-contained G418 provided a high efficiency of gene delivery for advanced selection. Neucleofected cells were plated on the top of fibronectin-coated Petri dishes. Addition of Ly294002 and raised the glucose in medium at 24 h before examination. The differentiation status of these cells was monitored by semi-quantitative PCR (SQ-PCR) detection of the expression of relative genes, such as oct-4, sox-17, foxa2, mixl1, pdx-1, insulin 1, glucagons and somatostatin. The percentage of IPC population on d 18 of the experiment was investigated by immunohistochemistry (IHC), and the content/secretion of insulin was estimated by ELISA assay. The mice with severe combined immunodeficiency disease (SCID) pretreated with streptozotocin (STZ) were used to eliminate plasma glucose restoration after pax4+ ES implantation. RESULTS: A high efficiency of gene delivery was demonstrated when neucleofection was used in the present study; approximately 70% cells showed DsRed expression 2 d after neucleofection. By selection of medium-contained G418, the percentage of DsRed expressing cells kept high till the end of study. The pancreatic differentiation seemed to be accelerated by pax4 nucleofection. When compared to the group of cells with mock control, foxa2, mixl1, pdx1, higher insulin and somatostatin levels were detected by SQ-PCR 4 d after nucleofection in the group of pax4

expressing plasmid delivery. Approximately 55% of neucleofected cells showed insulin expression 18 d after neucleofection, and only 18% of cells showed insulin expression in mock control. The disturbance was shown by nucleofected pax4 RNAi vector; only 8% of cells expressed insulin 18 d after nucleofection. A higher IPC population was also detected in the insulin content by ELISA assay, and the glucose dependency was demonstrated in insulin secretion level. In the animal model, improvement of average plasma glucose concentration was observed in the group of pax-4 expressed ES of SCID mice pretreated with STZ, but no significant difference was observed in the group of STZ-pretreated SCID mice who were transplanted ES with mock plasmid. CONCLUSION: Enhancement of IPC differentiation from EB-dissociated ES cells can be revealed by simply using pax4 expressing plasmid delivery. Not only more IPCs but also pancreatic differentiation-related genes can be detected by SQ-PCR. Expression of relative genes, such as foxa 2, mixl 1, pdx-1, insulin 1 and somatostatin after nucleofection, suggests that pax4 accelerates the whole differentiation progress. The higher insulin production with glucose dependent modulation suggests that pax4 expression can drive more mature IPCs. Although further determination of the entire mechanism is required, the potential of pax-4-nucleofected cells in medical treatment is promising.

Lin, I. Y., et al. (2014). "Suppression of the SOX2 neural effector gene by PRDM1 promotes human germ cell fate in embryonic stem cells." Stem Cell Reports **2**(2): 189-204.

The mechanisms of transcriptional regulation underlying human primordial germ cell (PGC) differentiation are largely unknown. The transcriptional repressor Prdm1/Blimp-1 is known to play a critical role in controlling germ cell specification in mice. Here, we show that PRDM1 is expressed in developing human gonads and contributes to the determination of germline versus neural fate in early development. We show that knockdown of PRDM1 in human embryonic stem cells (hESCs) impairs germline potential and upregulates neural genes. Conversely, ectopic expression of PRDM1 in hESCs promotes the generation of cells that exhibit phenotypic and transcriptomic features of early PGCs. Furthermore, PRDM1 suppresses transcription of SOX2. Overexpression of SOX2 in hESCs under conditions favoring germline differentiation skews cell fate from the germline to the neural lineage. Collectively, our results demonstrate that PRDM1 serves as a molecular switch to modulate the divergence of neural or germline fates through repression of SOX2 during human development.

Lin, J., et al. (2010). "Controlled major histocompatibility complex-T cell receptor signaling allows efficient generation of functional, antigen-specific CD8+ T cells from embryonic stem cells and thymic progenitors." Tissue Eng Part A **16**(9): 2709-2720.

Generation of early T cells by coculturing stem cells on notch-ligand-expressing OP9 stromal cells (OP9-DL1) has been widely reported. However, further differentiation of these cells into mature, antigen-specific, functional T cells, without retroviral transduction of T cell receptors (TcRs), is yet to be achieved. In the thymic niche this differentiation is controlled by the interaction of developing TcRs with major histocompatibility (MHC) molecules on stromal cells. We hypothesized that by providing exogenous antigen-specific MHC/TcR signals, stem and progenitor cells could be engineered into functional, effector T cells specific for the same antigen. Here we demonstrate that both thymus-derived immature T cells (double positive [DP]: CD4+CD8+) and mouse embryonic stem cells can be efficiently differentiated into antigen-specific CD8+ T cells using either MHC tetramers or peptide-loaded stromal cells. DP cells, following MHC/TcR signaling, retained elevated recombination activating gene-1 levels, suggesting continuing TcR gene rearrangement. Both DP and embryonic stem-cell-derived CD8+ T cells showed significant cytotoxic T lymphocytes activity against antigen-loaded target cells, indicating that these cells are functional. Such directed differentiation strategy could provide an efficient method for generating functional, antigen-specific T cells from stem cells for potential use in adoptive T cell therapy.

Lin, Y. D., et al. (2018). "The genes involved in asthma with the treatment of human embryonic stem cell-derived mesenchymal stem cells." Mol Immunol **95**: 47-55.

BACKGROUND: Asthma is affecting more than 300 million people worldwide, which represents the most common chronic disease among children. We previously found that mesenchymal stem cells (MSCs) derived from induced pluripotent stem cells (iPSCs) modulated the immune response on Th2-mediated asthma in vivo and in vitro. This study further evaluated the immunomodulatory effects of MSCs from human embryonic stem cells (hESCs) on asthma. METHODS: Multipotent hESC-MSCs were obtained using a feeder-free method. The hESC-MSCs were analysed for the expression of stem cell surface markers by flow cytometry, their differentiation potentials were analysed using in vitro trilineage differentiation methods hESC-MSCs were transplanted into the murine model with ovalbumin (OVA)-induced airway allergic inflammation. The expression levels of

allergic related genes were measured by the mRNA PCR arrays. RESULTS: The hESC-MSCs expressed classical MSC markers and held the capability of differentiation into multiple mesoderm-type cell lineages. hESC-MSCs were able to suppress allergic inflammation by modulating Th2 cells and eosinophils in the mice, and reversed the reduction of regulatory T cells. By using PCR array, 5 mRNAs- chemokine (C-C motif) ligand 11 (Ccl11), Ccl24, interleukin13 (Il13), Il33 and eosinophil-associated, ribonuclease A family, member 11 (Ear11) were identified the most relevant in murine airway allergic inflammation and hESC-MSCs treatment. CONCLUSIONS: The therapeutic effects of hESC-MSCs were identified in the murine model of airway allergic inflammation with key mRNAs involved. This study will provide a better understanding regarding the mechanisms underlying hESC-MSCs therapeutic application in airway allergic inflammation.

Lindgren, A. G., et al. (2015). "ETV2 expression increases the efficiency of primitive endothelial cell derivation from human embryonic stem cells." Cell Regen (Lond) **4**(1): 1.

BACKGROUND: Endothelial cells line the luminal surface of blood vessels and form a barrier between the blood and other tissues of the body. Ets variant 2 (ETV2) is transiently expressed in both zebrafish and mice and is necessary and sufficient for vascular endothelial cell specification. Overexpression of this gene in early zebrafish and mouse embryos results in ectopic appearance of endothelial cells. Ectopic expression of ETV2 in later development results in only a subset of cells responding to the signal. FINDINGS: We have examined the expression pattern of ETV2 in differentiating human embryonic stem cells (ESCs) to determine when the peak of ETV2 expression occurs. We show that overexpression of ETV2 in differentiating human ESC is able to increase the number of endothelial cells generated when administered during or after the endogenous peak of gene expression. CONCLUSIONS: Addition of exogenous ETV2 to human ESCs significantly increased the number of cells expressing angioblast genes without arterial or venous specification. This may be a viable solution to generate in vitro endothelial cells for use in research and in the clinic.

Lindskog, H., et al. (2006). "New insights to vascular smooth muscle cell and pericyte differentiation of mouse embryonic stem cells in vitro." Arterioscler Thromb Vasc Biol **26**(7): 1457-1464.

OBJECTIVE: The molecular mechanisms that regulate pericyte differentiation are not well understood, partly because of the lack of well-characterized in vitro systems that model this process. In this article, we develop a mouse embryonic stem (ES) cell-based angiogenesis/vasculogenesis assay and characterize the system for vascular smooth muscle cell (VSMC) and pericyte differentiation. METHODS AND RESULTS: ES cells that were cultured for 5 days on OP9 stroma cells upregulated their transcription of VSMC and pericyte selective genes. Other SMC marker genes were induced at a later time point, which suggests that vascular SMC/pericyte genes are regulated by a separate mechanism. Moreover, sequence analysis failed to identify any conserved CArG elements in the vascular SMC and pericyte gene promoters, which indicates that serum response factor is not involved in their regulation. Gleevec, a tyrosine kinase inhibitor that blocks platelet-derived growth factor (PDGF) spell-receptor signaling, and a neutralizing antibody against transforming growth factor (TGF) beta1, beta2, and beta3 failed to inhibit the induction of vascular SMC/pericyte genes. Finally, ES-derived vascular sprouts recruited cocultured MEF cells to pericyte-typical locations. The recruited cells activated expression of a VSMC- and pericyte-specific reporter gene. CONCLUSIONS: We conclude that OP9 stroma cells induce pericyte differentiation of cocultured mouse ES cells. The induction of pericyte marker genes is temporally separated from the induction of SMC genes and does not require platelet-derived growth factor B or TGFbeta1 signaling.

Liou, J. Y., et al. (2017). "An Efficient Transfection Method for Differentiation and Cell Proliferation of Mouse Embryonic Stem Cells." Methods Mol Biol **1622**: 139-147.

Embryonic stem (ES) cells are an important source of stem cells in tissue engineering and regenerative medicine because of their high self-renewal capacities and differentiation potentials. However, the detailed molecular mechanisms controlling the differentiation and renewal programs in ES cells remained unclear. One of the difficulties in understanding these mechanisms substantially results from the low efficacies of gene manipulation by delivering exogenous gene expression or knockdown of endogenous gene expression with small interfering RNA (siRNA) in ES cells. Here we describe an optimized protocol for efficiently transfecting mouse ES cells by Effectene, a liposome-based method. The high transfection efficiency in mouse ES cells is demonstrated in this chapter by (1) achieving a percentage of enhanced green fluorescence protein (EGFP) expression in >98% embryoid bodies after introducing plasmids encoding the protein; (2) decreased SOX-2 and Oct-3/4 expression and subsequent morphological evidences of cell differentiation after introducing siRNA expression for suppressing SOX-2 and Oct-3/4, which are known to

be essential for maintenance of stem cell properties in mouse ES cells; and (3) overexpression or attenuated expression of 14-3-3sigma to regulate cell proliferation of mouse ES cells.

Liour, S. S., et al. (2006). "Further characterization of embryonic stem cell-derived radial glial cells." Glia **53**(1): 43-56.

Previously, we showed that radial glia-like (RG) cells differentiated from embryonic stem (ES) cells after retinoic acid induction (Liour and Yu, 2003: Glia 42:109-117). In the present study, we demonstrate that the production of RG cells from ES cells is independent of the neural differentiation protocol used. These ES cell-derived RG (ES-RG) cells are similar in morphology to RG cells in vivo and express several characteristic RG cell markers. The processes of these ES-RG cells are organized into radial arrays similar to the RG scaffold in developing CNS. Expression of Pax6, along with other circumstantial data, suggests that at least some of these ES-RG cells are neural progenitors. The progression of neurogenesis into gliogenesis during the in vitro neural differentiation of ES cells recapitulates the in vivo developmental process. The identification of two cell surface markers, SSEA-1 and GM1, on both the native embryonic RG cells and ES-RG cells, may facilitate purification of radial glial cells for future studies and cell therapy. Overall, our study suggests that differentiation of radial glial cells is a common pathway during the neural differentiation of ES cells.

Liu, H., et al. (2010). "Folic Acid supplementation stimulates notch signaling and cell proliferation in embryonic neural stem cells." J Clin Biochem Nutr **47**(2): 174-180.

The present study investigated the effect of folic acid supplementation on the Notch signaling pathway and cell proliferation in rat embryonic neural stem cells (NSCs). The NSCs were isolated from E14-16 rat brain and grown as neurospheres in serum-free suspension culture. Individual cultures were assigned to one of 3 treatment groups that differed according to the concentration of folic acid in the medium: Control (baseline folic acid concentration of 4 mg/l), low folic acid supplementation (4 mg/l above baseline, Folate-L) and high folic acid supplementation (40 mg/l above baseline, Folate-H). NSCs were identified by their expression of immunoreactive nestin and proliferating cells by incorporation of 5'bromo-2'deoxyuridine. Cell proliferation was also assessed by methyl thiazolyl tetrazolium assay. Notch signaling was analyzed by real-time PCR and western blot analyses of the expression of Notch1 and hairy and enhancer of split 5 (Hes5). Supplementation of NSCs with folic acid increased the mRNA and protein expression levels of Notch1 and Hes5. Folic acid supplementation also stimulated NSC proliferation dose-dependently. Embryonic NSCs respond to folic acid supplementation with increased Notch signaling and cell proliferation. This mechanism may mediate the effects of folic acid supplementation on neurogenesis in the embryonic nervous system.

Liu, H., et al. (2016). "High-Efficient Transfection of Human Embryonic Stem Cells by Single-Cell Plating and Starvation." Stem Cells Dev **25**(6): 477-491.

Nowadays, the low efficiency of small interfering RNA (siRNA) or plasmid DNA (pDNA) transfection is a critical issue in genetic manipulation of human embryonic stem (hES) cells. Development of an efficient transfection method for delivery of siRNAs and plasmids into hES cells becomes more and more imperative. In this study, we tried to modify the traditional transfection protocol by introducing two crucial processes, single-cell plating and starvation, to increase the transfection efficiency in hES cells. Furthermore, we comparatively examined the transfection efficiency of some commercially available siRNA or pDNA transfection reagents in hES cells. Our results showed that the new developed method markedly enhanced the transfection efficiency without influencing the proliferation and pluripotency of hES cells. Lipofectamine RNAiMAX exhibited much higher siRNA transfection efficiency than the other reagents, and FuGENE HD was identified as the best suitable reagent for efficient pDNA transfection of hES cells among the tested reagents.

Liu, H., et al. (2014). "Systematically labeling developmental stage-specific genes for the study of pancreatic beta-cell differentiation from human embryonic stem cells." Cell Res **24**(10): 1181-1200.

The applications of human pluripotent stem cell (hPSC)-derived cells in regenerative medicine has encountered a long-standing challenge: how can we efficiently obtain mature cell types from hPSCs? Attempts to address this problem are hindered by the complexity of controlling cell fate commitment and the lack of sufficient developmental knowledge for guiding hPSC differentiation. Here, we developed a systematic strategy to study hPSC differentiation by labeling sequential developmental genes to encompass the major developmental stages, using the directed differentiation of pancreatic beta cells from hPSCs as a model. We therefore generated a large panel of pancreas-specific mono- and dual-reporter cell lines. With this unique platform, we visualized the kinetics of the entire differentiation process in real time for the first time by monitoring the expression dynamics of the reporter genes, identified desired cell populations at each differentiation stage and demonstrated the ability

to isolate these cell populations for further characterization. We further revealed the expression profiles of isolated NGN3-eGFP(+) cells by RNA sequencing and identified sushi domain-containing 2 (SUSD2) as a novel surface protein that enriches for pancreatic endocrine progenitors and early endocrine cells both in human embryonic stem cells (hESC)-derived pancreatic cells and in the developing human pancreas. Moreover, we captured a series of cell fate transition events in real time, identified multiple cell subpopulations and unveiled their distinct gene expression profiles, among heterogeneous progenitors for the first time using our dual reporter hESC lines. The exploration of this platform and our new findings will pave the way to obtain mature beta cells in vitro.

Liu, J., et al. (2012). "Role of miRNAs in neuronal differentiation from human embryonic stem cell-derived neural stem cells." Stem Cell Rev **8**(4): 1129-1137.

microRNAs (miRNAs) are important modulators in regulating gene expression at the post-transcriptional level and are therefore emerging as strong mediators in neural fate determination. Here, by use of the model of human embryonic stem cell (hESC)-derived neurogenesis, miRNAs involved in the differentiation from neural stem cells (hNSC) to neurons were profiled and identified. hNSC were differentiated into the neural lineage, out of which the neuronal subset was enriched through cell sorting based on select combinatorial biomarkers: CD15-/CD29(Low)/CD24(High). This relatively pure and viable subpopulation expressed the neuronal marker beta III-tubulin. The miRNA array demonstrated that a number of miRNAs were simultaneously induced or suppressed in neurons, as compared to hNSC. Real-time PCR further validated the decrease in levels of miR214, but increase in brain-specific miR7 and miR9 in the derived neurons. For functional studies, hNSC were stably transduced with lentiviral vectors carrying specific constructs to downregulate miR214 or to upregulate miR7. Manipulation of either miR214 or miR7 did not affect the expression of beta III-tubulin or neurofilament, however miR7 overexpression gave rise to enhanced synapsin expression in the derived neurons. This indicated that miR7 might play an important role in neurite outgrowth and synapse formation. In conclusion, our data demonstrate that miRNAs function as important modulators in neural lineage determination. These studies shed light on strategies to optimize in vitro differentiation efficiencies to mature neurons for use in drug discovery studies and potential future clinical applications.

Liu, M. L., et al. (2005). "[Directed differentiation of Balb/C mouse embryonic stem cells into pancreatic islet-like cell clusters in vitro: observation by atomic force microscope]." Di Yi Jun Yi Da Xue Xue Bao **25**(4): 377-379, 402.

OBJECTIVE: To observe the morphological changes of Balb/C mouse embryonic stem cells following directed differentiation into pancreatic islet-like cell clusters (PICC) in vitro using atomic force microscope (AFM). METHODS: Balb/C mouse embryonic stem cells were first cultured into embryonic bodies (EBs) and allowed to differentiate spontaneously for 4 days. The cells were then transferred to gelatin-coated dishes for the EBs to attach and spread on the tissue culture plates, in the course of which a series of cell growth factors such as basic fibroblast growth factor (bFGF), insulin-like growth factor 1 (IGF-1) and nicotinamide were added into the culture medium at specific time points to induce directed differentiation of the stem cells into PICC. Immunocytochemistry was employed to detect the cells positive for insulin and glucagon, which were observed with AFM. RESULTS: The embryonic stem cells developed into cell clusters of different sizes, in which the cells were tightly arranged. Islet B cells were numerous in the center of clusters and darkly stained, but fewer in the peripherals with lighter stains. Islet A cells expressing glucagon were relatively fewer in the cell clusters, found mainly in the peripherals. Scanning of the insulin-positive clusters by AFM revealed large quantity of tissue fibers resembling nerve fibers that formed a reticular structure in disorderly arrangement. Numerous round granules were observed in the cytoplasm of almost identical sizes ranging from 0.5 to 1.0 mum in diameter. CONCLUSION: The cell clusters obtained by directed differentiation are mature in both morphology and function with also well organized structures.

Liu, Q., et al. (2007). "Embryonic stem cells as a novel cell source of cell-based biosensors." Biosens Bioelectron **22**(6): 810-815.

To investigate the use of embryonic stem cells as biosensor elements, mouse embryoid bodies were cultured on the surface of the light-addressable potentiometric sensor and induce to in vitro differentiate into cardiomyocytes and neurons. Extracellular potentials of the cells were recorded by sensor, to detect stem cells potential applications in drugs screening. The experimental results show that known cardiac stimulants (isoproterenol) and relaxants (carbamylcholine) have characteristic effects on the cardiomyocytes in terms of the changes of beat frequency, amplitude and duration. Thus, the embryonic stem cells potentially represent a renewable cell source for the cell-based biosensors.

Liu, S., et al. (2014). "Effect of transplantation of human embryonic stem cell-derived neural progenitor cells on adult neurogenesis in aged hippocampus." Am J Stem Cells **3**(1): 21-26.

Adult neurogenesis occurs within the special microenvironment in the subgranular zone of the hippocampus and the subventricular zone of the lateral ventricle of the mammalian brain. The special microenvironment is known as neurogenic niches. Multiple cell types, including endothelial cells, astroglia, ependymal cells, immature progeny of neural stem cells, and mature neurons, comprise the neurogenic niche. Differentiation of embryonic stem cells towards the neural lineage results in the generation of different neuronal subtypes and non-neuronal cells (mainly astrocytes). Therefore, it is reasonable to hypothesize that transplantation of human embryonic stem cell-derived neural progenitor cells can be used to modify neurogenic niches for facilitating adult neurogenesis. Furthermore, if generated new neurons are functionally integrated into the existing circuits of the aged hippocampus, synaptic plasticity in the hippocampus and learning/memory functions in aged mice should be enhanced. In this article, we provide a comprehensive review of the concepts in the regulation of adult neurogenesis by neurogenic niches and discuss the molecular mechanisms underlying the effect of stem cell transplantation on adult neurogenesis in aged hippocampus.

Liu, Y., et al. (2010). "Enhancement of long-term proliferative capacity of rabbit corneal epithelial cells by embryonic stem cell conditioned medium." Tissue Eng Part C Methods **16**(4): 793-802.

Induction of autologous stem cells for directed differentiation has become a predominant method to obtain autologous cells for tissue reconstruction. However, the low inducing efficiency and contamination with other type of cells hinder its clinical utilization. Here we report a novel phenomenon that the corneal epithelial cells maintain long-term proliferative capacity and tissue-specific cell phenotype by factors secreted from murine embryonic stem cells (ESCs). The rabbit corneal epithelial cells grew very well in culture medium with addition of 40% ESC conditioned medium (ESC-CM). These corneal epithelial cells have been serially subcultured for more than 20 passages and maintained high cell purity, cobble-stone-like morphology, enhanced colony forming efficiency, normal diploid, and capacity to regenerate a functional stratified corneal epithelial equivalent. More importantly, these cells did not form tumor, and the cells lost their proliferative capacity after withdrawal of ESC-CM. The long-term proliferative capacity of corneal epithelial cells is partly resulted from enhancement of cell survival and colony formation, and mediated by ectopic expression of telomerase. Our findings indicate that this new ESC-CM culture system can generate low-immunogenic autologous cells sufficiently for use in regenerative medicine.

Liu, Y. X., et al. (2010). "Production of erythriod cells from human embryonic stem cells by fetal liver cell extract treatment." BMC Dev Biol **10**: 85.

BACKGROUND: We recently developed a new method to induce human stem cells (hESCs) differentiation into hematopoietic progenitors by cell extract treatment. Here, we report an efficient strategy to generate erythroid progenitors from hESCs using cell extract from human fetal liver tissue (hFLT) with cytokines. Human embryoid bodies (hEBs) obtained of human H1 hESCs were treated with cell extract from hFLT and co-cultured with human fetal liver stromal cells (hFLSCs) feeder to induce hematopoietic cells. After the 11 days of treatment, hEBs were isolated and transplanted into liquid medium with hematopoietic cytokines for erythroid differentiation. Characteristics of the erythroid cells were analyzed by flow cytometry, Wright-Giemsa staining, real-time RT-PCR and related functional assays. RESULTS: The erythroid cells produced from hEBs could differentiate into enucleated cells and expressed globins in a time-dependent manner. They expressed not only embryonic globins but also the adult-globin with the maturation of the erythroid cells. In addition, our data showed that the hEBs-derived erythroid cells were able to act as oxygen carriers, indicating that hESCs could generate functional mature erythroid cells. CONCLUSION: Cell extract exposure with the addition of cytokines resulted in robust erythroid -like differentiation of hEBs and these hEBs-derived erythroid cells possessed functions similar to mature red blood cells.

Lo, I. C., et al. (2016). "TRPV3 Channel Negatively Regulates Cell Cycle Progression and Safeguards the Pluripotency of Embryonic Stem Cells." J Cell Physiol **231**(2): 403-413.

Embryonic stem cells (ESCs) have tremendous potential for research and future therapeutic purposes. However, the calcium handling mechanism in ESCs is not fully elucidated. Aims of this study are (1) to investigate if transient receptor potential vanilloid-3 (TRPV3) channels are present in mouse ESCs (mESCs) and their subcellular localization; (2) to investigate the role of TRPV3 in maintaining the characteristics of mESCs. Western blot and immunocytochemistry showed that TRPV3 was present at the endoplasmic reticulum (ER) of mESCs. Calcium imaging showed that, in the absence of extracellular calcium, TRPV3 activators camphor and 6-tert-butyl-m-cresol increased the cytosolic calcium. However,

depleting the ER store in advance of activator addition abolished the calcium increase, suggesting that TRPV3 released calcium from the ER. To dissect the functional role of TRPV3, TRPV3 was activated and mESC proliferation was measured by trypan blue exclusion and MTT assays. The results showed that TRPV3 activation led to a decrease in mESC proliferation. Cell cycle analysis revealed that TRPV3 activation increased the percentage of cells in G2 /M phase; consistently, Western blot also revealed a concomitant increase in the expression of inactive form of cyclin-dependent kinase 1, suggesting that TRPV3 activation arrested mESCs at G2 /M phase. TRPV3 activation did not alter the expression of pluripotency markers Oct-4, Klf4 and c-Myc, suggesting that the pluripotency was preserved. Our study is the first study to show the presence of TRPV3 at ER. Our study also reveals the novel role of TRPV3 in controlling the cell cycle and preserving the pluripotency of ESCs.

Lo Nigro, A., et al. (2017). "PDGFRalpha(+) Cells in Embryonic Stem Cell Cultures Represent the In Vitro Equivalent of the Pre-implantation Primitive Endoderm Precursors." Stem Cell Reports **8**(2): 318-333.

In early mouse pre-implantation development, primitive endoderm (PrE) precursors are platelet-derived growth factor receptor alpha (PDGFRalpha) positive. Here, we demonstrated that cultured mouse embryonic stem cells (mESCs) express PDGFRalpha heterogeneously, fluctuating between a PDGFRalpha+ (PrE-primed) and a platelet endothelial cell adhesion molecule 1 (PECAM1)-positive state (epiblast-primed). The two surface markers can be co-detected on a third subpopulation, expressing epiblast and PrE determinants (double-positive). In vitro, these subpopulations differ in their self-renewal and differentiation capability, transcriptional and epigenetic states. In vivo, double-positive cells contributed to epiblast and PrE, while PrE-primed cells exclusively contributed to PrE derivatives. The transcriptome of PDGFRalpha(+) subpopulations differs from previously described subpopulations and shows similarities with early/mid blastocyst cells. The heterogeneity did not depend on PDGFRalpha but on leukemia inhibitory factor and fibroblast growth factor signaling and DNA methylation. Thus, PDGFRalpha(+) cells represent the in vitro counterpart of in vivo PrE precursors, and their selection from cultured mESCs yields pure PrE precursors.

Lobanok, E. S., et al. (2008). "[Effect of the stem cell factor on the morphology and functional state of mouse embryonic stem cells]." Biofizika **53**(4): 646-651.

The effect of the stem cell factor on the state of membranes and functional activity of mouse embryonic stem cells cultivated in LIF (Leukemia Inhibitory Factor) cytokine-free and LIF-containing media has been studied. It was shown that the stem cell factor induces changes in the viscosity of membrane lipid bilayer and increases the respiration rate, the ATP level, and the proliferation activity of embryonic stem cells. An intricate character of the LIF-dependent modification of biological effects of the stem cell factor was revealed.

Longo, L., et al. (1997). "The chromosome make-up of mouse embryonic stem cells is predictive of somatic and germ cell chimaerism." Transgenic Res **6**(5): 321-328.

Mouse pluripotent embryonic stem (ES) cells, once reintroduced into a mouse blastocyst, can contribute to the formation of all tissues, including the germline, of an organism referred to as a chimaeric. However, the reasons why this contribution often appears erratic are poorly understood. We have tested the notion that the chromosome make-up may be important in contributing both to somatic cell chimaerism and to germ line transmission. We found that the percentage of chimaerism of ES cell-embryo chimaeras, the absolute number of chimaeras and the ratio of chimaeras to total pups born all correlate closely with the percentage of euploid metaphases in the ES cell clones injected into the murine blastocyst. The majority of the ES cell clones that we tested, which were obtained from different gene targeting knockout experiments and harboured 50 to 100% euploid metaphases, did transmit to the germline; in contrast, none of the ES cell clones with more than 50% of chromosomally abnormal metaphases transmitted to the germline. Euploid ES cell clones cultured in vitro for more than 20 passages rapidly became severely aneuploid, and again this correlated closely with the percentage of chimaerism and with the number of ES cell-embryo chimaeras obtained per number of blastocysts injected. At the same time, the ability of these clones to contribute to the germline was lost when the proportion of euploid cells dropped below 50%. This study suggests that aneuploidy, rather than 'loss of totipotency', in ES cells, is the major cause of failure in obtaining contributions to all tissues of the adult chimaera, including the germline. Because euploidy is predictive of germline transmission, karyotype analysis is crucial and time/cost saving in any gene-targeting experiment.

Lorberbaum, D. S. and D. Gottlieb (2011). "Regulated expression of transgenes in embryonic stem cell-derived neural cells." Genesis **49**(2): 66-74.

Discovery and characterization of gene promoters, enhancers and repressor binding elements is an important research area in neuroscience. Here, the suitability of embryonic stem cells and their neural

derivatives as a model system for this research is investigated. Three neural transgenic constructs (from the Mnx1, Fabp7, and tuba1a genes) that have been validated in transgenic mice were inserted into embryonic stem cells as stable transgenes. These transgenic embryonic stem cells were differentiated into neural cultures and the pattern of transgene expression across a series of inducing conditions determined. The pattern of expression matched that predicted from transgenic mouse experiments for each of the three transgenes. The results show that embryonic stem cells and their neural derivatives comprise a promising model for investigating the mechanisms that control cell- and temporal-specific neural gene transcription.

Lorincz, M. T. (2006). "Optimized neuronal differentiation of murine embryonic stem cells: role of cell density." Methods Mol Biol **330**: 55-69.

Neuronally differentiated embryonic stem (ES) cells offer a flexible and extremely potent model to study nervous system development and disease. A variety of protocols have been described to facilitate neuronal differentiation. The density of ES cells used for neuronal differentiation has striking effects on the proportion and purity of the derived neuronal cells. Here, the protocols used to optimize ES cell density in neuronal differentiation with and without an initial aggregation step are described.

Loring, J. F., et al. (2001). "A gene expression profile of embryonic stem cells and embryonic stem cell-derived neurons." Restor Neurol Neurosci **18**(2-3): 81-88.

Embryonic stem (ES) cells have the ability to differentiate into a variety of cell lineages. We are examining ES cell differentiation in vitro by using cDNA microarrays to generate a molecular phenotype for each cell type. El4 ES cells induced by retinoic acid after forming embryoid bodies differentiate almost exclusively to neurons. We obtained expression patterns for about 8500 gene sequences by comparing mRNAs from undifferentiated ES cells and their differentiated derivatives in a competitive hybridization. Our results indicate that the genes expressed by ES cells change dramatically as they differentiate (58 gene sequences up-regulated, 34 down-regulated). Most notably, totipotent ES cells expressed high levels of a repressor of Hox expression (the polycomb homolog Mphl) and a co-repressor (CTBP2). Expression of these genes was undetectable in differentiated cells; the ES cell-derived neurons expressed a different set of transcriptional regulators, as weil as markers of neurogenesis. The gene expression profiles indicate that ES cells actively suppress differentiation by transcriptional repression; cell-cell contact in embryoid bodies and retinoic acid treatment may overcome this suppression, allowing expression of Hox genes and inducing a suite of neuronal genes. Gene expression profiles will be a useful outcome measure for comparing in vitro treatments of differentiating ES cells and other stem cells. Also, knowing the molecule phenotype of transplantable cells will allow correlation of phenotype with the success of the transplant.

Losino, N., et al. (2011). "Maintenance of murine embryonic stem cells' self-renewal and pluripotency with increase in proliferation rate by a bovine granulosa cell line-conditioned medium." Stem Cells Dev **20**(8): 1439-1449.

Murine embryonic stem cells (mESCs) are pluripotent cells that can be propagated in an undifferentiated state in continuous culture on a feeder layer or without feeders in the presence of leukemia inhibitory factor (LIF). Although there has been a great advance since their establishment, ESC culture is still complex and expensive. Therefore, finding culture conditions that maintain the self-renewal of ESCs, preventing their differentiation and promoting their proliferation, is still an area of great interest. In this work, we studied the effects of the conditioned medium from a bovine granulosa cell line (BGC-CM) on the maintenance of self-renewal and pluripotency of mESCs. We found that this medium is able to maintain mESCs' self-renewal while preserving its critical properties without LIF addition. mESCs cultured in BGC-CM expressed the stem cell markers Oct4, Sox2, Nanog, SSEA-1, Klf4, Rex1, and ECAT1. Moreover, mESCs cultured in BGC-CM gave rise to embryoid bodies and teratomas that differentiated effectively to diverse cell populations from endoderm, mesoderm, and ectoderm. Further, we found that mESCs cultured in BGC-CM have an increased proliferation rate compared with cells grown in the mESC standard culture medium supplemented with LIF. These findings may provide a powerful tool to culture mESCs for long periods of time with high proliferation rate while preserving its basic characteristics, contributing to the application of these cells to assess potential tissue engineering and cellular therapy applications.

Lotfinia, M., et al. (2016). "Effect of Secreted Molecules of Human Embryonic Stem Cell-Derived Mesenchymal Stem Cells on Acute Hepatic Failure Model." Stem Cells Dev **25**(24): 1898-1908.

Adult tissue-derived mesenchymal stem cells (MSCs) show tremendous promise for a wide array of therapeutic applications predominantly through paracrine activity. Recent reports showed that human embryonic stem cell (ESC)-derived MSCs are an alternative for regenerative cellular therapy due to manufacturing large quantities of MSCs from a single

donor. However, no study has been reported to uncover the secretome of human ESC-MSCs as treatment of an acute liver failure (ALF) mouse model. We demonstrated that human ESC-MSCs showed similar morphology and cell surface markers compared with bone marrow-derived MSCs. ESC-MSCs exhibited a higher growth rate during early in vitro expansion, along with adipogenic and osteogenic differentiation potential. Treatment with ESC-MSC-conditioned medium (CM) led to statistically significant enhancement of primary hepatocyte viability and increased immunomodulatory interleukin-10 secretion from lipopolysaccharide-induced human blood mononuclear cells. Analysis of the MSCs secretome by a protein array screen showed an association between higher frequencies of secretory proteins such as vascular endothelial growth factor (VEGF) and regulation of cell proliferation, cell migration, the development process, immune system process, and apoptosis. In this thioacetamide-induced mouse model of acute liver injury, we observed that systemic infusion of VEGF led to significant survival. These data have provided the first experimental evidence of the therapeutic potential of human ESC-MSC-derived molecules. These molecules show trophic support to hepatocytes, which potentially creates new avenues for the treatment of ALF, as an inflammatory condition.

Lotfinia, M., et al. (2017). "Hypoxia Pre-Conditioned Embryonic Mesenchymal Stem Cell Secretome Reduces IL-10 Production by Peripheral Blood Mononuclear Cells." Iran Biomed J **21**(1): 24-31.

BACKGROUND: Mesenchymal stem cells (MSCs) are important candidates for MSC-based cellular therapy. Current paradigm states that MSCs support local progenitor cells in damaged tissue through paracrine signaling. Therefore, study of paracrine effects and secretome of MSCs could lead to the appreciation of mechanisms and molecules associated with the therapeutic effects of these cells. This study analyzed anti-inflammatory and immune-modulatory effects of MSC secretomes derived from embryonic stem cells (ESCs) and bone marrow cells after hypoxia and normoxia preconditioning. METHODS: ESCs differentiated into MSCs and characterized by flow cytometry and differentiation into adipocytes and osteoblasts. The experimental groups consisted of individual groups of ESC-MSCs and BM-MSCs (bone marrow-derived mesenchymal stromal cells), which were preconditioned with either hypoxia or normoxia for 24, 48 and 72 h. After collecting the cell-free medium from each treatment, secretomes were concentrated by centrifugal filters. Using a peripheral blood mononuclear cell (PBMC) assay and ELISA, IL-10 concentration in PBMCs was evaluated after their incubation with different secretomes from preconditioned and non-preconditioned MSCs. RESULTS: A significant difference was observed between ESC-MSC normoxia and ESC-MSC hypoxia in IL-10 concentration, and normoxia secretomes increased IL-10 secretion from PBMCs. Moreover, the strongest IL-10 secretion from PBMCs could be detected after the stimulation by ESC-MSC conditioned secretomes, but not BM-MSC conditioned medium. CONCLUSIONS: Human hypoxia preconditioned ESC-MSC secretome indicated stronger immune-modulatory effects compared to BM-MSC conditioned medium. It could be suggested that induced MSCs confer less immune-modulatory effects but produce more inflammatory molecules such as tumor necrosis factor alpha, which needs further investigation.

Love, P. E., et al. (1992). "Targeting of the T-cell receptor zeta-chain gene in embryonic stem cells: strategies for generating multiple mutations in a single gene." Proc Natl Acad Sci U S A **89**(20): 9929-9933.

The T-cell receptor zeta chain is a member of a family of related proteins that play a critical role in coupling cell-surface receptors to intracellular signaling pathways. To study the role of zeta chain in T-cell ontogeny, we generated targeted mutations of the zeta-chain gene in murine embryonic stem cells. The mutant alleles are predicted to result either in a null phenotype or in the synthesis of a truncated protein capable of supporting T-cell-receptor surface expression but deficient in transmembrane signaling. Both of these targeting events were recovered in a single electroporation experiment with either coelectroporation or a combination deletion/truncation construct. Our results suggest that similar approaches could be used to generate multiple single mutations, modifications of more than one site within a gene, or subtle alterations that rely upon coconversion with the selectable marker gene.

Lu, J., et al. (2017). "Interactions of human embryonic stem cell-derived cardiovascular progenitor cells with immobilized extracellular matrix proteins." J Biomed Mater Res A **105**(4): 1094-1104.

Human embryonic stem cell-derived cardiovascular progenitor cells (hESC-CVPCs) hold great promise for cell-based therapies of heart diseases. However, little is known about their niche microenvironment and in particular the required extracellular matrix (ECM) components. Here we screened combinations of surface-immobilized ECM proteins to identify substrates that support the attachment and survival of hESC-CVPCs. Covalent immobilization of ECM proteins laminin (Lm), fibronectin (Fn), collagen I (CI), collagen III (CIII), and collagen IV (CIV) in multiple combinations and

concentrations was achieved by reductive amination on transparent acetaldehyde plasma polymer (AAPP) interlayer coatings. We identified that CI, CIII, CIV, and Fn and their combinations were important for hESC-CVPC attachment and survival, while Lm was dispensable. Moreover, for coatings displaying single ECM proteins, CI and CIII performed better than CIV and Fn, while coatings displaying the combined ECM proteins CIII + CIV and Fn + CIII + CIV at 100 microg/mL were comparable to Matrigel in regard to supporting hESC-CVPC attachment and viability. Our results identify ECM proteins required for hESC-CVPCs and demonstrate that coatings displaying multiple immobilized ECM proteins offer a suitable microenvironment for the attachment and survival of hESC-CVPCs. This knowledge contributes to the development of approaches for maintaining hESC-CVPCs and therefore to advances in cardiovascular regeneration. (c) 2017 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 105A: 1094-1104, 2017.

Lu, M., et al. (2009). "Enhanced generation of hematopoietic cells from human hepatocarcinoma cell-stimulated human embryonic and induced pluripotent stem cells." Exp Hematol **37**(8): 924-936.

OBJECTIVE: Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) constitute unique sources of pluripotent cells, although the molecular mechanisms involved in their differentiation into specific lineages are just beginning to be defined. Here we evaluated the ability of MEDII (medium conditioned by HepG2 cells, a human hepatocarcinoma cell line) to selectively enhance generation of mesodermal derivatives, including hematopoietic cells, from hESCs and hiPSCs. MATERIALS AND METHODS: Test cells were exposed to MEDII prior to being placed in conditions that promote embryoid body (EB) formation. Hematopoietic activity was measured by clonogenic assays, flow cytometry, quantitative real-time polymerase chain reaction of specific transcript complementary DNAs and the ability of cells to repopulate sublethally irradiated nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice for almost 1 year. RESULTS: Exposure of both hESCs and hiPSCs to MEDII induced a rapid and preferential differentiation of hESCs into mesodermal elements. Subsequently produced EBs showed a further enhanced expression of transcripts characteristic of multiple mesodermal lineages, and a concurrent decrease in endodermal and ectodermal cell transcripts. Frequency of all types of clonogenic hematopoietic progenitors in subsequently derived EBs was also increased. In vivo assays of MEDII-treated hESC-derived EBs also showed they contained cells able to undertake low-level but longterm multilineage repopulation of primary and secondary nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice. CONCLUSIONS: MEDII treatment of hESCs and hiPSCs alike selectively enhances their differentiation into mesodermal cells and allows subsequent generation of detectable levels of hematopoietic progenitors with in vitro and in vivo differentiating activity.

Lu, S., et al. (2010). "Engineered heart tissue graft derived from somatic cell nuclear transferred embryonic stem cells improve myocardial performance in infarcted rat heart." J Cell Mol Med **14**(12): 2771-2779.

The concept of regenerating diseased myocardium by implanting engineered heart tissue (EHT) is intriguing. Yet it was limited by immune rejection and difficulties to be generated at a size with contractile properties. Somatic cell nuclear transfer is proposed as a practical strategy for generating autologous histocompatible stem (nuclear transferred embryonic stem [NT-ES]) cells to treat diseases. Nevertheless, it is controversial as NT-ES cells may pose risks in their therapeutic application. EHT from NT-ES cell-derived cardiomyocytes was generated through a series of improved techniques in a self-made mould to keep the EHTs from contraction and provide static stretch simultaneously. After 7 days of static and mechanical stretching, respectively, the EHTs were implanted to the infarcted rat heart. Four weeks after transplantation, the suitability of EHT in heart muscle repair after myocardial infarction was evaluated by histological examination, echocardiography and multielectrode array measurement. The results showed that large (thickness/diameter, 2-4 mm/10 mm) spontaneously contracting EHTs was generated successfully. The EHTs, which were derived from NT-ES cells, inte grated and electrically coupled to host myocardium and exerted beneficial effects on the left ventricular function of infarcted rat heart. No teratoma formation was observed in the rat heart implanted with EHTs for 4 weeks. NT-ES cells can be used as a source of seeding cells for cardiac tissue engineering. Large contractile EHT grafts can be constructed in vitro with the ability to survive after implantation and improve myocardial performance of infarcted rat hearts.

Lu, S., et al. (2010). "Both the transplantation of somatic cell nuclear transfer- and fertilization-derived mouse embryonic stem cells with temperature-responsive chitosan hydrogel improve myocardial performance in infarcted rat hearts." Tissue Eng Part A **16**(4): 1303-1315.

The transplantation of embryonic stem cells could improve cardiac function but was limited by

immune rejection as well as low cell retention and survival within the ischemic tissues. The somatic cell nuclear transfer (SCNT) is practical to generate autologous histocompatible stem (nuclear-transferred embryonic stem [NTES]) cells for diseases, but NTES may be arguably unsafe for therapeutic application. The temperature-responsive chitosan hydrogel is a suitable matrix in cell transplantation. As the scaffold, chitosan hydrogel was coinjected with NTES cells into the left ventricular wall of rat infarction models. Detailed histological analysis and echocardiography were performed to determine the structure and functional consequences of transplantation. The myocardial performance in SCNT- and fertilization-derived mouse ES cell transplantation with chitosan hydrogel was also compared. The results showed that both the 24-h cell retention and 4-week graft size were significantly greater in the NTES + chitosan group than that of NTES + phosphate-buffered saline (PBS) group ($p < 0.01$). The NTES cells might differentiate into cardiomyocytes in vivo. The heart function improved significantly in the chitosan + NTES group (fractional shortening: 28.7% +/- 2.8%) compared with that of PBS + NTES group (fractional shortening: 25.2% +/- 2.9%) at 4 weeks after transplantation ($p < 0.01$). In addition, the arteriole/venule densities within the infarcted area improved significantly in the chitosan + NTES group (280 +/- 17/mm(2)) compared with that of PBS + NTES group (234 +/- 16/mm(2)) at 4 weeks after transplantation ($p < 0.01$). There was no difference in the myocardial performance in SCNT- and fertilization-derived mouse ES cell transplantation with chitosan hydrogel. The NTES cells with chitosan hydrogel have been proved to possess therapeutic potential to improve the function of infarcted heart. Thus the method of in situ injectable tissue engineering is promising clinically.

Ma, D. K., et al. (2008). "G9a and Jhdm2a regulate embryonic stem cell fusion-induced reprogramming of adult neural stem cells." Stem Cells **26**(8): 2131-2141.

Somatic nuclei can be reprogrammed to pluripotency through fusion with embryonic stem cells (ESCs). The underlying mechanism is largely unknown, primarily because of a lack of effective approaches to monitor and quantitatively analyze transient, early reprogramming events. The transcription factor Oct4 is expressed specifically in pluripotent stem cells, and its reactivation from somatic cell genome constitutes a hallmark for effective reprogramming. Here we developed a double fluorescent reporter system using engineered ESCs and adult neural stem cells/progenitors (NSCs) to simultaneously and independently monitor cell fusion and reprogramming-induced reactivation of transgenic Oct4-enhanced green fluorescent protein (EGFP) expression. We demonstrate that knockdown of a histone methyltransferase, G9a, or overexpression of a histone demethylase, Jhdm2a, promotes ESC fusion-induced Oct4-EGFP reactivation from adult NSCs. In addition, coexpression of Nanog and Jhdm2a further enhances the ESC-induced Oct4-EGFP reactivation. Interestingly, knockdown of G9a alone in adult NSCs leads to demethylation of the Oct4 promoter and partial reactivation of the endogenous Oct4 expression from adult NSCs. Our results suggest that ESC-induced reprogramming of somatic cells occurs with coordinated actions between erasure of somatic epigenome and transcriptional resetting to restore pluripotency. These mechanistic findings may guide more efficient reprogramming for future therapeutic applications of stem cells. Disclosure of potential conflicts of interest is found at the end of this article.

Ma, J., et al. (2007). "Treatment of hypoxic-ischemic encephalopathy in mouse by transplantation of embryonic stem cell-derived cells." Neurochem Int **51**(1): 57-65.

A 7-day-old hypoxic-ischemic encephalopathy (HIE) mouse model was used to study the effect of transplantation of embryonic stem (ES) cell-derived cells on the HIE. After the inducement in vitro, the ES cell-derived cells expressed Nestin and MAP-2, rather than GFAP mRNA. After transplantation, ES cell-derived cells can survive, migrate into the injury site, and specifically differentiate into neurons, showing improvement of the learning ability and memory of the HIE mouse at 8 months post-transplantation. The non-grafted HIE mouse brain showed typical pathological changes in the hippocampus and cerebral cortex, where the number of neurons was reduced, while in the cell graft group, number of the neurons increased in the same regions. Although further study is necessary to elucidate the precise mechanisms responsible for this functional recovery, we believe that ES cells have advantages for use as a donor source in HIE.

Ma, M., et al. (2010). "Major histocompatibility complex-I expression on embryonic stem cell-derived vascular progenitor cells is critical for syngeneic transplant survival." Stem Cells **28**(9): 1465-1475.

Donor-recipient cell interactions are essential for functional engraftment after nonautologous cell transplantation. During this process, transplant engraftment is characterized and defined by interactions between transplanted cells with local and recruited inflammatory cells. The outcome of these interactions determines donor cell fate. Here, we provide evidence that lineage-committed embryonic stem cell (ESC)-derived vascular progenitor cells are the target of major histocompatibility complex (MHC)

class I-dependent, natural killer (NK) cell-mediated elimination in vitro and in vivo. Treatment with interferon gamma was found to significantly upregulate MHC class I expression on ESC-derived vascular progenitor cells, rendering them less susceptible to syngeneic NK cell-mediated killing in vitro and enhancing their survival and differentiation potential in vivo. Furthermore, in vivo ablation of NK cells led to enhanced progenitor cell survival after transplantation into a syngeneic murine ischemic hindlimb model, providing additional evidence that NK cells mediate ESC-derived progenitor cell transplant rejection. These data highlight the importance of recipient immune-donor cell interactions, and indicate a functional role for MHC-I antigen expression during successful ESC-derived syngeneic transplant engraftment.

Ma, W., et al. (2008). "Cell-extracellular matrix interactions regulate neural differentiation of human embryonic stem cells." BMC Dev Biol **8**: 90.

BACKGROUND: Interactions of cells with the extracellular matrix (ECM) are critical for the establishment and maintenance of stem cell self-renewal and differentiation. However, the ECM is a complex mixture of matrix molecules; little is known about the role of ECM components in human embryonic stem cell (hESC) differentiation into neural progenitors and neurons. RESULTS: A reproducible protocol was used to generate highly homogenous neural progenitors or a mixed population of neural progenitors and neurons from hESCs. This defined adherent culture system allowed us to examine the effect of ECM molecules on neural differentiation of hESCs. hESC-derived differentiating embryoid bodies were plated on Poly-D-Lysine (PDL), PDL/fibronectin, PDL/laminin, type I collagen and Matrigel, and cultured in neural differentiation medium. We found that the five substrates instructed neural progenitors followed by neuronal differentiation to differing degrees. Glia did not appear until 4 weeks later. Neural progenitor and neuronal generation and neurite outgrowth were significantly greater on laminin and laminin-rich Matrigel substrates than on other 3 substrates. Laminin stimulated hESC-derived neural progenitor expansion and neurite outgrowth in a dose-dependent manner. The laminin-induced neural progenitor expansion was partially blocked by the antibody against integrin alpha6 or beta1 subunit. CONCLUSION: We defined laminin as a key ECM molecule to enhance neural progenitor generation, expansion and differentiation into neurons from hESCs. The cell-laminin interactions involve alpha6beta1 integrin receptors implicating a possible role of laminin/alpha6beta1 integrin signaling in directed neural differentiation of hESCs. Since laminin acts in concert with other ECM molecules in vivo, evaluating cellular responses to the composition of the ECM is essential to clarify further the role of cell-matrix interactions in neural derivation of hESCs.

Ma, X., et al. (2014). "Human amniotic fluid stem cells support undifferentiated propagation and pluripotency of human embryonic stem cell without b-FGF in a density dependent manner." Int J Clin Exp Pathol **7**(8): 4661-4673.

Human embryonic stem cells (hESCs) are pluripotent cells which can give rise to almost all adult cell lineages. Culture system of hESCs is complex, requiring exogenous b-FGF and feeder cell layer. Human mesenchymal stem cells (MSCs) not only synthesize soluble cytokines or factors such as b-FGF, but also provide other mechanism which might play positive role on sustaining hESCs propagation and pluripotency. Human amniotic fluid stem (AFS) cells, which share characteristics of both embryonic and adult stem cells, have been regarded as promising cells for regenerative medicine. Taking advantage by AFS cells, we studied the ability of AFS cells in supporting undifferentiated propagation and pluripotency of Chinese population derived X-01 hESCs. Human AF-type amniotic fluid stem cells (hAF-AFSCs) transcribed genes including Activin A, TGF-beta1, Noggin and b-FGF, which involved in maintaining pluripotency and self-renewal of hESCs. Compared to mouse embryonic fibroblasts (MEFs), hAF-AFSCs secreted higher concentration of b-FGF which was important in hESCs culture ($P < 0.05$). The hESCs were propagated more than 30 passages on hAF-AFSCs layer with exogenous b-FGF supplementation, keeping undifferentiated status. While exogenous b-FGF was obviated, propagation of hESCs with undifferentiated status was dependent on density of hAF-AFSC feeder layer. Lower density of hAF-AFSCs resulted in rapid decline in undifferentiated clone number, while higher ones hindered the growth of colonies. The most appropriate hAF-AFSCs feeder density to maintain the X-01 hESC line without exogenous b-FGF was 15-20x10(4)/well. To the best of our knowledge, this is the first study demonstrating that hAF-AFSCs could support undifferentiated propagation and pluripotency of Chinese population derived hESCs without exogenous b-FGF supplementation.

Maass, K., et al. (2015). "Isolation and characterization of embryonic stem cell-derived cardiac Purkinje cells." Stem Cells **33**(4): 1102-1112.

The cardiac Purkinje fiber network is composed of highly specialized cardiomyocytes responsible for the synchronous excitation and contraction of the ventricles. Computational modeling, experimental animal studies, and intracardiac electrical

recordings from patients with heritable and acquired forms of heart disease suggest that Purkinje cells (PCs) may also serve as critical triggers of life-threatening arrhythmias. Nonetheless, owing to the difficulty in isolating and studying this rare population of cells, the precise role of PC in arrhythmogenesis and the underlying molecular mechanisms responsible for their proarrhythmic behavior are not fully characterized. Conceptually, a stem cell-based model system might facilitate studies of PC-dependent arrhythmia mechanisms and serve as a platform to test novel therapeutics. Here, we describe the generation of murine embryonic stem cells (ESC) harboring pan-cardiomyocyte and PC-specific reporter genes. We demonstrate that the dual reporter gene strategy may be used to identify and isolate the rare ESC-derived PC (ESC-PC) from a mixed population of cardiogenic cells. ESC-PC display transcriptional signatures and functional properties, including action potentials, intracellular calcium cycling, and chronotropic behavior comparable to endogenous PC. Our results suggest that stem-cell derived PC are a feasible new platform for studies of developmental biology, disease pathogenesis, and screening for novel antiarrhythmic therapies.

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.

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.

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.

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.

Rodriguez-Gomez, J. A., et al. (2012). "T-type Ca2+ channels in mouse embryonic stem cells: modulation during cell cycle and contribution to self-renewal." Am J Physiol Cell Physiol **302**(3): C494-504.

Ion channels participate in cell homeostasis and are involved in the regulation of proliferation and differentiation in several cell types; however, their presence and function in embryonic stem (ES) cells are poorly studied. We have investigated the existence of voltage-dependent inward currents in mouse ES cells and their ability to modulate proliferation and self-renewal. Patch-clamped ES cells had inactivating tetrodotoxin (TTX)-sensitive Na(+) currents as well as transient Ca(2+) currents abolished by the external application of Ni(2+). Biophysical and pharmacological data indicated that the Ca(2+) current is predominantly mediated by T-type (Ca(v)3.2) channels. The number of cells expressing T-type channels and Ca(v)3.2 mRNA levels increased at the G1/S transition of the cell cycle. TTX had no effect on ES cell proliferation. However, blockade of T-type Ca(2+) currents with Ni(2+) induced a decrease in proliferation and alkaline phosphatase positive colonies as well as reduced expression of Oct3/4 and Nanog, all indicative of loss in self-renewal capacity. Decreased alkaline phosphatase and Oct3/4 expression were also observed in cells subjected to small interfering RNA-induced knockdown for T-type (Ca(v)3.2) Ca(2+) channels, thus partially recapitulating the pharmacological effects on self-renewal. These results indicate that Ca(v)3.2 channel expression in ES cells is modulated along the cell cycle being induced at late G1 phase. They also suggest that these channels are involved in the maintenance of the undifferentiated state of mouse ES cells. We propose that Ca(2+) entry mediated by Ca(v)3.2 channels might be one of the intracellular signals that participate in the complex network responsible for ES cell self-renewal.

Rohwedel, J., et al. (1994). "Muscle cell differentiation of embryonic stem cells reflects myogenesis in vivo: developmentally regulated expression of myogenic determination genes and functional expression of ionic currents." Dev Biol **164**(1): 87-101.

The mouse blastocyst-derived embryonic stem cell (ES cell) line BLC6 efficiently differentiates into myosin heavy chain-, desmin- and myogenin-positive skeletal muscle cells when cultivated in embryo-like aggregates (embryoid bodies). Here, we show that the muscle-specific determination genes myf5, myogenin, myoD, and myf6 are expressed in these embryoid bodies in a characteristic temporal pattern which precisely reflects the sequence observed during mouse development in vivo. Myf5 is the first gene to be expressed followed by myogenin, myoD, and myf6, in this order. In situ hybridization demonstrates transcripts for myogenin and myoD accumulating in mono- and multinucleated myogenic cells, while myf5 mRNA is already found in mononucleated myoblasts. The myocytes also express functional nicotinic cholinoceptors and exhibit T-type Ca2+ currents and later L-type Ca2+ currents, demonstrating physiological properties of skeletal muscle cells. During myocyte differentiation the density of L-type Ca2+ channels significantly increases while the density of T-type Ca2+ channels decreases. The effect of external signals on myogenic differentiation of BLC6 cells was demonstrated by cocultivation with visceral endodermal END-2 cells and the activin A-secreting WEHI-3 cells. END-2 cells essentially prevent skeletal muscle differentiation, whereas basic fibroblast growth factor, transforming growth factor-beta, and WEHI-3 cells have no or an attenuating effect, respectively. Our results suggest that ES cells recapitulate closely the early steps of muscle development in vivo and may serve as an excellent in vitro system to study this process.

Rohwedel, J., et al. (1996). "Primordial germ cell-derived mouse embryonic germ (EG) cells in vitro resemble undifferentiated stem cells with respect to differentiation capacity and cell cycle distribution." Cell Biol Int **20**(8): 579-587.

Embryonic germ (EG) cells of line EG-1 derived from mouse primordial germ cells were investigated for their in vitro differentiation capacity. By cultivation as embryo-like aggregates EG-1 cells differentiated into cardiac, skeletal muscle and neuronal cells accompanied by the expression of tissue-specific genes and proteins as shown by RT-PCR analysis and indirect immunofluorescence. In comparison to embryonic stem (ES) cells of line D3 the efficiency of differentiation into cardiac and muscle cells was comparatively low, whereas spontaneous neuronal differentiation was more efficient than in D3 cells. Furthermore, the distribution of cell cycle phases as a parameter for the differentiation state was analysed in undifferentiated EG cells and ES cells and compared to data obtained for embryonic carcinoma (EC) cells of line P19 and differentiated, epithelioid EPI-7 cells. Flow cytometric analysis revealed similar cell cycle phase distributions in EG, EC and ES cells. In contrast,

the somatic differentiated EPI-7 cells showed a longer G1-phase and shorter S- and G2/M-phases. Together, our results demonstrate that the differentiation state and capacity of EG cells in vitro resemble that of totipotent ES cells.

Rojas-Mayorquin, A. E., et al. (2008). "Microarray analysis of striatal embryonic stem cells induced to differentiate by ensheathing cell conditioned media." Dev Dyn **237**(4): 979-994.

The mammalian central nervous system contains well-defined regions of plasticity in which cells of the aldynoglia phenotype promote neuronal growth and regeneration. Only now are the factors that regulate the production of new cells from multipotential neural precursors (MNP) starting to be identified. We are interested in understanding how differentiation towards the aldynoglia phenotype is controlled, and to study these events we have induced the differentiation of embryonic MNP towards this phenotype in vitro. Accordingly, we have used microarrays to analyze gene expression in three different cell populations: olfactory bulb ensheathing cells (EC), a prototypic aldynoglia cell type; undifferentiated MNP; and MNP differentiated in vitro for 24 hr in EC-conditioned media. The expression profiles identified support the idea that the EC are more closely related to Schwann cells and astrocytes than to oligodendrocytes. Following MNP differentiation, more strongly expressed genes define a neuroglial cell phenotype. RT-PCR confirms that S100a6, Mtmr2, and Col5a were highly expressed by EC, whereas Pou3f3 were more strongly expressed in MNP than in EC, and SafB1 and Mash1 expression were induced in MNP by EC-conditioned media. The profile of gene expression after differentiation suggests that Wnt signaling may be inactivated during this process, while activation of the BMP pathway may be elicited through the BMPr1A. These results provide us with a starting point to study the genes involved in the induction of aldynoglia differentiation from MNP.

Rolletschek, A., et al. (2004). "Embryonic stem cell-derived cardiac, neuronal and pancreatic cells as model systems to study toxicological effects." Toxicol Lett **149**(1-3): 361-369.

Primary cultures or established cell lines of vertebrates are commonly used to analyse the cytotoxic potential of chemical factors, drugs and xenobiotics in vitro. An alternative approach will be provided by permanent lines of pluripotent embryonic stem (ES) cells, which are able to differentiate into specialised somatic cell types in vitro. Here, we demonstrate the capacity of ES cells to generate functional cardiac, neuronal and pancreatic cells. We show that during ES cell differentiation, tissue-specific genes, proteins as well as functional properties are expressed in a developmentally regulated manner recapitulating processes of early embryonic development. We present data that show the use of ES-derived cardiomyocytes and dopaminergic neurons in toxicological studies and the potential of ES-derived pancreatic beta-like cells in future in vitro assays. The application of these differentiation systems to human ES cells opens up new perspectives in basic and applied toxicology.

Romorini, L., et al. (2013). "Effect of antibiotics against Mycoplasma sp. on human embryonic stem cells undifferentiated status, pluripotency, cell viability and growth." PLoS One **8**(7): e70267.

Human embryonic stem cells (hESCs) are self-renewing pluripotent cells that can differentiate into specialized cells and hold great promise as models for human development and disease studies, cell-replacement therapies, drug discovery and in vitro cytotoxicity tests. The culture and differentiation of these cells are both complex and expensive, so it is essential to extreme aseptic conditions. hESCs are susceptible to Mycoplasma sp. infection, which is hard to detect and alters stem cell-associated properties. The purpose of this work was to evaluate the efficacy and cytotoxic effect of Plasmocin(TM) and ciprofloxacin (specific antibiotics used for Mycoplasma sp. eradication) on hESCs. Mycoplasma sp. infected HUES-5 884 (H5 884, stable hESCs H5-brachyury promoter-GFP line) cells were effectively cured with a 14 days Plasmocin(TM) 25 microg/ml treatment (curative treatment) while maintaining stemness characteristic features. Furthermore, cured H5 884 cells exhibit the same karyotype as the parental H5 line and expressed GFP, through up-regulation of brachyury promoter, at day 4 of differentiation onset. Moreover, H5 cells treated with ciprofloxacin 10 microg/ml for 14 days (mimic of curative treatment) and H5 and WA09 (H9) hESCs treated with Plasmocin(TM) 5 microg/ml (prophylactic treatment) for 5 passages retained hESCs features, as judged by the expression of stemness-related genes (TRA1-60, TRA1-81, SSEA-4, Oct-4, Nanog) at mRNA and protein levels. In addition, the presence of specific markers of the three germ layers (brachyury, Nkx2.5 and cTnT: mesoderm; AFP: endoderm; nestin and Pax-6: ectoderm) was verified in in vitro differentiated antibiotic-treated hESCs. In conclusion, we found that Plasmocin(TM) and ciprofloxacin do not affect hESCs stemness and pluripotency nor cell viability. However, curative treatments slightly diminished cell growth rate. This cytotoxic effect was reversible as cells regained normal growth rate upon antibiotic withdrawal.

Ronaghi, M., et al. (2010). "Challenges of stem cell therapy for spinal cord injury: human embryonic stem

cells, endogenous neural stem cells, or induced pluripotent stem cells?" Stem Cells **28**(1): 93-99.

Spinal cord injury (SCI) causes myelopathy, damage to white matter, and myelinated fiber tracts that carry sensation and motor signals to and from the brain. The gray matter damage causes segmental losses of interneurons and motoneurons and restricts therapeutic options. Recent advances in stem cell biology, neural injury, and repair, and the progress toward development of neuroprotective and regenerative interventions are the basis for increased optimism. This review summarizes the pathophysiological mechanisms following SCI and compares human embryonic, adult neural, and the induced pluripotent stem cell-based therapeutic strategies for SCI.

Ronaghi, M., et al. (2014). "Inner ear hair cell-like cells from human embryonic stem cells." Stem Cells Dev **23**(11): 1275-1284.

In mammals, the permanence of many forms of hearing loss is the result of the inner ear's inability to replace lost sensory hair cells. Here, we apply a differentiation strategy to guide human embryonic stem cells (hESCs) into cells of the otic lineage using chemically defined attached-substrate conditions. The generation of human otic progenitor cells was dependent on fibroblast growth factor (FGF) signaling, and protracted culture led to the upregulation of markers indicative of differentiated inner ear sensory epithelia. Using a transgenic ESC reporter line based on a murine Atoh1 enhancer, we show that differentiated hair cell-like cells express multiple hair cell markers simultaneously. Hair cell-like cells displayed protrusions reminiscent of stereociliary bundles, but failed to fully mature into cells with typical hair cell cytoarchitecture. We conclude that optimized defined conditions can be used in vitro to attain otic progenitor specification and sensory cell differentiation.

Ronay, V., et al. (2014). "Expression of embryonic stem cell markers and osteogenic differentiation potential in cells derived from periodontal granulation tissue." Cell Biol Int **38**(2): 179-186.

The aim of this study was to identify if cells obtained from periodontal granulation tissue possess embryonic stem cell properties and osteogenic capacities in vitro. Periodontal granulation tissue was removed from one furcation and one infrabony defect (FGTC/IGTC-furcation/infrabony defect derived granulation tissue cells) of six patients. The extracted tissues were treated with collagenase/dispase solution, cultured and passaged twice, while a fraction of them was bacteriologically analyzed. Upon reaching confluence, total RNA was extracted, followed by cDNA synthesis and real-time PCR analysis. Gene expression levels of collagen type I, alkaline phosphatase (ALP), and the embryonic stem cell markers Nanog, Oct-4, Rex-1 and Sox-2 were measured, calibrated against the housekeeping gene GAPDH. Further, osteogenic differentiation was induced. Mineralized matrix formation was confirmed by von Kossa staining, and ALP activity was measured colorimetrically. The total bacterial load amounted to 9.4 +/- 14.6 x 10(6) counts/mg of tissue for IGTC, and 11.1 +/- 6.1 x 10(6) counts/of tissue for FGTC. Among the embryonic stem cell markers (FGTC/IGTC), Nanog was most highly expressed (3.48 +/- 1.2/5.85 +/- 5.7), followed by Oct-4 (1.79 +/- 0.69/2.85 +/- 2.5), Sox-2 (0.66 +/- 0.3/1.26 +/- 1.4) and Rex-1 (0.06 +/- 0.0/0.04 +/- 0.0). The osteogenic differentiation process was positive in both FGTC and IGTC, judged by increased von Kossa staining, and elevated ALP activity and gene expression. This study provides evidence that infected periodontal granulation tissue harbors cells expressing embryonic stem cell markers, and exhibiting osteogenic capacities when in culture in vitro.

Ronay, V., et al. (2013). "Infected periodontal granulation tissue contains cells expressing embryonic stem cell markers. A pilot study." Schweiz Monatsschr Zahnmed **123**(1): 12-16.

The commonly practiced removal of granulation tissue during periodontal surgery, aiming to eliminate infection and optimize healing conditions, may also remove progenitor stem cells that could otherwise support periodontal regeneration. The present study aimed to investigate if cells with embryonic stem cell properties are present in periodontal granulation tissue. During the course of flap surgery inflammatory granulation tissue was obtained from four patients and five periodontal defects. Tissues were processed in a collagenase/dispase solution to release the cells. Part of the resulting suspension was processed for bacteriological analysis (IAI PadoTest 4.5), whereas the remaining cell suspension was cultured and passaged once. Upon reaching confluence, total RNA was extracted, followed by cDNA synthesis. PCR was then performed (SYBR Green-based protocols) to measure gene expression levels of Collagen type I, and embryonic stem cell markers Nanog, Oct4, Rex-1 and Sox2. Results are expressed as 2(-)Delta(Ct) values of the target gene, calibrated against a house-keeping gene (GAPDH). A high total bacterial load up to 20.6 +/- 11.0x10(6) counts/mg of tissue was found. Collagen type I was strongly expressed, confirming the predominance of mesenchymal/fibroblastic cells. Among the studied embryonic stem cells markers, Nanog was most highly expressed (2.3 +/- 1.2),

followed by Oct4 (1.1 +/- 0.5), Rex-1 (0.6 +/- 0.2) and Sox2 (0.3 +/- 0.2). This is the first study that demonstrates the presence of cells expressing embryonic stem cell markers among infected granulation tissue. This knowledge needs to be considered when devising future strategies to improve periodontal wound healing and regeneration.

Saito, M. and H. Matsuoka (2010). "Semi-quantitative analysis of transient single-cell gene expression in embryonic stem cells by femtoinjection." Methods Mol Biol **650**: 155-170.

Single-cell manipulation supporting robot (SMSR) has enabled femtoinjection, a high-throughput and semi-quantitative microinjection in the range of femtogram (fg) DNA and other molecules. An enhanced green fluorescent protein (EGFP) gene expression vector can be introduced directly into mouse embryonic stem cells at 100 cells/h with a 10% success rate. The intensity of EGFP fluorescence in single ES cells or single colonies of ES cells increases as the concentration of an EGFP gene expression vector in the injection capillary increases from 1 to 50 ng/muL. On the other hand, the knockdown of EGFP gene expression can be demonstrated by femtoinjection of siRNA against EGFP or an shRNA expression vector using an EGFP expressing ES cell line. Femotoinjection can provide a useful method for quantitative analysis of transient gene expression in single cells using RNAi.

Sakaguchi, T., et al. (2006). "Putative "stemness" gene jam-B is not required for maintenance of stem cell state in embryonic, neural, or hematopoietic stem cells." Mol Cell Biol **26**(17): 6557-6570.

Many genes have been identified that are specifically expressed in multiple types of stem cells in their undifferentiated state. It is generally assumed that at least some of these putative "stemness" genes are involved in maintaining properties that are common to all stem cells. We compared gene expression profiles between undifferentiated and differentiated embryonic stem cells (ESCs) using DNA microarrays. We identified several genes with much greater signal in undifferentiated ESCs than in their differentiated derivatives, among them the putative stemness gene encoding junctional adhesion molecule B (Jam-B gene). However, in spite of the specific expression in undifferentiated ESCs, Jam-B mutant ESCs had normal morphology and pluripotency. Furthermore, Jam-B homozygous mutant mice are fertile and have no overt developmental defects. Moreover, we found that neural and hematopoietic stem cells recovered from Jam-B mutant mice are not impaired in their ability to self-renew and differentiate. These results demonstrate that Jam-B is dispensable for normal mouse development and stem cell identity in embryonic, neural, and hematopoietic stem cells.

Salguero-Aranda, C., et al. (2016). "Differentiation of Mouse Embryonic Stem Cells toward Functional Pancreatic beta-Cell Surrogates through Epigenetic Regulation of Pdx1 by Nitric Oxide." Cell Transplant **25**(10): 1879-1892.

Pancreatic and duodenal homeobox 1 (Pdx1) is a transcription factor that regulates the embryonic development of the pancreas and the differentiation toward beta cells. Previously, we have shown that exposure of mouse embryonic stem cells (mESCs) to high concentrations of diethylenetriamine nitric oxide adduct (DETA-NO) triggers differentiation events and promotes the expression of Pdx1. Here we report evidence that Pdx1 expression is associated with release of polycomb repressive complex 2 (PRC2) and P300 from its promoter region. These events are accompanied by epigenetic changes in bivalent markers of histones trimethylated histone H3 lysine 27 (H3K27me3) and H3K4me3, site-specific changes in DNA methylation, and no change in H3 acetylation. On the basis of these findings, we developed a protocol to differentiate mESCs toward insulin-producing cells consisting of sequential exposure to DETA-NO, valproic acid, and P300 inhibitor (C646) to enhance Pdx1 expression and a final maturation step of culture in suspension to form cell aggregates. This small molecule-based protocol succeeds in obtaining cells that express pancreatic beta-cell markers such as PDX1, INS1, GCK, and GLUT2 and respond in vitro to high glucose and KCl.

Santolaya-Forgas, J., et al. (2007). "A study to determine if human umbilical cord hematopoietic stem cells can survive in baboon extra-embryonic celomic fluid: a prerequisite for determining the feasibility of in-utero stem cell xeno-transplantation via celocentesis." Fetal Diagn Ther **22**(2): 131-135.

OBJECTIVES: To determine if: (1) human umbilical cord stem cells could survive for 20 h in extra-embryonic celomic fluid obtained at 40 days of development from baboon pregnancies by ultrasound-guided celocentesis and, (2) human hematopoietic stem cell survival could be enhanced by adding increasing concentrations of hematopoietic stem cell culture medium to the celomic fluid. METHODS: CD34+ cells were isolated from umbilical cord blood using a magnetic activated cell sorter and flow cytometry. Cells were then cultured in five platforms containing different combinations of baboon extra-embryonic celomic fluid and hematopoietic stem cell culture medium to determine cell survival kinetics over a 20-hour period in each of the conditions. RESULTS: Human umbilical cord stem cells can survive for at

least 20 h in baboon's celomic fluid. Chi-square for linear trends demonstrated that the number of viable cells was significantly enhanced by adding the increasing concentrations of culture medium to the celomic fluid (29% cell survival in pure celomic fluid, 48% at 1:8, 50% at 1:2, 54.5% at 1:1, 61% in pure culture medium; $p < 0.001$). CONCLUSIONS: Human umbilical cord stem cells survive in baboon celomic fluid and cell survival improves when the celomic fluid is mixed with greater concentrations of hematopoietic stem cell culture medium. Based on these findings, future in-vivo experiments in the pregnant baboon animal model can be directed at determining whether in-utero injection of human hematopoietic stem cells prior to 10 weeks of pregnancy can lead to permanent chimeras.

Sauer, H., et al. (1998). "Spontaneous calcium oscillations in embryonic stem cell-derived primitive endodermal cells." Exp Cell Res **238**(1): 13-22.

In vitro differentiation of mouse embryonic stem cells within three-dimensional cell aggregates called embryoid bodies parallels the development of postimplantation embryos at the egg cylinder stage, where visceral and parietal endoderm diverge from the primitive endoderm. We have investigated spontaneous [Ca2+]i oscillations by means of confocal laser-scanning microscopy in primitive endodermal cell layers of embryoid bodies during their differentiation to parietal and visceral endoderm. The frequency of [Ca2+]i oscillations increased from day 4 to day 19 of development, whereas their duration decreased from day 3 to days 16-17. Oscillations depended on both extracellular Ca2+ and Ca2+ release from intracellular stores as they were abolished in Ca(2+)-free solution and in the prescence of Ni2+ and thapsigargin. Signal transduction operated via the phospholipase C (PLC)-mediated inositol 1,4,5-triphosphate (InsP3) pathway with a negative feedback loop via protein kinase C (PKC) as U73,122, a blocker of PLC; bisindolylmaleimide 1, staurosporine, and H-7, blockers of PKC; and 10 mM caffeine totally inhibited [Ca2+]i spiking. Thimerosal, which hypersensitizes the InsP3 receptor, as well as vasopressin and bradykinin, which act via the InsP3 pathway, increased the frequency of [Ca2+]i spikes. In the prescence of brefeldin A (50 microM) or monensin (20 microM), which both inhibit endo/exocytotic vesicle pathways, an immediate transient increase in spiking activity was followed by a decline within 1 to 2 h. In the presence of brefeldin A or thapsigargin or in the absence of extracellular Ca2+, endocytotic vesicles were absent, suggesting that oscillating [Ca2+]i transients are involved in the exo/endocytotic vesicle shuttle.

Schumacher, A., et al. (2003). "Staurosporine is a potent activator of neuronal, glial, and "CNS stem cell-like" neurosphere differentiation in murine embryonic stem cells." Mol Cell Neurosci **23**(4): 669-680.

Staurosporine (STS), a broad spectrum protein kinase inhibitor, was previously shown to induce neurite outgrowth in several neuroblastoma cell lines. However, data on the neurotrophic potential of this alkaloid in embryonic stem cell systems were not available. Therefore, three mouse ES cell lines, IB10, RW4, and Bruce 4, were induced to enter neurogenesis in culture at low concentrations of STS. These cells differentiated into epidermal growth factor-responsive neural precursor cells, formed neurospheres, and further developed to neurons and astrocytes. The clonally derived neurospheres consisted of multipotent cells which exhibited some of the classical characteristics of early CNS stem cells and could be propagated in vitro. STS was antagonistic in several ways to retinoic acid (RA), a vitamin A metabolite, which promotes neuritogenesis. Results from RT-PCR experiments and inhibition studies with RA provided evidence that staurosporine exerted its neurotrophic effects through the induction of very late levels of the nerve growth factor and protein kinase C neurogenesis pathways.

Scott, G. J., et al. (2018). "Trans-inner Cell Mass Injection of Embryonic Stem Cells Leads to Higher Chimerism Rates." J Vis Exp(135).

In an effort to increase efficiency in the creation of genetically modified mice via ES Cell methodologies, we present an adaptation to the current blastocyst injection protocol. Here we report that a simple rotation of the embryo, and injection through Trans-Inner cell mass (TICM) increased the percentage of chimeric mice from 31% to 50%, with no additional equipment or further specialized training. 26 different inbred clones, and 35 total clones were injected over a period of 9 months. There was no significant difference in either pregnancy rate or recovery rate of embryos between traditional injection techniques and TICM. Therefore, without any major alteration in the injection process and a simple positioning of the blastocyst and injecting through the ICM, releasing the ES cells into the blastocoel cavity can potentially improve the quantity of chimeric production and subsequent germline transmission.

Seiler, A., et al. (2002). "[Improving the embryonic stem cell test (EST) by establishing molecular endpoints of tissue specific development using murine embryonic stem cells (D3 cells)]." ALTEX **19 Suppl 1**: 55-63.

Blastocyst-derived pluripotent embryonic stem (ES) cells of the mouse can be induced to

differentiate in culture into a variety of cell types, including cardiac muscle cells. In the embryonic stem cell test (EST) the capacity of ES cells of the mouse cell line D3 to differentiate into contracting cardiomyocytes is used to assess the embryotoxic potential of test compounds and in addition, the effects on the viability of ES cells and differentiated mouse fibroblasts (cell line 3T3) are compared. The three endpoints are used to classify the embryotoxic potential of chemicals after 10 days of exposure: (i) the inhibition of differentiation of ES cells into cardiomyocytes (ID50) and (ii) the decrease of viability of 3T3 cells (IC503T3) and (iii) ES cells (IC50D3) in a MTT cytotoxicity test. Applying linear analysis of discriminance, a biostatistical prediction model (PM) was developed to assign test chemicals to three classes of embryotoxicity. In an international validation study funded by ECVAM it could be demonstrated that the EST can predict the embryotoxic potential of a test compound as good as frequently used mammalian systems based on pregnant animals. In a joint project with major German pharmaceutical companies we are attempting to improve the EST by establishing molecular endpoints of differentiation (e.g. cardiac, neuronal, chondrogenic) in cultured ES cells. We have studied the expression of tissue specific proteins in ES cell cultures in the presence of embryotoxic chemicals by immunofluorescent antibody techniques, e.g. FACS analysis. The other groups are focusing on endogenous gene expression in early development by RT-PCR methods or the DNA microarray technique. The results obtained recently using molecular markers specific for cardiac differentiation and employing intracellular flow cytometry for quantification will be presented. Molecular endpoints will allow improvement of the EST by measuring gene expression patterns in a small number of murine ES cells.

Seiler, A. E., et al. (2006). "Use of murine embryonic stem cells in embryotoxicity assays: the embryonic stem cell test." Methods Mol Biol **329**: 371-395.

The embryonic stem cell test (EST) takes advantage of the potential of murine embryonic stem (ES) cells to differentiate in culture to test embryotoxicity in vitro. The EST represents a scientifically validated in vitro system for the classification of compounds according to their teratogenic potential based on the morphological analysis of beating cardiomyocytes in embryoid body outgrowths compared to cytotoxic effects on murine ES cells and differentiated 3T3 fibroblasts. Through a number of prevalidation and validation studies, the EST has been demonstrated to be a reliable alternative method for embryotoxicity testing based on the most important mechanisms in embryotoxicity-cytotoxicity and differentiation--as well as on differences in sensitivity between differentiated and embryonic tissues. Improvements of the EST protocol using flow cytometry analysis showed that differential expression of sarcomeric myosin heavy chain and alpha-actinin proteins quantified under the influence of a test compound is a useful marker for detecting potential teratogenicity. The in vitro embryotoxicity test described in this chapter is rapid, simple, and sensitive and can be usefully employed as a component of the risk/hazard assessment process.

Semrau, S., et al. (2017). "Dynamics of lineage commitment revealed by single-cell transcriptomics of differentiating embryonic stem cells." Nat Commun **8**(1): 1096.

Gene expression heterogeneity in the pluripotent state of mouse embryonic stem cells (mESCs) has been increasingly well-characterized. In contrast, exit from pluripotency and lineage commitment have not been studied systematically at the single-cell level. Here we measure the gene expression dynamics of retinoic acid driven mESC differentiation from pluripotency to lineage commitment, using an unbiased single-cell transcriptomics approach. We find that the exit from pluripotency marks the start of a lineage transition as well as a transient phase of increased susceptibility to lineage specifying signals. Our study reveals several transcriptional signatures of this phase, including a sharp increase of gene expression variability and sequential expression of two classes of transcriptional regulators. In summary, we provide a comprehensive analysis of the exit from pluripotency and lineage commitment at the single cell level, a potential stepping stone to improved lineage manipulation through timing of differentiation cues.

Senju, S., et al. (2007). "Genetically manipulated human embryonic stem cell-derived dendritic cells with immune regulatory function." Stem Cells **25**(11): 2720-2729.

Genetically manipulated dendritic cells (DC) are considered to be a promising means for antigen-specific immune therapy. This study reports the generation, characterization, and genetic modification of DC derived from human embryonic stem (ES) cells. The human ES cell-derived DC (ES-DC) expressed surface molecules typically expressed by DC and had the capacities to stimulate allogeneic T lymphocytes and to process and present protein antigen in the context of histocompatibility leukocyte antigen (HLA) class II molecule. Genetic modification of human ES-DC can be accomplished without the use of viral vectors, by the introduction of expression vector plasmids into undifferentiated ES cells by electroporation and subsequent induction of

differentiation of the transfectant ES cell clones to ES-DC. ES-DC introduced with invariant chain-based antigen-presenting vectors by this procedure stimulated HLA-DR-restricted antigen-specific T cells in the absence of exogenous antigen. Forced expression of programmed death-1-ligand-1 in ES-DC resulted in the reduction of the proliferative response of allogeneic T cells cocultured with the ES-DC. Generation and genetic modification of ES-DC from nonhuman primate (cynomolgus monkey) ES cells was also achieved by the currently established method. ES-DC technology is therefore considered to be a novel means for immune therapy.

Serfozo, P., et al. (2006). "Selective migration of neuralized embryonic stem cells to stem cell factor and media conditioned by glioma cell lines." Cancer Cell Int **6**: 1.

BACKGROUND: Pluripotent mouse embryonic stem (ES) cells can be induced in vitro to become neural progenitors. Upon transplantation, neural progenitors migrate toward areas of damage and inflammation in the CNS. We tested whether undifferentiated and neuralized mouse ES cells migrate toward media conditioned by glioma cell lines (C6, U87 & N1321) or Stem Cell Factor (SCF). RESULTS: Cell migration assays revealed selective migration by neuralized ES cells to conditioned media as well as to synthetic SCF. Migration of undifferentiated ES cells was extensive, but not significantly different from that of controls (Unconditioned Medium). RT-PCR analysis revealed that all the three tumor cell lines tested synthesized SCF and that both undifferentiated and neuralized ES cells expressed c-kit, the receptor for SCF. CONCLUSION: Our results demonstrate that undifferentiated ES cells are highly mobile and that neural progenitors derived from ES cells are selectively attracted toward factors produced by gliomas. Given that the glioma cell lines synthesize SCF, SCF may be one of several factors that contribute to the selective migration observed.

Serrano, L., et al. (2011). "Homologous recombination conserves DNA sequence integrity throughout the cell cycle in embryonic stem cells." Stem Cells Dev **20**(2): 363-374.

The maintenance of genomic integrity is crucial to embryonic stem cells (ESC) considering the potential for propagating undesirable mutations to the resulting somatic and germ cell lineages. Indeed, mouse ESC (mESC) exhibit a significantly lower mutation frequency compared to differentiated cells. This could be due to more effective elimination of genetically damaged cells via apoptosis, or especially robust, sequence-conserving DNA damage repair mechanisms such as homologous recombination (HR). We used fluorescence microscopy and 3-dimensional image analysis to compare mESC and differentiated cells, with regard to HR-mediated repair of spontaneous and X-ray-induced double-strand breaks (DSBs). Microscopic analysis of repair foci, flow cytometry, and functional assays of the major DSB repair pathways indicate that HR is greater in mESC compared to fibroblasts. Strikingly, HR appears to be the predominant pathway choice to repair induced or spontaneous DNA damage throughout the ESC cycle in contrast to fibroblasts, where it is restricted to replicated chromatin. This suggests that alternative templates, such as homologous chromosomes, are more frequently used to repair DSB in ESC. Relatively frequent HR utilizing homolog chromosome sequences preserves genome integrity in ESC and has distinctive and important genetic consequences to subsequent somatic and germ cell lineages.

Shah, S. M., et al. (2015). "RETRACTED: Bone morphogenetic protein 4 (BMP4) induces buffalo (Bubalus bubalis) embryonic stem cell differentiation into germ cells." Biochimie **119**: 113-124.

This article has been retracted: please see Elsevier Policy on Article Withdrawal (http://www.elsevier.com/locate/withdrawalpolicy). This article has been retracted at the request of the Editor-in-Chief. Problems related to images published in this paper in Figure 12 were brought to the authors' attention. Unfortunately this figure contains duplicate images for ESC controls for VASA, GDF9, and ZP4, which display identical patterns superimposed on varying intensities of background. Therefore, the authors retract the paper with the agreement of the editors and deeply regret this situation and apologize for any inconvenience to the editors and readers of Biochimie.

Shah, S. M., et al. (2017). "Cumulus cell-conditioned medium supports embryonic stem cell differentiation to germ cell-like cells." Reprod Fertil Dev **29**(4): 679-693.

Cumulus cells provide cellular interactions and growth factors required for oogenesis. In vitro studies of oogenesis are limited primarily because of the paucity of their source, first trimester fetal gonads, and the small number of germ lineage precursor cells present within these tissues. In order to understand this obscure but vitally important process, the present study was designed to direct differentiation of embryonic stem (ES) cells into germ lineage cells. For this purpose, buffalo ES cells were differentiated, as embryoid bodies (EBs) and monolayer adherent cultures, in the presence of different concentrations of cumulus-conditioned medium (CCM; 10%, 20% and 40%) for different periods of culture (4, 8 and 14 days) to identify the optimum differentiation-inducing

concentration and time. Quantitative polymerase chain reaction analysis revealed that 20%-40% CCM induced the highest expression of primordial germ cell-specific (deleted in Azoospermia- like (Dazl), dead (Asp-Glu-Ala-Asp) box polypeptide 4 (Vasa also known as DDX4) and promyelocytic leukemia zinc finger protein (Plzf)); meiotic (synaptonemal complex protein 3 (Sycp3), mutl homolog I (Mlh1), transition protein 1/2 (Tnp1/2) and protamine 2 (Prm2); spermatocyte-specific boule-like RNA binding protein (Boule) and tektin 1 (Tekt1)) and oocyte-specific growth differentiation factor 9 (Gdf9) and zona pellucida 2 /3 (Zp2/3)) genes over 8-14 days in culture. Immunocytochemical analysis revealed expression of primordial germ cell (c-KIT, DAZL and VASA), meiotic (SYCP3, MLH1 and PROTAMINE 1), spermatocyte (ACROSIN and HAPRIN) and oocyte (GDF9 and ZP4) markers in both EBs and monolayer differentiation cultures. Western blotting revealed germ lineage-specific protein expression in Day 14 EBs. The significantly lower ($P<0.05$) concentration of 5-methyl-2-deoxycytidine in differentiated EBs compared to undifferentiated EBs suggests that methylation erasure may have occurred. Oocyte-like structures obtained in monolayer differentiation stained positive for ZONA PELLUCIDA protein 4 and progressed through various embryo-like developmental stages in extended cultures.

Shah, S. M., et al. (2016). "Testicular cell-conditioned medium supports embryonic stem cell differentiation toward germ lineage and to spermatocyte- and oocyte-like cells." Theriogenology **86**(3): 715-729.

Testicular cells are believed to secrete various growth factors that activate signaling pathways finally leading to gametogenesis. In vitro gametogenesis is an obscure but paramountly important task primarily because of paucity of the precursor cells and first trimester gonadal tissues. To overcome these limitations for development of in vitro gametes, the present study was designed to induce differentiation of buffalo embryonic stem (ES) cells into germ lineage cells on stimulation by testicular cell-conditioned medium (TCM), on the basis of the assumption that ES cells have the intrinsic property to differentiate into any cell type and TCM would provide the necessary growth factors for differentiation toward germ cell lineage. For this purpose, buffalo ES cells were differentiated as embryoid bodies (EB) in floating cultures and as monolayer adherent cultures in different doses (10%, 20%, and 40%) of TCM for different culture intervals (4, 8, and 14 days), to identify the optimum dose-and-time period. We observed that 40% TCM dose induces highest expression of primordial germ cell-specific (DAZL, VASA, and PLZF), meiotic (SYCP3, MLH1, TNP1/2, and PRM2), spermatocyte-specific (BOULE and TEKT1), and oocyte-specific genes (GDF9 and ZP2/3) for a culture period of 14 days under both floating and adherent differentiation. Immunocytochemical analysis of EBs and adherent cultures revealed presence of primordial germ cell markers (c-KIT, DAZL, and VASA), meiotic markers (SYCP3, MLH1 and PROTAMINE1), spermatocyte markers (ACROSIN and HAPRIN), and oocyte markers (GDF9 and ZP4), indicating progression into post-meiotic gametogenesis. The detection of germ cell-specific proteins in Day 14 EBs like VASA, GDF9, and ZP4 by Western blotting further confirmed germ lineage differentiation. The significantly lower ($P < 0.05$) concentration of 5-methyl-2-deoxycytidine in optimally differentiated EBs is suggestive of the process of methylation erasure. Oocyte-like structures obtained in monolayer differentiation had a big nucleus and a surrounding ZP4 coat, the unique attributes of a female gamete. These oocyte-like structures, in extended cultures, showed embryonic development and progressed through two-cell, four-cell, eight-cell, morula, and blastocyst-like structures, indicative of their developmental competence. This, as per our knowledge, is first such study in higher mammals, especially farm animals, and assumes significance for its potential use in transgenic animal production, elite animal conservation and propagation, augmentation of reproductive performance in poor breeding buffalo species, and as a model for understanding human germ cell formation.

Shao, J., et al. (2017). "Experimental Study of the Biological Properties of Human Embryonic Stem Cell-Derived Retinal Progenitor Cells." Sci Rep **7**: 42363.

Retinal degenerative diseases are among the leading causes of blindness worldwide, and cell replacement is considered as a promising therapeutic. However, the resources of seed cells are scarce. To further explore this type of therapy, we adopted a culture system that could harvest a substantial quantity of retinal progenitor cells (RPCs) from human embryonic stem cells (hESCs) within a relatively short period of time. Furthermore, we transplanted these RPCs into the subretinal spaces of Royal College of Surgeons (RCS) rats. We quantified the thickness of the treated rats' outer nuclear layers (ONLs) and explored the visual function via electroretinography (ERG). It was found that the differentiated cells expressed RPC markers and photoreceptor progenitor markers. The transplanted RPCs survived for at least 12 weeks, resulting in beneficial effects on the morphology of the host retina, and led to a significant improvement in the visual function of the treated animals. These therapeutic effects suggest that the hESCs-derived RPCs could delay degeneration of the retina and partially restore visual function.

Shapira-Schweitzer, K., et al. (2009). "A photopolymerizable hydrogel for 3-D culture of human embryonic stem cell-derived cardiomyocytes and rat neonatal cardiac cells." J Mol Cell Cardiol **46**(2): 213-224.

The purpose of this study was to assess the in vitro ability of two types of cardiomyocytes (cardiomyocytes derived from human embryonic stem cells (hESC-CM) and rat neonatal cardiomyocytes (rN-CM)) to survive and generate a functional cardiac syncytium in a three-dimensional in situ polymerizable hydrogel environment. Each cell type was cultured in a PEGylated fibrinogen (PF) hydrogel for up to two weeks while maturation and cardiac function were documented in terms of spontaneous contractile behavior and biomolecular organization. Quantitative contractile parameters including contraction amplitude and synchronization were measured by non-invasive image analysis. The rN-CM demonstrated the fastest maturation and the most significant spontaneous contraction. The hESC-CM maturation occurred between 10-14 days in culture, and exhibited less contraction amplitude and synchronization in comparison to the rN-CMs. The maturation of both cell types within the hydrogels was confirmed by cardiac-specific biomolecular markers, including alpha-sarcomeric actin, actinin, and connexin-43. Cellular responsiveness to isoproterenol, carbamylcholine and heptanol provided further evidence of the cardiac maturation in the 3-D PF hydrogel as well as identified a potential to use this system for in vitro drug screening. These findings indicate that the PF hydrogel biomaterial can be used as an in situ polymerizable biomaterial for stem cells and their cardiomyocyte derivatives.

Sharma, A. D., et al. (2008). "Murine embryonic stem cell-derived hepatic progenitor cells engraft in recipient livers with limited capacity of liver tissue formation." Cell Transplant **17**(3): 313-323.

Directed endodermal differentiation of murine embryonic stem (ES) cells gives rise to a subset of cells with a hepatic phenotype. Such ES cell-derived hepatic progenitor cells (ES-HPC) can acquire features of hepatocytes in vitro, but fail to form substantial hepatocyte clusters in vivo. In this study, we investigated whether this is due to inefficient engraftment or an immature phenotype of ES-HPC. ES cells engrafted into recipient livers of NOD/SCID mice with a similar efficacy as adult hepatocytes after 28 days. Because transplanted unpurified ES-HPC formed teratomas in the spleen and liver, we applied an albumin promoter/enhancer-driven reporter system to purify ES-HPC by cell sorting. RT-PCR analyses for hepatocyte-specific genes showed that the cells exhibited a hepatic phenotype, lacking the expression of the pluripotency marker Oct4, comparable to cells of day 11.5 embryos. Sorted ES-HPC derived from beta-galactosidase transgenic ES cells were injected into fumaryl-acetoacetate-deficient (FAH(-/-)) SCID mice and analyzed after 8 to 12 weeks. Staining with X-gal solution revealed the presence of engrafted cells throughout the liver. However, immunostaining for the FAH protein indicated hepatocyte formation at a very low frequency, without evidence for large hepatocyte cluster formation. In conclusion, the limited repopulation capacity of ES-HPC is not caused by a failure of primary engraftment, but may be due to an immature hepatic phenotype of the transplanted ES-HPC.

Sharma, R., et al. (2013). "ROCK inhibitor Y-27632 enhances the survivability of dissociated buffalo (Bubalus bubalis) embryonic stem cell-like cells." Reprod Fertil Dev **25**(2): 446-455.

This study investigated the effects of supplementation of culture medium with 10 muM Y-27632, a specific inhibitor of Rho kinase activity, for 6 days on self-renewal of buffalo embryonic stem (ES) cell-like cells at Passage 50-80. Y-27632 increased mean colony area ($P<0.05$) although it did not improve their survival. It decreased OCT4 expression ($P<0.05$), increased NANOG expression ($P<0.05$), but had no effect on SOX2 expression. It also increased expression of anti-apoptotic gene BCL-2 ($P<0.05$) and decreased that of pro-apoptotic genes BAX and BID ($P<0.05$). It increased plating efficiency of single-cell suspensions of ES cells ($P<0.05$). Following vitrification, the presence of Y-27632 in the vitrification solution or thawing medium or both did not improve ES cell colony survival. However, following seeding of clumps of ES cells transfected with pAcGFP1N1 carrying green fluorescent protein (GFP), Y-27632 increased colony formation rate ($P<0.01$). ES cell colonies that formed in all Y-27632-supplemented groups were confirmed for expression of pluripotency markers alkaline phosphatase, SSEA-4 and TRA-1-60, and for their ability to generate embryoid bodies containing cells that expressed markers of ectoderm, mesoderm and endoderm. In conclusion, Y-27632 improves survival of buffalo ES cells under unfavourable conditions such as enzymatic dissociation to single cells or antibiotic-assisted selection after transfection, without compromising their pluripotency.

Sharma, R., et al. (2012). "Growth factor expression pattern of homologous feeder layer for culturing buffalo embryonic stem cell-like cells." Reprod Fertil Dev **24**(8): 1098-1104.

The present study examined the expression profile of buffalo fetal fibroblasts (BFF) used as a feeder layer for embryonic stem (ES) cell-like cells.

The expression of important growth factors was detected in cells at different passages. Mitomycin-C inactivation increased relative expression levels of ACTIVIN-A, TGF-beta1, BMP-4 and GREMLIN but not of fibroblast growth factor-2 (FGF-2). The expression level of ACTIVIN-A, transforming growth factor-beta1 (TGF-beta1), bone morphogenetic protein-4 (BMP-4) and FGF-2 was similar in buffalo fetal fibroblast (BFF) cultured in stem cell medium (SCM), SCM+1000IU mL(-1) leukemia inhibitory factor (LIF), SCM+5 ngmL(-1) FGF-2 or SCM+LIF+FGF-2 for 24 h whereas GREMLIN expression was higher in FGF-2-supplemented groups. In spent medium, the concentration of ACTIVIN-A was higher in FGF-2-supplemented groups whereas that of TGF-beta1 was similar in SCM and LIF+FGF-2, which was higher than when either LIF or FGF-2 was used alone. Following culture of ES cell-like cells on a feeder layer for 24 h, the TGF-beta1 concentration was higher with LIF+FGF-2 than with LIF or FGF-2 alone which, in turn, was higher than that in SCM. In the LIF+FGF-2 group, the concentration of TGF-beta1 was lower and that of ACTIVIN-A was higher in spent medium at 24 h than at 48 h of culture. These results suggest that BFF produce signalling molecules that may help in self-renewal of buffalo ES cell-like cells.

Sharma, R., et al. (2011). "Optimization of culture conditions to support long-term self-renewal of buffalo (Bubalus bubalis) embryonic stem cell-like cells." Cell Reprogram **13**(6): 539-549.

A culture system capable of sustaining self-renewal of buffalo embryonic stem (ES) cell-like cells in an undifferentiated state over a long period of time was developed. Inner cell masses were seeded on KO-DMEM+15% KO-serum replacer on buffalo fetal fibroblast feeder layer. Supplementation of culture medium with 5 ng/mL FGF-2 and 1000 IU/mL mLIF gave the highest ($p<0.05$) rate of primary colony formation. The ES cell-like cells' colony survival rate and increase in colony size were highest ($p<0.05$) following supplementation with FGF-2 and LIF compared to other groups examined. FGF-2 supplementation affected the quantitative expression of NANOG, SOX-2, ACTIVIN A, BMP 4, and TGFbeta1, but not OCT4 and GREMLIN. Supplementation with SU5402, an FGFR inhibitor (>/=20 muM) increased ($p<0.05$) the percentage of colonies that differentiated. FGFR1-3 and ERK1, K-RAS, E-RAS, and SHP-2, key signaling intermediates of FGF signaling, were detected in ES cell-like cells. Under culture conditions described, three ES cell lines were derived that, to date, have been maintained for 135, 95, and 85 passages for over 27, 19, and 17 months, respectively, whereas under other conditions examined, ES cell-like cells did not survive beyond passage 10. The ES cell-like cells were regularly monitored for expression of pluripotency markers and their potency to form embryoid bodies.

Shaykhiev, R., et al. (2013). "Airway basal cells of healthy smokers express an embryonic stem cell signature relevant to lung cancer." Stem Cells **31**(9): 1992-2002.

Activation of the human embryonic stem cell (hESC) signature genes has been observed in various epithelial cancers. In this study, we found that the hESC signature is selectively induced in the airway basal stem/progenitor cell population of healthy smokers (BC-S), with a pattern similar to that activated in all major types of human lung cancer. We further identified a subset of 6 BC-S hESC genes, whose coherent overexpression in lung adenocarcinoma (AdCa) was associated with reduced lung function, poorer differentiation grade, more advanced tumor stage, remarkably shorter survival, and higher frequency of TP53 mutations. BC-S shared with hESC and a considerable subset of lung carcinomas a common TP53 inactivation molecular pattern which strongly correlated with the BC-S hESC gene expression. These data provide transcriptome-based evidence that smoking-induced reprogramming of airway BC toward the hESC-like phenotype might represent a common early molecular event in the development of aggressive lung carcinomas in humans.

Shen, S. C., et al. (2014). "Susceptibility of human embryonic stem cell-derived neural cells to Japanese encephalitis virus infection." PLoS One **9**(12): e114990.

Pluripotent human embryonic stem cells (hESCs) can be efficiently directed to become immature neuroepithelial precursor cells (NPCs) and functional mature neural cells, including neurotransmitter-secreting neurons and glial cells. Investigating the susceptibility of these hESCs-derived neural cells to neurotrophic viruses, such as Japanese encephalitis virus (JEV), provides insight into the viral cell tropism in the infected human brain. We demonstrate that hESC-derived NPCs are highly vulnerable to JEV infection at a low multiplicity of infection (MOI). In addition, glial fibrillary acid protein (GFAP)-expressing glial cells are also susceptible to JEV infection. In contrast, only a few mature neurons were infected at MOI 10 or higher on the third day post-infection. In addition, functional neurotransmitter-secreting neurons are also resistant to JEV infection at high MOI. Moreover, we discover that vimentin intermediate filament, reported as a putative neurovirulent JEV receptor, is highly expressed in NPCs and glial cells, but not mature neurons. These results indicate that the expression of vimentin in neural cells correlates to the cell tropism of JEV.

Finally, we further demonstrate that membranous vimentin is necessary for the susceptibility of hESC-derived NPCs to JEV infection.

Shetty, D. K. and M. S. Inamdar (2016). "Generation of a transgenic human embryonic stem cell line ectopically expressing the endosomal protein Asrij that regulates pluripotency in mouse embryonic stem cells: BJNhem20-Asrij." Stem Cell Res **16**(2): 331-333.

Asrij is an endocytic protein expressed in mouse embryonic stem cells (mESCs) and is essential for maintenance of stemness of mESCs (Mukhopadhyay et al., 2003; Sinha et al., 2013). Its human ortholog named Ovarian Carcinoma Immuno-reactive Antigen domain containing protein 1 (OCIAD1) is 85% identical. We ectopically expressed Asrij in epiblast stage equivalent-human embryonic stem cells (hESCs) to test for induction of naive pluripotency in primed pluripotent cells. The construct pCAG-Asrij was introduced into hESCs by microporation. Ectopic expression of Asrij in BJNhem20 hESC line was performed by selecting for plasmid transfection, followed by stable cell line generation.

Shi, C., et al. (2011). "Derivation and characterization of Chinese human embryonic stem cell line with high potential to differentiate into pancreatic and hepatic cells." Chin Med J (Engl) **124**(7): 1037-1043.

BACKGROUND: Human embryonic stem cells have prospective uses in regenerative medicine and drug screening. Every human embryonic stem cell line has its own genetic background, which determines its specific ability for differentiation as well as susceptibility to drugs. It is necessary to compile many human embryonic stem cell lines with various backgrounds for future clinical use, especially in China due to its large population. This study contributes to isolating new Chinese human embryonic stem cell lines with clarified directly differentiation ability. METHODS: Donated embryos that exceeded clinical use in our in vitro fertilization-embryo transfer (IVF-ET) center were collected to establish human embryonic stem cells lines with informed consent. The classic growth factors of basic fibroblast growth factor (bFGF) and recombinant human leukaemia inhibitory factor (hLIF) for culturing embryonic stem cells were used to capture the stem cells from the plated embryos. Mechanical and enzymetic methods were used to propagate the newly established human embryonic stem cells line. The new cell line was checked for pluripotent characteristics with detecting the expression of stemness genes and observing spontaneous differentiation both in vitro and in vivo. Finally similar step-wise protocols from definitive endoderm to target specific cells were used to check the cell line's ability to directly differentiate into pancreatic and hepatic cells. RESULTS: We generated a new Chinese human embryonic stem cells line, CH1. This cell line showed the same characteristics as other reported Chinese human embryonic stem cells lines: normal morphology, karyotype and pluripotency in vitro and in vivo. The CH1 cells could be directly differentiated towards pancreatic and hepatic cells with equal efficiency compared to the H1 cell line. CONCLUSIONS: This newly established Chinese cell line, CH1, which is pluripotent and has high potential to differentiate into pancreatic and hepatic cells, will provide a useful tool for embryo development research, along with clinical treatments for diabetes and some hepatic diseases.

Shinoyama, M., et al. (2013). "Cortical region-specific engraftment of embryonic stem cell-derived neural progenitor cells restores axonal sprouting to a subcortical target and achieves motor functional recovery in a mouse model of neonatal hypoxic-ischemic brain injury." Front Cell Neurosci **7**: 128.

Hypoxic-ischemic encephalopathy (HIE) at birth could cause cerebral palsy (CP), mental retardation, and epilepsy, which last throughout the individual's lifetime. However, few restorative treatments for ischemic tissue are currently available. Cell replacement therapy offers the potential to rescue brain damage caused by HI and to restore motor function. In the present study, we evaluated the ability of embryonic stem cell-derived neural progenitor cells (ES-NPCs) to become cortical deep layer neurons, to restore the neural network, and to repair brain damage in an HIE mouse model. ES cells stably expressing the reporter gene GFP are induced to a neural precursor state by stromal cell co-culture. Forty-hours after the induction of HIE, animals were grafted with ES-NPCs targeting the deep layer of the motor cortex in the ischemic brain. Motor function was evaluated 3 weeks after transplantation. Immunohistochemistry and neuroanatomical tracing with GFP were used to analyze neuronal differentiation and axonal sprouting. ES-NPCs could differentiate to cortical neurons with pyramidal morphology and expressed the deep layer-specific marker, Ctip2. The graft showed good survival and an appropriate innervation pattern via axonal sprouting from engrafted cells in the ischemic brain. The motor functions of the transplanted HIE mice also improved significantly compared to the sham-transplanted group. These findings suggest that cortical region specific engraftment of preconditioned cortical precursor cells could support motor functional recovery in the HIE model. It is not clear whether this is a direct effect of the engrafted cells or due to neurotrophic factors produced by these cells. These results suggest that cortical region-specific NPC engraftment is a promising therapeutic approach for brain repair.

Shirasawa, S., et al. (2011). "Pancreatic exocrine enzyme-producing cell differentiation via embryoid bodies from human embryonic stem cells." Biochem Biophys Res Commun **410**(3): 608-613.

Mouse embryonic stem cells (ESCs) can be induced to form pancreatic exocrine enzyme-producing cells in vitro in a stepwise fashion that recapitulates the development in vivo. However, there is no protocol for the differentiation of pancreatic-like cells from human ESCs (hESCs). Based upon the mouse ESC model, we have induced the in vitro formation of pancreatic exocrine enzyme-producing cells from hESCs. The protocol took place in four stages. In Stage 1, embryoid bodies (EBs) were formed from dissociated hESCs and then treated with the growth factor activin A, which promoted the expression of Foxa2 and Sox17 mRNAs, markers of definitive endoderm. In Stage 2, the cells were treated with all-trans retinoic acid which promoted the transition to cells that expressed gut tube endoderm mRNA marker HNF1b. In Stage 3, the cells were treated with fibroblast growth factor 7 (FGF7), which induced expression of Pdx1 typical of pancreatic progenitor cells. In Stage 4, treatment with FGF7, glucagon-like peptide 1, and nicotinamide induced the expression amylase (AMY) mRNA, a marker for mature pancreatic exocrine cells. Immunohistochemical staining showed the expression of AMY protein at the edges of cell clusters. These cells also expressed other exocrine secretory proteins including elastase, carboxypeptidase A, chymotrypsin, and pancreatic lipase in culture. Production of these hESC-derived pancreatic enzyme-producing cells represents a critical step in the study of pancreatic organogenesis and in the development of a renewable source of human pancreatic-like exocrine cells.

Simonstein, F. (2008). "Embryonic stem cells: the disagreement debate and embryonic stem cell research in Israel." J Med Ethics **34**(10): 732-734.

While some people claim that the present disagreement over embryonic stem (ES) cell research cannot be resolved, others argue that developing transparency and trust are key elements that could resolve the existing disagreements over such research. This paper reveals that transparency is not necessarily a requirement for advancing ES cell research, since in Israel, for instance, there is (almost) no transparency, and research nevertheless flourishes. Moreover, trust is not independent of cultural values and religious beliefs. Because of these beliefs, the environment in Israel for ES cell research has been pragmatic and liberal. The Israeli case illustrates the key role that culture and religion can play in biomedical research; it also suggests that as far as cultural values or religious beliefs of people in Western countries strongly oppose research on embryonic tissue, it would be very difficult, if not impossible, to overcome the disagreements.

Simpson, D. L., et al. (2012). "Use of human embryonic stem cell derived-mesenchymal cells for cardiac repair." Biotechnol Bioeng **109**(1): 274-283.

Human mesenchymal stem cells (hMSC) have proven beneficial in the repair and preservation of infarcted myocardium. Unfortunately, MSCs represent a small portion of the bone marrow and require ex vivo expansion. To further advance the clinical usefulness of cellular cardiomyoplasty, derivation of "MSC-like" cells that can be made available "off-the-shelf" are desirable. Recently, human embryonic stem cell-derived mesenchymal cells (hESC-MC) were described. We investigated the efficacy of hESC-MC for cardiac repair after myocardial infarction (MI) compared to hMSC. Because of increased efficacy of cell delivery, cells were embedded into collagen patches and delivered to infarcted myocardium. Culture of hMSC and hESC-MCs in collagen patches did not induce differentiation or significant loss in viability. Transplantation of hMSC and hES-MC patches onto infarcted myocardium of athymic nude rats prevented adverse changes in infarct wall thickness and fractional area change compared to a non-viable patch control. Hemodynamic assessment showed that hMSCs and hES-MC patch application improved end diastolic pressure equivalently. There were no changes in systolic function. hES-MC and hMSC construct application enhanced neovessel formation compared to a non-viable control, and each cell type had similar efficacy in stimulating endothelial cell growth in vitro. In summary, the use of hES-MC provides similar efficacy for cellular cardiomyoplasty as compared to hMSC and may be considered a suitable alternative for cell therapy.

Singh, H., et al. (2010). "Up-scaling single cell-inoculated suspension culture of human embryonic stem cells." Stem Cell Res **4**(3): 165-179.

We have systematically developed single cell-inoculated suspension cultures of human embryonic stem cells (hESC) in defined media. Cell survival was dependent on hESC re-aggregation. In the presence of the Rho kinase inhibitor Y-27632 (Ri) only approximately 44% of the seeded cells were rescued, but an optimized heat shock treatment combined with Ri significantly increased cell survival to approximately 60%. Mechanistically, our data suggest that E-cadherin plays a role in hESC aggregation and that dissociation and re-aggregation upon passaging functions as a purification step towards a pluripotency markers-enriched population. Mass expansion of hESC was readily achieved by up-scaling 2 ml cultures to serial passaging in 50 ml spinner flasks. A media

comparison revealed that mTeSR was superior to KnockOut-SR in supporting cell proliferation and pluripotency. Persistent expression of pluripotency markers was achieved for two lines (hES2, hES3) that were used at higher passages (>86). In contrast, rapid down regulation of Oct4, Tra-1-60, and SSEA4 was observed for ESI049, a clinically compliant line, used at passages 20-36. The up-scaling strategy has significant potential to provide pluripotent cells on a clinical scale. Nevertheless, our data also highlights a significant line-to-line variability and the need for a critical assessment of novel methods with numerous relevant cell lines.

Singh, N., et al. (2012). "Cloning and characterization of buffalo NANOG gene: alternative transcription start sites, splicing, and polyadenylation in embryonic stem cell-like cells." DNA Cell Biol **31**(5): 721-731.

NANOG is a critical homeodomain transcription factor responsible for maintaining embryonic stem cell (ESC) self-renewal and pluripotency. In the present study, we isolated, sequenced, and characterized the NANOG gene in buffalo ESC-like cells. Here, we demonstrated that NANOG mRNA is expressed as multiple isoforms and uses four alternative transcriptional start sites (TSSs) and five different polyadenylation sites. The TSSs identified by 5'-RNA ligase-mediated rapid amplification of cDNA ends (RLM-5'-RACE) were positioned at 182, 95, 35, and 17 nucleotides upstream relative to the translation initiation codon. 3'-RACE experiment revealed the presence of tandem polyadenylation signals, which leads to the expression of at least five different 3'-untranslated regions (269, 314, 560, 566, and 829 nucleotides). Expression analysis showed that these alternatively polyadenylated transcripts expressed differentially. Sequence analysis showed that the open reading frame of buffalo NANOG codes for a 300-amino-acid-long protein. Further, results showed that alternative splicing leads to the expression of two types of transcript variants encoded by four and five exons. In silico analysis of cloned 5'-flanking region (3366 nucleotides upstream of translation start codon) identified several putative transcription factors binding sites in addition to a TATA box and CAAT box at -30 and -139 bp (upstream to the distal most TSS), respectively, in the buffalo NANOG promoter.

Singh, S. A., et al. (2014). "p53-Independent cell cycle and erythroid differentiation defects in murine embryonic stem cells haploinsufficient for Diamond Blackfan anemia-proteins: RPS19 versus RPL5." PLoS One **9**(2): e89098.

Diamond Blackfan anemia (DBA) is a rare inherited bone marrow failure syndrome caused by ribosomal protein haploinsufficiency. DBA exhibits marked phenotypic variability, commonly presenting with erythroid hypoplasia, less consistently with non-erythroid features. The p53 pathway, activated by abortive ribosome assembly, is hypothesized to contribute to the erythroid failure of DBA. We studied murine embryonic stem (ES) cell lines harboring a gene trap mutation in a ribosomal protein gene, either Rps19 or Rpl5. Both mutants exhibited ribosomal protein haploinsufficiency and polysome defects. Rps19 mutant ES cells showed significant increase in p53 protein expression, however, there was no similar increase in the Rpl5 mutant cells. Embryoid body formation was diminished in both mutants but nonspecifically rescued by knockdown of p53. When embryoid bodies were further differentiated to primitive erythroid colonies, both mutants exhibited a marked reduction in colony formation, which was again nonspecifically rescued by p53 inhibition. Cell cycle analyses were normal in Rps19 mutant ES cells, but there was a significant delay in the G2/M phase in the Rpl5 mutant cells, which was unaffected by p53 knockdown. Concordantly, Rpl5 mutant ES cells had a more pronounced growth defect in liquid culture compared to the Rps19 mutant cells. We conclude that the defects in our RPS19 and RPL5 haploinsufficient mouse ES cells are not adequately explained by p53 stabilization, as p53 knockdown appears to increase the growth and differentiation potential of both parental and mutant cells. Our studies demonstrate that gene trap mouse ES cells are useful tools to study the pathogenesis of DBA.

Singla, D. K., et al. (2006). "Transplantation of embryonic stem cells into the infarcted mouse heart: formation of multiple cell types." J Mol Cell Cardiol **40**(1): 195-200.

Initial studies have suggested that transplantation of embryonic stem (ES) cells following myocardial infarction (MI) in animal models is beneficial; however, the mechanism of benefit is largely unknown. The present study investigated the fate of mouse ES cells transplanted post-MI to determine if the ES cells give rise to the range of major cell types present in the native myocardium. MI was produced by coronary artery ligation in C57BL/6 mice. Two different mouse ES cell lines, expressing eGFP and beta-galactosidase, respectively, were tested. Post-MI intramyocardial injection of 3 x 10(4) ES cells was compared to injection of media alone. Histochemistry and immunofluorescence were used to track the transplanted ES cells and identify the resulting cell types. Echocardiography assessed the cardiac size and function in a blinded fashion. Two weeks post-MI, engraftment of the transplanted ES cells was demonstrated by eGFP or beta-galactosidase-positive

cells in the infarct region without evidence for tumor formation. Co-immunolabeling demonstrated that the transplanted ES cells had become cardiomyocytes, vascular smooth muscle, and endothelial cells. Echocardiographic analysis showed that ES cell transplantation resulted in reduced post-MI remodeling of the heart and improved cardiac function. In conclusion, transplanted mouse ES cells can regenerate infarcted myocardium in part by becoming cardiomyocytes, vascular smooth muscle, and endothelial cells that result in an improvement in cardiac structure and function. Therefore, ES cells hold promise for myocardial cellular therapy.

Tirotta, E., et al. (2012). "IFN-gamma-induced apoptosis of human embryonic stem cell derived oligodendrocyte progenitor cells is restricted by CXCR2 signaling." Stem Cell Res **9**(3): 208-217.

Engraftment of human embryonic stem cell (hESC)-derived OPCs in animal models of demyelination results in remyelination and clinical recovery, supporting the feasibility of cell replacement therapies in promoting repair of damaged neural tissue. A critical gap in our understanding of the mechanisms associated with repair revolves around the effects of the local microenvironment on transplanted cell survival. We have determined that treatment of human ESC-derived OPCs with the pleiotropic cytokine IFN-gamma promotes apoptosis that is associated with mitochondrial cytochrome c released into the cytosol with subsequent caspase 3 activation. IFN-gamma-induced apoptosis is mediated, in part, by secretion of the CXC chemokine ligand 10 (CXCL10) from IFN-gamma-treated cells. Signaling through the chemokine receptor CXCR2 by the ligand CXCL1 functions in a tonic manner by muting apoptosis and this is associated with reduced levels of cytosolic cytochrome c and impaired cleavage of caspase 3. These findings support a role for both IFN-gamma and CXCL10 in contributing to neuropathology by promoting OPC apoptosis. In addition, these data suggest that hOPCs used for therapeutic treatment for human neurologic disease/damage are susceptible to death through exposure to local inflammatory cytokines present within the inflammatory milieu.

Toh, W. S., et al. (2010). "Cartilage repair using hyaluronan hydrogel-encapsulated human embryonic stem cell-derived chondrogenic cells." Biomaterials **31**(27): 6968-6980.

Human embryonic stem cells (hESCs) have the potential to offer a virtually unlimited source of chondrogenic cells for use in cartilage repair and regeneration. We have recently shown that expandable chondrogenic cells can be derived from hESCs under selective growth factor-responsive conditions. In this study, we explore the potential of these hESC-derived chondrogenic cells to produce an extracellular matrix (ECM)-enriched cartilaginous tissue construct when cultured in hyaluronic acid (HA)-based hydrogel, and further investigated the long-term reparative ability of the resulting hESC-derived chondrogenic cell-engineered cartilage (HCCEC) in an osteochondral defect model. We hypothesized that HCCEC can provide a functional template capable of undergoing orderly remodeling during the repair of critical-sized osteochondral defects (1.5 mm in diameter, 1 mm depth into the subchondral bone) in a rat model. In the process of repair, we observed an orderly spatial-temporal remodeling of HCCEC over 12 weeks into osteochondral tissue, with characteristic architectural features including a hyaline-like neocartilage layer with good surface regularity and complete integration with the adjacent host cartilage and a regenerated subchondral bone. By 12 weeks, the HCCEC-regenerated osteochondral tissue resembled closely that of age-matched unoperated native control, while only fibrous tissue filled in the control defects which left empty or treated with hydrogel alone. Here we demonstrate that transplanted hESC-derived chondrogenic cells maintain long-term viability with no evidence of tumorigenicity, providing a safe, highly-efficient and practical strategy of applying hESCs for cartilage tissue engineering.

Tong, W., et al. (2007). "Human Embryonic Stem Cells Undergo Osteogenic Differentiation in Human Bone Marrow Stromal Cell Microenvironments." J Stem Cells **2**(3): 139-147.

Human embryonic stem cells (hESCs) may offer an unlimited supply of cells that can be directed to differentiate into all cell types within the body and used in regenerative medicine for tissue and cell replacement therapies. Previous work has shown that exposing hESCs to exogenous factors such as dexamethasone, ascorbic acid and beta-glycerophosphate can induce osteogenesis. The specific factors that induce osteogenic differentiation of hESCs have not been identified yet, however, it is possible that differentiated human bone marrow stromal cells (hMBSCs) may secrete factors within the local microenvironment that promote osteogenesis. Here we report that the lineage progression of hESCs to osteoblasts is achieved in the presence of soluble signaling factors derived from differentiated hBMSCs. For 28 days, hESCs were grown in a transwell co-culture system with hBMSCs that had been previously differentiated in growth medium containing defined osteogenic supplements for 7-24 days. As a control. hESCs were co-cultured with undifferentiated hBMSCs and alone. Von Kossa and Alizarin Red staining as well as immunohistochemistry confirmed that the hESCs

co-cultured with differentiated hBMSCs formed mineralized bone nodules and secreted extracellular matrix protein osteocalcin (OCN). Quantitative Alizarin Red assays showed increased mineralization as compared to the control with undifferentiated hBMSCs. RT-PCR revealed the loss of pluripotent hESC markers with the concomitant gain of osteoblastic markers such as collagen type I, runx2, and osterix. We demonstrate that osteogenic growth factors derived from differentiated hBMSCs within the local microenvironment may help to promote hESC osteogenic differentiation.

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.

Tropepe, V., et al. (2001). "Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism." Neuron **30**(1): 65-78.

Little is known about how neural stem cells are formed initially during development. We investigated whether a default mechanism of neural specification could regulate acquisition of neural stem cell identity directly from embryonic stem (ES) cells. ES cells cultured in defined, low-density conditions readily acquire a neural identity. We characterize a novel primitive neural stem cell as a component of neural lineage specification that is negatively regulated by TGFbeta-related signaling. Primitive neural stem cells have distinct growth factor requirements, express neural precursor markers, generate neurons and glia in vitro, and have neural and non-neural lineage potential in vivo. These results are consistent with a default mechanism for neural fate specification and support a model whereby definitive neural stem cell formation is preceded by a primitive neural stem cell stage during neural lineage commitment.

Trott, J. and A. Martinez Arias (2013). "Single cell lineage analysis of mouse embryonic stem cells at the exit from pluripotency." Biol Open **2**(10): 1049-1056.

Understanding how interactions between extracellular signalling pathways and transcription factor networks influence cellular decision making will be crucial for understanding mammalian embryogenesis and for generating specialised cell types in vitro. To this end, pluripotent mouse Embryonic Stem (mES) cells have proven to be a useful model system. However, understanding how transcription factors and signalling pathways affect decisions made by individual cells is confounded by the fact that measurements are generally made on groups of cells, whilst individual mES cells differentiate at different rates and towards different lineages, even in conditions that favour a particular lineage. Here we have used single-cell measurements of transcription factor expression and Wnt/beta-catenin signalling activity to investigate their effects on lineage commitment decisions made by individual cells. We find that pluripotent mES cells exhibit differing degrees of heterogeneity in their expression of important regulators from pluripotency, depending on the signalling environment to which they are exposed. As mES cells differentiate, downregulation of Nanog and Oct4 primes cells for neural commitment, whilst loss of Sox2 expression primes cells for primitive streak commitment. Furthermore, we find that Wnt signalling acts through Nanog to direct cells towards a primitive streak fate, but that transcriptionally active beta-catenin is associated with both neural and primitive streak commitment. These observations confirm and extend previous suggestions that pluripotency genes influence lineage commitment and demonstrate how their dynamic expression affects the direction of lineage commitment, whilst illustrating two ways in which the Wnt signalling pathway acts on this network during cell fate assignment.

Trounson, A. (2002). "Human embryonic stem cells: mother of all cell and tissue types." Reprod Biomed Online **4 Suppl 1**: 58-63.

Pluripotential embryonic stem (ES) cells have been derived very efficiently from spare human embryos produced by IVF and grown in culture to the nascent blastocyst stage. The inner cell mass (ICM) is isolated by immunosurgery and grown on selected embryonic fibroblast monolayer cultures. ICM cells lose their memory for axis during formation of ES cell colonies and are then unable to integrate tissue formation with a body plan. ES cells form teratomas in vivo with cells and tissues representative of the three major embryonic lineages (ectoderm, mesoderm, endoderm). The ES cells are continuously renewable and can be directed to differentiate into early progenitors of neural stem cells (Noggin cells) and from there into mature neurons and glia (astrocytes and oligodendrocytes). The neural stem cells formed from human ES cells repopulate the brains of newborn mice when injected into the lateral cerebral ventricles, forming astrocytes dominantly in the parenchyma. The human neural cells can be observed migrating from the subventricular areas along the rostral migratory stream. Human neurons can be found in the olfactory bulb. Human ES cells can also be directed into cardiomyocytes when co-cultured with visceral endoderm-like cells (END-2). These observations provide further scope to explore stem cell therapies, gene therapies and drug discovery. For compatible transplantation, ES may need to be derived with a range of HLA types or by nuclear transplantation or stem cell fusion.

Troy, T. C. and K. Turksen (2005). "Commitment of embryonic stem cells to an epidermal cell fate and differentiation in vitro." Dev Dyn **232**(2): 293-300.

The epidermis develops from a stem cell population in the surface ectoderm that feeds a single vertical terminal differentiation pathway. To date, however, the limited capacity for the isolation or purification of epidermal stem or precursor cells has hampered studies on early commitment and differentiation events. We have developed a two-step culture scheme in which pluripotent mouse embryonic stem (ES) cells are induced first to a surface ectoderm phenotype and then are positively selected for putative epidermal stem cells. We show that the earliest stages of epidermal development follow an ordered sequence that is similar to that observed in vivo (expression of keratin 8, keratin 19, keratin 17, and keratin 14), suggesting that ES cell-derived surface ectoderm-like cells can be induced to follow the epidermal developmental pathway. At a low frequency, keratin 14-positive early epidermal cells progressed to keratin 1-positive and terminally differentiated cells producing a cornified envelope. This culturing protocol provides an invaluable system in which to study both the mechanisms that direct stem cells along the epidermal pathway as well as those that influence their subsequent epidermal differentiation.

Tsai, M., et al. (2002). "Mast cells derived from embryonic stem cells: a model system for studying the effects of genetic manipulations on mast cell development, phenotype, and function in vitro and in vivo." Int J Hematol **75**(4): 345-349.

Large quantities of highly enriched populations of mast cells can be generated from mouse embryonic stem (ES) cells using an in vitro differentiation system. These embryonic stem cell-derived mast cells (ESMCs) exhibit many similarities to mouse bone marrow-derived cultured mast cells (BMCMCs), including the abilities to survive and to orchestrate immunologically specific immunoglobulin E (IgE)-dependent reactions in vivo after transplantation into genetically mast cell-deficient KitW/KitW-v mice. Coupled with the current spectrum of techniques for genetically manipulating ES cells, ESMCs represent a unique model system to analyze the effects of specific alterations in gene structure, expression, or function, including embryonic lethal mutations, on mast cell development, phenotype, and function in vitro and in vivo.

Tsai, Z. Y., et al. (2011). "Proteomic comparison of human embryonic stem cells with their differentiated fibroblasts: Identification of 206 genes targeted by hES cell-specific microRNAs." Kaohsiung J Med Sci **27**(8): 299-306.

Human embryonic stem (hES)-T3 (T3ES) cells were spontaneously differentiated into autogeneic fibroblast-like T3DF cells, as feeder cells with the capacity to support the growth of undifferentiated hES cells. The proteomes of undifferentiated T3ES cells and their differentiated T3DF fibroblasts were quantitatively compared. Several heterogeneous nuclear ribonucleoproteins and glycolytic enzymes, including l-lactate dehydrogenase A (M), were found to be abundantly and differentially expressed in T3ES cells and T3DF fibroblasts, respectively. Both miRNA and mRNA profiles from the undifferentiated T3ES cells and their differentiated T3DF fibroblasts had been previously determined. In this investigation, 206 genes were found to be targets of the four hES cell-specific miRNAs of miR-302d, miR-372, miR-200c, and/or miR-367 by using two-fold differential expression and inverse expression levels (highly negative correlations) of miRNAs to their target mRNAs. That YWHAZ (14-3-3 zeta) is a target of miR-302d and miR-372 was further confirmed by proteomic comparison between T3ES cells and their differentiated T3DF fibroblasts.

According to GeneOntology analyses, almost 50% of these 206 target proteins are nuclear and are involved in gene transcription. Identifying the target mRNAs of hES cell-specific miRNAs will provide a better understanding of the complex regulatory networks in hES cells. Furthermore, these miRNA-targeted proteins play important roles in differentiation of hES cells and during embryo development.

Tsang, K. M., et al. (2017). "Embryonic Stem Cell Differentiation to Functional Arterial Endothelial Cells through Sequential Activation of ETV2 and NOTCH1 Signaling by HIF1alpha." Stem Cell Reports **9**(3): 796-806.

The generation of functional arterial endothelial cells (aECs) from embryonic stem cells (ESCs) holds great promise for vascular tissue engineering. However, the mechanisms underlying their generation and the potential of aECs in revascularizing ischemic tissue are not fully understood. Here, we observed that hypoxia exposure of mouse ESCs induced an initial phase of HIF1alpha-mediated upregulation of the transcription factor Etv2, which in turn induced the commitment to the EC fate. However, sustained activation of HIF1alpha in these EC progenitors thereafter induced NOTCH1 signaling that promoted the transition to aEC fate. We observed that transplantation of aECs mediated arteriogenesis in the mouse hindlimb ischemia model. Furthermore, transplantation of aECs in mice showed engraftment in ischemic myocardium and restored cardiac function in contrast to ECs derived under normoxia. Thus, HIF1alpha activation of Etv2 in ESCs followed by NOTCH1 signaling is required for the generation aECs that are capable of arteriogenesis and revascularization of ischemic tissue.

Wakao, H., et al. (2008). "In vitro induction of natural killer T cells from embryonic stem cells prepared using somatic cell nuclear transfer." FASEB J **22**(7): 2223-2231.

The ectopic expression of the Notch receptor ligand delta-like 1 on stromal cells allows the induction of T cells from embryonic stem cells (ESCs). However, these in vitro-generated T cells are not transplantable because they are too immature to mount an immune response in an immunocompromised animal. We efficiently generated a subset of T cells called invariant natural killer T (iNKT) cells from ESCs derived from peripheral iNKT cells using somatic cell nuclear transfer (ntESCs). These iNKT cells matured autonomously in vivo and exhibited an adjuvant effect accompanying the production of interferon-gamma in an antigen-specific manner. This adjuvant effect culminated in the inhibition of inoculated tumor cell growth. Our results indicate that ntESC-derived iNKT cells are transplantable lymphocytes that will be beneficial for the induction of immune tolerance and the treatment of autoimmune diseases, tumors, and infections.

Wakayama, S., et al. (2005). "Propagation of an infertile hermaphrodite mouse lacking germ cells by using nuclear transfer and embryonic stem cell technology." Proc Natl Acad Sci U S A **102**(1): 29-33.

Animals generated by systematic mutagenesis and routine breeding are often infertile because they lack germ cells, and maintenance of such lines of animals has been impossible. We found a hermaphrodite infertile mouse in our colony, a genetic male with an abnormal Y chromosome lacking developing germ cells. We tried to clone this mouse by conventional nuclear transfer but without success. ES cells produced from blastocysts, which had been cloned by using somatic cell nuclear transfer (ntES cells) from this mouse, were also unable to produce offspring when injected into enucleated oocytes. Although we were able to produce two chimeric offspring using these ntES cells by tetraploid complementation, they were infertile, because they also lacked developing germ cells. However, when such ntES cells were injected into normal diploid blastocysts, many chimeric offspring were produced. One such male offspring transmitted hermaphrodite mouse genes to fertile daughters via X chromosome-bearing sperm. Thus, ntES cells were used to propagate offspring from infertile mice lacking germ cells.

Wakayama, T. (2003). "Cloned mice and embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Oncol Res **13**(6-10): 309-314.

Mice can now be cloned from cultured and noncultured adult-, fetus-, male-, or female-derived cells. Using the mouse as a model, research is moving towards a comprehensive description of clones generated by somatic cell nuclear transfer. In addition, embryonic stem (ES) cell lines can be generated from adult somatic cells via nuclear transfer (ntES cells). ntES cells contribute to an extensive variety of cell types including neurons in vitro and germ cells in vivo. Recent advances in mouse cloning are reported to illustrate its strengths and promise in the study of mammalian biology and biomedicine.

Wakayama, T. (2006). "Establishment of nuclear transfer embryonic stem cell lines from adult somatic cells by nuclear transfer and its application." Ernst Schering Res Found Workshop(60): 111-123.

Nuclear transfer can be used to generate embryonic stem cell (ntESC) lines from a patient's own somatic cells. We have shown that ntESCs can be generated relatively easily from a variety of mouse

genotypes and cell types of both sexes, even though it may be more difficult to generate clones directly. Several reports have already demonstrated that ntESCs can be used in regenerative medicine in order to rescue immunodeficient or infertile phenotypes. However, it is unclear whether ntES cells are identical to fertilized embryonic stem cells (ESCs). This review seeks to describe the phenotype and possible abnormalities of ntESC lines.

Wakayama, T., et al. (2001). "Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Science **292**(5517): 740-743.

Embryonic stem (ES) cells are fully pluripotent in that they can differentiate into all cell types, including gametes. We have derived 35 ES cell lines via nuclear transfer (ntES cell lines) from adult mouse somatic cells of inbred, hybrid, and mutant strains. ntES cells contributed to an extensive variety of cell types, including dopaminergic and serotonergic neurons in vitro and germ cells in vivo. Cloning by transfer of ntES cell nuclei could result in normal development of fertile adults. These studies demonstrate the full pluripotency of ntES cells.

Walker, L. M., et al. (2018). "The Validated Embryonic Stem Cell Test with Murine Embryonic Stem Cells." Methods Mol Biol **1797**: 97-124.

Birth defects are the leading cause of infant mortality in the USA, yet the causes of most of these conditions are unknown. While a combination of genetic and environmental factors are suspected in most cases, little information exists about the health risks that prenatal exposure to many common chemicals poses for the fetus. Thus, development and refinement of procedures that can accurately predict embryotoxicity of compounds is important for curtailing the number of infants born with birth defects. The embryonic stem cell test (EST) is a procedure that utilizes comparison of cytotoxicity in embryonic and adult cells and inhibition of differentiation to predict embryotoxicity of compounds tested. Because of its use of existing cell lines, the EST dramatically reduces the need for animal test subjects in toxicity testing. In addition, because of its use of inhibition of differentiation as an endpoint, the EST is extremely versatile in the range of complications it can test for. In this chapter, procedures for use of the validated embryonic stem cell test with the traditional cardiomyocyte differentiation endpoint are explained. The protocol includes discussion of routine stem cell culture, the cardiomyocyte differentiation procedure, and methods for utilization of molecular endpoints for assessing embryotoxicity of compounds.

Walter, J. and M. Dihne (2012). "Species-dependent differences of embryonic stem cell-derived neural stem cells after Interferon gamma treatment." Front Cell Neurosci **6**: 52.

Pluripotent stem cell (pSC)-derived, neural stem cells (NSCs) are actually extensively explored in the field of neuroregeneration and to clarify disease mechanisms or model neurological diseases in vitro. Regarding the latter, proliferation and differentiation of pSC-derived NSCs are investigated under the influence of a variety of different substances among them key players of inflammation. However, results generated on a murine genetic background are not always representative for the human situation which increasingly leads to the application of human cell culture systems derived from human pSCs. We investigated here, if the recently described interferon gamma (IFNgamma)-induced dysregulated neural phenotype characterized by simultaneous expression of glial and neuronal markers on murine NSCs (Walter et al., 2011, 2012) can also be found on a human genetic background. For this purpose, we performed experiments with human embryonic stem cell-derived NSCs. We could show that the IFNgamma-induced dysregulated neural phenotype cannot be induced in human NSCs. This difference occurs, although typical genes like signal transducers and activators of transcription 1 (Stat 1) or interferon regulatory factor 9 (IRF-9) are similarly regulated by IFNgamma in both, murine and human populations. These results illustrate that fundamental differences between murine and human neural populations exist in vitro, independent of anatomical system-related properties.

Wan, Q., et al. (2014). "Oleanolic acid has similar effects as retinoic acid in inducing mouse embryonic stem cell 1B10 to differentiate towards germ cells." Hum Cell **27**(1): 5-11.

This study investigated the effects of the compound oleanolic acid (OA) in inducing mouse embryonic stem cells (MESC) to differentiate towards germ cells (GC). MESC 1B10 was used as the model cell. 1B10 was cultured, and embryoid bodies (EBs) were produced from 1B10. The EBs were allowed to attach to the bottoms of culturing disks and grow. OA was added into the medium to induce the EBs to differentiate. Retinoic acid (RA) was used as the positive drug. After 72 h, total RNA was extracted, cDNA was synthesized, and real-time fluorescence quantitative PCR was performed to measure the transcriptional expression profiles of 11 reproduction-related genes affected by OA and RA, respectively. When the data were compared, it was found that OA up-regulated the transcriptional levels of Oct-4, GDF-9, Stra8, Mvh, ZP2, ZP3, Itga6, and TP2, and down-regulated transcriptional levels of SCP3, ZP1, and

Itgb1; RA up-regulated the transcriptional levels of GDF-9, Stra8, Mvh, ZP2, ZP3, Itga6, Itgb1, and TP2, and down-regulated the transcriptional levels of Oct-4, SCP3, and ZP1. The data showed that OA and RA had similar effects in inducing differentiation of MESC towards GC.

Wan, Q., et al. (2014). "Retinoic acid can induce mouse embryonic stem cell R1/E to differentiate toward female germ cells while oleanolic acid can induce R1/E to differentiate toward both types of germ cells." Cell Biol Int **38**(12): 1423-1429.

Retinoic acid (RA) and oleanolic acid (OA) were studied about their potential to induce mouse embryonic stem cell R1/E (MESC-R1/E) to differentiate toward germ cells. Embryoid bodies (EBs) first formed from MESC-R1/E and EBs were allowed to attach to the bottoms of normal cell-culturing plate and grow. Then, different compounds including RA, OA and so on were respectively added to induce MESC-R1/E to differentiate. After 72 h, microscopy images were taken for all interventions, then total RNAs were extracted, cDNAs were synthesized and real-time fluorescence quantitative PCR (qPCR) was performed to detect the transcriptional expression patterns of 11 reproductive-differentiation-related genes for different compounds respectively. During the data analysis, it was found RA significantly up-regulated the expression levels of GDF-9, Stra8, SCP3, Mvh, ZP1, ZP2, and ZP3, while significantly down-regulated the levels of Itag6 and Itgb1, and the level of Oct-4 was down-regulated insignificantly, while the level of TP2 was up-regulated insignificantly; OA significantly up-regulated the expression levels of Stra8, SCP3, Mvh, ZP1, ZP2, Itgb1, and TP2, and the levels of Oct-4, GDF-9, ZP3, and Itga6 were up-regulated insignificantly. The data showed that RA can induce MESC-R1/E to differentiate toward female germ cells while OA can induce MESC-R1/E to differentiate toward male and female germ cells.

Wang, C., et al. (2017). "Basic fibroblast growth factor is critical to reprogramming buffalo (Bubalus bubalis) primordial germ cells into embryonic germ stem cell-like cells." Theriogenology **91**: 112-120.

Primordial germ cells (PGCs) are destined to form gametes in vivo, and they can be reprogrammed into pluripotent embryonic germ (EG) cells in vitro. Buffalo PGC have been reported to be reprogrammed into EG-like cells, but the identities of the major signaling pathways and culture media involved in this derivation remain unclear. Here, the effects of basic fibroblast growth factor (bFGF) and downstream signaling pathways on the reprogramming of buffalo PGCs into EG-like cells were investigated. Results showed bFGF to be critical to buffalo PGCs to dedifferentiate into EG-like cells (20 ng/mL is optimal) with many characteristics of pluripotent stem cells, including alkaline phosphatase (AP) activity, expression of pluripotency marker genes such as OCT4, NANOG, SOX2, SSEA-1, CDH1, and TRA-1-81, and the capacity to differentiate into all three embryonic germ layers. After chemically inhibiting pathways or components downstream of bFGF, data showed that inhibition of the PI3K/AKT pathway led to significantly lower EG cell derivation, while inhibition of P53 activity resulted in an efficiency of EG cell derivation comparable to that in the presence of bFGF. These results suggest that the role of bFGF in PGC-derived EG-like cell generation is mainly due to the activation of the PI3K/AKT/P53 pathway, in particular, the inhibition of P53 function.

Wang, D., et al. (2010). "Transplantation of human embryonic stem cell-derived alveolar epithelial type II cells abrogates acute lung injury in mice." Mol Ther **18**(3): 625-634.

Respiratory diseases are a major cause of mortality and morbidity worldwide. Current treatments offer no prospect of cure or disease reversal. Transplantation of pulmonary progenitor cells derived from human embryonic stem cells (hESCs) may provide a novel approach to regenerate endogenous lung cells destroyed by injury and disease. Here, we examine the therapeutic potential of alveolar type II epithelial cells derived from hESCs (hES-ATIICs) in a mouse model of acute lung injury. When transplanted into lungs of mice subjected to bleomycin (BLM)-induced acute lung injury, hES-ATIICs behaved as normal primary ATIICs, differentiating into cells expressing phenotypic markers of alveolar type I epithelial cells. Without experiencing tumorigenic side effects, lung injury was abrogated in mice transplanted with hES-ATIICs, demonstrated by recovery of body weight and arterial blood oxygen saturation, decreased collagen deposition, and increased survival. Therefore, transplantation of hES-ATIICs shows promise as an effective therapeutic to treat acute lung injury.

Wang, H., et al. (2016). "Germ Cell Nuclear Factor (GCNF) Represses Oct4 Expression and Globally Modulates Gene Expression in Human Embryonic Stem (hES) Cells." J Biol Chem **291**(16): 8644-8652.

Oct4 is considered a key transcription factor for pluripotent stem cell self-renewal. It binds to specific regions within target genes to regulate their expression and is downregulated upon induction of differentiation of pluripotent stem cells; however, the mechanisms that regulate the levels of human Oct4 expression remain poorly understood. Here we show that expression of human Oct4 is directly repressed by germ cell nuclear factor (GCNF), an orphan nuclear

receptor, in hES cells. Knockdown of GCNF by siRNA resulted in maintenance of Oct4 expression during RA-induced hES cell differentiation. While overexpression of GCNF promoted repression of Oct4 expression in both undifferentiated and differentiated hES cells. The level of Oct4 repression was dependent on the level of GCNF expression in a dose-dependent manner. mRNA microarray analysis demonstrated that overexpression of GCNF globally regulates gene expression in undifferentiated and differentiated hES cells. Within the group of altered genes, GCNF down-regulated 36% of the genes, and up-regulated 64% in undifferentiated hES cells. In addition, GCNF also showed a regulatory gene pattern that is different from RA treatment during hES cell differentiation. These findings increase our understanding of the mechanisms that maintain hES cell pluripotency and regulate gene expression during the differentiation process.

Wang, L. (2006). "Endothelial and hematopoietic cell fate of human embryonic stem cells." Trends Cardiovasc Med **16**(3): 89-94.

The endothelial cells, lining the inside of blood vessels, and the blood-forming hematopoietic cells play crucial roles in vasculogenesis. The establishment of human embryonic stem cells (hESCs) provides a unique tool to study the early development of endothelial and hematopoietic cells, opening new avenues of research to explore organ vascularization and regeneration. The current study demonstrates that a population of intermediate-stage precursors, which possesses primitive endothelial properties during hESC differentiation, is capable of giving rise to endothelial and hematopoietic cells. Single cell analysis reveals that these primitive endothelial-like precursors contain rare bipotent cells with hemangioblast properties, responsible for both endothelial and hematopoietic cell fates. These findings will facilitate the further study of cellular commitment, lineage restriction, and terminal differentiation of endothelial and hematopoietic compartments and may lead to novel regenerative therapies.

Wang, L., et al. (2004). "Endothelial and hematopoietic cell fate of human embryonic stem cells originates from primitive endothelium with hemangioblastic properties." Immunity **21**(1): 31-41.

The cellular organization and relationships among precursors that initiate embryonic angiogenesis and hematopoiesis in the human have yet to be characterized. Here, we identify a subpopulation of primitive endothelial-like cells derived from human embryonic stem cells (hESCs) that express PECAM-1, Flk-1, and VE-cadherin, but not CD45 (CD45negPFV cells), and that are uniquely responsible for endothelial and hematopoietic development. Molecular profiling of CD45negPFV cells is consistent with endothelial and hematopoietic competency. Clonal isolation demonstrates that the CD45negPFV population includes bipotent cells with endothelial and hematopoietic capacity. We suggest that human hematopoiesis and endothelial maturation originate exclusively from a subset of embryonic endothelium that possesses hemangioblastic properties and offers a model system to study these lineage relationships in the human.

Wang, Q., et al. (2013). "GASZ promotes germ cell derivation from embryonic stem cells." Stem Cell Res **11**(2): 845-860.

Primordial germ cells (PGCs) are the first germ-line population that forms from the proximal epiblast of the developing embryo. Despite their biological importance, the regulatory networks whereby PGCs arise, migrate, and differentiate into gametes during embryonic development remains elusive, largely due to the limited number of germ cells in the early embryo. To elucidate the molecular mechanisms that govern early germ cell development, we utilized an in vitro differentiation model of embryonic stem cells (ESCs) and screened a series of candidate genes with specific expression in the adult reproductive organs. We discovered that gain of function of Gasz, a gene previously reported to participate in meiosis of postnatal spermatocytes, led to the most robust upregulation of PGC formation from both human and murine ESCs. In contrast, Gasz deficiency resulted in pronounced reduction of germ cells during ESC differentiation and decreased expression of MVH and DAZL in genital ridges during early embryonic development. Further analyses demonstrated that GASZ interacted with DAZL, a key germ cell regulator, to synergistically promote germ cell derivation from ESCs. Thus, our data reveal a potential role of GASZ during embryonic germ cell development and provide a powerful in vitro system for dissecting the molecular pathways in early germ cell formation during embryogenesis.

Wang, R. and Y. L. Guo (2012). "Transient inhibition of cell proliferation does not compromise self-renewal of mouse embryonic stem cells." Exp Cell Res **318**(16): 2094-2104.

Embryonic stem cells (ESCs) have unlimited capacity for self-renewal and can differentiate into various cell types when induced. They also have an unusual cell cycle control mechanism driven by constitutively active cyclin dependent kinases (Cdks). In mouse ESCs (mESCs). It is proposed that the rapid cell proliferation could be a necessary part of mechanisms that maintain mESC self-renewal and pluripotency, but this hypothesis is not in line with the

finding in human ESCs (hESCs) that the length of the cell cycle is similar to differentiated cells. Therefore, whether rapid cell proliferation is essential for the maintenance of mESC state remains unclear. We provide insight into this uncertainty through chemical intervention of mESC cell cycle. We report here that inhibition of Cdks with olomoucine II can dramatically slow down cell proliferation of mESCs with concurrent down-regulation of cyclin A, B and E, and the activation of the Rb pathway. However, mESCs display can recover upon the removal of olomoucine II and are able to resume normal cell proliferation without losing self-renewal and pluripotency, as demonstrated by the expression of ESC markers, colony formation, embryoid body formation, and induced differentiation. We provide a mechanistic explanation for these observations by demonstrating that Oct4 and Nanog, two major transcription factors that play critical roles in the maintenance of ESC properties, are up-regulated via de novo protein synthesis when the cells are exposed to olomoucine II. Together, our data suggest that short-term inhibition of cell proliferation does not compromise the basic properties of mESCs.

Wang, Y. and R. Blelloch (2009). "Cell cycle regulation by MicroRNAs in embryonic stem cells." Cancer Res **69**(10): 4093-4096.

The cell cycle is tightly orchestrated during normal development. Embryonic stem (ES) cells have a unique cell cycle structure, in which the G1/S restriction is largely absent, enabling cells to rapidly move through the G1 phase and enter the S phase. This hastened cell cycle allows the early embryo to rapidly grow. Recent experiments suggest that small noncoding RNAs, the microRNAs (miRNAs), play a central role in achieving this unique cell cycle structure. The responsible miRNAs function by suppressing multiple inhibitors of the G1/S transition. Expression of these miRNAs drops dramatically as the ES cells differentiate and as the G1 phase extends. Some of the same miRNAs are overexpressed in cancers, in which they can promote tumor growth, suggesting common mechanisms of miRNA-regulated cell cycle control in ES cells and cancers. This review discusses these recent findings in the context of broader knowledge of cell cycle control in normal and abnormal development.

Wang, Y., et al. (2005). "Embryonic stem cell-derived hematopoietic stem cells." Proc Natl Acad Sci U S A **102**(52): 19081-19086.

Despite two decades of studies documenting the in vitro blood-forming potential of murine embryonic stem cells (ESCs), achieving stable long-term blood engraftment of ESC-derived hematopoietic stem cells in irradiated mice has proven difficult. We have exploited the Cdx-Hox pathway, a genetic program important for blood development, to enhance the differentiation of ESCs along the hematopoietic lineage. Using an embryonic stem cell line engineered with tetracycline-inducible Cdx4, we demonstrate that ectopic Cdx4 expression promotes hematopoietic mesoderm specification, increases hematopoietic progenitor formation, and, together with HoxB4, enhances multilineage hematopoietic engraftment of lethally irradiated adult mice. Clonal analysis of retroviral integration sites confirms a common stem cell origin of lymphoid and myeloid populations in engrafted primary and secondary mice. These data document the cardinal stem cell features of self-renewal and multilineage differentiation of ESC-derived hematopoietic stem cells.

Yildirimman, R., et al. (2011). "Human embryonic stem cell derived hepatocyte-like cells as a tool for in vitro hazard assessment of chemical carcinogenicity." Toxicol Sci **124**(2): 278-290.

Hepatocyte-like cells derived from the differentiation of human embryonic stem cells (hES-Hep) have potential to provide a human relevant in vitro test system in which to evaluate the carcinogenic hazard of chemicals. In this study, we have investigated this potential using a panel of 15 chemicals classified as noncarcinogens, genotoxic carcinogens, and nongenotoxic carcinogens and measured whole-genome transcriptome responses with gene expression microarrays. We applied an ANOVA model that identified 592 genes highly discriminative for the panel of chemicals. Supervised classification with these genes achieved a cross-validation accuracy of > 95%. Moreover, the expression of the response genes in hES-Hep was strongly correlated with that in human primary hepatocytes cultured in vitro. In order to infer mechanistic information on the consequences of chemical exposure in hES-Hep, we developed a computational method that measures the responses of biochemical pathways to the panel of treatments and showed that these responses were discriminative for the three toxicity classes and linked to carcinogenesis through p53, mitogen-activated protein kinases, and apoptosis pathway modules. It could further be shown that the discrimination of toxicity classes was improved when analyzing the microarray data at the pathway level. In summary, our results demonstrate, for the first time, the potential of human embryonic stem cell--derived hepatic cells as an in vitro model for hazard assessment of chemical carcinogenesis, although it should be noted that more compounds are needed to test the robustness of the assay.

Yin, X., et al. (2006). "Proteomic analysis reveals higher demand for antioxidant protection in embryonic

stem cell-derived smooth muscle cells." Proteomics **6**(24): 6437-6446.

Embryonic stem (ES) cells can differentiate into vascular smooth muscle cells (SMCs), but differences in protein composition, function and behaviour between stem cell-derived and mature SMCs remain to be characterized. Using differential in gel electrophoresis (DIGE) and MS, we identified 146 proteins that differed between ES cell-derived SMCs (esSMCs) and aortic SMCs, including proteins involved in DNA maintenance (higher in esSMCs), cytoskeletal proteins and calcium-binding proteins (higher in aortic SMCs). Notably, esSMCs showed decreased expression of mitochondrial, but a compensatory increase of cytosolic antioxidants. Subsequent experiments revealed that mitochondrial-derived reactive oxygen species (ROS) were markedly increased in esSMCs. Despite a three-fold rise in glutathione (GSH) reductase activity, esSMCs had lower levels of reduced GSH, and depletion of GSH by diethyl maleate or inhibition of GSH reductase by carmustine (BCNU) resulted in more pronounced cell death compared to aortic SMCs, while addition of antioxidants improved the viability of esSMCs. We present the first proteomic analysis of esSMCs demonstrating that stem cell-derived SMCs are more sensitive to oxidative stress due to increased generation of mitochondrial-derived ROS and require additional antioxidant protection for survival.

Yla-Outinen, L., et al. (2014). "Three-dimensional growth matrix for human embryonic stem cell-derived neuronal cells." J Tissue Eng Regen Med **8**(3): 186-194.

The future of tissue engineering applications for neuronal cells will require a supportive 3D matrix. This particular matrix should be soft, elastic and supportive for cell growth. In this study, we characterized the suitability of a 3D synthetic hydrogel matrix, PuraMatrix, as a growth platform for human embryonic stem cell (hESC)-derived neural cells. The viability of the cells grown on top of, inside and under the hydrogel was monitored. The maturation and electrical activity of the neuronal networks inside the hydrogel were further characterized. We showed that cells stayed viable on the top of the PuraMatrix surface and growth of the neural cells and neural processes was good. Further, hESC-derived neurons, astrocytes and oligodendrocytes all grew, matured and migrated when cultured inside the hydrogel. Importantly, neuronal cells were able to form electrically active connections that were verified using microelectrode array. Thus, PuraMatrix is a good supportive growth matrix for human neural cells and may serve as a matrix for neuronal scaffolds in neural tissue engineering.

Yoffe, Y., et al. (2016). "Cap-independent translation by DAP5 controls cell fate decisions in human embryonic stem cells." Genes Dev **30**(17): 1991-2004.

Multiple transcriptional and epigenetic changes drive differentiation of embryonic stem cells (ESCs). This study unveils an additional level of gene expression regulation involving noncanonical, cap-independent translation of a select group of mRNAs. This is driven by death-associated protein 5 (DAP5/eIF4G2/NAT1), a translation initiation factor mediating IRES-dependent translation. We found that the DAP5 knockdown from human ESCs (hESCs) resulted in persistence of pluripotent gene expression, delayed induction of differentiation-associated genes in different cell lineages, and defective embryoid body formation. The latter involved improper cellular organization, lack of cavitation, and enhanced mislocalized apoptosis. RNA sequencing of polysome-associated mRNAs identified candidates with reduced translation efficiency in DAP5-depleted hESCs. These were enriched in mitochondrial proteins involved in oxidative respiration, a pathway essential for differentiation, the significance of which was confirmed by the aberrant mitochondrial morphology and decreased oxidative respiratory activity in DAP5 knockdown cells. Further analysis identified the chromatin modifier HMGN3 as a cap-independent DAP5 translation target whose knockdown resulted in defective differentiation. Thus, DAP5-mediated translation of a specific set of proteins is critical for the transition from pluripotency to differentiation, highlighting the importance of cap-independent translation in stem cell fate decisions.

Yoneyama, M., et al. (2011). "Endogenous nitric oxide generation linked to ryanodine receptors activates cyclic GMP / protein kinase G pathway for cell proliferation of neural stem/progenitor cells derived from embryonic hippocampus." J Pharmacol Sci **115**(2): 182-195.

Nitric oxide (NO) activates the cyclic GMP (cGMP) / protein kinase G (PKG) pathway during physiological processes in numerous types of cells. Here, we evaluated whether this NO/cGMP/PKG pathway is involved in the proliferation of neural stem/progenitor cells (NPCs) derived from the hippocampus of embryonic mice. In culture, the exposure to the NO synthase inhibitor N(omega)-nitro-L-arginine methyl ester (L-NAME) significantly decreased the number of viable cells and 5-bromo-2'-deoxyuridine (BrdU) incorporation into the cells, as well as the levels of intracellular reactive oxygen species, extracellular NO(2), and intracellular cGMP. Like L-NAME, the soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and PKG inhibitor KT5823 also decreased cell viability

and BrdU incorporation. The membrane-permeable cGMP analogue 8-bromo-cGMP partially abolished the L-NAME-induced decrease in the BrdU incorporation. BrdU incorporation was decreased by Ca(2+)-channel blockers, including dantrolene, MK-801, ifenprodil, and nifedipine. Interestingly, the NO(2) level was decreased by dantrolene, but not by the other 3 blockers. L-NAME and ODQ attenuated phosphorylation of Akt, but not that of extracellular signal-regulated kinases or epidermal growth factor receptors. Our data suggest that endogenous NO generation linked to dantrolene-sensitive ryanodine receptors activates the cGMP/PKG signaling pathway for positive regulation of proliferation of hippocampal NPCs derived from embryonic mice.

Yoo, H., et al. (2016). "Ultrastructural comparison of porcine putative embryonic stem cells derived by in vitro fertilization and somatic cell nuclear transfer." J Reprod Dev **62**(2): 177-185.

The ultrastructure of porcine putative embryonic stem cells and porcine fetal fibroblasts (PFFs) was analyzed by transmission electron microscopy. The aim of this study was to compare the features of organelles in in vitro fertilization (IVF) derived porcine embryonic stem cells (IVF-pESCs) and somatic cell nuclear transfer (SCNT) derived pESCs (SCNT-pESCs). Also, the features of organelles in high-passage IVF-pESCs were compared with those in low-passage cells. The ultrastructure of PFFs showed rare microvilli on the cell surfaces, polygonal or irregular nuclei with one to two reticular-shaped nucleoli and euchromatin, low cytoplasm-to-nucleus ratios, rare ribosomes, rare rough endoplasmic reticulum, elongated mitochondria, rich lysosomes and rich phagocytic vacuoles. IVF-pESCs showed rare microvilli on the cell surfaces, round or irregular nuclei with one to two reticular-shaped nucleoli and euchromatin, low cytoplasm-to-nucleus ratios, rich ribosomes, long stacks of rough endoplasmic reticulum, elongated mitochondria, rare lysosomes and rare autophagic vacuoles. By contrast, SCNT-pESCs showed rich microvilli with various lengths and frequencies on the cell surfaces, polygonal nuclei with one reticular shaped nucleoli and heterochromatin, high cytoplasm-to-nucleus ratios, rare ribosomes, rare rough endoplasmic reticulum, round mitochondria, rich lysosomes and rich phagocytic vacuoles with clear intercellular junctions. Furthermore, high-passage IVF-pESCs showed irregularly shaped colonies, pyknosis and numerous lysosomes associated with autophagic vacuoles showing signs of apoptosis. In conclusion, this study confirms that the ultrastructural characteristics of pESCs differ depending on their origin. These ultrastructural characteristics might be useful in biomedical research using pESCs, leading to new insights regarding regenerative medicine and tissue repair.

Yoo, M., et al. (2014). "Analysis of human embryonic stem cells with regulatable expression of the cell adhesion molecule l1 in regeneration after spinal cord injury." J Neurotrauma **31**(6): 553-564.

Cell replacement therapy is one potential avenue for central nervous system (CNS) repair. However, transplanted stem cells may not contribute to long-term recovery of the damaged CNS unless they are engineered for functional advantage. To fine tune regenerative capabilities, we developed a human neural cell line expressing L1, a regeneration-conducive adhesion molecule, under the control of a doxycycline regulatable Tet-off promoter. Controlled expression of L1 is desired because overexpression after regenerative events may lead to adverse consequences. The regulated system was tested in several cell lines, where doxycycline completely eliminated green fluorescent protein or L1 expression by 3-5 days in vitro. Increased colony formation as well as decreased proliferation were observed in H9NSCs without doxycycline (hL1-on). To test the role of L1 in vivo after acute compression spinal cord injury of immunosuppressed mice, quantum dot labeled hL1-on or hL1-off cells were injected at three sites: lesion; proximal; and caudal. Mice transplanted with hL1-on cells showed a better Basso Mouse Scale score, when compared to those with hL1-off cells. As compared to the hL1-off versus hL1-on cell transplanted mice 6 weeks post-transplantation, expression levels of L1, migration of transplanted cells, and immunoreactivity for tyrosine hydroxylase were higher, whereas expression of chondroitin sulfate proteoglycans was lower. Results indicate that L1 expression is regulatable in human stem cells by doxycycline in a nonviral engineering approach. Regulatable expression in a prospective nonleaky Tet-off system could hold promise for therapy, based on the multifunctional roles of L1, including neuronal migration and survival, neuritogenesis, myelination, and synaptic plasticity.

Yoo, S. J., et al. (2005). "Efficient culture system for human embryonic stem cells using autologous human embryonic stem cell-derived feeder cells." Exp Mol Med **37**(5): 399-407.

Human embryonic stem cells (hESCs) need feeder cells for their maintenance in an undifferentiated state. In conventional culture systems, mouse embryonic fibroblasts (MEFs) serve as feeder cells to maintain hESCs. However, the use of MEFs elevates the risk of transmitting mouse pathogens and thus limits the potential of hESCs in cell replacement therapy. Consequently, the use of human feeder cells would be an important step forward in this in vitro

technology. To address this issue, we used fibroblast-like cells differentiated from the Miz-hES6 hESC line (DiffMiz-hES6) as feeder cells to support the in vitro growth of three hESC lines. Immunofluorescence microscopy and reverse transcription-PCR assessing the expression of undifferentiated hESC markers revealed all three hESC lines were maintained in an undifferentiated state. In vitro proliferation proceeded as efficiently as when the hESCs were cultured on MEFS. Moreover, karyotype analysis revealed the chromosomal normality of the hESC lines and the DiffMiz-hES6 feeders themselves after even 50 passages. Furthermore, the hESC lines maintained their pluripotency since they remained capable of forming embryoid bodies (EBs) in vitro. Thus, hESC-derived fibroblast-like cells successfully support in vitro hESC propagation.

Yoon, S. W., et al. (2014). "Rad51 regulates cell cycle progression by preserving G2/M transition in mouse embryonic stem cells." Stem Cells Dev **23**(22): 2700-2711.

Homologous recombination (HR) maintains genomic integrity against DNA replication stress and deleterious lesions, such as double-strand breaks (DSBs). Rad51 recombinase is critical for HR events that mediate the exchange of genetic information between parental chromosomes in eukaryotes. Additionally, Rad51 and HR accessory factors may facilitate replication fork progression by preventing replication fork collapse and repair DSBs that spontaneously arise during the normal cell cycle. In this study, we demonstrated a novel role for Rad51 during the cell cycle in mouse embryonic stem cells (mESCs). In mESCs, Rad51 was constitutively expressed throughout the cell cycle, and the formation of Rad51 foci increased as the cells entered S phase. Suppression of Rad51 expression caused cells to accumulate at G2/M phase and activated the DNA damage checkpoint, but it did not affect the self-renewal or differentiation capacity of mESCs. Even though Rad51 suppression significantly inhibited the proliferation rate of mESCs, Rad51 suppression did not affect the replication fork progression and speed, indicating that Rad51 repaired DNA damage and promoted DNA replication in S phase through an independent mechanism. In conclusion, Rad51 may contribute to G2/M transition in mESCs, while preserving genomic integrity in global organization of DNA replication fork.

Yoshie, S., et al. (2012). "Establishment of novel detection system for embryonic stem cell-derived hepatocyte-like cells based on nongenetic manipulation with indocyanine green." Tissue Eng Part C Methods **18**(1): 12-20.

Hepatocytes derived from embryonic stem cells (ESCs) are expected to be useful for basic research and clinical applications. However, in several studies, genetic methods used to detect and obtain them are difficult and pose major safety problems. Therefore, in this study, we established a novel detection system for hepatocytes by using indocyanine green (ICG), which is selectively taken up by hepatocytes, based on nongenetic manipulation. ICG has maximum light absorption near 780 nm, and it fluoresces between 800 and 900 nm. Making use of these properties, we developed flow cytometry equipped with an excitation lazer of 785 nm and specific bandpass filters and successfully detected ESC-derived ICG-positive cells that were periodic acid-Schiff positive and expressed hepatocyte phenotypic mRNAs. These results demonstrate that this detection system based on nongenetic manipulation with ICG will lead to isolate hepatocytes generated from ESCs and provide the appropriate levels of stability, quality, and safety required for cell source for cell-based therapy and pharmaceutical studies such as toxicology.

You, J. S., et al. (2009). "Depletion of embryonic stem cell signature by histone deacetylase inhibitor in NCCIT cells: involvement of Nanog suppression." Cancer Res **69**(14): 5716-5725.

The embryonic stem cell-like gene expression signature has been shown to be associated with poorly differentiated aggressive human tumors and has attracted great attention as a potential target for future cancer therapies. Here, we investigate the potential of the embryonic stem cell signature as molecular target for the therapy and the strategy to suppress the embryonic stem cell signature. The core stemness gene Nanog is abnormally overexpressed in human embryonic carcinoma NCCIT cells showing gene expression profiles similar to embryonic stem cells. Down-regulation of the gene by either small interfering RNAs targeting Nanog or histone deacetylase inhibitor apicidin causes reversion of expression pattern of embryonic stem cell signature including Oct4, Sox2, and their target genes, leading to cell cycle arrest, inhibition of colony formation in soft agar, and induction of differentiation into all three germ layers. These effects are antagonized by reintroduction of Nanog. Interestingly, embryonic carcinoma cells (NCCIT, NTERA2, and P19) exhibit a higher sensitivity to apicidin in down-regulation of Nanog compared with embryonic stem cells. Furthermore, the down-regulation of Nanog expression by apicidin is mediated by a coordinated change in recruitment of epigenetic modulators and transcription factors to the promoter region. These findings indicate that overexpression of stemness gene Nanog in NCCIT cells is associated with maintaining stem cell-like

phenotype and suggest that targeting Nanog might be an approach for improved therapy of poorly differentiated tumors.

Youngblood, B. A., et al. (2014). "CstF-64 supports pluripotency and regulates cell cycle progression in embryonic stem cells through histone 3' end processing." Nucleic Acids Res **42**(13): 8330-8342.

Embryonic stem cells (ESCs) exhibit a unique cell cycle with a shortened G1 phase that supports their pluripotency, while apparently buffering them against pro-differentiation stimuli. In ESCs, expression of replication-dependent histones is a main component of this abbreviated G1 phase, although the details of this mechanism are not well understood. Similarly, the role of 3' end processing in regulation of ESC pluripotency and cell cycle is poorly understood. To better understand these processes, we examined mouse ESCs that lack the 3' end-processing factor CstF-64. These ESCs display slower growth, loss of pluripotency and a lengthened G1 phase, correlating with increased polyadenylation of histone mRNAs. Interestingly, these ESCs also express the tauCstF-64 paralog of CstF-64. However, tauCstF-64 only partially compensates for lost CstF-64 function, despite being recruited to the histone mRNA 3' end-processing complex. Reduction of tauCstF-64 in CstF-64-deficient ESCs results in even greater levels of histone mRNA polyadenylation, suggesting that both CstF-64 and tauCstF-64 function to inhibit polyadenylation of histone mRNAs. These results suggest that CstF-64 plays a key role in modulating the cell cycle in ESCs while simultaneously controlling histone mRNA 3' end processing.

Yu, G. and Q. Wen (2018). "Expression of embryonic liver fodrin (ELF) and stem cell markers in CD13 liver cancer stem cells." Eur Rev Med Pharmacol Sci **22**(6): 1653-1657.

OBJECTIVE: Investigating the molecular signaling pathways using CD13 as a marker for Cancer Stem Cells (CSCs) in human liver cancer. PATIENTS AND METHODS: In the present study, liver carcinoma biopsies were obtained from the liver cancer patients, as well as healthy controls. Immunohistochemistry and Immunoblotting experiments were performed accordingly to conclude the data. RESULTS: Immunohistochemistry along with immunoblot data showed the expression of Oct-4, STAT3 and interestingly Embryonic Liver Fodrin (ELF) in the CD13 positive liver cancer stem cells. Though embryonic liver fodrin (ELF) is a pro-differentiation protein, it was expressed in the CD13 positive liver cancer stem cells along with stem cell markers such as Oct-4 and STAT3. CONCLUSIONS: Our finding concludes that an association of ELF expression was noted in liver cancer patients. Hence, ELF may have value as prognostic indicators and may facilitate the development of novel therapeutics for liver cancer.

Zwaka, T. P. and J. A. Thomson (2005). "Differentiation of human embryonic stem cells occurs through symmetric cell division." Stem Cells **23**(2): 146-149.

Embryonic (ES) stem cells can be expanded indefinitely, yet retain the ability to form all cell types of the body. Here we report that human ES cells differentiate exclusively by symmetric cell division in each of four distinct differentiation conditions examined. This suggests that, in some respects, ES cells more closely resemble precursor or transit amplifying cells rather than adult stem cells.

Zwaka, T. P. and J. A. Thomson (2005). "A germ cell origin of embryonic stem cells?" Development **132**(2): 227-233.

Because embryonic stem (ES) cells are generally derived by the culture of inner cell mass (ICM) cells, they are often assumed to be the equivalent of ICM cells. However, various evidence indicates that ICM cells transition to a different cell type during ES-cell derivation. Historically, ES cells have been believed to most closely resemble pluripotent primitive ectoderm cells derived directly from the ICM. However, differences between ES cells and primitive ectoderm cells have caused developmental biologists to question whether ES cells really have an in vivo equivalent, or whether their properties merely reflect their tissue culture environment. Here, we review recent evidence that the closest in vivo equivalent of an ES cell is an early germ cell.

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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