Mouse Stem Cell Literatures

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on mouse stem cell.
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Key words: stem cell; life; gene; DNA; protein; mouse

Ahn, J. I., K. H. Lee, et al. (2004). "Temporal expression changes during differentiation of neural stem cells derived from mouse embryonic stem cell." J Cell Biochem **93**(3): 563-78.

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.

Aiba, K., T. Nedorezov, 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.

Ali Owchi, M., M. Salehnia, et al. (2009). "The effect of bone morphogenetic protein 4 on the differentiation of mouse embryonic stem cell to erythroid lineage in serum free and serum supplemented media." Int J Biomed Sci **5**(3): 275-82.

This study was done to compare the effects of bone morphogenetic protein-4 (BMP-4) on mouse embryonic stem cells (ESC) differentiation to erythroid lineage in serum free and serum

supplemented media. The embryoid bodies (EBs) cells were seeded in semisolid serum free and serum supplemented media in the presence of different concentrations of BMP-4. The erythroid colonies were assessed morphologically, ultrastructurally and by benzidine staining. The expression of the epsilon (epsilon), betaH1 and betamajor globins, Runx1 and beta2m genes was evaluated by Real time PCR. The colony size and the percent of benzidine-positive colonies increased in both BMP-4 supplementd groups but the number of colonies were lower in these groups than control. Erythropoiesis related genes were expressed in both serum free and serum supplemented groups. There were not significant differences between the ratios of genes expression to beta2m in these groups except the ratio of Runx1 was significantly higher in serum free group ($P<0.05$). The ratio of epsilon and betaH1 to beta2m in EBs was higher than both BMP-4 containing groups ($P<0.05$) and betamajor was not expressed in EB cells. These findings showed in serum free condition the effects of BMP-4 on the erythroid differentiation was prominent than serum supplemented group.

Allard, P., M. J. Champigny, et al. (2002). "Stem-loop binding protein accumulates during oocyte maturation and is not cell-cycle-regulated in the early mouse embryo." J Cell Sci **115**(Pt 23): 4577-86.

The stem-loop binding protein (SLBP) binds to the 3' end of histone mRNA and participates in 3'-processing of the newly synthesized transcripts, which protects them from degradation, and probably also promotes their translation. In proliferating cells, translation of SLBP mRNA begins at G1/S and the protein is degraded following DNA replication. These post-transcriptional mechanisms closely couple SLBP expression to S-phase of the cell cycle, and play a key role in restricting synthesis of replication-dependent histones to S-phase. In contrast to somatic cells, replication-dependent histone mRNAs accumulate and are translated independently of DNA replication in oocytes and early embryos. We report here that SLBP expression and activity also differ in mouse oocytes and early embryos compared with somatic cells. SLBP is present in oocytes that are arrested at prophase of G2/M, where it is concentrated in the nucleus. Upon entry into M-phase of meiotic maturation, SLBP begins to accumulate rapidly, reaching a very high level in mature oocytes arrested at metaphase II. Following fertilization, SLBP remains abundant in the nucleus and the cytoplasm throughout the first cell cycle, including both G1 and G2 phases. It declines during the second and third cell cycles, reaching a relatively low level by the late 4-cell stage. SLBP can bind the histone mRNA-stem-loop at all stages of the cell cycle in oocytes and early embryos, and it is the only stem-loop binding activity detectable in these cells. We also report that SLBP becomes phosphorylated rapidly following entry into M-phase of meiotic maturation through a mechanism that is sensitive to roscovitine, an inhibitor of cyclin-dependent kinases. SLBP is rapidly dephosphorylated following fertilization or parthenogenetic activation, and becomes newly phosphorylated at M-phase of mitosis. Phosphorylation does not affect its stem-loop binding activity. These results establish that, in contrast to Xenopus, mouse oocytes and embryos contain a single SLBP. Expression of SLBP is uncoupled from S-phase in oocytes and early embryos, which indicates that the mechanisms that impose cell-cycle-regulated expression of SLBP in somatic cells do not operate in oocytes or during the first embryonic cell cycle. This distinctive pattern of SLBP expression may be required for accumulation of histone proteins required for sperm chromatin remodelling and assembly of newly synthesized embryonic DNA into chromatin.

Anderson, P. D., V. R. Nelson, et al. (2009). "Genetic factors on mouse chromosome 18 affecting susceptibility to testicular germ cell tumors and permissiveness to embryonic stem cell derivation." Cancer Res **69**(23): 9112-7.

Despite strong heritability, little is known about the genetic control of susceptibility to testicular germ cell tumors (TGCT) in humans or mice. Although the mouse model of spontaneous TGCTs has been extensively studied, conventional linkage analysis has failed to locate the factors that control teratocarcinogenesis in the susceptible 129 family of inbred strains. As an alternative approach, we used both chromosome substitution strains (CSS) to identify individual chromosomes that harbor susceptibility genes and a panel of congenic strains derived from a selected CSS to determine the number and location of susceptibility variants on the substituted chromosome. We showed that 129-Chr 18(MOLF) males are resistant to spontaneous TGCTs and that at least four genetic variants control susceptibility in males with this substituted chromosome. In addition, early embryonic cells from this strain fail to establish embryonic stem cell lines as efficiently as those from the parental 129/Sv strain. For the first time, 129-derived genetic variants that control TGCT susceptibility and fundamental aspects of embryonic stem cell biology have been localized in a genetic context in which the genes can be identified and functionally characterized.

Auerbach, W., J. H. Dunmore, et al. (2000). "Establishment and chimera analysis of 129/SvEv-

and C57BL/6-derived mouse embryonic stem cell lines." Biotechniques **29**(5): 1024-8, 1030, 1032.

Hundreds of new mutant mouse lines are being produced annually using gene targeting and gene trap approaches in embryonic stem (ES) cells, and the number is expected to continue to grow as the human and mouse genome projects progress. The availability of robust ES cell lines and a simple technology for making chimeras is more attractive now than ever before. We established several new ES cell lines from 129/SvEv and C57BL/6 mice and tested their ability to contribute to the germline following blastocyst injections and/or the less expensive and easier method of morula-ES cell aggregation. Using morula aggregation to produce chimeras, five newly derived 129/SvEv and two C57BL/6 ES cell lines tested at early passages were found to contribute extensively to chimeras and produce germline-transmitting male chimeras. Furthermore, the two 129S/vEv ES cell lines that were tested and one of the C57BL/6 ES cell lines were able to maintain these characteristics after many passages in vitro. Our results indicate that the ability of ES cells to contribute strongly to chimeras following aggregation with outbred embryos is a general property of early passage ES cells and can be maintained for many passages. C56BL/6-derived ES cell lines, however, have a greater tendency than 129-derived ES cell lines to lose their ability to colonize the germline.

Baharvand, H., A. Fathi, et al. (2008). "Identification of mouse embryonic stem cell-associated proteins." J Proteome Res **7**(1): 412-23.

Over the past few years, there has been a growing interest in discovering the molecular mechanisms controlling embryonic stem cells' (ESCs) proliferation and differentiation. Proteome analysis has proven to be an effective approach to comprehensively unravel the regulatory network of differentiation. We applied a two-dimensional electrophoresis based proteomic approach followed by mass spectrometry to analyze the proteome of two mouse ESC lines, Royan B1 and D3, at 0, 6, and 16 days after differentiation initiation. Out of 97 ESC-associated proteins commonly expressed in two ESC lines, 72 proteins were identified using MALDI TOF-TOF mass spectrometry analysis. The expression pattern of four down-regulated proteins including Hspd1, Hspa8, beta-Actin, and Tpt1 were further confirmed by Western blot and immunofluorescence analyses in Royan B1 and D3 as well as two other mouse ESC lines, Royan C1 and Royan C4. Differential mRNA expression analysis of 20 genes using quantitative real-time reverse transcription PCR revealed a low correlation between mRNA and protein levels during differentiation. We also observed that the mRNA level of Tpt1 increased significantly in differentiating cells, whereas its protein level decreased. Several novel ESC-associated proteins have been presented in this study which warrants further investigation with respect to the etiology of stemness.

Baharvand, H. and K. I. Matthaei (2004). "Culture condition difference for establishment of new embryonic stem cell lines from the C57BL/6 and BALB/c mouse strains." In Vitro Cell Dev Biol Anim **40**(3-4): 76-81.

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocysts. These cells are appropriate for creation of animal models of human genetic diseases, the study of gene function in vivo and differentiation into specific types as potential therapeutic agents for several human diseases. We describe here, the production of new ES cell lines from blastocysts recovered from the C57BL/6 and BALB/c mouse strains by changing the concentration of leukemia inhibitory factor (LIF) and primary culture conditions. The established cell lines were analyzed by simple karyotype, C banding, alkaline phosphatase activity, and Oct-4 expression as well as for the presence of the SRY gene. Two ES cell lines from C57BL/6 and three from the BALB/c were produced. The two C57BL/6 ES cell lines were established with either 1000 or 5000 IU LIF, whereas the BALB/c ES cell lines required 5000 IU LIF. Four of the ES cell lines had a normal karyotype. C banding and sex-determining region of Y chromosome-polymerase chain reaction showed that all cell lines had an XY sex chromosome composition. All five of the cell lines expressed alkaline phosphatase activity and Oct-4. One of the BALB/c ES cell lines, when injected into C57BL/6 blastocysts, produced high rates of chimerism as assessed by coat color, and the male chimera produced germ-line offspring when mated with BALB/c females. These results indicate that ES cells from inbred strains can be isolated using commercially available reagents and that the establishment of BALB/c ES cell lines may require different culture conditions to the 129 or C57BL/6 strains.

Baharvand, H., A. Piryaei, et al. (2006). "Ultrastructural comparison of developing mouse embryonic stem cell- and in vivo-derived cardiomyocytes." Cell Biol Int **30**(10): 800-7.

Embryonic stem cells (ESCs) are expected to become a powerful tool for future regenerative medicine and developmental biology due to their capacity for self-renewal and pluripotency. The present study involves characterization and

particularly, the ultrastructure of ESC-derived cardiomyocytes (ESC-CMs). Spontaneously differentiated murine (C57BL/6) ESC-CMs were cultured for 21 days. At different stages, growth characteristics of the CMs were assessed by immunocytochemistry, RT-PCR, transmission electron microscopy, and by addition of chronotropic drugs. EB-derived spontaneously beating cells expressed markers characteristic of CMs including alpha-actinin, desmin, troponin I, sarcomeric myosin heavy chain (MHC), pan-cadherin, connexin 43, cardiac alpha-MHC, cardiac beta-MHC, atrial natriuretic factor (ANF), and myosin light chain isoform-2V (MLC-2V) and responded to drugs in a maturation- and dose-dependent manner. At the ultrasructural level, maturation proceeded with increasing time in culture. In 7+21 days CMs, all sarcomeric components, such as Z-discs, A-, I- and H-bands as well as M-lines, T-tubules, intercalated discs, and the sarcoplasmic reticulum were present. Our data suggest that ESCs can differentiate into functional mature CMs in vitro. Furthermore, ESC-CMs may provide an ideal model for the study of cardiomyocytic development and may be useful for cell therapy of various cardiac diseases.

Baker, R. K., M. A. Haendel, et al. (1997). "In vitro preselection of gene-trapped embryonic stem cell clones for characterizing novel developmentally regulated genes in the mouse." Dev Biol **185**(2): 201-14.

We have developed an in vitro gene trap screen for novel murine genes that allows one to determine, prior to making chimeric or transgenic animals, if these genes are expressed in one or more specific embryonic tissues. Totipotent embryonic stem (ES) cells are infected with a retroviral gene trap construct encoding a selectable lacZ/neo fusion gene, which is expressed only if the gene trap inserts within an active transcription unit. G418-resistant ES cell clones are induced to differentiate in vitro, and neurons, glia, myocytes, and chondrocytes are screened for expression of beta-galactosidase (beta-gal). cDNAs of the gene trap transcripts are obtained by 5' rapid amplification of cDNA ends and are sequenced to determine if they represent novel genes. In situ hybridization analyses show that trapped genes are expressed in vivo within the cell types that express beta-gal in vitro. Gene traps and their wild-type alleles are characterized in terms of copy number, alternate splicing of their transcripts, and the proportion of endogenous mRNA sequence that is replaced by lacZ/neo in the hybrid gene trap transcript. This approach, which we term "in vitro preselection," is more economical than standard in vivo gene trap screening because tissue-specific expression of probable knockout alleles is verified before transgenic animals are generated. These results also highlight the utility of ES cell differentiation in vitro as a method with which to study the molecular mechanisms regulating the specification and commitment of a variety of cell and tissue types.

Bee, T., E. L. Ashley, et al. (2009). "The mouse Runx1 +23 hematopoietic stem cell enhancer confers hematopoietic specificity to both Runx1 promoters." Blood **113**(21): 5121-4.

The transcription factor Runx1 plays a pivotal role in hematopoietic stem cell (HSC) emergence, and studies into its transcriptional regulation should give insight into the critical steps of HSC specification. Recently, we identified the Runx1 +23 enhancer that targets reporter gene expression to the first emerging HSCs of the mouse embryo when linked to the heterologous hsp68 promoter. Endogenous Runx1 is transcribed from 2 alternative promoters, P1 and P2. Here, we examined the in vivo cis-regulatory potential of these alternative promoters and asked whether they act with and contribute to the spatiotemporal specific expression of the Runx1 +23 enhancer. Our results firmly establish that, in contrast to zebrafish runx1, mouse Runx1 promoter sequences do not confer any hematopoietic specificity in transgenic embryos. Yet, both mouse promoters act with the +23 enhancer to drive reporter gene expression to sites of HSC emergence and colonization, in a +23-specific pattern.

Bensidhoum, M., A. Chapel, et al. (2004). "Homing of in vitro expanded Stro-1- or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment." Blood **103**(9): 3313-9.

The Stro-1 antigen potentially defines a mesenchymal stem cell (MSC) progenitor subset. We here report on the role of human ex vivo-expanded selected Stro-1(+) or Stro-1(-) MSC subsets on the engraftment of human CD34(+) cord blood cells in the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mouse model. The data show that cotransplantation of expanded Stro-1(-) cells with CD34(+) cells resulted in a significant increase of human CD45, CD34, CD19, and CD11b cells detected in blood or in bone marrow (BM) and spleen as compared with the infusion of CD34(+) cells alone. Infusion into mice of expanded Stro-1(+) and Stro-1(-) cells (without CD34(+) cells) showed that the numbers of Stro-1(+)-derived (as assessed by DNA analysis of human beta-globin with quantitative polymerase chain reaction [PCR]) were higher than Stro-1(-)-derived cells in spleen, muscles, BM, and kidneys, while more Stro-1(-)-derived than Stro-1(+)-

derived cells were found in lungs. The transduction of expanded Stro-1(+) cells with an enhanced green fluorescent protein (eGFP) gene did not modify their cytokine release and their homing in NOD/SCID mouse tissues. The difference between the hematopoietic support and the homing capabilities of expanded Stro-1(+) and Stro-1(-) cells may be of importance for clinical therapeutic applications: Stro-1(+) cells may rather be used for gene delivery in tissues while Stro-1(-) cells may rather be used to support hematopoietic engraftment.

Benveniste, R. J., G. Keller, et al. (2005). "Embryonic stem cell-derived astrocytes expressing drug-inducible transgenes: differentiation and transplantion into the mouse brain." J Neurosurg **103**(1): 115-23.

OBJECT: Embryonic stem cell (ESC)-derived astrocytes have many theoretical and practical advantages as vectors for delivery of gene therapy to the central nervous system (CNS). The aim of this study was to generate highly pure populations of ESC-derived astrocytes expressing drug-inducible transgenes, while minimizing contamination by undifferentiated ESCs METHODS: Embryonic stem cells carrying a doxycycline-inducible green fluorescent protein (GFP) transgene were induced to differentiate into astrocytes by using feeder cell-free conditions that are completely defined. More than 95% of these cells expressed the astrocyte markers glial fibrillary acidic protein and GLT-1 glutamate transporter, and the morphological characteristics of these cells were typical of astrocytes. The expression of additional astrocyte markers was detected using reverse transcription-polymerase chain reaction. Undifferentiated ESCs comprised fewer than 0.1% of the cells after 10 days in this culture. Positive and negative selection techniques based on fluorescence-activated cell sorting were successfully used to decrease further the numbers of undifferentiated ESCs. Fully differentiated astrocytes expressed a GFP transgene under the tight control of a doxycycline-responsive promoter, and maintained their astrocytic phenotype 24 hours after transplantation into the mouse brain. CONCLUSIONS: This study shows that transgenic ESCs can be induced to differentiate into highly pure populations of astrocytes. The astrocytes continue to express the transgene under the tight control of a drug-inducible promoter and are suitable for transplantation into the mouse brain. The number of potentially hazardous ESCs can be minimized using cell-sorting techniques. This strategy may be used to generate cellular vectors for delivering gene therapy to the CNS.

Bigger, B. W., E. K. Siapati, et al. (2006). "Permanent partial phenotypic correction and tolerance in a mouse model of hemophilia B by stem cell gene delivery of human factor IX." Gene Ther **13**(2): 117-26.

Immune responses against an introduced transgenic protein are a potential risk in many gene replacement strategies to treat genetic disease. We have developed a gene delivery approach for hemophilia B based on lentiviral expression of human factor IX in purified hematopoietic stem cells. In both normal C57Bl/6J and hemophilic 129/Sv recipient mice, we observed the production of therapeutic levels of human factor IX, persisting for at least a year with tolerance to human factor IX antigen. Secondary and tertiary recipients also demonstrate long-term production of therapeutic levels of human factor IX and tolerance, even at very low levels of donor chimerism. Furthermore, in hemophilic mice, partial functional correction of treated mice and phenotypic rescue is achieved. These data show the potential of a stem cell approach to gene delivery to tolerize recipients to a secreted foreign transgenic protein and, with appropriate modification, may be of use in developing treatments for other genetic disorders.

Bilodeau, M., T. MacRae, et al. (2009). "Analysis of blood stem cell activity and cystatin gene expression in a mouse model presenting a chromosomal deletion encompassing Csta and Stfa2l1." PLoS One **4**(10): e7500.

The cystatin protein superfamily is characterized by the presence of conserved sequences that display cysteine protease inhibitory activity (e.g., towards cathepsins). Type 1 and 2 cystatins are encoded by 25 genes of which 23 are grouped in 2 clusters localized on mouse chromosomes 16 and 2. The expression and essential roles of most of these genes in mouse development and hematopoiesis remain poorly characterized. In this study, we describe a set of quantitative real-time PCR assays and a global expression profile of cystatin genes in normal mouse tissues. Benefiting from our collection of DelES embryonic stem cell clones harboring large chromosomal deletions (to be reported elsewhere), we selected a clone in which a 95-kb region of chromosome 16 is missing (Del(16qB3Delta/+)). In this particular clone, 2 cystatin genes, namely Csta and Stfa2l1 are absent along with 2 other genes (Fam162a, Ccdc58) and associated intergenic regions. From this line, we established a new homozygous mutant mouse model (Del(16qB3Delta/16qB3Delta)) to assess the in vivo biological functions of the 2 deleted cystatins. Stfa2l1 gene expression is high in wild-type fetal liver, bone marrow, and spleen, while Csta is ubiquitously expressed. Homozygous Del(16qB3Delta/16qB3Delta) animals are phenotypically normal, fertile, and not overtly susceptible to spontaneous or irradiation-induced

tumor formation. The hematopoietic stem and progenitor cell activity in these mutant mice are also normal. Interestingly, quantitative real-time PCR expression profiling reveals a marked increase in the expression levels of Stfa2l1/Csta phylogenetically-related genes (Stfa1, Stfa2, and Stfa3) in Del(16qB3Delta/16qB3Delta) hematopoietic tissues, suggesting that these candidate genes might be contributing to compensatory mechanisms. Overall, this study presents an optimized approach to globally monitor cystatin gene expression as well as a new mouse model deficient in Stfa2l1/Csta genes, expanding the available tools to dissect cystatin roles under normal and pathological conditions.

Bodine, D. M., N. E. Seidel, et al. (1994). "Efficient retrovirus transduction of mouse pluripotent hematopoietic stem cells mobilized into the peripheral blood by treatment with granulocyte colony-stimulating factor and stem cell factor." Blood **84**(5): 1482-91.

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

Boer, P. H. (1994). "Activation of the gene for type-b natriuretic factor in mouse stem cell cultures induced for cardiac myogenesis." Biochem Biophys Res Commun **199**(2): 954-61.

We assessed the temporal transcriptional activity profiles of the genes for type-B natriuretic factor, BNF, the isoform ANF, and other cardiac muscle proteins in differentiating cultures derived from multipotential mouse cell lines. P19 embryonal carcinoma cells and D3 embryonic stem cells were induced for in vitro cardiac myogenesis; RNA was isolated at regular intervals throughout the differentiation programs, and mRNAs were detected by reverse transcriptase mediated polymerase chain reactions. The transcriptional activation profiles of the ANF and BNF gene were similar, but there were quantitative differences that were best assayed by use of competitive internal DNA standards. The levels of induced BNF transcripts were highest in the P19 developmental system reaching approximately 10% of adult mouse ventricular muscle levels; those for ANF were lower, but also readily detected. The cell lines may be used to define the regulatory control elements for natriuretic factor gene expression, in stably transfected cell lines, during cardiac muscle growth.

Boiani, M., L. Gentile, et al. (2005). "Variable reprogramming of the pluripotent stem cell marker Oct4 in mouse clones: distinct developmental potentials in different culture environments." Stem Cells **23**(8): 1089-104.

A prevailing view of cloning by somatic-cell nuclear transfer is that reprogramming of gene expression occurs during the first few hours after injection of the nucleus into an oocyte, that the process is stochastic, and that the type of reprogramming needed for cloning success is foreign and unlikely to be readily achieved in the ooplasm. Here, we present evidence that the release of reprogramming capacity is contingent on the culture environment of the clone while the contribution of aneuploidy to altered gene expression is marginal. In particular, the rate of blastocyst formation in clones and the regional distribution of mRNA for the pluripotent stem cell marker Oct4 in clonal blastocysts was highly dependent on the culture environment after cumulus cell nuclear transfer, unlike that in genetically equivalent zygotes. Epigenetic modifications of genetically identical somatic nuclei continue after the first cell division of the clones and are amenable to a degree of experimental control, and their development to the blastocyst stage and appropriate expression of Oct4 predict further outcome, such as derivation of embryonic stem (ES) cells, but not fetal development. This observation indicates that development to the blastocyst stage is not equivalent to full reprogramming and lends support to the novel concept that ES cells are not the equivalent of the inner cell mass, hence the

discrepancy between ES cell derivability and fetal development of clones.

Bourne, S., J. M. Polak, 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., D. C. Wu, et al. (2008). "A comparison of protocols used to generate insulin-producing cell clusters from mouse embryonic stem cells." Stem Cells **26**(5): 1128-37.

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.

Brenner, M. K., G. G. Wulf, et al. (2003). "Complement-fixing CD45 monoclonal antibodies to facilitate stem cell transplantation in mouse and man." Ann N Y Acad Sci **996**: 80-8.

Broadening the applicability of stem cell therapies requires safer preparative regimens for patients. The CD45 antigen is present on all cells of the hematopoietic lineage, and using a murine model, we determined whether a lytic CD45 monoclonal antibody could produce persistent aplasia and whether it could facilitate syngeneic or allogeneic stem cell engraftment. After its systemic administration, we found that all leukocyte subsets in peripheral blood were markedly diminished, but only the effect on the lymphoid compartment was sustained and marrow progenitor cells were spared from destruction. Given the transient effects of the monoclonal antibody on myelopoiesis and the more persistent effects on lymphopoiesis, we asked whether this agent could contribute to donor hemopoietic engraftment after subablative transplantation. Treatment with anti-CD45 alone did not enhance syngeneic engraftment, consistent with its inability to destroy progenitor cells and permit competitive repopulation with syngeneic donor stem cells. By contrast, the combination of anti-CD45 and an otherwise inactive dose of total-body irradiation allowed engraftment of H2 fully allogeneic donor stem cells. We attribute this result to the recipient immunosuppression produced by depletion of CD45-positive lymphocytes. We next assessed a pair of unconjugated rat anti-human CD45 monoclonal antibodies (MAbs), YTH54.12 and YTH25.4, in a clinical trial in patients who were to receive stem cell transplantation for acute leukemia. The maximum tolerated dose of these MAbs, 400 microg/kg/day, produced a pattern of response identical to that seen in the mice, with marked reductions in circulating lymphoid and myeloid cells and sparing of early marrow progenitors. In two of

three patients with active leukemia, the MAbs also produced a decrease in the percentage of leukemic blast cells in bone marrow. These pre-clinical and clinical results warrant further evaluation of anti-CD45 MAbs in subablative preparative regimens for stem cell transplantation.

Brown, J., M. F. Greaves, et al. (1991). "The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in haemopoietic progenitor cell lines, brain, and embryonic fibroblasts." Int Immunol **3**(2): 175-84.

The human haemopoietic cell surface antigen, CD34, is a 105 - 120 kd cell surface glycoprotein whose stage-specific expression by stem cells and lineage-specific progenitor cells suggests a role in regulating early events in blood cell differentiation. A murine gene and cDNA encoding a closely homologous protein have been isolated. The gene is organized in eight exons in 22 kb of DNA. The first exon lies in a GC- and CpG-rich island. The sequence of the gene and the cDNA predict a 382 amino acid-long protein containing an N-terminal signal peptide and one transmembrane region 73 amino acids from the C-terminus. The extracellular part of the protein contains: a 140 amino acid-long-N-terminal region, 40% of whose residues are serine or threonine potential attachment sites for O-linked carbohydrate, as well as five potential attachment sites for N-linked carbohydrate. Proximal to the extracellular membrane there is a 79 amino acid-long cysteine-rich region. The homology with the human sequence is highest in the intracellular domain (90% amino acid identity) and lowest in the N-terminal region (43% amino acid identity). The protein is not homologous with any other proteins currently in the databases. The expression of the murine gene by a number of haemopoietic progenitor cell lines suggests that the CD34 function in haemopoiesis may be conserved between man and mouse. The high level of expression in a number of embryonic fibroblast cell lines and in brain imply a function outside of haemopoiesis.

Campbell, T. B., S. Basu, et al. (2009). "Overexpression of Rheb2 enhances mouse hematopoietic progenitor cell growth while impairing stem cell repopulation." Blood **114**(16): 3392-401.

Molecular mechanisms preserving hematopoietic stem cell (HSC) self-renewal by maintaining a balance between proliferation, differentiation, and other processes are not fully understood. Hyperactivation of the mammalian target of rapamycin (mTOR) pathway, causing sustained proliferative signals, can lead to exhaustion of HSC repopulating ability. We examined the role of the novel ras gene Rheb2, an activator of the mTOR kinase, in colony-forming ability, survival, and repopulation of immature mouse hematopoietic cells. In a cell line model of mouse hematopoietic progenitor cells (HPCs), we found enhanced proliferation and mTOR signaling in cells overexpressing Rheb2. In addition, overexpression of Rheb2 enhanced colony-forming ability and survival of primary mouse bone marrow HPCs. Expansion of phenotypic HSCs in vitro was enhanced by Rheb2 overexpression. Consistent with these findings, Rheb2 overexpression transiently expanded phenotypically defined immature hematopoietic cells after in vivo transplantation; however, these Rheb2-transduced cells were significantly impaired in overall repopulation of primary and secondary congenic transplantation recipients. Our findings suggest that HPCs and HSCs behave differently in response to growth-promoting signals stimulated by Rheb2. These results may have value in elucidating mechanisms controlling the balance between proliferation and repopulating ability, a finding of importance in clinical uses of HPCs/HSCs.

Carter, M. G., T. Hamatani, et al. (2003). "In situ-synthesized novel microarray optimized for mouse stem cell and early developmental expression profiling." Genome Res **13**(5): 1011-21.

Applications of microarray technologies to mouse embryology/genetics have been limited, due to the nonavailability of microarrays containing large numbers of embryonic genes and the gap between microgram quantities of RNA required by typical microarray methods and the miniscule amounts of tissue available to researchers. To overcome these problems, we have developed a microarray platform containing in situ-synthesized 60-mer oligonucleotide probes representing approximately 22,000 unique mouse transcripts, assembled primarily from sequences of stem cell and embryo cDNA libraries. We have optimized RNA labeling protocols and experimental designs to use as little as 2 ng total RNA reliably and reproducibly. At least 98% of the probes contained in the microarray correspond to clones in our publicly available collections, making cDNAs readily available for further experimentation on genes of interest. These characteristics, combined with the ability to profile very small samples, make this system a resource for stem cell and embryogenomics research.

Challen, G. A., N. Boles, et al. (2009). "Mouse hematopoietic stem cell identification and analysis." Cytometry A **75**(1): 14-24.

Hematopoietic stem cells (HSCs) remain by far the most well-characterized adult stem cell

population both in terms of markers for purification and assays to assess functional potential. However, despite over 40 years of research, working with HSCs in the mouse remains difficult because of the relative abundance (or lack thereof) of these cells in the bone marrow. The frequency of HSCs in bone marrow is about 0.01% of total nucleated cells and approximately 5,000 can be isolated from an individual mouse depending on the age, sex, and strain of mice as well as purification scheme utilized. This prohibits the study of processes in HSCs, which require large amounts of starting material. Adding to the challenge is the continual reporting of new markers for HSC purification, which makes it difficult for the uninitiated in the field to know which purification strategies yield the highest proportion of long-term, multilineage HSCs. This report will review different hematopoietic stem and progenitor purification strategies and compare flow cytometry profiles for HSC sorting and analysis on different instruments. We will also discuss methods for rapid flow cytometric analysis of peripheral blood cell types, and novel strategies for working with rare cell populations such as HSCs in the analysis of cell cycle status by BrdU, Ki-67, and Pyronin Y staining. The purpose of this review is to provide insight into some of the recent experimental and technical advances in mouse hematopoietic stem cell biology.

Chaudhry, G. R., C. Fecek, et al. (2009). "Fate of embryonic stem cell derivatives implanted into the vitreous of a slow retinal degenerative mouse model." Stem Cells Dev **18**(2): 247-58.

Stem cell therapy may be used potentially to treat retinal degeneration and restore vision. Since embryonic stem cells (ESCs) can differentiate into almost any cell types, including those found in the eye, they can be transplanted to repair or replace damaged or injured retinal tissue resulting from inherited diseases or traumas. In this investigation, we explored the potential of ESCs and ESC-derived neuroprogenitors to proliferate and integrate into the diseased retinal tissue of rd12 mice. These rd12 mice mimic the slow and progressive retinal degeneration seen in humans. Both ESCs and ESC-derived neuroprogenitors from ESCs survived and proliferated as evidenced from an increase in yellow fluorescent protein fluorescence. Quantification analysis of cryosectioned retinal tissue initially revealed that both ESCs and neuroprogenitors differentiated into cells expressing neural markers. However, ESC proliferation was robust and resulted in the disruption of the retinal structure and the eventual formation of teratomas beyond 6 weeks postimplantation. In contrast, the neuroprogenitors proliferated slowly, but differentiated further and integrated into the retinal layers of the eye. The differentiation of neuroprogenitors represented various retinal cell types, as judged from the expression of cell-specific markers including Nestin, Olig1, and glial fibrillary acidic protein. These results suggest that ESC-derived neuroprogenitors can survive, proliferate, and differentiate when implanted into the eyes of experimental mice and may be used potentially as cell therapy for treating degenerated or damaged retinal tissue.

Chen, S., A. Choo, et al. (2006). "TGF-beta2 allows pluripotent human embryonic stem cell proliferation on E6/E7 immortalized mouse embryonic fibroblasts." J Biotechnol **122**(3): 341-61.

In this study we report observations that mouse embryonic fibroblasts (MEF) capable of supporting expansion of pluripotent, human embryonic stem cells (hESC) fail to support after immortalization using E6/E7 oncogenes in serum conditions; however this can be reversed following addition of exogenous TGF-beta2. Microarray analysis of immortalized and non-immortalized MEF revealed differential gene expression of several TGF-beta related genes. By supplementing TGF-beta2 into E6/E7 immortalized MEF cultures, this enabled proliferation of undifferentiated, pluripotent hESC as demonstrated by marker expression (Oct-4, SSEA-4, alkaline phosphatase) and teratoma formation representing three germ layers following hESC injection into immuno-deficient mice. Subsequent investigation using quantitative real-time PCR highlighted differential gene expression of several extracellular matrix related transcripts in primary and immortal (+/-TGF-beta2) feeder cells including the induction of osteopontin following addition of TGF-beta2. Our results demonstrate that TGF-beta2 and its related genes in MEF play a role in the support of pluripotent hESC expansion.

Chen, U., M. Kosco, et al. (1992). "Establishment and characterization of lymphoid and myeloid mixed-cell populations from mouse late embryoid bodies, "embryonic-stem-cell fetuses"." Proc Natl Acad Sci U S A **89**(7): 2541-5.

Mouse embryonic stem (ES) cells have the potential to differentiate into embryoid bodies in vitro and mimic normal embryonic development. The "ES fetus" is a specific development at a late stage seen under our culture conditions. We have established several mixed populations from ES fetuses by using combinations of retroviruses carrying different oncogenes (v-abl, v-raf, c-myc), interleukins 2 and 3, and Con A. Six groups of mixed populations were characterized by immunophenotyping. For some groups, transfer of cells into sublethally irradiated

mice resulted in the development of macrophages, mature T and B lymphocytes, and plasma cells of donor origin. Thus, these mixed populations may contain immortalized precursors of hematopoietic lineages. These mixed populations should be valuable for defining hematopoietic stem cells and their committed progenitors.

Chen, X., X. L. Gong, et al. (2009). "Hematopoietic stem cell engraftment by early-stage in utero transplantation in a mouse model." Exp Mol Pathol **87**(3): 173-7.

A novel intrauterine transplantation (IUT) approach was developed to improve the efficiency of engraftment of hematopoietic stem cells (HSCs). HSCs with a green fluorescent protein (GFP) reporter gene were transplanted in utero on days 12.5, 13.5 and 14.5 post coitum (p.c.). The degree of chimerism of donor cells in recipient newborn mice was examined using fluorescent microscopy, polymerase chain reaction (PCR), fluorescence-activated cell sorting (FACS), and fluorescence in situ hybridization (FISH) analyses. Microscopic examination revealed the presence of green fluorescent signal in the peripheral blood of the chimeric mice. The highest survival rate (47%) as well as the highest chimerism rate (73%) were achieved by our new approach in the newborn mice that were subjected to in utero transplantation (IUT) on day 12.5 p.c. (E12.5) compared to the conventional IUT method. FACS analysis indicated that 1.55+/-1.10% of peripheral blood cells from the newborn mice were GFP-positive donor cells. FISH showed that cells containing the donor-specific GFP sequence were present in the bone marrow (BM) of the chimeric mice. Thus, the efficiency of chimera production with this new method of IUT was significantly improved over the existing IUT techniques and instruments.

Chepko, G., R. Slack, et al. (2005). "Differential alteration of stem and other cell populations in ducts and lobules of TGFalpha and c-Myc transgenic mouse mammary epithelium." Tissue Cell **37**(5): 393-412.

Genes associated with proliferation are active in stem and progenitor cells, and their over-expression can promote cancer. Two such genes, c-Myc and TGFalpha, promote morphologically dissimilar mammary tumors in transgenic mice. We investigated whether their over-expression affects population size and cell cycle activity in stem and other cell populations in non-neoplastic mammary epithelia. Results indicated that both cell population and cell cycle regulation are cell type- and microenvironment-specific. To create a tool for identifying and categorizing the five cellular phenotypes by light microscopy, we adapted previously established ultrastructural criteria. Using nulliparous MMTV-c-myc or MT-tgfalpha mice, we determined and compared the relative sizes the putative stem, progenitor and differentiated cell populations. PCNA staining was used to compare the portion of each cell population in the cell cycle. Cell population sizes were analyzed relative to: (1) their location in ducts versus lobules (microenvironment), (2) genotype, and (3) cell type. Population sizes differed significantly by genotype, depending on microenvironment ($p=0.0008$), by genotype, depending on cell type ($p<0.0001$), and by microenvironment, depending on cell type ($p=0.03$). The number of cycling cells was also affected by all three factors, confirming that the interplay of cell type, gene expression and three-dimensional organization are very important in tissue morphogenesis and function. We describe a structure in mammary epithelium consistent with that of a stem cell niche, and show that it is altered in MMTV-c-myc and likely altered in MT TGFalpha transgenic epithelia.

Chinzei, R., Y. Tanaka, et al. (2002). "Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes." Hepatology **36**(1): 22-9.

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.

Chung, S., T. Andersson, et al. (2002). "Analysis of different promoter systems for efficient transgene expression in mouse embryonic stem cell lines." Stem Cells **20**(2): 139-45.

Mouse embryonic stem (ES) cells are derived from the inner cell mass of the preimplantation embryo and have the developmental capacity to generate all cell types of the body. Combined with efficient genetic manipulation and in vitro differentiation procedures, ES cells are a useful system for the molecular analysis of developmental pathways. We analyzed and compared the transcriptional activities of a cellular polypeptide chain elongation factor 1 alpha (EF), a cellular-virus hybrid (cytomegalo-virus [CMV] immediate early enhancer fused to chicken beta-actin [CBA]), and a viral CMV promoter system in two ES cell lines. When transiently transfected, the EF and CBA promoters robustly drove reporter gene expression, while the CMV promoter was inactive. We also demonstrated that the EF and CBA promoters effectively drove gene expression in different stages of cell development: naive ES cells, embryoid bodies (EBs), and neuronal precursor cells. In contrast, the CMV promoter did not have transcriptional activity in either ES cells or EB but had significant activity once ES cells differentiated into neuronal precursors. Our data show that individual promoters have different abilities to express reporter gene expression in the ES and other cell types tested.

Conley, B. J., M. Denham, et al. (2005). "Mouse embryonic stem cell derivation, and mouse and human embryonic stem cell culture and differentiation as embryoid bodies." Curr Protoc Cell Biol **Chapter 23**: Unit 23 2.

Embryonic stem (ES) cells are pluripotent cells derived from developing mouse blastocysts in vitro that maintain long-term self renewal and the capacity to give rise to all cell types in the adult body (including some extraembryonic cell types) when subjected to the appropriate conditions. It is envisaged that the development of methods enabling controlled differentiation of mouse ES cell counterparts from human blastocysts would enable the provision of an unlimited supply of tissue for cell and tissue transplantation therapies for the repair and replacement of diseased, injured, and senescent tissue. Furthermore, derivation of mouse ES cells has allowed for the generation of thousands of gene-targeted mouse mutants. Culture of mouse ES cells as embryoid bodies (EBs) has provided a convenient system for studying early mouse developmental processes, including several aspects of extraembryonic lineage and axis formation associated with the pre- and peri-gastrulating mouse embryo. Relatively little is known regarding the corresponding development of the early human embryo due to limitations associated with the acquisition of relevant tissue material for study. The transfer of methods such as EB formation to human systems should, by association, facilitate a more advanced understanding of similar processes associated with early human development. This unit describes protocols for isolating mouse embryonic stem cells and methods for propagating, freezing, and producing EBs from both mouse and human embryonic stem cells.

Constantinescu, D., H. L. Gray, et al. (2006). "Lamin A/C expression is a marker of mouse and human embryonic stem cell differentiation." Stem Cells **24**(1): 177-85.

Nuclear lamins comprise the nuclear lamina, a scaffold-like structure that lines the inner nuclear membrane. B-type lamins are present in almost all cell types, but A-type lamins are expressed predominantly in differentiated cells, suggesting a role in maintenance of the differentiated state. Previous studies have shown that lamin A/C is not expressed during mouse development before day 9, nor in undifferentiated mouse embryonic carcinoma cells. To further investigate the role of lamins in cell phenotype maintenance and differentiation, we examined lamin expression in undifferentiated mouse and human embryonic stem (ES) cells. Wide-field and confocal immunofluorescence microscopy and semiquantitative reverse transcription-polymerase chain reaction analysis revealed that undifferentiated mouse and human ES cells express lamins B1 and B2 but not lamin A/C. Mouse ES cells display high levels of lamins B1 and B2 localized both at the nuclear periphery and throughout the nucleoplasm, but in human ES cells, B1 and B2 expression is dimmer and localized primarily at the nuclear periphery. Lamin A/C expression is activated during human ES cell differentiation before downregulation of the pluripotency marker Oct-3/4 but not before the downregulation of the pluripotency markers Tra-1-60, Tra-1-81, and SSEA-4. Our results identify the absence of A-type lamin expression as a novel marker for undifferentiated ES cells and further support a role for nuclear lamins in cell maintenance and differentiation.

Cooper, G. E., D. L. DiMartino, et al. (1991). "Molecular analysis of APRT deficiency in mouse P19 teratocarcinoma stem cell line." Somat Cell Mol Genet **17**(2): 105-16.

We have used four gene probes specific for mouse chromosome 8, including adenine phosphoribosyltransferase (aprt), to demonstrate that the P19 teratocarcinoma stem cell line contains two distinct chromosome 8 homologs. One represents the common laboratory mouse C3H (Mus musculus domesticus) homolog while the second homolog was presumably contributed by a feral Mus musculus musculus animal. Six cell lines with APRT heterozygous deficiencies were isolated from P19 subclones. A molecular analysis of these heterozygotes demonstrated that three arose by deletion of the Mus musculus musculus aprt allele and three arose by aprt gene inactivation. APRT homozygous deficient cell lines were isolated from both classes of heterozygote; most contained little or no detectable APRT activity. When the heterozygous deficiency was due to deletion of the Mus musculus musculus aprt allele, the most frequent event yielding homozygous deficient cell lines was associated with loss of heterozygosity for all tested markers on the Mus musculus domesticus homolog indicating chromosome loss. In contrast, when the initial event resulting in APRT heterozygous deficiency was gene inactivation, homozygotes arose predominantly from gene deletion or a second inactivation event. These results suggest a potential relationship between the first- and second-step events resulting in APRT deficiencies.

Corti, S., M. Nizzardo, et al. (2008). "Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy." J Clin Invest **118**(10): 3316-30.

Spinal muscular atrophy (SMA), a motor neuron disease (MND) and one of the most common genetic causes of infant mortality, currently has no cure. Patients with SMA exhibit muscle weakness and hypotonia. Stem cell transplantation is a potential therapeutic strategy for SMA and other MNDs. In this study, we isolated spinal cord neural stem cells (NSCs) from mice expressing green fluorescent protein only in motor neurons and assessed their therapeutic effects on the phenotype of SMA mice. Intrathecally grafted NSCs migrated into the parenchyma and generated a small proportion of motor neurons. Treated SMA mice exhibited improved neuromuscular function, increased life span, and improved motor unit pathology. Global gene expression analysis of laser-capture-microdissected motor neurons from treated mice showed that the major effect of NSC transplantation was modification of the SMA phenotype toward the wild-type pattern, including changes in RNA metabolism proteins, cell cycle proteins, and actin-binding proteins. NSC transplantation positively affected the SMA disease phenotype, indicating that transplantation of NSCs may be a possible treatment for SMA.

Dinger, M. E., P. P. Amaral, et al. (2008). "Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation." Genome Res **18**(9): 1433-45.

The transcriptional networks that regulate embryonic stem (ES) cell pluripotency and lineage specification are the subject of considerable attention. To date such studies have focused almost exclusively on protein-coding transcripts. However, recent transcriptome analyses show that the mammalian genome contains thousands of long noncoding RNAs (ncRNAs), many of which appear to be expressed in a developmentally regulated manner. The functions of these remain untested. To identify ncRNAs involved in ES cell biology, we used a custom-designed microarray to examine the expression profiles of mouse ES cells differentiating as embryoid bodies (EBs) over a 16-d time course. We identified 945 ncRNAs expressed during EB differentiation, of which 174 were differentially expressed, many correlating with pluripotency or specific differentiation events. Candidate ncRNAs were identified for further characterization by an integrated examination of expression profiles, genomic context, chromatin state, and promoter analysis. Many ncRNAs showed coordinated expression with genomically associated developmental genes, such as Dlx1, Dlx4, Gata6, and Ecsit. We examined two novel developmentally regulated ncRNAs, Evx1as and Hoxb5/6as, which are derived from homeotic loci and share similar expression patterns and localization in mouse embryos with their associated protein-coding genes. Using chromatin immunoprecipitation, we provide evidence that both ncRNAs are associated with trimethylated H3K4 histones and histone methyltransferase MLL1, suggesting a role in epigenetic regulation of homeotic loci during ES cell differentiation. Taken together, our data indicate that long ncRNAs are likely to be important in processes directing pluripotency and alternative differentiation programs, in some cases through engagement of the epigenetic machinery.

Dobrovolsky, V. N., D. A. Casciano, et al. (1996). "Development of a novel mouse tk+/- embryonic stem cell line for use in mutagenicity studies." Environ Mol Mutagen **28**(4): 483-9.

A tk+/- mouse embryonic stem (ES) cell line, designated 1G2, has been created in which one allele of the thymidine kinase (tk) gene was inactivated by targeted homologous recombination. This line is an analog of the mouse lymphoma tk+/- L5178Y cell line, which is used widely to assess the mutagenicity

of chemical agents. Treatment of 1G2 cells with the alkylating agent N-ethyl-N-nitrosourea (ENU) resulted in a dose-related increase in trifluorothymidine-resistant colonies. Mutant frequencies of 152 and 296 per 10(6) cells were determined for 0.1 and 0.3 mg/ml doses of ENU, compared with a spontaneous mutant frequency of 15 per 10(6) cells. The data indicate that tk+/- 1G2 ES cells may be useful for the creation of a transgenic mouse model for assessing in vivo mutation using an endogenous autosomal gene.

Downing, G. J. and J. F. Battey, Jr. (2004). "Technical assessment of the first 20 years of research using mouse embryonic stem cell lines." Stem Cells **22**(7): 1168-80.

This review assesses the effect that mouse embryonic stem (ES) cells have had on biomedical research during the 20 years that followed their isolation in 1981. Notable scientific discoveries enabled by these cell lines--including insights into cell cycle regulation, spatial and temporal relationships during development, and the roles of transcription factors and homeobox genes in developmental pathways--are discussed. The acceleration of basic discovery of gene function and the genetic basis of disease using a breakthrough technology (homologous recombination between modified gene constructs and the ES cell genome) became the principal enabling method to establish transgenic laboratory animals with single targeted genetic change. This review also examines the widespread influence of mouse ES cells as an enabling technology by highlighting their effect on drug development paradigms, directed differentiation to treat specific diseases, nuclear transfer protocols used in cloning, and establishment of methodologies for isolating non-rodent ES cells. This review concludes with a brief analysis of the most influential mouse ES cell lines of the first 20 years as viewed within the twin contexts of human disease application and contributions to the primary literature.

Dunnwald, M., S. Chinnathambi, et al. (2003). "Mouse epidermal stem cells proceed through the cell cycle." J Cell Physiol **195**(2): 194-201.

The epidermis is a continuously renewing tissue maintained by undifferentiated stem cells. For decades it has been assumed that epidermal stem cells (ESCs) were held in the G0 phase of the cell cycle and that they only entered the cell cycle when needed. Previously, we showed that ESCs retained nuclear label for long periods, indicating that these cells did not proceed through the cell cycle at the same rate as the other proliferative basal cells. However, their exact cell-cycle profile has not been determined because a pure population of ESCs has not been available. In this study, we sorted stem and transient amplifying (TA) cells from murine neonatal back skin, and adult ear, footpad, and back skin, using our recently developed method. We found that neonatal back skin had two times the number of ESCs as the adult tissues. Despite the age and anatomical difference, these ESC populations exhibited similar cell cycle profiles with approximately 96% in G0/G1 and 4% in S-G2/M. The cell cycle profiles of the TA cells from neonatal back skin and adult footpad also showed a profile similar to each other (85% in G1 and 15% in S-G2/M). Examination of genes on a cell cycle chip showed that proliferation associated genes and only p57 were upregulated in the TA cell and ESC population, respectively. We found BrdU positive and cyclin B1 positive cells in all groups, confirming that both ESCs and TA cells were cycling. These data demonstrate that there are more TA cells dividing than ESCs, that the cell cycle profile of adult TA cells is related to the proliferative state of the tissue in which they reside, and that ESC proceed through the cell cycle.

Ebata, K. T., X. Zhang, et al. (2005). "Expression patterns of cell-surface molecules on male germ line stem cells during postnatal mouse development." Mol Reprod Dev **72**(2): 171-81.

Spermatogonial stem cells (SSCs) are stem cells of the male germ line. In mice, SSCs are quiescent at birth but actively proliferate during the first postnatal week, while they rarely divide in adult, suggesting an age-dependent difference in SSC characteristics. As an approach to evaluate this possibility, we studied the expression pattern of cell-surface molecules on neonatal, pup, and adult mouse SSCs. Using immunomagnetic cell sorting, testis cells were selected for the expression of alpha(6) integrin, alpha(v) integrin, c-kit receptor tyrosine kinase (Kit), or a binding subunit of glial-cell-line-derived neurotrophic factor (GDNF) receptor, GFRalpha1. Selected cells were assayed for their stem cell activity using spermatogonial transplantation. The results showed that SSCs expressed alpha(6) integrin, but not alpha(v) integrin and Kit, regardless of age. The SSC activity in pup GFRalpha1(+) cells was higher than that in adult and neonatal cells, indicating that the expression pattern of GFRalpha1 varied age-dependently. To evaluate if SSCs show an age-dependent difference in their response to GDNF, we cultured highly enriched pup and adult SSCs with GDNF: we could not observe such an age-dependent difference in vitro. In addition, we failed to immunologically detect the expression of two types of GDNF receptor signaling subunits on SSCs. These results indicate that SSCs may change the expression

patterns of cell-surface molecules during postnatal development, and suggest that GDNF receptor molecules may not be abundantly or specifically expressed in the in vivo population of mouse SSCs.

Eistetter, H. R. (1988). "A mouse pluripotent embryonal stem cell line stage-specifically regulates expression of homeo-box containing DNA sequences during differentiation in vitro." Eur J Cell Biol **45**(2): 315-21.

Mouse embryonal stem (ES) cells have been shown to provide a new model system suitable for the analysis of different aspects of murine development. This report gives evidence that ES cell lines are also most useful for the study of developmentally regulated gene expression in vitro. Homeo-box containing genes which are suggested to play a key role in the regulation of differentiation steps occurring during embryogenesis are stage-specifically transcribed in differentiating murine ES cells: (i) A mouse embryonal stem cell line (ES-12957) was isolated and characterized with respect to its differentiation potential. When injected subcutaneously into syngeneic mice, ES-12957 cells formed fully differentiated teratomas representing derivatives of all three germ layers. When allowed to grow in suspension cultures in vitro, the cells followed a reproducible developmental pathway forming complex organized 'embryoid bodies' which resembled mouse early postimplantation embryos. (ii) A mouse DNA sequence with homeo-box homology (MH-121) was isolated and structurally analyzed. Transcription of a 1.7 kb RNA species from this DNA sequence was demonstrated in ES-12957 cells which were differentiated in vitro. A second, previously described homeo-box gene (Mo-10) was also shown to be expressed in ES-12957 cells in a stage-specific manner. A 4-kb transcript could be identified exclusively in RNA of cells which were allowed to differentiate for 9 days. These findings support the suggestion that the homeo-box genes of mammals, like those of Drosophila, may have important functions during embryonic development.

Fok, E. Y. and P. W. Zandstra (2005). "Shear-controlled single-step mouse embryonic stem cell expansion and embryoid body-based differentiation." Stem Cells **23**(9): 1333-42.

To facilitate the exploitation of embryonic stem cells (ESCs) and ESC-derived cells, scale-up of cell production and optimization of culture conditions are necessary. Conventional ESC culture methods are impractical for large-scale cell production and lack robust microenvironmental control. We developed two stirred-suspension culture systems for the propagation of undifferentiated ESCs--microcarrier and aggregate cultures--and compared them with tissue-culture flask and Petri dish controls. ESCs cultured on glass microcarriers had population doubling times (approximately 14-17 hours) comparable to tissue-culture flask controls. ESC growth could be elicited in shear-controlled stirred-suspension culture, with population doubling times ranging between 24 and 39 hours at 100 rpm impeller speed. Upon removal of leukemia inhibitory factor, the size-controlled ESC aggregates developed into embryoid bodies (EBs) capable of multilineage differentiation. A comprehensive analysis of ESC developmental potential, including flow cytometry for Oct-4, SSEA-1, and E-cadherinprotein expression, reverse transcription-polymerase chain reaction for Flk-1, HNF3-beta, MHC, and Sox-1 gene expression, and EB differentiation analysis, demonstrated that the suspension-cultured ESCs retained the developmental potential of the starting cell population. Analysis of E-cadherin-/- and E-cadherin+/- cells using both systems provided insight into the mechanisms behind the role of cell aggregation control, which is fundamental to these observations. These cell-culture tools should prove useful for both the production of ESCs and ESC-derived cells and for investigations into adhesion, survival, and differentiation phenomena during ESC propagation and differentiation.

Francini, F., H. Del Zotto, et al. (2009). "Selective effect of INGAP-PP upon mouse embryonic stem cell differentiation toward islet cells." Regul Pept **153**(1-3): 43-8.

We evaluated the effect of islet neogenesis-associated protein pentadecapeptide (INGAP-PP) upon islet beta- and non-beta cell differentiation from mouse embryonic stem (mES) cells. ES-D3 cell lines were cultured following Lumelsky's protocol with or without INGAP-PP (5 microg/ml) at different stages. Gene expression was quantified using qPCR. mES cells were fixed and immunostained using anti insulin-, somatostatin-, glucagon-, Pdx-1-, Ngn-3-, Nkx-6.1 and PGP9.5 specific antibodies. PCNA was used to measure replication rate. Bcl(2) (immunostaining) and caspase-3 (enzyme activity and gene expression) were determined as apoptosis markers. INGAP-PP increased IAPP, Glut-2, Kir-6.2, SUR-1 and insulin gene expression, and the percentage of insulin-immunostained cells. Conversely, INGAP-PP reduced significantly glucagon and somatostatin gene expression and immunopositivity. While nestin gene expression was not affected, there was a significant reduction in the percentage of PGP9.5-immunostained cells. Pdx-1 gene expression increased by 115% in INGAP-PP treated cells, as well as the percentage of Pdx-1, Ngn-3 and Nkx-6.1 immunopositive cells. Neither caspase-3 (expression and activity) nor Bcl(2)

positively immunostained cells were affected by INGAP-PP. Accordingly, INGAP-PP would promote stem cell differentiation into a beta-like cell phenotype, simultaneously decreasing its differentiation toward non-beta-cell precursors. Therefore, INGAP-PP would be potentially useful to obtain beta-cells from stem cells for replacement therapy.

Fujii-Yamamoto, H., J. M. Kim, et al. (2005). "Cell cycle and developmental regulations of replication factors in mouse embryonic stem cells." J Biol Chem **280**(13): 12976-87.

Embryonic stem (ES) cells can grow rapidly and permanently while maintaining their differentiation capacity. To gain insight into how the cell cycle progression of undifferentiated murine ES cells is regulated, we have examined the expression patterns of various replication and cell cycle regulators. Most factors including cyclins, Cdc6, and geminin are rather constitutively expressed during the cell cycle of ES cells. Furthermore, the transcript levels of almost all the cell cycle regulators we investigated except for p21 and p27 are higher in undifferentiated ES cells than in murine embryonic fibroblasts (MEFs), and the increased stability of mRNA in ES cells may be partially responsible for this at least with some of the factors. More strikingly, the transcriptional levels of these factors are strongly correlated with the acetylated state of histone H3 at their promoter regions. However, the methylation state of histone or CpG methylation of the promoter region is not generally correlated significantly with the expression pattern of these factors in both cell types. On the protein level, Cdc6, ASK, cyclin A2, and cyclin B1 are extremely abundant in ES cells compared with MEFs. Furthermore, they are rapidly down-regulated upon induction of differentiation of ES cells. The significance of these findings is discussed in relation to the unusual proliferative properties of ES cells in an undifferentiated state.

Fukumitsu, K., T. Ishii, 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): 3847-56.

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.

Furusawa, T., M. Ikeda, et al. (2006). "Gene expression profiling of mouse embryonic stem cell subpopulations." Biol Reprod **75**(4): 555-61.

We previously demonstrated that mouse embryonic stem (ES) cells show a wide variation in the expression of platelet endothelial cell adhesion molecule 1 (PECAM1) and that the level of expression is positively correlated with the pluripotency of ES cells. We also found that PECAM1-positive ES cells could be divided into two subpopulations according to the expression of stage-specific embryonic antigen (SSEA)-1. ES cells that showed both PECAM1 and SSEA-1 predominantly differentiated into epiblast after the blastocyst stage. In the present study, we performed pairwise oligo microarray analysis to characterize gene expression profiles in PECAM1-positive and -negative subpopulations of ES cells. The microarray analysis identified 2034 genes with a more than 2-fold difference in expression levels between the PECAM1-positive and -negative cells. Of these genes, 803 were more highly expressed in PECAM1-positive cells and 1231 were more highly expressed in PECAM1-negative cells. As expected, genes known to function in ES cells, such as Pou5f1(Oct3/4)and Nanog, were found to be upregulated in PECAM1-positive cells. We also isolated 23 previously uncharacterized genes. A comparison of gene expression profiles in PECAM1-positive cells that were either positive or negative for SSEA-1 expression identified only 53 genes that showed a more than 2-fold greater difference in expression levels between these

subpopulations. However, many genes that are under epigenetic regulation, such as globins, Igf2, Igf2r, andH19, showed differential expression. Our results suggest that in addition to differences in gene expression profiles, epigenetic status was altered in the three cell subpopulations.

Gambaro, K., E. Aberdam, et al. (2006). "BMP-4 induces a Smad-dependent apoptotic cell death of mouse embryonic stem cell-derived neural precursors." Cell Death Differ **13**(7): 1075-87.

Embryonic ectoderm is fated to become either neural or epidermal, depending on patterning processes that occur before and during gastrulation. It has been stated that epidermal commitment proceeds from a bone morphogenetic protein-4 (BMP-4)-dependent inhibition of dorsal ectoderm neuralization. We recently demonstrated that murine embryonic stem (ES) cells treated with BMP-4 undergo effective keratinocyte commitment and epidermogenesis. Focusing on the precise role of BMP-4 in the early choice between neural and epidermal commitment, we show here that BMP-4 treatment of ES cells leads to a dramatic apoptotic death of Sox-1+ neural precursors with concomitant epidermal engagement. In addition, neutralization of the Smad pathway prevents both the BMP-4 apoptotic process and the inhibition of neural differentiation. Our results suggest that, in mammals, BMP-4, as an active inducer of epidermal commitment, interferes with the survival of neural precursors through induction of their apoptotic cell death.

Gangloff, Y. G., M. Mueller, et al. (2004). "Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development." Mol Cell Biol **24**(21): 9508-16.

The mammalian target of rapamycin (mTOR) is a key component of a signaling pathway which integrates inputs from nutrients and growth factors to regulate cell growth. Recent studies demonstrated that mice harboring an ethylnitrosourea-induced mutation in the gene encoding mTOR die at embryonic day 12.5 (E12.5). However, others have shown that the treatment of E4.5 blastocysts with rapamycin blocks trophoblast outgrowth, suggesting that the absence of mTOR should lead to embryonic lethality at an earlier stage. To resolve this discrepancy, we set out to disrupt the mTOR gene and analyze the outcome in both heterozygous and homozygous settings. Heterozygous mTOR (mTOR(+/-)) mice do not display any overt phenotype, although mouse embryonic fibroblasts derived from these mice show a 50% reduction in mTOR protein levels and phosphorylation of S6 kinase 1 T389, a site whose phosphorylation is directly mediated by mTOR. However, S6 phosphorylation, raptor levels, cell size, and cell cycle transit times are not diminished in these cells. In contrast to the situation in mTOR(+/-) mice, embryonic development of homozygous mTOR(-/-) mice appears to be arrested at E5.5; such embryos are severely runted and display an aberrant developmental phenotype. The ability of these embryos to implant corresponds to a limited level of trophoblast outgrowth in vitro, reflecting a maternal mRNA contribution, which has been shown to persist during preimplantation development. Moreover, mTOR(-/-) embryos display a lesion in inner cell mass proliferation, consistent with the inability to establish embryonic stem cells from mTOR(-/-) embryos.

Garcia-Rivera, M. F., L. E. Colvin-Wanshura, et al. (2007). "Characterization of an immunodeficient mouse model of mucopolysaccharidosis type I suitable for preclinical testing of human stem cell and gene therapy." Brain Res Bull **74**(6): 429-38.

Mucopolysaccharidosis type I (MPS-I or Hurler syndrome) is an inherited deficiency of the lysosomal glycosaminoglycan (GAG)-degrading enzyme alpha-l-iduronidase (IDUA) in which GAG accumulation causes progressive multi-system dysfunction and death. Early allogeneic hematopoietic stem cell transplantation (HSCT) ameliorates clinical features and extends life but is not available to all patients, and inadequately corrects its most devastating features including mental retardation and skeletal deformities. To test novel therapies, we characterized an immunodeficient MPS-I mouse model less likely to develop immune reactions to transplanted human or gene-corrected cells or secreted IDUA. In the liver, spleen, heart, lung, kidney and brain of NOD/SCID/MPS-I mice IDUA was undetectable, and reduced to half in heterozygotes. MPS-I mice developed marked GAG accumulation (3-38-fold) in these organs. Neuropathological examination showed GM(3) ganglioside accumulation in the striatum, cerebral peduncles, cerebellum and ventral brainstem of MPS-I mice. Urinary GAG excretion (6.5-fold higher in MPS-I mice) provided a non-invasive and reliable method suitable for serially following the biochemical efficacy of therapeutic interventions. We identified and validated using rigorous biostatistical methods, a highly reproducible method for evaluating sensorimotor function and motor skills development. This Rotarod test revealed marked abnormalities in sensorimotor integration involving the cerebellum, striatum, proprioceptive pathways, motor cortex, and in acquisition of motor coordination. NOD/SCID/MPS-I mice exhibit many of the clinical, skeletal, pathological and behavioral abnormalities of human MPS-I, and provide an

extremely suitable animal model for assessing the systemic and neurological effects of human stem cell transplantation and gene therapeutic approaches, using the above techniques to measure efficacy.

Gautrey, H., J. McConnell, et al. (2008). "Staufen1 is expressed in preimplantation mouse embryos and is required for embryonic stem cell differentiation." Biochim Biophys Acta **1783**(10): 1935-42.

Pluripotent mouse embryonic stem (mES) cells derived from the blastocyst of the preimplantation embryo can be induced to differentiate in vitro along different cell lineages. However the molecular and cellular factors that signal and/or determine the expression of key genes, and the localisation of the encoded proteins, during the differentiation events are poorly understood. One common mechanism by which proteins can be targeted to specific regions of the cell is through the asymmetric localisation of mRNAs and Staufen, a double-stranded RNA binding protein, is known to play a direct role in mRNA transport and localisation. The aims of the present study were to describe the expression of Staufen in preimplantation embryos and mES cells and to use RNA interference (RNAi) to investigate the roles of Staufen1 in mES cell lineage differentiation. Western blotting and immunocytochemistry demonstrated that Staufen is present in the preimplantation mouse embryo, pluripotent mES cells and mES cells stimulated to differentiate into embryoid bodies, but the Staufen staining patterns did not support asymmetric distribution of the protein. Knockdown of Staufen1 gene expression in differentiating mES cells reduced the synthesis of lineage-specific markers including Brachyury, alpha-fetoprotein (AFP), PAX-6, and Vasa. There was however no significant change in either the gene expression of Nanog and Oct4, or in the synthesis of SSEA-1, all of which are key markers of pluripotency. These data indicate that inhibition of Staufen1 gene expression by RNAi affects an early step in mES cell differentiation and suggest a key role for Staufen in the cell lineage differentiation of mES cells.

Gelain, F., D. Bottai, et al. (2006). "Designer self-assembling peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures." PLoS One **1**: e119.

Biomedical researchers have become increasingly aware of the limitations of conventional 2-dimensional tissue cell culture systems, including coated Petri dishes, multi-well plates and slides, to fully address many critical issues in cell biology, cancer biology and neurobiology, such as the 3-D microenvironment, 3-D gradient diffusion, 3-D cell migration and 3-D cell-cell contact interactions. In order to fully understand how cells behave in the 3-D body, it is important to develop a well-controlled 3-D cell culture system where every single ingredient is known. Here we report the development of a 3-D cell culture system using a designer peptide nanofiber scaffold with mouse adult neural stem cells. We attached several functional motifs, including cell adhesion, differentiation and bone marrow homing motifs, to a self-assembling peptide RADA16 (Ac-RADARADARADARADA-COHN2). These functionalized peptides undergo self-assembly into a nanofiber structure similar to Matrigel. During cell culture, the cells were fully embedded in the 3-D environment of the scaffold. Two of the peptide scaffolds containing bone marrow homing motifs significantly enhanced the neural cell survival without extra soluble growth and neurotrophic factors to the routine cell culture media. In these designer scaffolds, the cell populations with beta-Tubulin(+), GFAP(+) and Nestin(+) markers are similar to those found in cell populations cultured on Matrigel. The gene expression profiling array experiments showed selective gene expression, possibly involved in neural stem cell adhesion and differentiation. Because the synthetic peptides are intrinsically pure and a number of desired function cellular motifs are easy to incorporate, these designer peptide nanofiber scaffolds provide a promising controlled 3-D culture system for diverse tissue cells, and are useful as well for general molecular and cell biology.

Glover, C. H., M. Marin, et al. (2006). "Meta-analysis of differentiating mouse embryonic stem cell gene expression kinetics reveals early change of a small gene set." PLoS Comput Biol **2**(11): e158.

Stem cell differentiation involves critical changes in gene expression. Identification of these should provide endpoints useful for optimizing stem cell propagation as well as potential clues about mechanisms governing stem cell maintenance. Here we describe the results of a new meta-analysis methodology applied to multiple gene expression datasets from three mouse embryonic stem cell (ESC) lines obtained at specific time points during the course of their differentiation into various lineages. We developed methods to identify genes with expression changes that correlated with the altered frequency of functionally defined, undifferentiated ESC in culture. In each dataset, we computed a novel statistical confidence measure for every gene which captured the certainty that a particular gene exhibited an expression pattern of interest within that dataset. This permitted a joint analysis of the datasets, despite the different experimental designs. Using a ranking scheme that favored genes exhibiting patterns of interest, we

focused on the top 88 genes whose expression was consistently changed when ESC were induced to differentiate. Seven of these (103728_at, 8430410A17Rik, Klf2, Nr0b1, Sox2, Tcl1, and Zfp42) showed a rapid decrease in expression concurrent with a decrease in frequency of undifferentiated cells and remained predictive when evaluated in additional maintenance and differentiating protocols. Through a novel meta-analysis, this study identifies a small set of genes whose expression is useful for identifying changes in stem cell frequencies in cultures of mouse ESC. The methods and findings have broader applicability to understanding the regulation of self-renewal of other stem cell types.

Goan, S. R., I. Fichtner, et al. (1995). "The severe combined immunodeficient-human peripheral blood stem cell (SCID-huPBSC) mouse: a xenotransplant model for huPBSC-initiated hematopoiesis." Blood **86**(1): 89-100.

Mononuclear cells (MNCs) containing peripheral blood stem cells (PBSCs) were obtained from solid-tumor patients undergoing mobilizing chemotherapy followed by granulocyte colony-stimulating factor for PBSC transplantation-supported dose-intensified anticancer chemotherapy and were transplanted into unconditioned "nonleaky" young severe combined immunodeficient mice. Multilineage engraftment was shown by flow cytometry and immunocytochemistry using monoclonal antibodies to various human cell surface antigens as well as identification of human immunoglobulin in murine sera. Within a dose range of MNCs suitable for transplantation (10 to 36 x 10(6) cells/graft) the number of CD34+ cells injected (optimal at > 0.7 x 10(6)/graft) determined the yield of human cells produced in recipient animals. Engraftment of hu PBSC preparations resulted in prolonged generation of physiologic levels of human cytokines including interleukin-3 (IL-3), IL-6, and granulocyte-macrophage colony-stimulating factor, which were detectable in the murine blood over a period of at least 4 months. In vivo survival of immature human progenitor cells was preserved even 9 months after transplantation. Because human IL-3 is known to stimulate early hematopoiesis, a rat fibroblast cell line was stably transfected with a retroviral vector carrying the human IL-3 gene and cotransplanted subcutaneously as additional source of growth factor. Cotransplants of this cell line producing sustained in vivo levels of circulating human IL-3 for at least 12 weeks significantly accelerated the process of engraftment of huPBSC and spurred the spread of mature human cells to the murine spleen, liver, thymus, and peripheral blood. Cotransplants of allogeneic human bone marrow stromal cells derived from long-term cultures resulted in a comparable--though less prominent--support of engraftment.

Golestaneh, N., Y. Tang, et al. (2006). "Cell cycle deregulation and loss of stem cell phenotype in the subventricular zone of TGF-beta adaptor elf-/- mouse brain." Brain Res **1108**(1): 45-53.

The mammalian forebrain subependyma contains neural stem cells and other proliferating progenitor cells. Recent studies have shown the importance of TGF-beta family members and their adaptor proteins in the inhibition of proliferation in the nervous system. Previously, we have demonstrated that TGF-beta induces phosphorylation and association of ELF (embryonic liver fodrin) with Smad3 and Smad4 resulting in nuclear translocation. Elf(-/-) mice manifest abnormal neuronal differentiation, with loss of neuroepithelial progenitor cell phenotype in the subventricular zone (SVZ) with dramatic marginal cell hyperplasia and loss of nestin expression. Here, we have analyzed the expression of cell cycle-associated proteins cdk4, mdm2, p21, and pRb family members in the brain of elf(-/-) mice to verify the role of elf in the regulation of neural precursor cells in the mammalian brain. Increased proliferation in SVZ cells of the mutant mice coincided with higher levels of cdk4 and mdm2 expression. A lesser degree of apoptosis was observed in the mutant mice compared to the wild-type control. Elf(-/-) embryos showed elevated levels of hyperphosphorylated forms of pRb, p130 and p107 and decreased level of p21 compared to the wild-type control. These results establish a critical role for elf in the development of a SVZ neuroepithelial stem cell phenotype and regulation of neuroepithelial cell proliferation, suggesting that a mutation in the elf locus renders the cells susceptible to a faster entry into S phase of cell cycle and resistance to senescence and apoptotic stimuli.

Gouon-Evans, V., L. Boussemart, et al. (2006). "BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm." Nat Biotechnol **24**(11): 1402-11.

When differentiated in the presence of activin A in serum-free conditions, mouse embryonic stem cells efficiently generate an endoderm progenitor population defined by the coexpression of either Brachyury, Foxa2 and c-Kit, or c-Kit and Cxcr4. Specification of these progenitors with bone morphogenetic protein-4 in combination with basic fibroblast growth factor and activin A results in the development of hepatic populations highly enriched (45-70%) for cells that express the alpha-fetoprotein and albumin proteins. These cells also express transcripts of Afp, Alb1, Tat, Cps1, Cyp7a1 and

Cyp3a11; they secrete albumin, store glycogen, show ultrastructural characteristics of mature hepatocytes, and are able to integrate into and proliferate in injured livers in vivo and mature into hepatocytes expressing dipeptidyl peptidase IV or fumarylacetoacetate hydrolase. Together, these findings establish a developmental pathway in embryonic stem cell differentiation cultures that leads to efficient generation of cells with an immature hepatocytic phenotype.

Greber, B., H. Tandara, et al. (2005). "Comparison of PCR-based mutation detection methods and application for identification of mouse Sult1a1 mutant embryonic stem cell clones using pooled templates." Hum Mutat **25**(5): 483-90.

Reverse genetic approaches to generate mutants of model species are useful tools to assess functions of unknown genes. Recent work has demonstrated the feasibility of such strategies in several organisms, exploiting the power of chemical mutagenesis to disrupt genes randomly throughout the genome. To increase the throughput of gene-driven mutant identification, efficient mutation screening protocols are needed. Given the availability of sequence information for large numbers of unknown genes in many species, mutation detection protocols are preferably based on PCR. Using a set of defined mutations in the Hprt1 gene of mouse embryonic stem (ES) cells, we have systematically compared several PCR-based point mutation and deletion detection methods available for their ability to identify lesions in pooled samples, which is a major criterion for an efficient large-scale mutation screening assay. Results indicate that point mutations are most effectively identified by heteroduplex cleavage using CEL I endonuclease. Small deletions can most effectively be detected employing the recently described "poison" primer PCR technique. Further, we employed the CEL I assay followed by conventional agarose gel electrophoresis analysis for screening a library of chemically mutagenized ES cell clones. This resulted in the isolation of several clones harboring mutations in the mouse Sult1a1 locus, demonstrating the high-throughput compatibility of this approach using simple and inexpensive laboratory equipment.

Gussoni, E., Y. Soneoka, et al. (1999). "Dystrophin expression in the mdx mouse restored by stem cell transplantation." Nature **401**(6751): 390-4.

The development of cell or gene therapies for diseases involving cells that are widely distributed throughout the body has been severely hampered by the inability to achieve the disseminated delivery of cells or genes to the affected tissues or organ. Here we report the results of bone marrow transplantation studies in the mdx mouse, an animal model of Duchenne's muscular dystrophy, which indicate that the intravenous injection of either normal haematopoietic stem cells or a novel population of muscle-derived stem cells into irradiated animals results in the reconstitution of the haematopoietic compartment of the transplanted recipients, the incorporation of donor-derived nuclei into muscle, and the partial restoration of dystrophin expression in the affected muscle. These results suggest that the transplantation of different stem cell populations, using the procedures of bone marrow transplantation, might provide an unanticipated avenue for treating muscular dystrophy as well as other diseases where the systemic delivery of therapeutic cells to sites throughout the body is critical. Our studies also suggest that the inherent developmental potential of stem cells isolated from diverse tissues or organs may be more similar than previously anticipated.

Hailesellasse Sene, K., C. J. Porter, et al. (2007). "Gene function in early mouse embryonic stem cell differentiation." BMC Genomics **8**: 85.

BACKGROUND: Little is known about the genes that drive embryonic stem cell differentiation. However, such knowledge is necessary if we are to exploit the therapeutic potential of stem cells. To uncover the genetic determinants of mouse embryonic stem cell (mESC) differentiation, we have generated and analyzed 11-point time-series of DNA microarray data for three biologically equivalent but genetically distinct mESC lines (R1, J1, and V6.5) undergoing undirected differentiation into embryoid bodies (EBs) over a period of two weeks. RESULTS: We identified the initial 12 hour period as reflecting the early stages of mESC differentiation and studied probe sets showing consistent changes of gene expression in that period. Gene function analysis indicated significant up-regulation of genes related to regulation of transcription and mRNA splicing, and down-regulation of genes related to intracellular signaling. Phylogenetic analysis indicated that the genes showing the largest expression changes were more likely to have originated in metazoans. The probe sets with the most consistent gene changes in the three cell lines represented 24 down-regulated and 12 up-regulated genes, all with closely related human homologues. Whereas some of these genes are known to be involved in embryonic developmental processes (e.g. Klf4, Otx2, Smn1, Socs3, Tagln, Tdgf1), our analysis points to others (such as transcription factor Phf21a, extracellular matrix related Lama1 and Cyr61, or endoplasmic reticulum related Sc4mol and Scd2) that have not been previously related to mESC function. The majority of identified functions were related to transcriptional regulation, intracellular

signaling, and cytoskeleton. Genes involved in other cellular functions important in ESC differentiation such as chromatin remodeling and transmembrane receptors were not observed in this set. CONCLUSION: Our analysis profiles for the first time gene expression at a very early stage of mESC differentiation, and identifies a functional and phylogenetic signature for the genes involved. The data generated constitute a valuable resource for further studies. All DNA microarray data used in this study are available in the StemBase database of stem cell gene expression data 1 and in the NCBI's GEO database.

Harada, H., T. Toyono, et al. (2002). "FGF10 maintains stem cell compartment in developing mouse incisors." Development **129**(6): 1533-41.

Mouse incisors are regenerative tissues that grow continuously throughout life. The renewal of dental epithelium-producing enamel matrix and/or induction of dentin formation by mesenchymal cells is performed by stem cells that reside in cervical loop of the incisor apex. However, little is known about the mechanisms of stem cell compartment formation. Recently, a mouse incisor was used as a model to show that fibroblast growth factor (FGF) 10 regulates mitogenesis and fate decision of adult stem cells. To further illustrate the role of FGF10 in the formation of the stem cell compartment during tooth organogenesis, we have analyzed incisor development in Fgf10-deficient mice and have examined the effects of neutralizing anti-FGF10 antibody on the developing incisors in organ cultures. The incisor germs of FGF10-null mice proceeded to cap stage normally. However, at a later stage, the cervical loop was not formed. We found that the absence of the cervical loop was due to a divergence in Fgf10 and Fgf3 expression patterns at E16. Furthermore, we estimated the growth of dental epithelium from incisor explants of FGF10-null mice by organ culture. The dental epithelium of FGF10-null mice showed limited growth, although the epithelium of wild-type mice appeared to grow normally. In other experiments, a functional disorder of FGF10, caused by a neutralizing anti-FGF10 antibody, induced apoptosis in the cervical loop of developing mouse incisor cultures. However, recombinant human FGF10 protein rescued the cervical loop from apoptosis. Taken together, these results suggest that FGF10 is a survival factor that maintains the stem cell population in developing incisor germs.

Haupt, S., F. Edenhofer, et al. (2007). "Stage-specific conditional mutagenesis in mouse embryonic stem cell-derived neural cells and postmitotic neurons by direct delivery of biologically active Cre recombinase." Stem Cells **25**(1): 181-8.

Conditional mutagenesis using Cre/loxP recombination is a powerful tool to investigate genes involved in neural development and function. However, the efficient delivery of biologically active Cre recombinase to neural cells, particularly to postmitotic neurons, represents a limiting factor. In this study, we devised a protocol enabling highly efficient conditional mutagenesis in ESC-derived neural progeny. Using a stepwise in vitro differentiation paradigm, we demonstrate that recombinant cell-permeable Cre protein can be used to efficiently induce recombination at defined stages of neural differentiation. Recombination rates of more than 90% were achieved in multipotent pan-neural and glial precursors derived from the Z/EG reporter mouse ESC line, in which Cre recombination activates enhanced green fluorescent proteinexpression. Recombined precursor cells displayed a normal phenotype and were able to differentiate into neurons and/or glial cells, indicating that Cre treatment has no overt side effects on proliferation and neural differentiation. Our data further demonstrate that recombination via Cre protein transduction is not restricted to dividing cells but can even be applied to postmitotic neurons. The ability to conduct Cre/loxP recombination at defined stages of stem cell differentiation in an expression-independent manner provides new prospects for studying the role of individual genes under stringent temporal control.

He, Z., J. Jiang, et al. (2008). "Gdnf upregulates c-Fos transcription via the Ras/Erk1/2 pathway to promote mouse spermatogonial stem cell proliferation." Stem Cells **26**(1): 266-78.

Glial cell line-derived neurotrophic factor (GDNF) plays a crucial role in regulating the proliferation of spermatogonial stem cells (SSC). The signaling pathways mediating the function of GDNF in SSC remain unclear. This study was designed to determine whether GDNF signals via the Ras/ERK1/2 pathway in the C18-4 cells, a mouse SSC line. The identity of this cell line was confirmed by the expression of various markers for germ cells, proliferating spermatogonia, and SSC, including GCNA1, Vasa, Dazl, PCNA, Oct-4, GFRalpha1, Ret, and Plzf. Western blot analysis revealed that GDNF activated Ret tyrosine phosphorylation. All 3 isoforms of Shc were phosphorylated upon GDNF stimulation, and GDNF induced the binding of the phosphorylated Ret to Shc and Grb2 as indicated by immunoprecipitation and Western blotting. The active Ras was induced by GDNF, which further activated ERK1/2 phosphorylation. GDNF stimulated the phosphorylation of CREB-1, ATF-1, and CREM-1,

and c-fos transcription. Notably, the increase in ERK1/2 phosphorylation, c-fos transcription, bromodeoxyuridine incorporation, and metaphase counts induced by GDNF, was completely blocked by pretreatment with PD98059, a specific inhibitor for MEK1, the upstream regulator of ERK1/2. GDNF stimulation eventually upregulated cyclin A and CDK2 expression. Together, these data suggest that GDNF induces CREB/ATF-1 family member phosphorylation and c-fos transcription via the Ras/ERK1/2 pathway to promote the proliferation of SSC. Unveiling GDNF signaling cascades in SSC has important implications in providing attractive targets for male contraception as well as for the regulation of stem cell renewal vs. differentiation.

Hernandez, D., P. J. Mee, et al. (1999). "Transchromosomal mouse embryonic stem cell lines and chimeric mice that contain freely segregating segments of human chromosome 21." Hum Mol Genet **8**(5): 923-33.

At least 8% of all human conceptions have major chromosome abnormalities and the frequency of chromosomal syndromes in newborns is >0.5%. Despite these disorders making a large contribution to human morbidity and mortality, we have little understanding of their aetiology and little molecular data on the importance of gene dosage to mammalian cells. Trisomy 21, which results in Down syndrome (DS), is the most frequent aneuploidy in humans (1 in 600 live births, up to 1 in 150 pregnancies world-wide) and is the most common known genetic cause of mental retardation. To investigate the molecular genetics of DS, we report here the creation of mice that carry different human chromosome 21 (Hsa21) fragments as a freely segregating extra chromosome. To produce these 'transchromosomal' animals, we placed a selectable marker into Hsa21 and transferred the chromosome from a human somatic cell line into mouse embryonic stem (ES) cells using irradiation microcell-mediated chromosome transfer (XMMCT). 'Transchromosomal' ES cells containing different Hsa21 regions ranging in size from approximately 50 to approximately 0.2 Mb have been used to create chimeric mice. These mice maintain Hsa21 sequences and express Hsa21 genes in multiple tissues. This novel use of the XMMCT protocol is applicable to investigations requiring the transfer of large chromosomal regions into ES or other cells and, in particular, the modelling of DS and other human aneuploidy syndromes.

Hirst, C. E., E. S. Ng, et al. (2006). "Transcriptional profiling of mouse and human ES cells identifies SLAIN1, a novel stem cell gene." Dev Biol **293**(1): 90-103.

We analyzed the transcriptional profiles of differentiating mouse embryonic stem cells (mESCs) and show that embryoid bodies (EBs) sequentially expressed genes associated with the epiblast, primitive streak, mesoderm and endoderm of the developing embryo, validating ESCs as a model system for identifying cohorts of genes marking specific stages of embryogenesis. By comparing the transcriptional profiles of undifferentiated ESCs to those of their differentiated progeny, we identified 503 mESC and 983 hESC genes selectively expressed in undifferentiated ES cells. Over 75% of the mESC genes were expressed in hESC and vice versa, attesting to the underlying similarity of mESCs and hESCs. The expression of a cohort of 68 genes decreased greater than 2-fold during differentiation in both mESCs and hESCs. As well as containing many validated ESC genes such as Oct4 [Pou5f1], Nanog and Nodal, this cohort included an uncharacterised gene (FLJ30046), which we designated SLAIN1/Slain1. Slain1 was expressed at the stem cell and epiblast stages of ESC differentiation and in the epiblast, nervous system, tailbud and somites of the developing mouse embryo. SLAIN1 and its more widely expressed homologue SLAIN2 comprise a new family of structurally unique genes conserved throughout vertebrate evolution.

Hoshi, N., T. Kusakabe, et al. (2007). "Side population cells in the mouse thyroid exhibit stem/progenitor cell-like characteristics." Endocrinology **148**(9): 4251-8.

Side population (SP) cells are characterized by their ability to efflux the vital dye Hoechst 33342 (Sigma-Aldrich, St. Louis, MO) due to expression of the ATP binding cassette (ABC)-dependent transporter ABCG2, and are highly enriched for stem/progenitor cell activity. In this study we identified SP cells in murine thyroid, which are composed of two populations of cells: CD45(-)/c-kit(-)/Sca1(+) and CD45(-)/c-kit(-)/Sca1(-) cells. Quantitative RT-PCR analysis revealed that SP cells highly express ABCG2 and the stem cell marker genes encoding nucleostemin and Oct4, whereas the expression of genes encoding the thyroid differentiation markers, thyroid peroxidase, thyroglobulin (TG), and TSH receptor, and two transcription factors, thyroid transcription factor 1 (TITF1) and paired PAX8, critical for thyroid specific gene expression, are low in SP cells as compared with the main population cells. In situ hybridization and double immunofluorescence demonstrated that cells expressing Abcg2 gene reside in the interfollicular space of the thyroid gland. Approximately half and a small percentage of the ABCG2-positive cells were also positive for vimentin and calcitonin, respectively.

After 9 wk under three-dimensional thyroid primary culture conditions, main population cells formed an epithelial arrangement and follicle-like structures that are immunoreactive for TITF1 and TG. In contrast, SP cells demonstrated very few morphological changes without any epithelial or follicle-like structure and negative immunostaining for TITF1 and TG. These results demonstrate that thyroid possesses SP cells that may represent stem/progenitor cells.

Huang, Z., J. Li, et al. (2009). "Inorganic arsenic modulates the expression of selenoproteins in mouse embryonic stem cell." Toxicol Lett **187**(2): 69-76.

At least 25 selenoproteins in humans and 24 homologues in rodents have been identified. They play important roles in antioxidation, redox regulation and detoxification. The modulation of the expression of selenoproteins by inorganic arsenic (iAs) exposure may highlight the molecular mechanism for the arsenic toxicity. To investigate the effects of iAs exposure on the expression of selenoproteins, we determined how addition of iAs to culture medium affected all known selenoproteins in the mouse embryonic stem (ES) cells. Separated groups of ES cells were treated with arsenite (iAsIII) (0.25-0.5microM), arsenate (iAsV) (1.0-2.0microM) and co-treatment with sodium selenite (SeIV) (0.5microM). The mRNA levels of all selenoproteins were detected by real time quantitative PCR. The up-regulated selenoproteins were confirmed by immunoblotting analysis and enzymatic activity detection. Results showed that CGR8 cells treated with iAsIII (0.25-0.5microM) and iAsV (2.0microM) displayed significant increases of cellular reactive oxygen species (ROS) generation and nuclear accumulation of the transcription factor NF-E2-related factor 2 (Nrf2). Treatments of iAsIII (0.5microM) or iAsV (2.0microM) for 24h caused significant increases in the expression of the antioxidant selenoproteins (Gpx1, Gpx4, and Tr1), whereas led to significant decreases in the mRNA levels of selenoprotein H and some endoplasmic reticulum (ER) located selenoproteins (15-Sep, SelK, SelM, and SelS). Additionally, supplement of SeIV (0.5microM) could restore most of the down-regulated selenoproteins. These results suggested that iAs exposure modulated not only the antioxidant selenoproteins but also the ER stress associated selenoproteins. Further studies are required to clarify whether these modulated selenoproteins genes are targets for selenium supplement in the defense against the toxicity of iAs.

Ishizuka, T., K. Chayama, et al. (1999). "Mitogen-activated protein kinase activation through Fc epsilon receptor I and stem cell factor receptor is differentially regulated by phosphatidylinositol 3-kinase and calcineurin in mouse bone marrow-derived mast cells." J Immunol **162**(4): 2087-94.

Aggregation of high affinity FcR for IgE (Fc epsilon RI) on mast cells activates intracellular signal transduction pathways, including the activation of protein tyrosine kinases, phosphatidylinositol 3-kinase (PI3-kinase), and protein kinase C. Binding of stem cell factor (SCF) to its receptor (SCFR, c-Kit) on mast cells also induces increases in intrinsic tyrosine kinase activity and activation of PI3-kinase. Although ligation of both receptors induces Ras and Raf-1 activation, the downstream consequences of these early activation events are not well defined, except for the activation of extracellular signal-regulated kinases (ERK). Addition of Ag (OVA) to mouse bone marrow-derived mast cells (BMMC) sensitized with anti-OVA IgE triggers the activation of three members of the mitogen-activated protein (MAP) kinase family, c-Jun amino-terminal kinase (JNK), p38 MAP kinase (p38), and extracellular signal-regulated kinases. SCF similarly activates all three MAP kinases. Wortmannin, an inhibitor of PI3-kinase, inhibited both Fc epsilon RI- and SCFR-mediated JNK activation and partially inhibited Fc epsilon RI, but not SCFR-mediated p38 activation. Cyclosporin A inhibited Fc epsilon RI-mediated JNK and p38 activation, but did not affect the activation of these kinases when stimulated through the SCFR. Wortmannin and cyclosporin A inhibited Fc epsilon RI-mediated production of TNF-alpha and IL-4 in addition to serotonin release in BMMC. These results indicate that both PI3-kinase and calcineurin may contribute to the regulation of cytokine gene transcription and the degranulation response by modulating JNK activity in BMMC.

Iuchi, S., M. Marsch-Moreno, 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): 160-6.

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., A. Muth, et al. (2008). "MicroRNA regulation of cell lineages in mouse and human embryonic stem cells." Cell Stem Cell **2**(3): 219-29.

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.

Jafary, H., B. Larijani, 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): 278-86.

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.

Jamieson, B. D., G. M. Aldrovandi, et al. (1996). "The SCID-hu mouse: an in-vivo model for HIV-1 pathogenesis and stem cell gene therapy for AIDS." Semin Immunol **8**(4): 215-21.

Animal models are critical to the investigation of human immunodeficiency virus type 1 (HIV-1) pathogenesis. However, normal animal models are either uninfectable with HIV-1, or if infected, do not display HIV-1 induced pathology. Here, we describe how the severe combined immunodeficient mouse (SCID), implanted with human fetal thymus and liver, has been used to model HIV-1 pathogenesis and anti-retroviral gene therapy. Unable to reject the human tissue, these chimeric mice provide the investigator with a human hematolymphoid organ which, following infection by HIV-1, may more closely mimic the situation seen in humans than standard in-vitro culture systems.

Jasmin, J. F., M. Yang, et al. (2009). "Genetic ablation of caveolin-1 increases neural stem cell proliferation in the subventricular zone (SVZ) of the adult mouse brain." Cell Cycle **8**(23): 3978-83.

Adult neural stem cells are self-renewing multipotent cells that have the potential to replace dysfunctional and/or dying neuronal cells at the site of brain injury or degeneration. Caveolins are well-known tumor-suppressor genes that were recently found to be involved in the regulation of stem cell proliferation. For instance, ablation of the caveolin-1 (Cav-1) gene in mice markedly increases the proliferation of intestinal and mammary stem cells. However, the roles of caveolins in the proliferation of adult neural stem cells still remain unknown. In this study, dual-label immunofluorescence analysis of the proliferation marker, Ki67, and the stem cell markers, nestin and Sox2, was performed on brains of 8 week-old wild-type (WT) and Cav-1 knockout (KO) mice. Our results demonstrate an increased number of Ki67-positive nuclei in the subventricular zone (SVZ) of Cav-1 KO brains. Importantly, our dual-label immunofluorescence analyses demonstrate increased co-localization of Ki67 with both nestin and Sox2 in the SVZ of Cav-1 KO brains. Remarkably similar results were also obtained with Cav-2 and Cav-3 KO

mouse brains as well, with increased proliferation of adult neural stem cells. Thus, the SVZ of caveolin KO mouse brains displays an increased proliferation of adult neural stem cells. Caveolin proteins might represent new crucial regulators of adult neural stem cell proliferation.

Jiang, H., B. Sun, et al. (2007). "Activation of paternally expressed imprinted genes in newly derived germline-competent mouse parthenogenetic embryonic stem cell lines." Cell Res **17**(9): 792-803.

Parthenogenetic embryonic stem (pES) cells provide a valuable in vitro model system for studying the molecular mechanisms that underlie genomic imprinting. However, the pluripotency of pES cells and the expression profiles of paternally expressed imprinted genes have not been fully explored. In this study, three mouse pES cell lines were established and the differentiation potential of these cells in extended culture was evaluated. The undifferentiated cells had a normal karyotype and homozygous genome, and expressed ES-cell-specific molecular markers. The cells remained undifferentiated after more than 50 passages and exhibited pluripotent differentiation capacity. All three lines of the established ES cells produced teratomas; two lines of ES cells produced chimeras and germline transmission. Furthermore, activation of the paternally expressed imprinted genes Snrpn, U2af1-rs1, Peg3, Impact, Zfp127, Dlk1 and Mest in these cells was detected. Some paternally expressed imprinted genes were found to be expressed in the blastocyst stage of parthenogenetically activated embryos in vitro and their expression level increased with extended pES cell culture. Furthermore, our data show that the activation of these paternally expressed imprinted genes in pES cells was associated with a change in the methylation of the related differentially methylated regions. These findings provide direct evidence for the pluripotency of pES cells and demonstrate the association between the DNA methylation pattern and the activation of paternally expressed imprinted genes in pES cells. Thus, the established ES cell lines provide a valuable model for studying epigenetic regulation in mammalian development.

Jincho, Y., Y. Sotomaru, et al. (2008). "Identification of genes aberrantly expressed in mouse embryonic stem cell-cloned blastocysts." Biol Reprod **78**(4): 568-76.

During development, cloned embryos often undergo embryonic arrest at any stage of embryogenesis, leading to diverse morphological abnormalities. The long-term effects resulting from embryo cloning procedures would manifest after birth as early death, obesity, various functional disorders, and so forth. Despite extensive studies, the parameters affecting the developmental features of cloned embryos remain unclear. The present study carried out extensive gene expression analysis to screen a cluster of genes aberrantly expressed in embryonic stem cell-cloned blastocysts. Differential screening of cDNA subtraction libraries revealed 224 differentially expressed genes in the cloned blastocysts: eighty-five were identified by the BLAST search as known genes performing a wide range of functions. To confirm their differential expression, quantitative gene expression analyses were performed by real-time PCR using single blastocysts. The genes Skp1a, Canx, Ctsd, Timd2, and Psmc6 were significantly up-regulated, whereas Aqp3, Ak3l1, Rhot1, Sf3b3, Nid1, mt-Rnr2, mt-Nd1, mt-Cytb, and mt-Co2 were significantly down-regulated in the majority of embryonic stem cell-cloned embryos. Our results suggest that an extraordinarily high frequency of multiple functional disorders caused by the aberrant expression of various genes in the blastocyst stage is involved in developmental arrest and various other disorders in cloned embryos.

Jung, C. G., H. Hida, et al. (2004). "Pleiotrophin mRNA is highly expressed in neural stem (progenitor) cells of mouse ventral mesencephalon and the product promotes production of dopaminergic neurons from embryonic stem cell-derived nestin-positive cells." Faseb J **18**(11): 1237-9.

Neural stem cells are promising candidates for donor cells in neural transplantation. However, the mechanism by which neural stem cells differentiate into neurons is not well understood. In the present study, a serial analysis of gene expression (SAGE) was carried out to generate a gene file of neural stem (progenitor) cells from the mouse ventral mesencephalon. Among the 15,815 tags investigated, the mRNA of the housekeeping genes (elongation factor 1-alpha, ATPase subunit 6, GAPDH, actin), laminin receptor 1, HSP 70, pleiotrophin, and nestin were highly expressed. Because pleiotrophin (PTN) exhibits mitogenic and trophic effects on neural development and exhibits trophic effects on survival of dopaminergic (DAergic) neurons, we investigated the role of PTN in neurogenesis, especially to DAergic neurons. Here, we show that PTN increased the production of tyrosine hydroxylase (TH)-positive neurons from embryonic stem (ES) cell-derived nestin-positive cells. The expression of Nurr1 mRNA was enhanced by PTN. L-dopa in the culture medium was increased by PTN. This effect was as strong as with sonic hedgehog. Data suggest that PTN mRNA is highly expressed in neural stem (progenitor) cells of mouse ventral mesencephalon, and PTN promotes the

production of DAergic neurons from ES cell-derived nestin-positive cells.

Kadokawa, Y., H. Suemori, et al. (1990). "Cell lineage analyses of epithelia and blood vessels in chimeric mouse embryos by use of an embryonic stem cell line expressing the beta-galactosidase gene." Cell Differ Dev **29**(3): 187-94.

We have established an embryonic stem (ES) cell line, MS1-EL4, which has the potential to make various tissues in chimeric embryos and, at the same time, expresses the beta-galactosidase gene which was introduced as a good cell marker. To examine cell behavior and lineage during embryogenesis, we injected MS1-EL4 cells into host blastocysts and recovered chimeric embryos at various developmental stages. We examined the distribution of the MS1-EL4 cell derivatives by staining whole embryos with X-gal and by making serial paraffin sections. So far we have obtained the following results: (1) the MS1-EL4 cell line is useful for studying cell lineages because of its ubiquitous expression at least until the mid-gestation stage; (2) cells of the primitive ectoderm and its derivative epithelial tissues continue to intermingle with each other until the late primitive streak stage. Then, at early somite stages, cells of various epithelia stop intermingling and give rise to small coherent clones; (3) blood vessels of the yolk sac are formed by local aggregation of the ancestor cells and those of the embryo proper by proliferation and sprouting from fewer angiogenic cells.

Katona, R. L., I. Sinko, et al. (2008). "A combined artificial chromosome-stem cell therapy method in a model experiment aimed at the treatment of Krabbe's disease in the Twitcher mouse." Cell Mol Life Sci **65**(23): 3830-8.

Mammalian artificial chromosomes (MACs) are safe, stable, non-integrating genetic vectors with almost unlimited therapeutic transgene-carrying capacity. The combination of MAC and stem cell technologies offers a new strategy for stem cell-based therapy, the efficacy of which was confirmed and validated by using a mouse model of a devastating monogenic disease, galactocerebrosidase deficiency (Krabbe's disease). Therapeutic MACs were generated by sequence-specific loading of galactocerebrosidase transgenes into a platform MAC, and stable, pluripotent mouse embryonic stem cell lines were established with these chromosomes. The transgenic stem cells were thoroughly characterized and used to produce chimeric mice on the mutant genetic background. The lifespan of these chimeras was increased twofold, verifying the feasibility of the development of MAC-stem cell systems for the delivery of therapeutic genes in stem cells to treat genetic diseases and cancers, and to produce cell types for cell replacement therapies.

Kawakami, T., Y. Soma, et al. (2002). "Transforming growth factor beta1 regulates melanocyte proliferation and differentiation in mouse neural crest cells via stem cell factor/KIT signaling." J Invest Dermatol **118**(3): 471-8.

Stem cell factor is essential to the migration and differentiation of melanocytes during embryogenesis based on the observation that mutations in either the stem cell factor gene, or its ligand, KIT, result in defects in coat pigmentation in mice. Stem cell factor is also required for the survival of melanocyte precursors while they are migrating towards the skin. Transforming growth factor beta1 has been implicated in the regulation of both cellular proliferation and differentiation. NCC-melb4, an immortal cloned cell line, was cloned from a mouse neural crest cell. NCC-melb4 cells provide a model to study the specific stage of differentiation and proliferation of melanocytes. They also express KIT as a melanoblast marker. Using the NCC-melb4 cell line, we investigated the effect of transforming growth factor beta1 on the differentiation and proliferation of immature melanocyte precursors. Immunohistochemically, NCC-melb4 cells showed transforming growth factor beta1 expression. The anti-transforming growth factor beta1 antibody inhibited the cell growth, and downregulated the KIT protein and mRNA expression. To investigate further the activation of autocrine transforming growth factor beta1, NCC-melb4 cells were incubated in nonexogenous transforming growth factor beta1 culture medium. KIT protein decreased with anti-transforming growth factor beta1 antibody concentration in a concentration-dependent manner. We concluded that in NCC-melb4 cells, transforming growth factor beta1 promotes melanocyte precursor proliferation in autocrine and/or paracrine regulation. We further investigated the influence of transforming growth factor beta1 in vitro using a neural crest cell primary culture system from wild-type mice. Anti-transforming growth factor beta1 antibody decreased the number of KIT positive neural crest cell. In addition, the anti-transforming growth factor beta1 antibody supplied within the wild-type neural crest explants abolished the growth of the neural crest cell. These results indicate that transforming growth factor beta1 affect melanocyte precursor proliferation and differentiation in the presence of stem cell factor/KIT in an autocrine/paracrine manner.

Kawazoe, S., N. Ikeda, 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.

Kidder, B. L., L. Oseth, et al. (2008). "Embryonic stem cells contribute to mouse chimeras in the absence of detectable cell fusion." Cloning Stem Cells **10**(2): 231-48.

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.

Kim, D. W., S. Chung, 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): 557-67.

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, G. D., G. S. Rhee, 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): 719-27.

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.

Kim, J. H., H. S. Jung-Ha, et al. (1997). "Development of a positive method for male stem cell-mediated gene transfer in mouse and pig." Mol Reprod Dev **46**(4): 515-26.

Classical approaches for producing transgenic livestock require labor-intensive, time-consuming, and expensive methods with low efficiency of transgenic production. A promising approach for producing transgenic animals by using male stem cells was recently reported by Brinster and Zimmermann (1994; Proc Natl Acad Sci 91:11298-11302) and by Brinster and Avarbock (1994: Proc Natl Acad Sci USA 91:11303-11307). However, in order to apply this technique to producing transgenic animals, some difficulties have to be overcome. These include a satisfactory method for short-term in vitro culture for drug selection after transfection with exogenous DNA, and methods for the use of livestock such as pigs. We developed a new method for transferring foreign DNA into male germ cells. Mice and pigs were treated with busulfan, an alkylating agent, to destroy the developing male germ cells, and liposome/bacterial LacZ gene complexes were introduced into each seminiferous tubule by using a microinjection needle. As a control, lipofectin was dissolved in phosphate-buffered saline at a ratio of 1:1, and then injected into seminiferous tubules. In mice, 8.0-14.8% of seminiferous tubule expressed the introduced LacZ gene, and 7-13% of epididymal spermatozoa were confirmed as having foreign DNA by polymerase chain reaction. The liposome-injected testes were all negative for X-gal staining. These results indicate that some spermatozoa were successfully transformed in their early stages by liposome/DNA complexes. In pigs, foreign DNA was also incorporated efficiently into male germ cells, and 15.3-25.1% of the seminiferous tubules containing germ cells expressed the LacZ gene. The data suggest that these techniques can be used as a powerful tool for producing transgenic livestock.

Kim, T. S., S. Misumi, et al. (2008). "Increase in dopaminergic neurons from mouse embryonic stem cell-derived neural progenitor/stem cells is mediated by hypoxia inducible factor-1alpha." J Neurosci Res **86**(11): 2353-62.

A reliable method to induce neural progenitor/stem cells (NPCs) into dopaminergic (DAergic) neurons has not yet been established. As well, the mechanism involved remains to be elucidated. To induce DAergic differentiation from NPCs, a cytokine mixture (C-Mix) of interleukin (IL)-1beta, IL-11, leukemia-inhibitory factor (LIF), and glial-derived neurotrophic factor or low oxygen (3.5% O(2): L-Oxy) was used to treat embryonic stem (ES) cell-derived NPCs. Treatment with C-Mix increased the number of tyrosine hydroxylase (TH)-positive cells compared with controls (2.20-fold of control). The C-Mix effect was induced by mainly LIF or IL-1beta treatment. Although L-Oxy caused an increase in TH-positive cells (1.34-fold), the combination of L-Oxy with C-Mix did not show an additive effect. Increases in DA in the medium were shown in the presence of C-Mix, LIF, and L-Oxy by high-performance liquid chromatography. Gene expression patterns of neural markers [tryptophan hydroxylase (TPH), GAD67, GluT1, beta-tubulin III, glial fibrillary acidc protein, and TH] were different in C-Mix and L-Oxy treatments. Because increases in hypoxia-inducible factor (HIF)-1alpha protein were found in both treatments, we investigated the effect of HIF-1alpha on differentiation of NPCs to DAergic neurons. Inhibition of HIF-1alpha by the application of antisense oligodeoxynucleotides (ODNs) to NPCs caused a decrease in TH-positive cells induced by LIF treatment. Gene expressions of TH, GAD67, and GluT1 were decreased, and those of TPH, beta-tubulin III, and S-100beta were increased by treatment with just ODNs, indicating the importance of the endogenous effect of HIF-1alpha on neuronal differentiation. These data suggest that enhanced

differentiation into DAergic neurons from ES cell-derived NPCs was induced by C-Mix or L-Oxy mediated by HIF-1alpha.

Kim, Y. H. and H. J. Han (2008). "High-glucose-induced prostaglandin E(2) and peroxisome proliferator-activated receptor delta promote mouse embryonic stem cell proliferation." Stem Cells **26**(3): 745-55.

Peroxisome proliferator-activated receptor is a nuclear receptor that has been implicated in blastocyst implantation, cell cycle, and pathogenesis of diabetes. However, the signal cascades underlying this effect are largely unknown in embryo stem cells. This study examined whether or not there is an association between the reactive oxygen species-mediated prostaglandin E(2) (PGE(2))/peroxisome proliferator-activated receptor (PPAR) delta and the growth response to high glucose levels in mouse ESCs. A high concentration of glucose (25 mM) significantly increased the level of [3H]thymidine incorporation, the level of 5-bromo-2'-deoxyuridine incorporation, and the number of cells. Moreover, 25 mM glucose increased the intracellular reactive oxygen species, phosphorylation of the cytosolic phospholipase A(2) (cPLA(2)), and the release of [3H]arachidonic acid ([3H]AA). In addition, 25 mM glucose also increased the level of cyclooxygenase-2 (COX-2) protein expression, which stimulated the synthesis of PGE(2). Subsequently, high glucose-induced PGE(2) stimulated PPARdelta expression directly or through Akt phosphorylation indirectly through the E type prostaglandin receptor receptors. The PPARdelta antagonist inhibited the 25 mM glucose-induced DNA synthesis. Moreover, transfection with a pool of PPARdelta-specific small interfering RNA inhibited the 25 mM glucose-induced DNA synthesis and G1/S phase progression. Twenty-five millimolar glucose also increased the level of the cell cycle regulatory proteins (cyclin E/cyclin-dependent kinase [CDK] 2 and cyclin D1/CDK 4) and decreased p21(WAF1/Cip1) and p27(Kip1), which were blocked by the inhibition of the cPLA(2), COX-2, or PPARdelta pathways. In conclusion, high glucose promotes mouse ESC growth in part through the cPLA(2)-mediated PGE(2) synthesis and in part through PPARdelta pathways.

Kitano, M., M. Kakinuma, et al. (2006). "Gene expression profiling of mouse embryonic stem cell progeny differentiated by Lumelsky's protocol." Cells Tissues Organs **183**(1): 24-31.

Successful conversion of embryonic stem (ES) cells into insulin-producing cells has been reported by Lumelsky et al. (Science 2001;292:1389-1394); however, it remains controversial. In this study, we investigated the properties of ES cell progeny-induced differentiation according to Lumelsky's protocol by immunocytochemistry, oligonucleotide microarray and real-time RT-PCR. Insulin-positive cells were observed at stages 3, 4 and 5. Microarray analysis demonstrated upregulation and appearance of some genes involved in pancreatic development but not beta-cell-specific functional genes in cells at stage 5. Similarly, real-time RT-PCR revealed that expression of beta-cell-specific functional genes such as islet amyloid polypeptide, insulin I and II was not increased in cells at stage 5. These results suggest that terminal differentiation of ES cell progeny toward functional pancreatic beta-cell is insufficient. This study also demonstrates the usefulness of multiple time-course expression profiles for validating differentiation fates of ES cell progeny.

Koch, K. S., K. H. Son, 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.

Koster, M. I., K. A. Huntzinger, et al. (2002). "Epidermal differentiation: transgenic/knockout mouse models reveal genes involved in stem cell fate decisions and commitment to differentiation." J Investig Dermatol Symp Proc **7**(1): 41-5.

Epidermal development and differentiation are similar processes and therefore the study of one is likely to provide insight into the other. The signaling cascades required for epidermal differentiation are largely unknown. Recent evidence, however, has implicated two proteins, p63 and c-Myc, in different stages of epidermal development and differentiation. p63 was shown to be required for embryonic epidermal development. Mice lacking p63 do not develop stratified epithelia and appendages suggesting a role for p63 in the commitment to squamous epithelial lineages. Subsequent stem cell fate decisions are required to form the different structures of stratified epithelia including hair follicles, sebaceous glands, and epidermis. Several genes of the Wnt signaling pathway have been implicated in this process, including c-Myc, a downstream target of the Wnt pathway. Interestingly, targeted overexpression of c-Myc in the basal layer of the epidermis results in an increase in sebaceous gland size and number at the expense of hair follicles. This suggests that c-Myc promotes differentiation of epidermal stem cells into sebaceous glands. In this review, we discuss transgenic/knockout mouse models that have provided evidence linking c-Myc and p63 to different stages of epidermal development and differentiation.

Koyanagi-Katsuta, R., N. Akimitsu, et al. (2000). "Apoptosis of mouse embryonic stem cells induced by single cell suspension." Tissue Cell **32**(1): 66-70.

Embryonic stem cells (ES cells) are pluripotential, and are therefore used to construct gene knock-out mice. We found that the apoptosis of mouse ES cells was induced when the cells were dispersed as single cells, whereas this process was suppressed when they proliferated in aggregates. The apoptosis of ES cells was repressed when the cells were cultured on feeders prepared from STO cells, a cell line established from embryonic fibroblasts. Culture supernatants from STO cells did not block the apoptosis of ES cells, which suggests that a direct interaction between ES cells and STO cells is required for the suppression of apoptosis. The viability of ES cells examined by the trypan blue exclusion test or by the MTT ((3-4,5-dimethyithiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay decreased dramatically when the cells were dispersed in phosphate-buffered saline PBS. Cellular activity was restored by the addition of culture medium for ES cells. Glucose in the medium was found to be a major factor responsible for the restoration. Amino acids also restored the decrease in reduction of MTT. Suspension of the ES cells in PBS(-) caused leakage of the nucleosome into cytoplasm. Results indicate that the single cell suspension of ES cells leads to leakage of substrates for oxidative phosphorylation from the mitochondria, and that these cells finally become committed to apoptosis.

Kress, C., S. Vandormael-Pournin, et al. (1998). "Nonpermissiveness for mouse embryonic stem (ES) cell derivation circumvented by a single backcross to 129/Sv strain: establishment of ES cell lines bearing the Omd conditional lethal mutation." Mamm Genome **9**(12): 998-1001.

The inbred mouse strain DDK carries a conditional early embryonic lethal mutation that is manifested when DDK females are crossed to males of other inbred strains but not in the corresponding reciprocal crosses. It has been shown that embryonic lethality could be assigned to a single genetic locus called Ovum mutant (Om), on Chromosome (Chr) 11 near Syca 1. In the course of our study of the molecular mechanisms underlying the embryonic lethality, we were interested in deriving an embryonic stem cell bearing the Om mutation in the homozygous state (Omd/Omd). However, it turned out that DDK is nonpermissive for ES cell establishment, with a standard protocol. Here we show that permissiveness could be obtained using Omd/Omd blastocysts with a 75% 129/Sv and 25% DDK genetic background. Several germline-competent Omd/Omd ES cell lines have been derived from blastocysts of this genotype. Such a scenario could be extended to the generation of ES cell lines bearing any mutation present in an otherwise nonpermissive mouse strain.

Kubikova, I., H. Konecna, et al. (2009). "Proteomic profiling of human embryonic stem cell-derived microvesicles reveals a risk of transfer of proteins of bovine and mouse origin." Cytotherapy **11**(3): 330-40, 1 p following 340.

BACKGROUND AIMS: Microvesicles (MV) shed from the plasma membrane of eukaryotic

cells, including human embryonic stem cells (hESC), contain proteins, lipids and RNA and serve as mediators of cell-to-cell communication. However, they may also contain immunogenic membrane domains and infectious particles acquired from xenogenic components of the culture milieu. Therefore, MV represent a potential risk for clinical application of cell therapy. METHODS: We tested the ability of hESC and their most commonly used feeder cells, mouse embryonic fibroblasts (MEF), to produce MV. We found that hESC are potent producers of MV, whereas mitotically inactivated MEF do not produce any detectable MV. We therefore employed a combined proteomic approach to identify the molecules that constitute the major components of MV from hESC maintained in a standard culture setting with xenogenic feeder cells. RESULTS: In purified MV fractions, we identified a total of 22 proteins, including five unique protein species that are known to be highly expressed in invasive cancers and participate in cellular activation, metastasis and inhibition of apoptosis. Moreover, we found that hESC-derived MV contained the immunogenic agents apolipoprotein and transferrin, a source of Neu5Gc, as well as mouse retroviral Gag protein. CONCLUSIONS: These findings indicate that MV represent a mechanism by which hESC communicate; however, they also serve as potential carriers of immunogenic and pathogenic compounds acquired from environment. Our results highlight a potential danger regarding the use of hESC that have previously been exposed to animal proteins and cells.

Kubonishi, I., S. Takeuchi, et al. (1995). "Direct transplantation of chronic myelogenous leukemia cells into nude mice and establishment of a leukemic stem cell (Ph1+, CD34+) line dependent on mouse bone marrow stromal cells in vitro." Jpn J Cancer Res **86**(5): 451-9.

Peripheral blood cells from a female patient with Ph1-positive chronic myelogenous leukemia (CML) in blast crisis were serially transplanted in BALB/c nude mice for 16 passages. This in vivo cell line, designated CML-N-1, had Ph1 chromosome abnormality and BCR gene rearrangement. The cells expressed CD11b, CD13, CD33, CD34, CD38, and HLA-DR antigens until the 11th passage and subcutaneous tumors produced by these passages were composed of admixtures of immature and maturing cells that differentiated to basophils when cultured in vitro. From the 12th passage on, the tumors became composed mainly of immature cells expressing CD13, CD34, and HLA-DR, and no longer differentiated to basophils even upon in vitro culture. In contrast to the vigorous proliferation in vivo, CML-N-1 cells from any passage failed to proliferate in vitro under standard liquid culture conditions with or without growth factors, such as granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, monocyte colony-stimulating factor, interleukin 3, interleukin 6 and stem cell factor. However, a continuously growing cell line, designated CML-C-1, was established by culturing CML-N-1 cells on feeder layers of mouse bone marrow stromal cells. This mouse bone marrow stromal cell-dependent cell line showed immature cell morphology and expressed early myeloid phenotype positive for CD13, CD34, and HLA-DR. These results indicate that mouse bone marrow stromal cells provide a certain growth factor(s) active on human leukemia cells.

Kunarso, G., K. Y. Wong, et al. (2008). "Detailed characterization of the mouse embryonic stem cell transcriptome reveals novel genes and intergenic splicing associated with pluripotency." BMC Genomics **9**: 155.

BACKGROUND: Transcriptional control of embryonic stem (ES) cell pluripotency has been a subject of intense study. Transcriptional regulators including Oct4 (Oct3/4 index), Sox2 and Nanog are fundamental for maintaining the undifferentiated state. However, the ES cell transcriptome is not limited to their targets, and exhibits considerable complexity when assayed with microarray, MPSS, cDNA/EST sequencing, and SAGE technologies. To identify novel genes associated with pluripotency, we globally searched for ES transcripts not corresponding to known genes, validated their sequences, determined their expression profiles, and employed RNAi to test their function. RESULTS: Gene Identification Signature (GIS) analysis, a SAGE derivative distinguished by paired 5' and 3' transcript end tags, identified 153 candidate novel transcriptional units (TUs) distinct from known genes in a mouse E14 ES mRNA library. We focused on 16 TUs free of artefacts and mapping discrepancies, five of which were validated by RTPCR product sequencing. Two of the TUs were revealed by annotation to represent novel protein-coding genes: a PRY-domain cluster member and a KRAB-domain zinc finger. The other three TUs represented intergenic splicing events involving adjacent, functionally unrelated protein-coding genes transcribed in the same orientation, with one event potentially encoding a fusion protein containing domains from both component genes (Clk2 and Scamp3). Expression profiling using embryonic samples and adult tissue panels confirmed that three of the TUs were unique to or most highly expressed in ES cells. Expression levels of all five TUs dropped dramatically during three distinct chemically induced differentiation treatments of ES cells in culture. However, siRNA knockdowns of the TUs did not alter

mRNA levels of pluripotency or differentiation markers, and did not affect cell morphology. CONCLUSION: Transcriptome libraries retain considerable potential for novel gene discovery despite massive recent cDNA and EST sequencing efforts; cDNA and EST evidence for these ES cell TUs had been limited or absent. RTPCR and full-length sequencing remain essential in resolving the bottleneck between numerous candidate novel transcripts inferred from high-throughput sequencing and the small fraction that can be validated. RNAi results indicate that, despite their strong association with pluripotency, these five transcriptomic novelties may not be required for maintaining it.

Kutsuzawa, K., T. Akaike, et al. (2008). "The influence of the cell-adhesive proteins E-cadherin and fibronectin embedded in carbonate-apatite DNA carrier on transgene delivery and expression in a mouse embryonic stem cell line." Biomaterials **29**(3): 370-6.

Stem cells have the potential to be differentiated to a specific cell type through genetic manipulation and therefore, represent a new and versatile source of cell replacement in regenerative medicine. However, conventional ways of gene transfer to these progenitor cells, suffer from a number of disadvantages particularly involving safety and efficacy issues. We have recently reported on the development of a bio-functionalized DNA carrier of carbonate apatite by embedding fibronectin and E-cadherin chimera on the carrier, leading to its high-affinity interactions with embryonic stem cell surface and accelerated transgene delivery for subsequent expression. Here, we show the molecular basis of synthesizing highly functional composite particles utilizing DNA, cell-adhesive proteins and inorganic crystals, and finally establish a superior transfection system for a mouse stem cell line having potential applications in cell-based therapy.

Labosky, P. A., D. P. Barlow, et al. (1994). "Mouse embryonic germ (EG) cell lines: transmission through the germline and differences in the methylation imprint of insulin-like growth factor 2 receptor (Igf2r) gene compared with embryonic stem (ES) cell lines." Development **120**(11): 3197-204.

Primordial germ cells of the mouse cultured on feeder layers with leukemia inhibitory factor, Steel factor and basic fibroblast growth factor give rise to cells that resemble undifferentiated blastocyst-derived embryonic stem cells. These primordial germ cell-derived embryonic germ cells can be induced to differentiate extensively in culture, form teratocarcinomas when injected into nude mice and contribute to chimeras when injected into host blastocysts. Here, we report the derivation of multiple embryonic germ cell lines from 8.5 days post coitum embryos of C57BL/6 inbred mice. Four independent embryonic germ cell lines with normal male karyotypes have formed chimeras when injected into BALB/c host blastocysts and two of these lines have transmitted coat color markers through the germline. We also show that pluripotent cell lines capable of forming teratocarcinomas and coat color chimeras can be established from primordial germ cells of 8.0 days p.c. embryos and 12.5 days p.c. genital ridges. We have examined the methylation status of the putative imprinting box of the insulin-like growth factor type 2 receptor gene (Igf2r) in these embryonic germ cell lines. No correlation was found between methylation pattern and germline competence. A significant difference was observed between embryonic stem cell and embryonic germ cell lines in their ability to maintain the methylation imprint of the Igf2r gene in culture. This may illustrate a fundamental difference between these two cell types.

Lamoury, F. M., J. Croitoru-Lamoury, et al. (2006). "Undifferentiated mouse mesenchymal stem cells spontaneously express neural and stem cell markers Oct-4 and Rex-1." Cytotherapy **8**(3): 228-42.

BACKGROUND: Previous adult stem cells studies have provided evidence that BM mesenchymal stem cells (MSC) exhibit multilineage differentiation capacity. These properties of MSC prompted us to explore the neural potential of MSC with a view to their use for the treatment of demyelinating disorders, such as multiple sclerosis. Indeed, issues such as the identification of a subset of stem cells that is neurally fated, methods of expansion and optimal stage of differentiation for transplantation remain poorly understood. METHODS: In order to isolate mouse (m) MSC from BM, we used and compared the classic plastic-adhesion method and one depleting technique, the magnetic-activated cell sorting technique. RESULTS: We established and optimized culture conditions so that mMSC could be expanded for more than 360 days and 50 passages. We also demonstrated that undifferentiated mMSC express the neural markers nestin, MAP2, A2B5, GFAP, MBP, CNPase, GalC, O1 under standard culture conditions before transplantation. The pluripotent stem cell marker Oct-4 and the embryonic stem cell marker Rex-1 are spontaneously expressed by untreated mMSC. The lineage-negative mMSC (CD5- CD11b- Ly-6G-Ter119- CD45R- c-kit/CD117-) overexpressed Oct-4, O1 and A2B5 in the first days of culture compared with the non-sorted MSC. Finally, we identified a distinct subpopulation of mMSC that is primed towards a neural fate, namely Sca-1+/nestin+ mMSC. DISCUSSION: These results should facilitate the

optimal timing of harvesting a neurally fated subpopulation of mMSC for transplantation into animal models of human brain diseases.

Lannutti, B. J., A. Epp, et al. (2009). "Incomplete restoration of Mpl expression in the mpl-/- mouse produces partial correction of the stem cell-repopulating defect and paradoxical thrombocytosis." Blood **113**(8): 1778-85.

Expression of Mpl is restricted to hematopoietic cells in the megakaryocyte lineage and to undifferentiated progenitors, where it initiates critical cell survival and proliferation signals after stimulation by its ligand, thrombopoietin (TPO). As a result, a deficiency in Mpl function in patients with congenital amegakaryocytic thrombocytopenia (CAMT) and in mpl(-/-) mice produces profound thrombocytopenia and a severe stem cell-repopulating defect. Gene therapy has the potential to correct the hematopoietic defects of CAMT by ectopic gene expression that restores normal Mpl receptor activity. We rescued the mpl(-/-) mouse with a transgenic vector expressing mpl from the promoter elements of the 2-kb region of DNA just proximal to the natural gene start site. Transgene rescued mice exhibit thrombocytosis but only partial correction of the stem cell defect. Furthermore, they show very low-level expression of Mpl on platelets and megakaryocytes, and the transgene-rescued megakaryocytes exhibit diminished TPO-dependent kinase phosphorylation and reduced platelet production in bone marrow chimeras. Thrombocytosis is an unexpected consequence of reduced Mpl expression and activity. However, impaired TPO homeostasis in the transgene-rescued mice produces elevated plasma TPO levels, which serves as an unchecked stimulus to drive the observed excessive megakaryocytopoiesis.

Lee, E., J. Yook, et al. (2005). "Induction of Ym1/2 in mouse bone marrow-derived mast cells by IL-4 and identification of Ym1/2 in connective tissue type-like mast cells derived from bone marrow cells cultured with IL-4 and stem cell factor." Immunol Cell Biol **83**(5): 468-74.

Mast cells play an important role in allergic inflammation by releasing various bioactive mediators. The function of mast cells is enhanced by various stimuli, partly due to the induction of specific genes and their products. Although many inducible genes have been identified, a significant number of genes remain to be identified. Therefore, this study used PCR-selected cDNA subtraction to establish the profile of induced genes in the connective tissue (CT) type-like mast cells derived from bone marrow cells cultured in the presence of IL-4 and stem cell factor. Two hundred and fifty cDNA clones were obtained from the CT type-like mast cells by PCR-selected cDNA subtraction. Among them, Ym1/2, a chitinase-like protein, is one of the most abundantly induced genes. Ym1 is produced by activated macrophages in a parasitic infection, whereas its isotype, Ym2, is highly upregulated in allergic lung disease. In order to differentiate which isotype is expressed in bone marrow cells, specific primers for bone marrow-derived mast cells (BMMC), and CT type-like mast cells were used for RT-PCR. The results showed that Ym1 was constitutively expressed in bone marrow cells and gradually decreased in the presence of IL-3, whereas Ym2 was induced only in the presence of IL-4. CT type-like mast cells from bone marrow cells expressed Ym1 throughout the culture period and Ym2 was induced only by the addition of IL-4 into BMMC, indicating that IL-4 is essential for the expression of Ym1/2 genes.

Lee, J., M. Kanatsu-Shinohara, et al. (2009). "Genetic reconstruction of mouse spermatogonial stem cell self-renewal in vitro by Ras-cyclin D2 activation." Cell Stem Cell **5**(1): 76-86.

Spermatogonial stem cells (SSCs) undergo self-renewal division and support spermatogenesis. Although several cytokines coordinate to drive SSC self-renewal, little is known about the mechanisms underlying this process. We investigated the molecular mechanism by reconstructing SSC self-renewal in vitro without exogenous cytokines. Activation of Ras or overexpression of cyclins D2 and E1, both of which were induced by Ras, enabled long-term self-renewal of cultured spermatogonia. SSCs with activated Ras responded properly to differentiation signals and underwent spermatogenesis, whereas differentiation was abrogated in cyclin transfectants after spermatogonial transplantation. Both Ras- and cyclin-transfected cells produced seminomatous tumors, suggesting that excessive self-renewing stimulus induces oncogenic transformation. In contrast, cells that overexpressed cyclin D1 or D3 failed to make germ cell colonies after transplantation, which indicated that cyclin expression pattern is an important determinant to long-term SSC recolonization. Thus, the Ras-cyclin D2 pathway regulates the balance between tissue maintenance and tumorigenesis in the SSC population.

Lee, S. H., J. S. Heo, et al. (2007). "Effect of hypoxia on 2-deoxyglucose uptake and cell cycle regulatory protein expression of mouse embryonic stem cells: involvement of Ca2+ /PKC, MAPKs and HIF-1alpha." Cell Physiol Biochem **19**(5-6): 269-82.

This study investigated the signal molecules linking the alteration in 2-dexoyglucose (2-DG) uptake and DNA synthesis in mouse embryonic stem

(ES) cells under hypoxia. Hypoxia increased the 2-DG uptake and GLUT-1 protein expression level while the undifferentiated state of ES cells and cell viability were not affected by the hypoxia (1 - 48h). Subsequently, [(3)H] thymidine incorporation was significantly increased at 12 hours of hypoxic exposure. Hypoxia increased the Ca(2+) uptake and PKC beta (I), epsilon, and zeta translocation from the cytosol to the membrane fraction. Moreover, hypoxia increased the level of p44/42 mitogen-activated protein kinases (MAPKs) phosphorylation and hypoxia inducible factor-1alpha (HIF-1alpha) in a time-dependent manner. On the other hand, inhibition of these pathways blocked the hypoxia-induced increase in the 2-DG uptake and GLUT-1 protein expression level. Under hypoxia, cell cycle regulatory protein expression [cyclin D1, cyclin E, cyclin-dependent kinase (CDK) 2, and CDK 4] were increased in a time-dependent manner, which were blocked by PD 98059. pRB protein was also increased in a time-dependent manner. In conclusion, under hypoxia, there might be a parallel relationship between the expression of GLUT1 and DNA synthesis, which is mediated by the Ca(2+) /PKC, MAPK, and the HIF-1alpha signal pathways in mouse ES cells.

Lei, Y., H. Tang, et al. (2008). "Applications of mesenchymal stem cells labeled with Tat peptide conjugated quantum dots to cell tracking in mouse body." Bioconjug Chem **19**(2): 421-7.

Fluorescent quantum dots have great potential in cellular labeling and tracking. Here, PEG encapsulated CdSe/ZnS quantum dots have been conjugated with Tat peptide, and introduced into living mesenchymal stem cells. The Tat peptide conjugated quantum dots in mesenchymal stem cells were assessed by fluorescent microscopy, laser confocal microscope and. flow cytometry. The result shows that Tat peptide conjugated quantum dots could enter mesenchymal stem cells efficiently. The Tat-quantum dots labeled stem cells were further injected into the tail veins of NOD/SCID beta2 M null mice, and the tissue distribution of these labeled cells in nude mice were examined with fluorescence microscope. The result shows that characteristic fluorescence of quantum dots was observed primarily in the liver, the lung and the spleen, with little or no quantum dots accumulation in the brain, the heart, or the kidney.

Lengner, C. J., F. D. Camargo, et al. (2007). "Oct4 expression is not required for mouse somatic stem cell self-renewal." Cell Stem Cell **1**(4): 403-15.

The Pou domain containing transcription factor Oct4 is a well-established regulator of pluripotency in the inner cell mass of the mammalian blastocyst as well as in embryonic stem cells. While it has been shown that the Oct4 gene is inactivated through a series of epigenetic modifications following implantation, recent studies have detected Oct4 activity in a variety of somatic stem cells and tumor cells. Based on these observations it has been suggested that Oct4 may also function in maintaining self-renewal of somatic stem cells and, in addition, may promote tumor formation. We employed a genetic approach to determine whether Oct4 is important for maintaining pluripotency in the stem cell compartments of several somatic tissues including the intestinal epithelium, bone marrow (hematopoietic and mesenchymal lineages), hair follicle, brain, and liver. Oct4 gene ablation in these tissues revealed no abnormalities in homeostasis or regenerative capacity. We conclude that Oct4 is dispensable for both self-renewal and maintenance of somatic stem cells in the adult mammal.

Levasseur, D. N., T. M. Ryan, et al. (2003). "Correction of a mouse model of sickle cell disease: lentiviral/antisickling beta-globin gene transduction of unmobilized, purified hematopoietic stem cells." Blood **102**(13): 4312-9.

Although sickle cell anemia was the first hereditary disease to be understood at the molecular level, there is still no adequate long-term treatment. Allogeneic bone marrow transplantation is the only available cure, but this procedure is limited to a minority of patients with an available, histocompatible donor. Autologous transplantation of bone marrow stem cells that are transduced with a stably expressed, antisickling globin gene would benefit a majority of patients with sickle cell disease. Therefore, the development of a gene therapy protocol that corrects the disease in an animal model and is directly translatable to human patients is critical. A method is described in which unmobilized, highly purified bone marrow stem cells are transduced with a minimum amount of self-inactivating (SIN) lentiviral vector containing a potent antisickling beta-globin gene. These cells, which were transduced in the absence of cytokine stimulation, fully reconstitute irradiated recipients and correct the hemolytic anemia and organ pathology that characterize the disease in humans. The mean increase of hemoglobin concentration was 46 g/L (4.6 g/dL) and the average lentiviral copy number was 2.2; therefore, a 21-g/L /vector copy increase (2.1-g/dL) was achieved. This transduction protocol may be directly translatable to patients with sickle cell disease who cannot tolerate current bone marrow mobilization procedures and may not safely be exposed to large viral loads.

Levi, B. P., O. H. Yilmaz, et al. (2009). "Aldehyde dehydrogenase 1a1 is dispensable for stem cell function in the mouse hematopoietic and nervous systems." Blood **113**(8): 1670-80.

High levels of aldehyde dehydrogenase (ALDH) activity have been proposed to be a common feature of stem cells. Adult hematopoietic, neural, and cancer stem cells have all been reported to have high ALDH activity, detected using Aldefluor, a fluorogenic substrate for ALDH. This activity has been attributed to Aldh1a1, an enzyme that is expressed at high levels in stem cells and that has been suggested to regulate stem cell function. Nonetheless, Aldh1a1 function in stem cells has never been tested genetically. We observed that Aldh1a1 was preferentially expressed in mouse hematopoietic stem cells (HSCs) and expression increased with age. Hematopoietic cells from Aldh1a1-deficient mice exhibited increased sensitivity to cyclophosphamide in a non-cell-autonomous manner, consistent with its role in cyclophosphamide metabolism in the liver. However, Aldh1a1 deficiency did not affect hematopoiesis, HSC function, or the capacity to reconstitute irradiated recipients in young or old adult mice. Aldh1a1 deficiency also did not affect Aldefluor staining of hematopoietic cells. Finally, Aldh1a1 deficiency did not affect the function of stem cells from the adult central or peripheral nervous systems. Aldh1a1 is not a critical regulator of adult stem cell function or Aldefluor staining in mice.

Lieu, D. K., J. Liu, et al. (2009). "Absence of transverse tubules contributes to non-uniform Ca(2+) wavefronts in mouse and human embryonic stem cell-derived cardiomyocytes." Stem Cells Dev **18**(10): 1493-500.

Mouse (m) and human embryonic stem cell-derived cardiomyocytes (hESC-CMs) are known to exhibit immature Ca(2+) dynamics such as small whole-cell peak amplitude and slower kinetics relative to those of adult. In this study, we examined the maturity and efficiency of Ca(2+)-induced Ca(2+) release in m and hESC-CMs, the presence of transverse (t) tubules and its effects on the regional Ca(2+) dynamics. In m and hESC-CMs, fluorescent staining and atomic force microscopy (AFM) were used to detect the presence of t-tubules, caveolin-3, amphiphysin-2 and colocalization of dihydropyridine receptors (DHPRs) and ryanodine receptors (RyRs). To avoid ambiguities, regional electrically-stimulated Ca(2+) dynamics of single ESC-CMs, rather than spontaneously beating clusters, were measured using confocal microscopy. m and hESC-CMs showed absence of dyads, with neither t-tubules nor colocalization of DHPRs and RyRs. Caveolin-3 and amphiphysin-2, crucial for the biogenesis of t-tubules with robust expression in adult CMs, were also absent. Single m and hESC-CMs displayed non-uniform Ca(2+) dynamics across the cell that is typical of CMs deficient of t-tubules. Local Ca(2+) transients exhibited greater peak amplitude at the peripheral than at the central region for m (3.50 +/- 0.42 vs. 3.05 +/- 0.38) and hESC-CMs (2.96 +/- 0.25 vs. 2.72 +/- 0.25). Kinetically, both the rates of rise to peak amplitude and transient decay were faster for the peripheral relative to the central region. Immature m and hESC-CMs display unsynchronized Ca(2+) transients due to the absence of t-tubules and gene products crucial for their biogenesis. Our results provide insights for driving the maturation of ESC-CMs.

Lin, H., A. Shabbir, et al. (2007). "Stem cell regulatory function mediated by expression of a novel mouse Oct4 pseudogene." Biochem Biophys Res Commun **355**(1): 111-6.

Multiple pseudogenes have been proposed for embryonic stem (ES) cell-specific genes, and their abundance suggests that some of these potential pseudogenes may be functional. ES cell-specific expression of Oct4 regulates stem cell pluripotency and self-renewing state. Although Oct4 expression has been reported in adult tissues during gene reprogramming, the detected Oct4 signal might be contributed by Oct4 pseudogenes. Among the multiple Oct4 transcripts characterized here is a approximately 1 kb clone derived from P19 embryonal carcinoma stem cells, which shares a approximately 87% sequence homology with the parent Oct4 gene, and has the potential of encoding an 80-amino acid product (designated as Oct4P1). Adenoviral expression of Oct4P1 in mesenchymal stem cells promotes their proliferation and inhibits their osteochondral differentiation. These dual effects of Oct4P1 are reminiscent of the stem cell regulatory function of the parent Oct4, and suggest that Oct4P1 may be a functional pseudogene or a novel Oct4-related gene with a unique function in stem cells.

Lindskog, H., E. Athley, 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): 1457-64.

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.

Liu, Y., B. Clem, et al. (2009). "Mouse fibroblasts lacking RB1 function form spheres and undergo reprogramming to a cancer stem cell phenotype." Cell Stem Cell **4**(4): 336-47.

Activation of the RB1 pathway triggers the cell-cycle arrest that mediates cell-cell contact inhibition. Accordingly, mutation of all three RB1 family members leads to loss of contact inhibition and outgrowth of fibroblasts into spheres where cell-cell contacts predominate. We present evidence that such outgrowth triggers reprogramming to generate cells with properties of cancer stem cells. Fibroblasts with only a single RB1 mutation remain contact inhibited; however, if this contact inhibition is bypassed by forcing the RB1(-/-) cells to form spheres in suspension, cells with properties of cancer stem cells are also generated. These cells not only form tumors in nude mice but also generate differentiated cells. We propose that contact inhibition imposed by the RB1 pathway performs an unexpected tumor suppressor function by preventing cell outgrowth into structures where cells with properties of cancer stem cells can be generated from differentiated somatic cells in advancing cancers.

Longo, L., A. Bygrave, et al. (1997). "The chromosome make-up of mouse embryonic stem cells is predictive of somatic and germ cell chimaerism." Transgenic Res **6**(5): 321-8.

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.

Lukaszewicz, A., P. Savatier, et al. (2002). "Contrasting effects of basic fibroblast growth factor and neurotrophin 3 on cell cycle kinetics of mouse cortical stem cells." J Neurosci **22**(15): 6610-22.

Basic fibroblast growth factor (bFGF) exerts a mitogenic effect on cortical neuroblasts, whereas neurotrophin 3 (NT3) promotes differentiation in these cells. Here we provide evidence that both the mitogenic effect of bFGF and the differentiation-promoting effect of NT3 are linked with modifications of cell cycle kinetics in mouse cortical precursor cells. We adapted an in vitro assay, which makes it possible to evaluate (1) the speed of progression of the cortical precursors through the cell cycle, (2) the duration of individual phases of the cell cycle, (3) the proportion of proliferative versus differentiative divisions, and (4) the influence on neuroglial differentiation. Contrary to what has been claimed previously, bFGF promotes proliferation via a change in cell cycle kinetics by simultaneously decreasing G1 duration and increasing the proportion of proliferative divisions. In contrast, NT3 lengthens G1 and promotes differentiative divisions. We investigated the

molecular foundations of these effects and show that bFGF downregulates p27(kip1) and upregulates cyclin D2 expression. This contrasts with NT3, which upregulates p27(kip1) and downregulates cyclin D2 expression. Neither bFGF nor NT3 influences the proportion of glia or neurons in short to medium term cultures. The data point to links between the length of the G1 phase and the type of division of cortical precursors: differentiative divisions are correlated with long G1 durations, whereas proliferative divisions correlate with short G1 durations. The present results suggest that concerted mechanisms control the progressive increase in the cell cycle duration and proportion of differentiative divisions that is observed as corticogenesis proceeds.

Lupu, F., A. Alves, et al. (2008). "Nuclear pore composition regulates neural stem/progenitor cell differentiation in the mouse embryo." Dev Cell **14**(6): 831-42.

Serving as the primary conduit for communication between the nucleus and the cytoplasm, nuclear pore complexes (NPCs) impact nearly every cellular process. The extent to which NPC composition varies and the functional significance of such variation in mammalian development has not been investigated. Here we report that a null allele of mouse nucleoporin Nup133, a structural subunit of the NPC, disrupts neural differentiation. We find that expression of Nup133 is cell type and developmental stage restricted, with prominent expression in dividing progenitors. Nup133-deficient epiblast and ES cells abnormally maintain features of pluripotency and differentiate inefficiently along the neural lineage. Neural progenitors achieve correct spatial patterning in mutant embryos; however, they are impaired in generating terminally differentiated neurons, as are Nup133 null ES cells. Our results reveal a role for structural nucleoporins in coordinating cell differentiation events in the developing embryo.

Magin, T. M., J. McWhir, et al. (1992). "A new mouse embryonic stem cell line with good germ line contribution and gene targeting frequency." Nucleic Acids Res **20**(14): 3795-6.

Martin, G. R. (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells." Proc Natl Acad Sci U S A **78**(12): 7634-8.

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

Martin, G. R., L. M. Silver, et al. (1987). "Establishment of embryonic stem cell lines from preimplantation mouse embryos homozygous for lethal mutations in the t-complex." Dev Biol **121**(1): 20-8.

We have determined the frequency at which embryonic stem cell (ESC) lines can be established from inner cell masses (ICMs) isolated from blastocysts homozygous for lethal mutations in the mouse t-complex. Approximately one-third of the expected number, 3/29, of the ESC lines established from embryos obtained by inter-se mating of +/tw18 mice are homozygous for the tw18 haplotype. These tw18/tw18 ESC lines form a variety of cell types in vitro and in vivo, including mesodermal derivatives such as cartilage and muscle. On the basis of these and data from other studies, we suggest that the normal function of the gene represented by the tw18 lethal allele is required for multiplication/survival of mesodermal precursors in the embryo rather than the specification of the mesodermal lineage, and that the lethal effects of this mutation are expressed in only the highly structured environment of the early postimplantation embryo. In studies of the lethal tw5 haplotype, we found that 2/2 ESC lines obtained are mutant homozygotes. Analysis of these data, in conjunction with the results of our earlier study (Magnuson, T., Epstein, C. J., Silver, L. M., and Martin, G. R. (1982), Nature (London) 298, 750-753), suggests that homozygosity for the genes found in the tw5 haplotype does not reduce cell viability. By contrast, 0/16 ESC lines isolated from embryos obtained from matings of +/t0 mice are mutant homozygotes. Analysis of the genotypes of ICM-derived primary stem cell colonies suggests that t0 homozygous ICM cells are unable to undergo sufficient proliferation in vitro to give rise to ESC lines.

Masaki, H., T. Nishida, et al. (2007). "Developmental pluripotency-associated 4 (DPPA4) localized in active chromatin inhibits mouse embryonic stem cell differentiation into a primitive ectoderm lineage." J Biol Chem **282**(45): 33034-42.

Because embryonic stem (ES) cells can proliferate indefinitely in an undifferentiated state and differentiate into various cell types, ES cells are expected to be useful for cell replacement therapy and basic research on early embryogenesis. Although molecular mechanisms of ES cell self-renewal have been studied, many uncharacterized genes expressed in ES cells remain to be clarified. Developmental pluripotency associated 4 (Dppa4) is one such gene highly expressed in both ES cells and early embryos. Here, we investigated the role of Dppa4 in mouse ES cell self-renewal and differentiation. We generated Dppa4-overexpressing ES cells under the control of tetracycline. Dppa4 overexpression suppressed cell proliferation and formation of embryoid bodies and caused massive cell death in differentiating ES cells. Quantitative reverse transcription-PCR analysis showed that Dppa4 overexpression does not support ES cell self-renewal but partially inhibits ES cell differentiation. Suppression of Dppa4 expression by short hairpin RNA induced ES cell differentiation into a primitive ectoderm lineage. DPPA4 protein was localized in the ES cell nucleus associated with chromatin. Micrococcal nuclease digestion analysis and immunocytochemistry revealed that DPPA4 is associated with transcriptionally active chromatin. These findings indicate that DPPA4 is a nuclear factor associated with active chromatin and that it regulates differentiation of ES cells into a primitive ectoderm lineage.

Mathur, D., T. W. Danford, et al. (2008). "Analysis of the mouse embryonic stem cell regulatory networks obtained by ChIP-chip and ChIP-PET." Genome Biol **9**(8): R126.

BACKGROUND: Genome-wide approaches have begun to reveal the transcriptional networks responsible for pluripotency in embryonic stem (ES) cells. Chromatin Immunoprecipitation (ChIP) followed either by hybridization to a microarray platform (ChIP-chip) or by DNA sequencing (ChIP-PET), has identified binding targets of the ES cell transcription factors OCT4 and NANOG in humans and mice, respectively. These studies have provided an outline of the transcriptional framework involved in maintaining pluripotency. Recent evidence with comparing multiple technologies suggests that expanding these datasets using different platforms would be a useful resource for examining the mechanisms underlying pluripotency regulation. RESULTS: We have now identified OCT4 and NANOG genomic targets in mouse ES cells by ChIP-chip and provided the means to compare these data with previously reported ChIP-PET results in mouse ES cells. We have mapped the sequences of OCT4 and NANOG binding events from each dataset to genomic coordinates, providing a valuable resource to facilitate a better understanding of the ES cell regulatory circuitry. Interestingly, although considerable differences are observed in OCT4 and NANOG occupancy as identified by each method, a substantial number of targets in both datasets are enriched for genes that have known roles in cell-fate specification and that are differentially expressed upon Oct4 or Nanog knockdown. CONCLUSION: This study suggests that each dataset is a partial representation of the overall ES cell regulatory circuitry, and through integrating binding data obtained by ChIP-chip and ChIP-PET, the methods presented here provide a useful means for integrating datasets obtained by different techniques in the future.

Matsuoka, H., S. Shimoda, 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): 341-50.

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.

Matzner, U., D. Hartmann, et al. (2002). "Bone marrow stem cell-based gene transfer in a mouse model for metachromatic leukodystrophy: effects on visceral and nervous system disease manifestations." Gene Ther **9**(1): 53-63.

Arylsulfatase A (ASA) knockout mice represent an animal model for the lysosomal storage disease metachromatic leukodystrophy (MLD). Stem cell gene therapy with bone marrow overexpressing

the human ASA cDNA from a retroviral vector resulted in the expression of high enzyme levels in various tissues. Treatment partially reduces sulfatide storage in livers exceeding 18 ng ASA/mg tissue, while complete reduction was observed in livers exceeding 50 ng ASA/mg tissue. This corresponds to about 80% and 200% of normal enzyme activity. Similar values seem to apply for kidney. A partial correction of the lipid metabolism was detectable in the brain where the galactoerebroside/sulfatide ratio, which is diminished in ASA-deficient mice, increased upon treatment. This partial correction was accompanied by amelioration of neuropathology; axonal cross-sectional areas, which are reduced in deficient mice, were significantly increased in the saphenic and sciatic nerve but not in the optic nerve. Behavioral tests suggest some improvement of neuromotor abilities. The gene transfer did not delay the degeneration occurring in the acoustic ganglion of ASA-deficient animals. The limited success of the therapy appears to be due to the requirement of unexpected high levels of ASA for correction of the metabolic defect.

Mazurier, F., A. Fontanellas, et al. (1999). "A novel immunodeficient mouse model--RAG2 x common cytokine receptor gamma chain double mutants--requiring exogenous cytokine administration for human hematopoietic stem cell engraftment." J Interferon Cytokine Res **19**(5): 533-41.

Gene transduction into immature human hematopoietic cells collected from umbilical cord blood, bone marrow, or mobilized peripheral blood cells could be useful for the treatment of genetic and acquired disorders of the hematopoietic system. Immunodeficient mouse models have been used frequently as recipients to assay the growth and differentiation of human hematopoietic stem/progenitor cells. Indeed, high levels of human cell engraftment were first reported in human/murine chimeras using NOD/SCID mice, which now are considered as the standard for these types of experiments. However, NOD/SCID mice have some clear disadvantages (including spontaneous tumor formation) that limit their general use. We have developed a new immunodeficient mouse model by combining recombinase activating gene-2 (RAG2) and common cytokine receptor gamma chain (gamma c) mutations. The RAG2-/-/gamma c- double mutant mice are completely alymphoid (T-, B-, NK-), show no spontaneous tumor formation, and exhibit normal hematopoietic parameters. Interestingly, human cord blood cell engraftment in RAG2-/-/gamma c- mice was greatly enhanced by the exogenous administration of human cytokines interleukin-(IL-3) granulocyte-macrophage colony-stimulating factor, (GM-CSF), and erythropoietin in contrast to the NOD/SCID model. This unique feature of the RAG2-/-/gamma c-mouse model should be particularly well suited for assessing the role of different cytokines in human lymphopoiesis and stem/progenitor cell function in vivo.

McCoy, E. L., R. Iwanaga, et al. (2009). "Six1 expands the mouse mammary epithelial stem/progenitor cell pool and induces mammary tumors that undergo epithelial-mesenchymal transition." J Clin Invest **119**(9): 2663-77.

Six1 is a developmentally regulated homeoprotein with limited expression in most normal adult tissues and frequent misexpression in a variety of malignancies. Here we demonstrate, using a bitransgenic mouse model, that misexpression of human Six1 in adult mouse mammary gland epithelium induces tumors of multiple histological subtypes in a dose-dependent manner. The neoplastic lesions induced by Six1 had an in situ origin, showed diverse differentiation, and exhibited progression to aggressive malignant neoplasms, as is often observed in human carcinoma of the breast. Strikingly, the vast majority of Six1-induced tumors underwent an epithelial-mesenchymal transition (EMT) and expressed multiple targets of activated Wnt signaling, including cyclin D1. Interestingly, Six1 and cyclin D1 coexpression was found to frequently occur in human breast cancers and was strongly predictive of poor prognosis. We further show that Six1 promoted a stem/progenitor cell phenotype in the mouse mammary gland and in Six1-driven mammary tumors. Our data thus provide genetic evidence for a potent oncogenic role for Six1 in mammary epithelial neoplasia, including promotion of EMT and stem cell-like features.

McLenachan, S., J. P. Sarsero, et al. (2007). "Flow-cytometric analysis of mouse embryonic stem cell lipofection using small and large DNA constructs." Genomics **89**(6): 708-20.

Using the lipofection reagent LipofectAMINE 2000 we have examined the delivery of plasmid DNA (5-200 kb) to mouse embryonic stem (mES) cells by flow cytometry. To follow the physical uptake of lipoplexes we labeled DNA molecules with the fluorescent dye TOTO-1. In parallel, expression of an EGFP reporter cassette in constructs of different sizes was used as a measure of nuclear delivery. The cellular uptake of DNA lipoplexes is dependent on the uptake competence of mES cells, but it is largely independent of DNA size. In contrast, nuclear delivery was reduced with increasing plasmid size. In addition, linear DNA is transfected with lower efficiency than circular DNA. Inefficient cytoplasmic trafficking

appears to be the main limitation in the nonviral delivery of large DNA constructs to the nucleus of mES cells. Overcoming this limitation should greatly facilitate functional studies with large genomic fragments in embryonic stem cells.

Miller, C. L., S. Imren, et al. (2002). "Feasibility of using autologous transplantation to evaluate hematopoietic stem cell-based gene therapy strategies in transgenic mouse models of human disease." Mol Ther **6**(3): 422-8.

Histoincompatibility between murine donors and recipients of bone marrow (BM) transplants reduces engraftment, and this compromises assessment of hematopoietic stem cells (HSCs) in certain transgenic mice. To study HSCs in the S+S-Antilles mouse model of human sickle cell disease (SCD), we developed an autotransplant protocol. Initial experiments showed no differences between S+S-Antilles mice and normal C57BL/6 (+/+) mice in their radiosensitivity or baseline hematopoietic progenitor numbers. The kinetics of red blood cell (RBC) replacement post-transplant in +/+ recipients of mixtures of transgenic and +/+ BM cells also showed no competitive advantage of the +/+ cells. BM cells were then aspirated from mice 4 days after 5-fluorouracil treatment, transduced with a green fluorescent protein (GFP)-encoding retrovirus, and transplanted into the same recipients that, just before transplant, were irradiated with 800 cGy. We subsequently detected high levels of GFP(+) RBCs (21-79%) and white blood cells (WBCs; 35-88%) in the blood for 11 months and showed that transduced HSCs regenerated in the primary mice also repopulated secondary mice. These findings provide a generally applicable protocol for performing autotransplants in mice and forecast the potential utility of this approach in assessing HSC-based gene therapy protocols in transgenic mouse models of many human diseases.

Mio, H., N. Kagami, et al. (1998). "Isolation and characterization of a cDNA for human mouse, and rat full-length stem cell growth factor, a new member of C-type lectin superfamily." Biochem Biophys Res Commun **249**(1): 124-30.

cDNA encoding stem cell growth factor (SCGF; 245 aa), a novel human growth factor for primitive hematopoietic progenitor cells, has been previously reported (Hiraoka, A., Sugimura, A., Seki, T., Nagasawa, T., Ohta, N., Shimonishi, M., Hagiya, M. and Shimizu, S. Proc. Natl. Acad. Sci. USA 94, 7577-7582, 1997). Here we report the cloning and characterization of a full-length SCGF cDNA. This protein consists of 323, 328 and 328 aa in the human, murine and rat forms, the latter two of which share 85.1% and 83.3% aa identity, and 90.4% and 90.4% aa similarity to the human protein, respectively. Because the newly identified human clone encodes the protein longer by 78 aa than that previously identified, we term the longer clone as hSCGF-alpha and the shorter one as hSCGF-beta. The computer-assisted homology search reveals that SCGF is a new member of the C-type lectin superfamily, and that SCGF shows the greatest homology to tetranectin among the members of the family (27.2-33.7% aa identity and 46.0-53.6% aa similarity). SCGF transcripts are detected in spleen, thymus, appendix, bone marrow and fetal liver. Fluorescent in situ hybridization mapping indicates that the SCGF gene is located on chromosome 19 at position q13.3 for human form and on chromosome 7 at position B3-B5 for murine form, which are close to flk-2/flt3 ligand and interleukin-11 genes of both human and murine species.

Miranda, S. R., S. Erlich, et al. (2000). "Hematopoietic stem cell gene therapy leads to marked visceral organ improvements and a delayed onset of neurological abnormalities in the acid sphingomyelinase deficient mouse model of Niemann-Pick disease." Gene Ther **7**(20): 1768-76.

Types A and B Niemann-Pick disease (NPD) result from the deficient activity of acid sphingomyelinase (ASM). Currently, no treatment is available for either form of NPD. Using the ASM knockout (ASMKO) mouse model, we evaluated the effects of ex vivo hematopoietic stem cell gene therapy on the NPD phenotype. Thirty-two newborn ASMKO mice were preconditioned with low dose radiation (200 cGy) and transplanted with ASMKO bone marrow cells which had been transduced with an ecotropic retroviral vector encoding human ASM. Engraftment of donor-derived cells ranged from 15 to 60% based on Y-chromosome in situ hybridization analysis of peripheral white blood cells, and was achieved in 92% of the transplanted animals. High levels of ASM activity (up to five-fold above normal) were found in the engrafted animals for up to 10 months after transplantation, and their life-span was extended from a mean of 5 to 9 months by the gene therapy procedure. Biochemical and histological analysis of tissues obtained 4-5 months after transplantation indicated that the ASM activities were increased and the sphingomyelin storage was significantly reduced in the spleens, livers and lungs of the treated mice, major sites of pathology in type B NPD. The presence of Purkinje cell neurons was also markedly increased in the treatment group as compared with non-treated animals at 5 months after transplantation, and a reduction of storage in spinal cord neurons was observed. However, all of the transplanted mice eventually developed ataxia and

died earlier than normal mice. Overall, these results indicated that hematopoietic stem cell gene therapy should be effective for the treatment of non-neurological type B NPD, but improved techniques for targeting the transplanted cells and/or expressed enzyme to specific sites of pathology in the central nervous system must be developed in order to achieve effective treatment for type A NPD.

Mitsunari, M., T. Harada, et al. (1999). "The potential role of stem cell factor and its receptor c-kit in the mouse blastocyst implantation." Mol Hum Reprod **5**(9): 874-9.

Embryo implantation is a complex process that requires the interaction of embryo and endometrium. Several growth factors and cytokines appear to be involved in this process. Stem cell factor (SCF) and its receptor c-kit regulate the proliferation and survival of germ cells and play an important role in follicular development. However, little information is available on the role of SCF and c-kit in the process of blastocyst implantation. In the present study, we examined the expression of SCF and c-kit mRNA in mouse embryos and in the stromal and epithelial cells of the uterine endometrium by reverse transcription-polymerase chain reaction (RT-PCR). SCF mRNA was expressed in the spreading blastocysts and endometrial cells, with especially strong expression occurring in the stromal cells. Expression of c-kit mRNA was detected in the blastocysts and spreading blastocysts, as well as in the endometrial cells. By immunocytochemical studies, staining for c-kit protein was observed in the in-vitro spreading trophoblasts. We found that 50-100 ng/ml SCF significantly promoted the expansion of the surface area of the spreading blastocysts ($P < 0.01$). These results are consistent with the hypothesis that SCF derived from endometrial cells and the implanting embryo exerts paracrine and/or autocrine action on the process of implantation by stimulating trophoblast outgrowth through its receptor c-kit.

Moliner, A., P. Enfors, et al. (2008). "Mouse embryonic stem cell-derived spheres with distinct neurogenic potentials." Stem Cells Dev **17**(2): 233-43.

Mouse embryonic stem (ES) cells grown in feeder-free suspension cultures in the presence of leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) form spheres that retain pluripotency after multiple passages. ES cell-derived spheres of any passage acquired increased competence to differentiate into neurons over time in culture. Eight-day-old spheres produced many neurons upon plating in differentiation conditions whereas 3-day-old spheres produce none, even after monolayer expansion or treatment with blockers of inhibitory signals, indicating the acquisition of a reversible, proto-neurogenic state during sphere development. Gene expression profiling with oligonucleotide microarrays was used to identify the transcriptional changes accompanying this process. Sphere growth was characterized by down-regulation of a subset of ES cell-expressed genes during the first few days of sphere formation, and progressive up-regulation of novel genes over the course of 1 week in culture. Differential gene expression between 3-day-old and 8 day-old spheres was verified by quantitative real-time PCR experiments. Gene Set Enrichment Analysis (GSEA) of microarray data indicated that neurogenic potential in the late stages of sphere development correlated predominantly with up-regulation of pathways related to mitochondrial function, cell metabolism, oxidative stress, hypoxia, and down-regulation of RNA transcription and proteasome machineries, as well as pathways induced by myc and repressed by retinoic acid. We propose that differences in cellular metabolic state brought about by cell-cell contact and paracrine interactions in the sphere niche may play crucial roles in biasing the early stages of ES cell differentiation toward a neuronal phenotype.

Moller, C., M. Karlberg, et al. (2007). "Bcl-2 and Bcl-XL are indispensable for the late phase of mast cell development from mouse embryonic stem cells." Exp Hematol **35**(3): 385-93.

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

Montini, E., D. Cesana, et al. (2006). "Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration." Nat Biotechnol **24**(6): 687-96.

Insertional mutagenesis represents a major hurdle to gene therapy and necessitates sensitive preclinical genotoxicity assays. Cdkn2a-/- mice are susceptible to a broad range of cancer-triggering genetic lesions. We exploited hematopoietic stem cells from these tumor-prone mice to assess the oncogenicity of prototypical retroviral and lentiviral vectors. We transduced hematopoietic stem cells in matched clinically relevant conditions, and compared integration site selection and tumor development in transplanted mice. Retroviral vectors triggered dose-dependent acceleration of tumor onset contingent on long terminal repeat activity. Insertions at oncogenes and cell-cycle genes were enriched in early-onset tumors, indicating cooperation in tumorigenesis. In contrast, tumorigenesis was unaffected by lentiviral vectors and did not enrich for specific integrants, despite the higher integration load and robust expression of lentiviral vectors in all hematopoietic lineages. Our results validate a much-needed platform to assess vector safety and provide direct evidence that prototypical lentiviral vectors have low oncogenic potential, highlighting a major rationale for application to gene therapy.

Moon, J. H., B. S. Yoon, et al. (2008). "Induction of neural stem cell-like cells (NSCLCs) from mouse astrocytes by Bmi1." Biochem Biophys Res Commun **371**(2): 267-72.

Recently, Bmi1 was shown to control the proliferation and self-renewal of neural stem cells (NSCs). In this study, we demonstrated the induction of NSC-like cells (NSCLCs) from mouse astrocytes by Bmi1 under NSC culture conditions. These NSCLCs exhibited the morphology and growth properties of NSCs, and expressed NSC marker genes, including nestin, CD133, and Sox2. In vitro differentiation of NSCLCs resulted in differentiated cell populations containing astrocytes, neurons, and oligodendrocytes. Following treatment with histone deacetylase inhibitors (trichostatin A and valproic acid), the potential of NSCLCs for proliferation, dedifferentiation, and self-renewal was significantly inhibited. Our data indicate that multipotent NSCLCs can be generated directly from astrocytes by the addition of Bmi1.

Motomura, Y., S. Senju, 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): 2414-22.

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.

Muller, A. M., A. Medvinsky, et al. (1994). "Development of hematopoietic stem cell activity in the mouse embryo." Immunity **1**(4): 291-301.

The precise time of appearance of the first hematopoietic stem cell activity in the developing mouse embryo is unknown. Recently the aorta-gonad-mesonephros region of the developing mouse embryo has been shown to possess hematopoietic colony-forming activity (CFU-S) in irradiated recipient mice. To determine whether the mouse embryo possesses definitive hematopoietic stem cell activity in the analogous AGM region and to determine the order of appearance of stem cells in the yolk sac, AGM region, and liver, we transferred these embryonic tissues into adult irradiated recipients. We report here the long-term, complete, and functional hematopoietic repopulation of primary and serial recipients with AGM-derived cells. We observe potent hematopoietic stem cell activity in the AGM region before the appearance of yolk sac and liver stem cell activity and

discuss a model for the maturation of stem cell activity in mouse embryogenesis.

Munsie, M. J., A. E. Michalska, et al. (2000). "Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei." Curr Biol **10**(16): 989-92.

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.

Natale, D. R., M. Hemberger, et al. (2009). "Activin promotes differentiation of cultured mouse trophoblast stem cells towards a labyrinth cell fate." Dev Biol **335**(1): 120-31.

Prolonged maintenance of trophoblast stem (TS) cells requires fibroblast growth factor (FGF) 4 and embryonic fibroblast feeder cells or feeder cell-conditioned medium. Previous studies have shown that TGF-beta and Activin are sufficient to replace embryonic fibroblast-conditioned medium. Nodal, a member of the TGF-beta superfamily, is also known to be important in vivo for the maintenance of TS cells in the developing placenta. Our current studies indicate that TS cells do not express the Nodal co-receptor, Cripto, and do not respond directly to active Nodal in culture. Conversely, Activin subunits and their receptors are expressed in the placenta and TS cell cultures, with Activin predominantly expressed by trophoblast giant cells (TGCs). Differentiation of TS cells in the presence of TGC-conditioned medium or exogenous Activin results in a reduction in the expression of TGC markers. In line with TGC-produced Activin representing the active component in TGC-conditioned medium, this differentiation-inhibiting effect can be reversed by the addition of follistatin. Additional experiments in which TS cells were differentiated in the presence or absence of exogenous Activin or TGF-beta show that Activin but not TGF-beta results in the maintenance of expression of TS cell markers, prolongs the expression of syncytiotrophoblast markers, and significantly delays the expression of spongiotrophoblast and TGC markers. These results suggest that Activin rather than TGF-beta (or Nodal) acts directly on TS cells influencing both TS cell maintenance and cell fate, depending on whether the cells are also exposed to FGF4.

Naujok, O., F. Francini, et al. (2008). "An efficient experimental strategy for mouse embryonic stem cell differentiation and separation of a cytokeratin-19-positive population of insulin-producing cells." Cell Prolif **41**(4): 607-24.

OBJECTIVES: Embryonic stem cells are a potential source for insulin-producing cells, but existing differentiation protocols are of limited efficiency. Here, the aim has been to develop a new one, which drives development of embryonic stem cells towards insulin-producing cells rather than to neuronal cell types, and to combine this with a strategy for their separation from insulin-negative cells. MATERIALS AND METHODS: The cytokeratin-19 (CK19) promoter was used to control the expression of enhanced yellow fluorescence protein in mouse embryonic stem cells during their differentiation towards insulin-producing cells, using a new optimized four-stage protocol. Two cell populations, CK19(+) and CK19(-) cells, were successfully fluorescence sorted and analysed. RESULTS: The new method reduced neuronal progeny and suppressed differentiation into glucagon- and somatostatin-producing cells. Concomitantly, beta-cell like characteristics of insulin-producing cells were strengthened, as documented by high gene expression of the Glut2 glucose transporter and the transcription factor Pdx1. This novel protocol was combined with a cell-sorting technique. Through the combined procedure, a fraction of glucose-responsive insulin-secreting CK19(+) cells was obtained with 40-fold higher insulin gene expression and 50-fold higher

insulin content than CK19(-) cells. CK19(+) cells were immunoreactive for C-peptide and had ultrastructural characteristics of an insulin-secretory cell. CONCLUSION: Differentiated CK19(+) cells reflect an endocrine precursor cell type of ductal origin, potentially suitable for insulin replacement therapy in diabetes.

Neri, T., M. Monti, et al. (2007). "Mouse fibroblasts are reprogrammed to Oct-4 and Rex-1 gene expression and alkaline phosphatase activity by embryonic stem cell extracts." Cloning Stem Cells **9**(3): 394-406.

A recent remarkable study has shown that when mouse NIH-3T3 fibroblasts are exposed to an embryonic stem cell (ESC) extract, the majority of them expresses the Oct-4 gene, form ESC-like colonies, and embryoid-like bodies that differentiate into cells of the three germ layers. The use of cell extracts for inducing cell dedifferentiation could be a powerful system to obtain large quantities of pluripotent cells. It is thus of crucial importance that the robustness of this method of cell transdifferentiation is tested by other laboratories before it is advanced to a more ambitious use in cell therapy programs. We report here our experimental observations using the same reprogramming protocol on STO and NIH-3T3 mouse fibroblasts. Three are the main results: first, we confirmed an enduring reprogramming activity of the ESC extract, although on a much smaller number of cells that varies from approximately 0.003 to 0.04% of the total population of fibroblasts and with an effect limited to the induction of Oct-4 and Rex-1 gene expression and alkaline phosphatase activity. Second, the expression of OCT-4, SSEA-1, and Forssman antigen proteins was never detected. Third, our work has clearly demonstrated that ESCs may survive the procedure of extract preparation, may be source of contamination that is expanded in culture and give false positive results.

Nevozhay, D. and A. Opolski (2006). "Key factors in experimental mouse hematopoietic stem cell transplantation." Arch Immunol Ther Exp (Warsz) **54**(4): 253-69.

The first mouse model of hematopoietic stem cell transplantation (HSCT) was developed more than 50 years ago. HSCT is currently being widely used in a broad range of research areas, which include studies of the engraftment process, the pathogenesis of graft-versus-host disease and possible ways of its treatment and prophylaxis, attempts to use the graft-versus-leukemia/tumor effect in treating hematological and oncological malignancies, cancer vaccine development, induction of transplanted organ tolerance, and gene therapy. However, although this model is widely distributed, many laboratories use different protocols for the procedure. There are a number of papers discussing different HSCT protocols in clinical work, but no articles summarizing mouse laboratory models are available. This review attempts to bring together different details about HSCT in the mouse model, such as the types of transplantation, possible pretreatment regimens and their combinations, methods and sources of graft harvesting and preparation for the transplantation procedure, the influence of graft cell dose and content on the engraftment process, the transplantation method itself, possible complications, symptoms and techniques of their prophylaxis or treatment, as well as follow-up and engraftment assessment. We have also tried to reflect current knowledge of the biology of the engraftment.

Noguchi, H., K. Oishi, et al. (2009). "Establishment of mouse pancreatic stem cell line." Cell Transplant **18**(5): 563-71.

beta-Cell replacement therapy via islet transplantation is a promising possibility for the optimal treatment of type 1 diabetes. However, such an approach is severely limited by the shortage of donor organs. Pancreatic stem/progenitor cells could become a useful target for beta-cell replacement therapy in diabetic patients because the cells are abundantly available in the pancreas of these patients and in donor organs. In this study, we established a mouse pancreatic stem cell line without genetic manipulation. The duct-rich population after islet isolation was inoculated into 96-well plates in limiting dilution. From over 200 clones, 15 clones were able to be cultured for over 3 months. The HN#13 cells, which had the highest expression of insulin mRNA after induction, expressed PDX-1 transcription factor, glucagon-like peptide-1 (GLP-1) receptor, and cytokeratin-19 (duct-like cells). These cells continue to divide actively beyond the population doubling level (PDL) of 300. Exendin-4 treatment and transduction of PDX-1 and NeuroD proteins by protein transduction technology in HN#13 cells induced insulin and pancreas-related gene expression. This cell line could be useful for analyzing pancreatic stem cell differentiation. Moreover, the isolation technique might be useful for identification and isolation of human pancreatic stem/progenitor cells.

Novosadova, E. V., E. S. Manuilova, et al. (2005). "Different effects of enhanced and reduced expression of pub gene on the formation of embryoid bodies by cultured embryonic mouse stem cell." Bull Exp Biol Med **140**(1): 153-8.

The effects of pub gene on proliferation and initial stages of differentiation of embryonic mouse stem cells were studied in vitro. To this end we used enhanced expression of human pub gene (hpub) and suppression of expression of mouse endogenous pub gene with RNA-interference in embryonic stem cells. Proliferative activity of genetically modified polyclonal lines of the embryonic stem cells transfected with plasmids carrying expressing hpub gene or plasmids generating small interference RNA to this gene did not differ from that of the control cells. Inhibition of expression of endogenous pub gene in embryonic stem cells using small interference RNA 2-fold decreased the formation of embryoid bodies, at the same time additional expression of exogenous hpub gene almost 2-fold increased their number in comparison with the control. It was hypothesized that pub gene participates in early stages of differentiation of embryonic stem cells leading to the formation of embryoid bodies.

Oatley, J. M., M. R. Avarbock, et al. (2007). "Glial cell line-derived neurotrophic factor regulation of genes essential for self-renewal of mouse spermatogonial stem cells is dependent on Src family kinase signaling." J Biol Chem **282**(35): 25842-51.

Self-renewal and differentiation by spermatogonial stem cells (SSCs) is the foundation for continual spermatogenesis. SSC self-renewal is dependent on glial cell line-derived neurotrophic factor (GDNF); however, intracellular mechanisms stimulated by GDNF in SSCs are unknown. To investigate these mechanisms we utilized a culture system that maintains a mouse undifferentiated germ cell population enriched for self-renewing SSCs. In these cultures mRNA for the transcription factors Bcl6b, Erm, and Lhx1 are up-regulated by GDNF and decreased in its absence. The expression of all three molecules was further identified in undifferentiated spermatogonia in vivo. Using small interfering RNA to reduce expression and transplantation to quantify stem cell numbers, Bcl6b, Erm, and Lhx1 were shown to be important for SSC maintenance in vitro. Next, GDNF was shown to activate both Akt and Src family kinase (SFK) signaling in SSCs, and culture of SSCs with inhibitors to Akt or SFKs followed by transplantation analysis showed significant impairment of SSC maintenance in vitro. Apoptosis analysis revealed a significant increase in the percentage of apoptotic cells when Akt, but not SFK, signaling was impaired, indicating that multiple signaling pathways are responsible for SSC self-renewal and survival. Biochemical and gene expression experiments revealed that GDNF up-regulated expression of Bcl6b, Erm, and Lhx1 transcripts is dependent on SFK signaling. Overall, these data demonstrate that GDNF up-regulation of Bcl6b, Erm, and Lhx1 expression through SFK signaling is a key component of the intracellular mechanism for SSC self-renewal.

Oatley, J. M., M. J. Oatley, et al. (2009). "Colony stimulating factor 1 is an extrinsic stimulator of mouse spermatogonial stem cell self-renewal." Development **136**(7): 1191-9.

Self-renewal and differentiation of spermatogonial stem cells (SSCs) provide the foundation for testis homeostasis, yet mechanisms that control their functions in mammals are poorly defined. We used microarray transcript profiling to identify specific genes whose expressions are augmented in the SSC-enriched Thy1(+) germ cell fraction of mouse pup testes. Comparisons of gene expression in the Thy1(+) germ cell fraction with the Thy1-depleted testis cell population identified 202 genes that are expressed 10-fold or higher in Thy1(+) cells. This database provided a mining tool to investigate specific characteristics of SSCs and identify novel mechanisms that potentially influence their functions. These analyses revealed that colony stimulating factor 1 receptor (Csf1r) gene expression is enriched in Thy1(+) germ cells. Addition of recombinant colony stimulating factor 1 (Csf1), the specific ligand for Csf1r, to culture media significantly enhanced the self-renewal of SSCs in heterogeneous Thy1(+) spermatogonial cultures over a 63-day period without affecting total germ cell expansion. In vivo, expression of Csf1 in both pre-pubertal and adult testes was localized to clusters of Leydig cells and select peritubular myoid cells. Collectively, these results identify Csf1 as an extrinsic stimulator of SSC self-renewal and implicate Leydig and myoid cells as contributors of the testicular stem cell niche in mammals.

Ohta, H., S. Aizawa, et al. (2003). "Functional analysis of the p53 gene in apoptosis induced by heat stress or loss of stem cell factor signaling in mouse male germ cells." Biol Reprod **68**(6): 2249-54.

Apoptosis plays an important role in controlling germ cell numbers and restricting abnormal cell proliferation during spermatogenesis. The tumor suppressor protein, p53, is highly expressed in the testis, and is known to be involved in apoptosis, which suggests that it is one of the major causes of germ cell loss in the testis. Mice that are c-kit/SCF mutant (Sl/Sld) and cryptorchid show similar testicular phenotypes; they carry undifferentiated spermatogonia and Sertoli cells in their seminiferous tubules. To investigate the role of p53-dependent apoptosis in infertile testes, we transplanted p53-deficient spermatogonia that were labeled with

enhanced green fluorescence protein into cryptorchid and Sl/Sld testes. In cryptorchid testes, transplanted p53-deficient spermatogonia differentiated into spermatocytes, but not into haploid spermatids. In contrast, no differentiated germ cells were observed in Sl/Sld mutant testes. These results indicate that the mechanism of germ cell loss in the c-kit/SCF mutant is not dependent on p53, whereas the apoptotic mechanism in the cryptorchid testis is quite different (i.e., although the early stage of differentiation of spermatogonia and the meiotic prophase is dependent on p53-mediated apoptosis, the later stage of spermatids is not).

Okada, Y., T. Shimazaki, et al. (2004). "Retinoic-acid-concentration-dependent acquisition of neural cell identity during in vitro differentiation of mouse embryonic stem cells." Dev Biol **275**(1): 124-42.

Retinoic acid (RA) is one of the most important morphogens, and its embryonic distribution correlates with neural differentiation and positional specification in the developing central nervous system. To investigate the concentration-dependent effects of RA on neural differentiation of mouse embryonic stem cells (ES cells), we investigated the precise expression profiles of neural and regional specific genes by ES cells aggregated into embryoid bodies (EBs) exposed to various concentrations of RA or the BMP antagonist Noggin. RA promoted both neural differentiation and caudalization in a concentration-dependent manner, and the concentration of RA was found to regulate dorso-ventral identity, i.e., higher concentrations of RA induced a dorsal phenotype, and lower concentrations of RA induced a more ventral phenotype. The induction of the more ventral phenotype was due to the higher expression level of the N-terminus of sonic hedgehog protein (Shh-N) when treated with low concentration RA, as it was abrogated by an inhibitor of Shh signaling, cyclopamine. These findings suggest that the concentration of RA strictly and simultaneously regulates the neuralization and positional specification during differentiation of mouse ES cells and that it may be possible to use it to establish a strategy for controlling the identity of ES-cell-derived neural cells.

Orlic, D., L. J. Girard, et al. (1998). "Identification of human and mouse hematopoietic stem cell populations expressing high levels of mRNA encoding retrovirus receptors." Blood **91**(9): 3247-54.

One obstacle to retrovirus-mediated gene therapy for human hematopoietic disorders is the low efficiency of gene transfer into pluripotent hematopoietic stem cells (HSC). We have previously shown a direct correlation between retrovirus receptor mRNA levels in mouse HSC and the efficiency with which they are transduced. In the present study, we assayed retrovirus receptor mRNA levels in a variety of mouse and human HSC populations to identify HSC which may be more competent for retrovirus transduction. The highest levels of amphotropic retrovirus receptor (amphoR) mRNA were found in cryopreserved human cord blood HSC. The level of amphoR mRNA in Lin- CD34(+) CD38(-) cells isolated from frozen cord blood was 12-fold higher than the level in fresh cord blood Lin- CD34(+) CD38(-) cells. In mice, the level of amphoR mRNA in HSC from the bone marrow (BM) of mice treated with stem cell factor and granulocyte-colony stimulating factor was 2.8- to 7.8-fold higher than in HSC from the BM of untreated mice. These findings suggest that HSC from frozen cord blood and cytokine-mobilized BM may be superior targets for amphotropic retrovirus transduction compared with HSC from untreated adult BM.

Paulis, M., M. Bensi, et al. (2007). "Transfer of a human chromosomal vector from a hamster cell line to a mouse embryonic stem cell line." Stem Cells **25**(10): 2543-50.

Two transchromosomic mouse embryonic stem (ES) sublines (ESMClox1.5 and ESMClox2.1) containing a human minichromosome (MC) were established from a sample of hybrid colonies isolated in fusion experiments between a normal diploid mouse ES line and a Chinese hamster ovary line carrying the MC. DNA cytometric and chromosome analyses of ESMClox1.5 and ESMClox2.1 indicated a mouse chromosome complement with a heteroploid constitution in a subtetraploid range; the karyotypes showed various degrees of polysomy for different chromosomes. A single copy of the MC was found in the majority of cells in all the isolated hybrid colonies and was stably maintained in the established sublines for more than 100 cell generations either with or without the selective agent. No significant differences from the ES parental cells were observed in growth characteristics of the transchromosomic ES sublines. ESMClox1.5 cells were unable to grow in soft agar; when cultured in hanging drops, they formed embryoid bodies, and when inoculated in nude mice, they produced teratomas. They were able to express the early development markers Oct4 and Nanog, as demonstrated by reverse transcription-polymerase chain reaction assay. All these features are in common with the ES parental line. Further research using the transchromosomic ES sublines described here may allow gene expression studies on transferred human minichromosomes and could shed light on the relationships among ploidy, pluripotency, cell transformation, and tumorigenesis. Disclosure of

potential conflicts of interest is found at the end of this article.

Peitz, M., R. Jager, et al. (2007). "Enhanced purification of cell-permeant Cre and germline transmission after transduction into mouse embryonic stem cells." Genesis **45**(8): 508-17.

Continuous expression of Cre recombinase has the potential to yield toxic side effects in various cell types, thereby limiting applications of the Cre/loxP system for conditional mutagenesis. In this study, we investigate the potential of Cre protein transduction to overcome this limitation. COS-7, CV1-5B, and mouse embryonic stem (ES) cells treated with cell-permeant Cre (HTNCre) maintain a normal growth behavior employing Cre concentrations sufficient to induce recombination in more than 90% of the cells, whereas continuous application of high doses resulted in markedly reduced proliferation. HTNCre-treated ES cells maintain a normal karyotype and are still able to contribute to the germline. Moreover, we present an enhanced HTNCre purification protocol that allows the preparation of a concentrated glycerol stock solution, thereby enabling a considerable simplification of the Cre protein transduction procedure. The protocol described here allows rapid and highly efficient conditional mutagenesis of cultured cells.

Perez-Campo, F. M., H. L. Spencer, et al. (2007). "Novel vectors for homologous recombination strategies in mouse embryonic stem cells: an ES cell line expressing EGFP under control of the 5T4 promoter." Exp Cell Res **313**(16): 3604-15.

The use of gene mutation/knock-out strategies in mouse embryonic stem (ES) cells has revolutionized the study of gene function in ES cells and embryonic development. However, the construction of vectors for homologous recombination strategies requires considerable expertise and time. We describe two novel vectors that can generate site specific knock-out or EGFP knock-in ES cells within 6 weeks from construct design to identification of positive ES cell clones. As proof-of-principle, we have utilized the knock-out targeting vector to modify the NEIL2 locus in ES cells. In addition, using the knock-in vector, we have inserted EGFP downstream of the 5T4 oncofetal antigen promoter in ES cells (5T4-GFP ES cells). Undifferentiated 5T4-GFP ES cells lack EGFP and maintain expression of the pluripotent markers OCT-4 and NANOG. Upon differentiation, EGFP expression is increased in 5T4-GFP ES cells and this correlates with 5T4 transcript expression of the unmodified allele, loss of Nanog and Oct-4 transcripts and upregulation of differentiation-associated transcripts. Furthermore, we demonstrate that fluorescent activated cell sorting of 5T4-GFP ES cells allows isolation of pluripotent or differentiated cells from a heterogeneous population. These vectors provide researchers with a rapid method of modifying specific ES cell genes to study cellular differentiation and embryonic development.

Pignataro, G., F. E. Studer, et al. (2007). "Neuroprotection in ischemic mouse brain induced by stem cell-derived brain implants." J Cereb Blood Flow Metab **27**(5): 919-27.

Protective mechanisms of the brain may reduce the extent of injury after focal cerebral ischemia. Here, we explored in a mouse model of focal cerebral ischemia potential synergistic neuroprotective effects of two mediators of neuroprotection: (i) neuronal or glial precursor cells and (ii) the inhibitory neuromodulator adenosine. Embryonic stem (ES) cells, engineered to release adenosine by biallelic disruption of the adenosine kinase gene, and respective wild-type cells were induced to differentiate into either neural or glial precursor cells and were injected into the striatum of mice 1 week before middle cerebral artery occlusion. All stem cell-derived graft recipients were characterized by a significant reduction in infarct volume, an effect that was augmented by the release of adenosine. Neuroprotection was strongest in adenosine-releasing glial precursor cell recipients, which were characterized by an 85% reduction of the infarct area. Graft-mediated neuroprotection correlated with a significant improvement of general and focal neurologic scores. Histologic analysis before and after ischemia revealed clusters of implanted cells within the striatum of all treated mice. We conclude that ES cell derived adenosine-releasing brain implants provide neuroprotection by synergism of endogenous precursor cell-mediated effects and paracrine adenosine release.

Pomper, M. G., H. Hammond, et al. (2009). "Serial imaging of human embryonic stem-cell engraftment and teratoma formation in live mouse models." Cell Res **19**(3): 370-9.

Two new types of lentiviral vectors expressing a reporter transgene encoding either firefly luciferase (fLuc) for bioluminescence imaging or the HSV1 thymidine kinase (HSV1-TK) for radiopharmaceutical-based imaging were constructed to monitor human embryonic stem cell (hESC) engraftment and proliferation in live mice after transplantation. The constitutive expression of either transgene did not alter the properties of hESCs in the culture. We next monitored the formation of teratomas in SCID mice to test (1) whether the gene-modified hESCs maintain their developmental pluripotency, and

(2) whether sustained reporter gene expression allows noninvasive, whole-body imaging of hESC derivatives in a live mouse model. We observed teratoma formation from both types of gene-modified cells as well as wild-type hESCs 2-4 months after inoculation. Using an optical imaging system, bioluminescence from the fLuc-transduced hESCs was easily detected in mice bearing teratomas long before palpable tumors could be detected. To develop a noninvasive imaging method more readily translatable to the clinic, we also utilized HSV1-TK and its specific substrate, 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-[(125)I]iodouracil ([(125)I]FIAU), as a reporter/probe pair. After systemic administration, [(125)I]FIAU is phosphorylated only by the transgene-encoded HSV1-TK enzyme and retained within transduced (and transplanted) cells, allowing sensitive and quantitative imaging by single-photon emission computed tomography. Noninvasive imaging methods such as these may enable us to monitor the presence and distribution of transplanted human stem cells repetitively within live recipients over a long term through the expression of a reporter gene.

Popova, N. V. and R. J. Morris (2004). "Genetic regulation of mouse stem cells: identification of two keratinocyte stem cell regulatory loci." Curr Top Microbiol Immunol **280**: 111-37.

It is well documented that the bulge of hair follicle is a 'niche' for a significant population of mouse keratinocyte stem cells, and 95% of rodent clonogenic keratinocytes originate from the bulge region. The ability to form colonies in vitro is a well recognized test for keratinocyte stem cells. We analyzed the epidermis of seven mouse strains and their segregating crosses [(BALB/c x C57BL/6)F1; (BALB/c x CB6F1); (C57BL/ 6 x CB6F1); (CBF1 x CBF1)F2] for their clonogenic activity in vitro. We found that keratinocyte colony (KC) number is a new quantitative multigenic trait. The analysis of KC size in two parental strains (C57BL/6 and BALB/c), the F1 generation and the segregating crosses demonstrated that the size of KC is a quantitative complex trait also. We determined that mouse epidermis has at least two subpopulations of keratinocytes that gave small (< 2 mm2) and large (> 2 mm2) colonies. The differences in the number of small and large colonies between parental strains (C57BL/6, BALB/c) were significant ($P < 0.01$). A genome-wide scan of the intercross and the two backcrosses maps the number of small KC to the central region of mouse Chromosome 9 (genomewide P value = 0.01). We define this locus as Ksc1. The proximal region of chromosome 4 is associated with the high number of large KC. We defined this locus as Ksc2. We found that Ksc1 and minor loci on chromosomes 6 and 7 map close, if not equal to, loci associated with mouse skin carcinogenesis. We conclude that mouse epidermis has at least two subpopulations of clonogenic keratinocyte stem cells that are regulated by different genes. We suggest that keratinocyte stem cells responsible for small colonies may play a major role in the regulation of resistance or sensitivity to skin carcinogenesis. Investigation of the genes regulating the stem cell number should provide new insight into the mechanisms of skin carcinogenesis, and should help to develop new approaches for therapies not only against active proliferating tumor cells but also quiescent tumor stem cells.

Pralong, D., M. L. Lim, et al. (2005). "Tetraploid embryonic stem cells contribute to the inner cell mass of mouse blastocysts." Cloning Stem Cells **7**(4): 272-8.

The demonstration that mouse somatic cells can be reprogrammed following fusion with embryonic stem (ES) cells may provide an alternative to somatic cell nuclear transfer (therapeutic cloning) to generate autologous stem cells. In an attempt to produce cells with an increased pool of reprogramming factors, tetraploid ES cells were produced by polyethylene glycol mediated fusion of two ES cell lines transfected with plasmids carrying puromycin or neomycin resistance cassettes, respectively, followed by double antibiotic selection. Tetraploid ES cells retain properties characteristic of diploid ES cells, including the expression of pluripotent gene markers Oct4 and Rex1. On injection into the testis capsule of severe combined immunodeficient (SCID) mice, tetraploid ES cells are able to form teratomas containing cells representative of all three germ layers. Further, these cells demonstrated the ability to integrate into the inner cell mass of blastocysts. This study indicates that tetraploid ES cells are promising candidates as cytoplasm donors for reprogramming studies.

Prandini, M. H., A. Desroches-Castan, et al. (2007). "No evidence for vasculogenesis regulation by angiostatin during mouse embryonic stem cell differentiation." J Cell Physiol **213**(1): 27-35.

During embryogenesis, the formation of blood vessels proceeds by both vasculogenesis and angiogenesis. Both processes appear to be finely regulated. To date, factors and genes involved in the negative regulation of embryonic vasculogenesis remain largely unknown. Angiostatin is a proteolytic fragment of plasminogen that acts as an inhibitor of angiogenesis. In this study, we analyzed the potential role of angiostatin during early stages of embryonic stem (ES) cell endothelial in vitro differentiation, as a model of vasculogenesis. We found an early

expression of the known angiostatin binding sites (angiomotin, alphav integrin and c-met oncogene) during ES cell differentiation. Nevertheless, we did not detect any significant effect of angiostatin on mesoderm induction and on differentiation commitment into cells of the endothelial lineage. In both control and angiostatin-treated conditions, the temporal and extent of formation of the Flk1 positive and Flk-1/CD31 (PECAM-1) positive cell populations were not significantly different. Quantitative RT-PCR experiments of endothelial gene expression (Flk-1, PECAM-1 and tie-2) confirm a lack of interference with early steps of endothelial differentiation in embryoid bodies. No evidence for an angiostatin effect on endothelial cord-like formation could be detected at later differentiation stages. On the other hand, angiostatin inhibits vascular endothelial growth factor-induced endothelial sprouting from embryoid bodies cultured in three dimensional type I collagen gels. Taken together, these findings support a selective inhibitory effect on the sprouting angiogenesis response for angiostatin during embryonic vascular development.

Ralston, A. and J. Rossant (2005). "Genetic regulation of stem cell origins in the mouse embryo." Clin Genet **68**(2): 106-12.

'Stem cell' has practically become a household term, but what is a stem cell and where does it come from? Insight into these questions has come from the early mouse embryo, or blastocyst, from which three kinds of stem cells have been derived: embryonic stem (ES) cells, trophoblast stem (TS) cells, and extraembryonic endoderm (XEN) cells. These stem cells appear to derive from three distinct tissue lineages within the blastocyst: the epiblast, the trophectoderm, and the extraembryonic endoderm. Understanding how these lineages arise during development will illuminate efforts to understand the establishment and maintenance of the stem cell state and the mechanisms that restrict stem cell potency. Genetic analysis has enabled the identification of several genes important for lineage decisions in the mouse blastocyst. Among these, Oct4, Nanog, Cdx2, and Gata6 encode transcription factors required for the three lineages of the blastocyst and for the maintenance their respective stem cell types. Interestingly, genetic manipulation of several of these factors can cause lineage switching among these stem cells, suggesting that knowledge of key lineage-determining genes could help control differentiation of stem cells more generally. Pluripotent stem cells have also been isolated from the human blastocyst, but the relationship between these cells and stem cells of the mouse blastocyst remains to be explored. This review describes the genetic regulation of lineage allocation during blastocyst formation and discusses similarities and differences between mouse and human ES cells.

Reddy, P., L. Shen, et al. (2005). "Activation of Akt (PKB) and suppression of FKHRL1 in mouse and rat oocytes by stem cell factor during follicular activation and development." Dev Biol **281**(2): 160-70.

Although communications between mammalian oocytes and their surrounding granulosa cells mediated by the Kit-Kit ligand (KL, or stem cell factor, SCF) system have been proven to be crucial for follicular development, Kit downstream signaling pathways in mammalian oocytes are largely unknown. In this study, by using ovaries and isolated oocytes from postnatal mice and rats, we demonstrated for the first time that components of the PI3 kinase pathway, the serine/threonine kinase Akt (PKB) which enhances cellular proliferation and survival, and an Akt substrate FKHRL1 which is a transcription factor that leads to apoptosis and cell cycle arrest, are expressed in mammalian oocytes. By using an in vitro oocytes culture system, we found that oocytes-derived Akt and FKHRL1 are regulated by SCF. Treatment of cultured oocytes with SCF cannot only rapidly phosphorylate and activate Akt, but also simultaneously phosphorylate and may therefore functionally suppress FKHRL1, through the action of PI3 kinase. Together with our in situ hybridization and immunohistochemistry data that Akt and FKHRL1 are mostly expressed in oocytes in primordial and primary ovaries and reports that FKHRL1 gene-deficient mice exhibited excessive activation from primordial to primary follicles as well as enlarged oocyte sizes, we suppose that in mammalian oocytes, actions of granulosa cell derived SCF on primordial to primary follicle transition and subsequent follicle development may involve activation of Akt and inhibition of FKHRL1 activities in oocytes. The role of oocyte's Akt may be to enhance follicle development and the role of oocyte's FKHRL1 may be to inhibit follicle development. We propose that the cascade from granulosa cell SCF to oocyte Kit-PI3 kinase-Akt-FKHRL1 may play an important role to regulate the growth rate of mammalian oocytes and hypothetically also the oocyte secretion of factors that may regulate the activation and early development of ovarian follicles.

Reumers, V., C. M. Deroose, et al. (2008). "Noninvasive and quantitative monitoring of adult neuronal stem cell migration in mouse brain using bioluminescence imaging." Stem Cells **26**(9): 2382-90.

It is now generally accepted that continuous neurogenesis occurs in the adult mammalian brain, including that of humans. Modulation of adult

neurogenesis can provide therapeutic benefits for various brain disorders, including stroke and Parkinson's disease. The subventricular zone-olfactory bulb pathway is one of the preferred model systems by which to study neural stem cell proliferation, migration, and differentiation in adult rodent brain. Research on adult neurogenesis would greatly benefit from reliable methods for long-term noninvasive in vivo monitoring. We have used lentiviral vectors encoding firefly luciferase to stably mark endogenous neural stem cells in the mouse subventricular zone. We show that bioluminescence imaging (BLI) allows quantitative follow-up of the migration of adult neural stem cells into the olfactory bulb in time. Moreover, we propose a model to fit the kinetic data that allows estimation of migration and survival times of the neural stem cells using in vivo BLI. Long-term expression of brain-derived neurotrophic factor in the subventricular zone attenuated neurogenesis, as detected by histology and BLI. In vivo monitoring of the impact of drugs or genes on adult neurogenesis is now within reach.

Ro, S. (2004). "Magnifying stem cell lineages: the stop-EGFP mouse." Cell Cycle **3**(10): 1246-9.

Cell fate mapping techniques which can label clonal cell lineages are of importance because they allow one to investigate the distribution and types of daughter cells arising from single precursor cells. Thus, the potential of precursor cells to generate various types of descendent cells can be studied at the single-cell level. The stop-EGFP transgenic mouse carries a premature stop codon-containing enhanced green fluorescent protein (EGFP) gene as a target gene for mutations. A cell having undergone a mutation at the premature stop codon and its descendant cell lineage will express EGFP, thus a clonal cell lineage can be traced in vivo using a fluorescent microscope. Using the stop-EGFP mouse, stem cell clonal lineages in the mouse dorsal epidermis can be investigated in vivo and repeated analyses of the same cell lineages can be performed over time. In vivo imaging studies possible with the stop-EGFP mouse provide new insights into the structure of epidermal proliferative units (EPUs). The stop-EGFP system provides a novel tool for investigating clonal cell lineages in developmental studies as well as in stem cell biology.

Rodenburg, M., M. Fischer, et al. (2007). "Importance of receptor usage, Fli1 activation, and mouse strain for the stem cell specificity of 10A1 murine leukemia virus leukemogenicity." J Virol **81**(2): 732-42.

Murine leukemia viruses (MuLV) induce leukemia through a multistage process, a critical step being the activation of oncogenes through provirus integration. Transcription elements within the long terminal repeats (LTR) are prime determinants of cell lineage specificity; however, the influence of other factors, including the Env protein that modulates cell tropism through receptor recognition, has not been rigorously addressed. The ability of 10A1-MuLV to use both PiT1 and PiT2 receptors has been implicated in its induction of blast cell leukemia. Here we show that restricting receptor usage of 10A1-MuLV to PiT2 results in loss of blast cell transformation capacity. However, the pathogenicity was unaltered when the env gene is exchanged with Moloney MuLV, which uses the Cat1 receptor. Significantly, the leukemic blasts express erythroid markers and consistently contain proviral integrations in the Fli1 locus, a target of Friend MuLV (F-MuLV) during erythroleukemia induction. Furthermore, an NB-tropic variant of 10A1 was unable to induce blast cell leukemia in C57BL/6 mice, which are also resistant to F-MuLV transformation. We propose that 10A1- and F-MuLV actually induce identical (erythro)blastic leukemia by a mechanism involving Fli1 activation and cooperation with inherent genetic mutations in susceptible mouse strains. Furthermore, we demonstrate that deletion of the Icsbp tumor suppressor gene in C57BL/6 mice is sufficient to confer susceptibility to 10A1-MuLV leukemia induction but with altered specificity. In summary, we validate the significance of the env gene in leukemia specificity and underline the importance of a complex interplay of cooperating oncogenes and/or tumor suppressors in determining the pathogenicity of MuLV variants.

Rohwedel, J., V. Horak, et al. (1995). "M-twist expression inhibits mouse embryonic stem cell-derived myogenic differentiation in vitro." Exp Cell Res **220**(1): 92-100.

The mouse M-twist gene codes for a basic helix-loop-helix protein which was shown to be inhibitory for differentiation of myogenic cells in culture. Mouse embryonic stem (ES) cells of line BLC6 efficiently differentiating into skeletal muscle cells when cultivated as embryo-like aggregates (embryoid bodies) were stably transfected with the plasmid pME18s-twist containing the M-twist gene under the control of the modified SV40 early promoter SR alpha. Two pME18s-twist-expressing clones showed delayed and reduced skeletal muscle cell differentiation depending on the level of exogenous M-twist expression compared to control cells. By morphological analysis using phase contrast microscopy and hematoxylin-eosin staining, the development of first myocytes and formation of myotubes in embryoid body outgrowths of these clones were found to be delayed for about 3 days in comparison to control cells. Immunofluorescence

studies with a monoclonal antibody against sarcomeric myosin heavy chain revealed that myogenic cells appeared in so-called myogenic centers showing a reduced number of myocytes and myotubes in the M-twist-expressing clones. Using RT-PCR analysis the expression of the skeletal muscle determination genes myf5, myogenin, and MyoD as well as muscle-specific genes coding for the gamma-subunit of the nicotinic acetylcholine receptor and the cell adhesion molecule M-cadherin were found to appear with a delay of at least 1 to 4 days in the pME18s-twist-transfected cells during the development of embryoid bodies. We conclude that the constitutive expression of the mouse M-twist gene during ES-cell-derived differentiation has an inhibitory effect on skeletal muscle cell development depending on the level of exogenous M-twist expression.

Rohwedel, J., U. Sehlmeyer, 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): 579-87.

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.

Roth, T. M., P. Ramamurthy, et al. (2007). "A mouse embryonic stem cell model of Schwann cell differentiation for studies of the role of neurofibromatosis type 1 in Schwann cell development and tumor formation." Glia **55**(11): 1123-33.

The neurofibromatosis Type 1 (NF1) gene functions as a tumor suppressor gene. One known function of neurofibromin, the NF1 protein product, is to accelerate the slow intrinsic GTPase activity of Ras to increase the production of inactive rasGDP, with wide-ranging effects on p21ras pathways. Loss of neurofibromin in the autosomal dominant disorder NF1 is associated with tumors of the peripheral nervous system, particularly neurofibromas, benign lesions in which the major affected cell type is the Schwann cell (SC). NF1 is the most common cancer predisposition syndrome affecting the nervous system. We have developed an in vitro system for differentiating mouse embryonic stem cells (mESC) that are NF1 wild type (+/+), heterozygous (+/-), or null (-/-) into SC-like cells to study the role of NF1 in SC development and tumor formation. These mES-generated SC-like cells, regardless of their NF1 status, express SC markers correlated with their stage of maturation, including myelin proteins. They also support and preferentially direct neurite outgrowth from primary neurons. NF1 null and heterozygous SC-like cells proliferate at an accelerated rate compared to NF1 wild type; this growth advantage can be reverted to wild type levels using an inhibitor of MAP kinase kinase (Mek). The mESC of all NF1 types can also be differentiated into neuron-like cells. This novel model system provides an ideal paradigm for studies of the role of NF1 in cell growth and differentiation of the different cell types affected by NF1 in cells with differing levels of neurofibromin that are neither transformed nor malignant.

Rust, E. M., M. V. Westfall, et al. (1997). "Gene transfer into mouse embryonic stem cell-derived cardiac myocytes mediated by recombinant adenovirus." In Vitro Cell Dev Biol Anim **33**(4): 270-6.

The main purpose of this study was to examine, for the first time, the ability of recombinant adenovirus to mediate gene transfer into cardiac myocytes derived from mouse embryonic stem (ES) cells differentiating in vitro. In addition, observations were made on the effect of adenovirus infection on cardiac myocyte differentiation and contractility in this in vitro system of cardiogenesis. ES cell cultures were infected at various times of differentiation with a recombinant adenovirus vector (AdCMVlacZ) containing the bacterial lacZ gene under the control of the cytomegalovirus (CMV) promoter. Expression of the lacZ reporter gene was determined by histochemical staining for beta-galactosidase activity. LacZ expression was not detected in undifferentiated ES cells infected with AdCMVlacZ. In contrast, infection of differentiating ES cell cultures showed increasing transgene expression with continued time

in culture. Expression in ES-cell-derived cardiac myocytes was demonstrated by codetection of beta-galactosidase activity and troponin T with indirect immunofluorescence. At 24 h postinfection, approximately 27% of the cardiac myocytes were beta-galactosidase positive, and lacZ gene expression appeared to be stable for up to 21 d postinfection. Adenovirus infection had no apparent effect on the onset, extent, or duration of spontaneously contracting ES-cell-derived cardiomyocytes, indicating that cardiac differentiation and contractile function were not significantly altered in the infected cultures. The demonstration of adenovirus-mediated gene transfer into ES-cell-derived cardiac myocytes will aid studies of gene expression with this in vitro model of cardiogenesis and may facilitate future studies involving the use of these myocytes for grafting experiments in vivo.

Rybak, A., H. Fuchs, et al. (2009). "The let-7 target gene mouse lin-41 is a stem cell specific E3 ubiquitin ligase for the miRNA pathway protein Ago2." Nat Cell Biol **11**(12): 1411-20.

The let-7 miRNA and its target gene Lin-28 interact in a regulatory circuit controlling pluripotency. We investigated an additional let-7 target, mLin41 (mouse homologue of lin-41), as a potential contributor to this circuit. We demonstrate the presence of mLin41 protein in several stem cell niches, including the embryonic ectoderm, epidermis and male germ line. mLin41 colocalized to cytoplasmic foci with P-body markers and the miRNA pathway proteins Ago2, Mov10 and Tnrc6b. In co-precipitation assays, mLin41 interacted with Dicer and the Argonaute proteins Ago1, Ago2 and Ago4. Moreover, we show that mLin41 acts as an E3 ubiquitin ligase in an auto-ubiquitylation assay and that mLin41 mediates ubiquitylation of Ago2 in vitro and in vivo. Overexpression and depletion of mLin41 led to inverse changes in the level of Ago2 protein, implicating mLin41 in the regulation of Ago2 turnover. mLin41 interfered with silencing of target mRNAs for let-7 and miR-124, at least in part by antagonizing Ago2. Furthermore, mLin41 cooperated with the pluripotency factor Lin-28 in suppressing let-7 activity, revealing a dual control mechanism regulating let-7 in stem cells.

Ryu, B. Y., H. Kubota, et al. (2005). "Conservation of spermatogonial stem cell self-renewal signaling between mouse and rat." Proc Natl Acad Sci U S A **102**(40): 14302-7.

Self-renewal of spermatogonial stem cells (SSCs) is the foundation for maintenance of spermatogenesis throughout life in males and for continuation of a species. The molecular mechanism underlying stem cell self-renewal is a fundamental question in stem cell biology. Recently, we identified growth factors necessary for self-renewal of mouse SSCs and established a serum-free culture system for their proliferation in vitro. To determine whether the stimulatory signals for SSC replication are conserved among different species, we extended the culture system to rat SSCs. Initially, a method to assess in vitro expansion of SSCs was developed by using flow cytometric analysis, and, subsequently, we found that a combination of glial cell line-derived neurotrophic factor, soluble glial cell line-derived neurotrophic factor-family receptor alpha-1 and basic fibroblast growth factor supports proliferation of rat SSCs. When cultured with the three factors, stem cells proliferated continuously for >7 months, and transplantation of the cultured SSCs to recipient rats generated donor stem cell-derived progeny, demonstrating that the cultured stem cells are normal. The growth factor requirement for replication of rat SSCs is identical to that of mouse; therefore, the signaling factors for SSC self-renewal are conserved in these two species. Because SSCs from many mammals, including human, can replicate in mouse seminiferous tubules after transplantation, the growth factors required for SSC self-renewal may be conserved among many different species. Furthermore, development of a long-term culture system for rat SSCs has established a foundation for germ-line modification of the rat by gene targeting technology.

Sakakibara, S., T. Imai, et al. (1996). "Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell." Dev Biol **176**(2): 230-42.

There is increasing interest in the role of RNA-binding proteins during neural development. Drosophila Musashi is one of the neural RNA-binding proteins essential for neural development and required for asymmetric cell divisions in the Drosophila adult sensory organ development. Here, a novel mammalian neural RNA-binding protein, mouse-Musashi-1, was identified based on the homology to Drosophila Musashi and Xenopus NRP-1. In the developing CNS, mouse-Musashi-1 protein was highly enriched in the CNS stem cell. Single-cell culture experiments indicated that mouse-Musashi-1 expression is associated with neural precursor cells that are capable of generating neurons and glia. In contrast, in fully differentiated neuronal and glial cells mouse-Musashi-1 expression is lost. This expression pattern of mouse-Musashi-1 is complementary to that of another mammalian neural RNA-binding protein, Hu (a mammalian homologue of a Drosophila neuronal RNA-binding protein Elav), that is expressed in

postmitotic neurons within the CNS. In vitro studies indicated that mouse-Musashi-1 possesses binding preferences on poly(G) RNA homopolymer, whereas Hu is known to preferentially bind to short A/U-rich regions in RNA. Based on their differential expression patterns and distinct preferential target RNA sequences, we believe that the mouse-Musashi-1 and Hu proteins may play distinct roles in neurogenesis, either through sequential regulatory mechanisms or differential sorting of mRNA populations during asymmetric division of neural precursor cells.

Satoh, M., H. Mioh, et al. (1997). "Mouse bone marrow stromal cell line MC3T3-G2/PA6 with hematopoietic-supporting activity expresses high levels of stem cell antigen Sca-1." Exp Hematol **25**(9): 972-9.

The murine clonal preadipose cell line, MC3T3-G2/PA6 (PA6), has the ability to support in vitro proliferation of hematopoietic stem cells defined as colony-forming units in spleen (CFU-S). In order to ascertain the relationship between the hematopoietic-supporting activity of PA6 cells and their expression, we cultured a number of these cells for over 45 weeks and investigated the level at which they expressed several cell surface markers and membrane-bound growth factors. Besides expressing stem cell factor (SCF) and macrophage colony-stimulating factor (M-CSF), PA6 cells were found by flow cytometry analysis to express high levels of stem cell antigen-1 (Sca-1). The expression level of Sca-1 in PA6 cells correlated with the ability of the latter to support hematopoiesis, whereas no such correlation was observed in the case of SCF and M-CSF expression. A cDNA clone encoding the protein recognized by anti-Sca-1 antibody was isolated from PA6 cells by expression cloning, so that its nucleotide sequence encoded the protein identical to mouse alloantigen Ly-6A.2. Genetically engineered COS-7 cells, transformed by the expression vector carrying the Ly-6A.2 gene, suppressed proliferation of murine lineage marker-negative (Lin) bone marrow cells by themselves and synergistically augmented proliferation of these cells in the presence of SCF. These results suggest that Ly-6A.2 regulates the proliferation of hematopoietic progenitor cells, and is one of the molecules organizing the hematopoietic microenvironment provided by stromal cells.

Sautin, Y. Y., M. Jorgensen, et al. (2002). "Hepatic oval (stem) cell expression of endothelial differentiation gene receptors for lysophosphatidic acid in mouse chronic liver injury." J Hematother Stem Cell Res **11**(4): 643-9.

Growth factor lysophosphatidic acid (LPA) regulates cell proliferation and differentiation and increases motility and survival in several cell types, mostly via G-protein-coupled receptors encoded by endothelial differentiation genes (EDG). We show herein that hepatic oval (stem) cell proliferation, induced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) in a mouse model of chronic liver injury, was associated with the expression of LPA1, LPA2, and LPA3 receptor subtypes; only LPA1 receptor protein was detectable in normal liver by western blot. In the injured liver, enhanced LPA1 receptor was identified predominantly in oval cells along the portal tract, proliferating ductular epithelial cells, and small cells, which were located in the nearby parenchyma and formed clusters. Interestingly, the LPA1 receptor was co-expressed in DDC-treated livers with the stem cell antigen SCA-1, suggesting that this receptor may be associated with bone marrow-derived progenitors. All three receptors for LPA were detected mostly in small cells in the vicinity of the portal tract, and co-localized with the A6 antigen, a marker of ductular oval cells. In addition, hepatic levels of endogenous LPA were significantly higher in DDC-fed mice compared to normal animals. We propose that the expression of diverse LPA receptors may be a necessary part of the mechanism responsible for activation of oval cells during liver injury. As a result, LPA and its analogs may represent critical endogenous mediators, which regulate survival, increase motility, and modulate proliferation and differentiation of hepatocyte progenitors in regenerating liver.

Scherr, M., K. Battmer, et al. (2003). "Inhibition of GM-CSF receptor function by stable RNA interference in a NOD/SCID mouse hematopoietic stem cell transplantation model." Oligonucleotides **13**(5): 353-63.

RNA interference (RNAi) describes a highly conserved mechanism of sequence-specific posttranscriptional gene silencing triggered by double-stranded RNA (dsRNA). Whereas RNAi is applied to study gene function in different organisms and in variant cell types, little is known about RNAi in human hematopoietic stem and progenitor cells and their myeloid progeny. To address this issue, short hairpin RNAs (shRNA) were designed to target the common beta-chain of the human receptors for granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5 (betaGMR). These receptors regulate proliferation, survival, differentiation, and functional activity of hematopoietic cells. In addition to markedly inhibiting mRNA and protein expression, anti-beta-GMR shRNAs were also found to inhibit receptor function in a cell culture model. Furthermore, lentiviral gene transfer of shRNA expression cassettes into primary normal CD34+ cells selectively inhibited colony

formation of transduced progenitors when stimulated with GM-CSF/IL-3 but not when stimulated with cytokines that do not signal via beta-GMR. Finally, anti-beta-GMR shRNAs had no detectable effect on engraftment or lineage composition of lentivirally transduced human CD34+ cells transplanted into NOD/SCID mice. However, the growth defect of transduced colony-forming cells under stimulation with GM-CSF/IL-3 remains unchanged in bone marrow cells harvested from individual NOD/SCID mice 6 weeks after transplantation. These data indicate that lentiviral gene transfer of shRNA expression cassettes may be used to induce long-term RNAi in human hematopoietic stem and progenitor cells for functional genetics and potential therapeutic intervention.

Schoonjans, L., V. Kreemers, et al. (2003). "Improved generation of germline-competent embryonic stem cell lines from inbred mouse strains." Stem Cells **21**(1): 90-7.

Genetically altered mice may exhibit highly variable phenotypes due to the variation in genetic background, which can only be circumvented by generation of inbred, isogenic gene-targeted and control mice. Here we report that an embryonic stem (ES) cell culture medium conditioned by a rabbit fibroblast cell line transduced with genomic rabbit leukemia inhibitory factor allows efficient derivation and maintenance of ES cell lines from all of 10 inbred mouse strains tested, including some that were presumed to be nonpermissive for ES cell derivation (129/SvEv, 129/SvJ, C57BL/6N, C57BL/6JOla, CBA/CaOla, DBA/2N, DBA/1Ola, C3H/HeN, BALB/c, and FVB/N). Germline transmission was established by blastocyst injection of established ES cell lines after 10 or more passages from all of seven strains tested (129/SvJ, C57BL/6N, C57BL/6JOla, DBA/2N, DBA/1Ola, BALB/c, and FVB/N), by diploid aggregation of ES cell lines from all of four strains tested (129/SvEv, C57BL/6N, CBA/ CaOla, and FVB/N), or by tetraploid aggregation of ES cell lines from all of three strains tested (129/SvEv, C57BL/6N, and CBA/CaOla). Thus, these inbred ES cell lines may constitute useful tools to derive gene-targeted mice and isogenic controls in selected genetic backgrounds.

Schulz, H., R. Kolde, et al. (2009). "The FunGenES database: a genomics resource for mouse embryonic stem cell differentiation." PLoS One **4**(9): e6804.

Embryonic stem (ES) cells have high self-renewal capacity and the potential to differentiate into a large variety of cell types. To investigate gene networks operating in pluripotent ES cells and their derivatives, the "Functional Genomics in Embryonic Stem Cells" consortium (FunGenES) has analyzed the transcriptome of mouse ES cells in eleven diverse settings representing sixty-seven experimental conditions. To better illustrate gene expression profiles in mouse ES cells, we have organized the results in an interactive database with a number of features and tools. Specifically, we have generated clusters of transcripts that behave the same way under the entire spectrum of the sixty-seven experimental conditions; we have assembled genes in groups according to their time of expression during successive days of ES cell differentiation; we have included expression profiles of specific gene classes such as transcription regulatory factors and Expressed Sequence Tags; transcripts have been arranged in "Expression Waves" and juxtaposed to genes with opposite or complementary expression patterns; we have designed search engines to display the expression profile of any transcript during ES cell differentiation; gene expression data have been organized in animated graphs of KEGG signaling and metabolic pathways; and finally, we have incorporated advanced functional annotations for individual genes or gene clusters of interest and links to microarray and genomic resources. The FunGenES database provides a comprehensive resource for studies into the biology of ES cells.

Selfridge, J., A. M. Pow, et al. (1992). "Gene targeting using a mouse HPRT minigene/HPRT-deficient embryonic stem cell system: inactivation of the mouse ERCC-1 gene." Somat Cell Mol Genet **18**(4): 325-36.

A convenient system for gene targeting that uses hypoxanthine phosphoribosyltransferase (HPRT) minigenes as the selectable marker in HPRT-deficient mouse embryonic stem (ES) cells is described. Improvements to the expression of HPRT minigenes in ES cells were achieved by promoter substitution and the provision of a strong translational initiation signal. The use of minigenes in the positive-negative selection strategy for gene targeting was evaluated and the smaller minigenes were found to be as effective as a more conventional marker--the herpes simplex virus thymidine kinase gene. Minigenes were used to target the DNA repair gene ERCC-1 in ES cells. A new HPRT-deficient ES cell line was developed that contributes with high frequency to the germ line of chimeric animals. The ability to select for and against HPRT minigene expression in the new HPRT-deficient ES cell line will make this system useful for a range of gene-targeting applications.

Shafi, R., S. P. Iyer, et al. (2000). "The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny." Proc Natl Acad Sci U S A **97**(11): 5735-9.

Nuclear and cytoplasmic protein glycosylation is a widespread and reversible posttranslational modification in eukaryotic cells. Intracellular glycosylation by the addition of N-acetylglucosamine (GlcNAc) to serine and threonine is catalyzed by the O-GlcNAc transferase (OGT). This "O-GlcNAcylation" of intracellular proteins can occur on phosphorylation sites, and has been implicated in controlling gene transcription, neurofilament assembly, and the emergence of diabetes and neurologic disease. To study OGT function in vivo, we have used gene-targeting approaches in male embryonic stem cells. We find that OGT mutagenesis requires a strategy that retains an intact OGT gene as accomplished by using Cre-loxP recombination, because a deletion in the OGT gene results in loss of embryonic stem cell viability. A single copy of the OGT gene is present in the male genome and resides on the X chromosome near the centromere in region D in the mouse spanning markers DxMit41 and DxMit95, and in humans at Xq13, a region associated with neurologic disease. OGT RNA expression in mice is comparably high among most cell types, with lower levels in the pancreas. Segregation of OGT alleles in the mouse germ line with ZP3-Cre recombination in oocytes reveals that intact OGT alleles are required for completion of embryogenesis. These studies illustrate the necessity of conditional gene-targeting approaches in the mutagenesis and study of essential sex-linked genes, and indicate that OGT participation in intracellular glycosylation is essential for embryonic stem cell viability and for mouse ontogeny.

Shibayama, M., S. Ohno, et al. (2009). "Polypyrimidine tract-binding protein is essential for early mouse development and embryonic stem cell proliferation." Febs J **276**(22): 6658-68.

Polypyrimidine tract-binding protein (PTB) is a widely expressed RNA-binding protein with multiple roles in RNA processing, including the splicing of alternative exons, mRNA stability, mRNA localization, and internal ribosome entry site-dependent translation. Although it has been reported that increased expression of PTB is correlated with cancer cell growth, the role of PTB in mammalian development is still unclear. Here, we report that a homozygous mutation in the mouse Ptb gene causes embryonic lethality shortly after implantation. We also established Ptb(-/-) embryonic stem (ES) cell lines and found that these mutant cells exhibited severe defects in cell proliferation without aberrant differentiation in vitro or in vivo. Furthermore, cell cycle analysis and a cell synchronization assay revealed that Ptb(-/-) ES cells have a prolonged G(2)/M phase. Thus, our data indicate that PTB is essential for early mouse development and ES cell proliferation.

Shimizu, N., K. Yamamoto, et al. (2008). "Cyclic strain induces mouse embryonic stem cell differentiation into vascular smooth muscle cells by activating PDGF receptor beta." J Appl Physiol **104**(3): 766-72.

Embryonic stem (ES) cells are exposed to fluid-mechanical forces, such as cyclic strain and shear stress, during the process of embryonic development but much remains to be elucidated concerning the role of fluid-mechanical forces in ES cell differentiation. Here, we show that cyclic strain induces vascular smooth muscle cell (VSMC) differentiation in murine ES cells. Flk-1-positive (Flk-1+) ES cells seeded on flexible silicone membranes were subjected to controlled levels of cyclic strain and examined for changes in cell proliferation and expression of various cell lineage markers. When exposed to cyclic strain (4-12% strain, 1 Hz, 24 h), the Flk-1+ ES cells significantly increased in cell number and became oriented perpendicular to the direction of strain. There were dose-dependent increases in the VSMC markers smooth muscle alpha-actin and smooth muscle-myosin heavy chain at both the protein and gene expression level in response to cyclic strain, whereas expression of the vascular endothelial cell marker Flk-1 decreased, and there were no changes in the other endothelial cell markers (Flt-1, VE-cadherin, and platelet endothelial cell adhesion molecule 1), the blood cell marker CD3, or the epithelial marker keratin. The PDGF receptor beta (PDGFR beta) kinase inhibitor AG-1296 completely blocked the cyclic strain-induced increase in cell number and VSMC marker expression. Cyclic strain immediately caused phosphorylation of PDGFR beta in a dose-dependent manner, but neutralizing antibody against PDGF-BB did not block the PDGFR beta phosphorylation. These results suggest that cyclic strain activates PDGFR beta in a ligand-independent manner and that the activation plays a critical role in VSMC differentiation from Flk-1+ ES cells.

Shimizukawa, R., A. Sakata, et al. (2005). "Establishment of a new embryonic stem cell line derived from C57BL/6 mouse expressing EGFP ubiquitously." Genesis **42**(1): 47-52.

Transgenic mice ubiquitously expressing enhanced green fluorescent protein (EGFP) are useful as marker lines in chimera experiments. We established a new embryonic stem (ES) cell line (named B6G-2) from a C57BL/6 blastocyst showing ubiquitous EGFP expression. Undifferentiated B6G-2 cells showed strong green fluorescence and mRNAs of pluripotent marker genes. B6G-2 cells were

transferred into a C57BL/6 blastocyst to generate a germline chimera, the progeny of which inherited ubiquitous EGFP expression. Mice derived completely from B6G-2 cells were also developed from the ES cells; these were tetraploid chimeras. The established B6G-2 cells were shown to be pluripotent and to be capable of differentiating into cells of all lineages. Thus, the new ES cell line expressing EGFP ubiquitously is useful for basic research in the field of regenerative medicine. The B6G-2 cell line is freely available from the BioResource Center, RIKEN Tsukuba Institute (http://www.brc.riken.jp/lab/cell/english/).

Silva, C., J. R. Wood, et al. (2009). "Expression profile of male germ cell-associated genes in mouse embryonic stem cell cultures treated with all-trans retinoic acid and testosterone." Mol Reprod Dev **76**(1): 11-21.

Cells that morphologically and functionally resemble male germ cells can be spontaneously derived from ES cells. However, this process is inefficient and unpredictable suggesting that the expression pattern of male germ cell associated genes during spontaneous ES cell differentiation does not mimic the in vivo profiles of the genes. Thus, in the present study, the temporal profile of genes expressed at different stages of male germ cell development was examined in differentiating ES cells. The effect of all-trans retinoic acid (RA) which is a known inducer of primordial germ cell (PGC) proliferation/survival in vitro and testosterone which is required for spermatogenesis in vivo on the expression of these genes was also determined. Each of the 12 genes analyzed exhibited one of four temporal expression patterns in untreated differentiating ES cells: progressively decreased (Dppa3, Sycp3, Msy2), initially low and then increased (Stra8, Sycp1, Dazl, Act, Prm1), initially decreased and then increased (Piwil2, Tex14), or relatively unchanged (Akap3, Odf2). RA-treated cells exhibited increased expression of Stra8, Dazl, Act, and Prm1 and suppressed expression of Dppa3 compared to untreated controls. Furthermore, testosterone increased expression of Stra8 while the combination of RA and testosterone synergistically increased expression of Act. Our findings establish a comprehensive profile of male germ cell gene expression during spontaneous differentiation of murine ES cells and describe the capacity of RA and testosterone to modulate the expression of these genes. Furthermore, these data represent an important first step in designing a plausible directed differentiation protocol for male germ cells.

Soncin, F., L. Mohamet, et al. (2009). "Abrogation of E-cadherin-mediated cell-cell contact in mouse embryonic stem cells results in reversible LIF-independent self-renewal." Stem Cells **27**(9): 2069-80.

We have previously demonstrated that differentiation of embryonic stem (ES) cells is associated with downregulation of cell surface E-cadherin. In this study, we assessed the function of E-cadherin in mouse ES cell pluripotency and differentiation. We show that inhibition of E-cadherin-mediated cell-cell contact in ES cells using gene knockout (Ecad(-/-)), RNA interference (EcadRNAi), or a transhomodimerization-inhibiting peptide (CHAVC) results in cellular proliferation and maintenance of an undifferentiated phenotype in fetal bovine serum-supplemented medium in the absence of leukemia inhibitory factor (LIF). Re-expression of E-cadherin in Ecad(-/-), EcadRNAi, and CHAVC-treated ES cells restores cellular dependence to LIF supplementation. Although reversal of the LIF-independent phenotype in Ecad(-/-) ES cells is dependent on the beta-catenin binding domain of E-cadherin, we show that beta-catenin null (betacat(-/-)) ES cells also remain undifferentiated in the absence of LIF. This suggests that LIF-independent self-renewal of Ecad(-/-) ES cells is unlikely to be via beta-catenin signaling. Exposure of Ecad(-/-), EcadRNAi, and CHAVC-treated ES cells to the activin receptor-like kinase inhibitor SB431542 led to differentiation of the cells, which could be prevented by re-expression of E-cadherin. To confirm the role of transforming growth factor beta family signaling in the self-renewal of Ecad(-/-) ES cells, we show that these cells maintain an undifferentiated phenotype when cultured in serum-free medium supplemented with Activin A and Nodal, with fibroblast growth factor 2 required for cellular proliferation. We conclude that transhomodimerization of E-cadherin protein is required for LIF-dependent ES cell self-renewal and that multiple self-renewal signaling networks subsist in ES cells, with activity dependent upon the cellular context.

Sorrentino, E., V. Nazzicone, et al. (2007). "Comparative transcript profiles of cell cycle-related genes in mouse primordial germ cells, embryonic stem cells and embryonic germ cells." Gene Expr Patterns **7**(6): 714-21.

We used cDNA array to compare the relative transcript levels of 96 cell cycle-related genes in mouse primordial germ cells (PGCs), embryonic germ (EG) cells and embryonic stem (ES) cells. Among 38 genes of the G1 phase analysed, Ccnd3 (CyclinD3), Cdkn1c (p57(kip2)), Rb1, and Tceb1l (Skip1-like) were expressed at significantly higher levels in PGCs than in EG and ES cells; Ccnd1 (CyclinD1) was more

abundant in EG cells than in PGCs. Except for higher mRNA levels of Ccng (CyclinG1) in EG and ES cells in comparison to PGCs, no difference among 20 genes of the S and 12 genes of G2/M phases was found. Less than half of the 26 genes regarded as DNA damage checkpoint/Trp53/Atm pathway genes showed significant transcript levels in all three cell populations. Among these, the transcript levels of Ube1x and Atm were significantly higher in PGCs than in EG and ES cells while that of Ube3a was higher in these latter. In addition, relatively high mRNA levels of Timp3 characterizes EG cells while transcripts of this gene were very low in PGCs and barely detectable in ES cells. With the exception of Tceb1l, differential transcript levels found in the cDNA array assay were confirmed by real time RT-PCR. Using this method, we also analysed the transcripts of two genes not present in the cDNA array: c-myc, known to be critical for the control of cell cycle in many cell types, and Eras, specifically expressed in ES cells and involved in the control of ES cell proliferation and their tumorigenic properties. While c-myc transcripts were present at similar levels in all three cell types examined, Eras was expressed at high levels in ES cells (10-fold) and even more so in EG cells (almost 40-fold) in comparison to PGCs. Taken together, these results indicate that despite similarities between PGCs and ES or EG cells, their cell cycles are differently regulated. In particular, it appears that PGCs, like most mitotic cells, possess a more regulatable control of G1 phase than EG and ES cells. Moreover, our data provide useful clues for further studies aimed at identifying cell cycle genes critical for PGC growth and their transformation in tumorigenic cells.

Sottile, V., M. Li, et al. (2006). "Stem cell marker expression in the Bergmann glia population of the adult mouse brain." Brain Res **1099**(1): 8-17.

Recent evidence suggests that the postnatal cerebellum contains cells with characteristics of neural stem cells, which had so far only been identified in the subventricular zone of the lateral ventricles and the subdentate gyrus of the hippocampus. In order to investigate the identity of these cells in the adult cerebellum, we have analyzed the expression of Sox1, a transcription factor from the SoxB1 subgroup and widely used marker of neural stem cells. In situ hybridization and the use of a transgenic mouse model show that, in the adult cerebellum, Sox 1 is only expressed in the Bergmann glia, a population of radial glia present in the Purkinje cell layer. Furthermore, another neural stem cell marker, Sox2 (also member of the SoxB1 subgroup), is also expressed in the Bergmann glia. We have previously shown that these same cells express Sox9, a member of the SoxE subgroup known for its role in glial development. Here we show that Sox9 is in fact also expressed in other regions harboring adult neural stem cells, suggesting that Sox9 represents a novel stem cell marker. Finally, using a Sox1-null mouse, we show that the formation of this Sox2/Sox9 positive Bergmann glia population does not require the presence of a functional Sox1. Our results identify these radial glia as a previously unreported Sox1/Sox2/Sox9 positive adult cell population, suggesting that these cells may represent the recently reported stem cells in the adult cerebellum.

Stevenson, A. J., D. Clarke, et al. (2000). "Herpesvirus saimiri-based gene delivery vectors maintain heterologous expression throughout mouse embryonic stem cell differentiation in vitro." Gene Ther **7**(6): 464-71.

In order to achieve a high efficiency of gene delivery into rare cell types like stem cells the use of viral vectors is presently without alternative. An ideal stem cell gene therapy vector would be able to infect primitive progenitor cells and sustain or activate gene expression in differentiated progeny. However, many viral vectors are inactivated when introduced in developing systems where cell differentiation occurs. To this end, we have developed a mouse in vitro model for testing herpesvirus saimiri (HVS)-based gene therapy vectors. We demonstrate here for the first time that HVS is able to infect totipotent mouse embryonic stem (ES) cells with high efficiency. We have transduced ES cells with a recombinant virus carrying the enhanced green fluorescent protein (EGFP) gene and the neomycin resistance gene (NeoR) driven by a CMV promoter and the SV40 promoter, respectively. ES cells maintain the viral episomal genome and can be terminally differentiated into mature haematopoietic cells. Moreover, heterologous gene expression is maintained throughout in vitro differentiation. Besides its obvious use in gene therapy, this unique expression system has wide ranging applications in studies aimed at understanding gene function and expression in cell differentiation and development.

Strubing, C., J. Rohwedel, et al. (1997). "Development of G protein-mediated Ca2+ channel regulation in mouse embryonic stem cell-derived neurons." Eur J Neurosci **9**(4): 824-32.

Besides other mechanisms, the influx of Ca2+ into embryonic neurons controls growth and differentiation processes. To study the expression and regulation of voltage-gated Ca2+ channels during early neurogenesis, we measured whole-cell Ca2+ currents (I(Ca)) in neurons developing from pluripotent embryonic stem cells. Various receptor

agonists, including somatostatin and baclofen, reversibly inhibited I(Ca) in embryonic stem cell-derived neurons. The effects of somatostatin and baclofen were abolished by pretreatment of cells with pertussis toxin and mimicked by intracellular infusion of guanosine 5'-O-(3-thiotriphosphate), suggesting the involvement of pertussis toxin-sensitive G proteins in I(Ca) inhibition. Investigations at different stages of neuronal differentiation showed that somatostatin efficiently suppressed L- and N-type Ca2+ channels in immature as well as mature neurons. In contrast, inhibition of L- and N-type channels by baclofen was rarely observed at the early stage. In terminally differentiated neurons, responses to baclofen were as prominent as those to somatostatin but were confined to N-type Ca2+ channels. The stage-dependent sensitivity of voltage-gated Ca2+ channels to somatostatin and baclofen was not due to differential expression of G alpha(o) isoforms, as revealed by reverse transcription-polymerase chain reaction and immunofluorescence microscopy. These findings demonstrate that specific neurotransmitters such as somatostatin regulate voltage-gated Ca2+ channels via G proteins during the early stages of neurogenesis, thus providing a mechanism for the epigenetic control of neuronal differentiation.

Suemori, H., Y. Kadodawa, et al. (1990). "A mouse embryonic stem cell line showing pluripotency of differentiation in early embryos and ubiquitous beta-galactosidase expression." Cell Differ Dev **29**(3): 181-6.

For analysis of chimeric mice made by injecting embryonic stem (ES) cells into host blastocysts, it is very desirable if the ES cells have a good cell marker that can distinguish them from host cells. It is ideal if the marker can be easily visualized in every type of cell and tissue throughout the embryogenesis. We tried to produce such ES cell lines by introducing an E. coli beta-galactosidase (beta-gal) gene construct by electroporation. One of the transformant lines (MS1-EL4) showed beta-gal activity in every undifferentiated stem cell. After being induced to differentiate in vitro, cells with various morphologies showed beta-gal activity. We also detected beta-gal activity in a wide variety of tissue elements in solid tumors made by injecting the MS1-EL4 cells into syngeneic mice. Then we produced chimeric embryos by injecting the MS1-EL4 cells into blastocysts and recovering the embryos at various developmental stages. We found that the MS1-EL4 cells contributed to various tissues and expressed beta-gal activity, including not only descendants of the inner cell mass but also the trophectoderm-derived extraembryonic ectoderm.

Sukoyan, M. A., A. Y. Kerkis, et al. (2002). "Establishment of new murine embryonic stem cell lines for the generation of mouse models of human genetic diseases." Braz J Med Biol Res **35**(5): 535-42.

Embryonic stem cells are totipotent cells derived from the inner cell mass of blastocysts. Recently, the development of appropriate culture conditions for the differentiation of these cells into specific cell types has permitted their use as potential therapeutic agents for several diseases. In addition, manipulation of their genome in vitro allows the creation of animal models of human genetic diseases and for the study of gene function in vivo. We report the establishment of new lines of murine embryonic stem cells from preimplantation stage embryos of 129/Sv mice. Most of these cells had a normal karyotype and an XY sex chromosome composition. The pluripotent properties of the cell lines obtained were analyzed on the basis of their alkaline phosphatase activity and their capacity to form complex embryoid bodies with rhythmically contracting cardiomyocytes. Two lines, USP-1 and USP-3, with the best in vitro characteristics of pluripotency were used in chimera-generating experiments. The capacity to contribute to the germ line was demonstrated by the USP-1 cell line. This cell line is currently being used to generate mouse models of human diseases.

Szabo, E., J. Soboloff, et al. (2009). "Tamoxifen-inducible Cre-mediated calreticulin excision to study mouse embryonic stem cell differentiation." Stem Cells Dev **18**(1): 187-93.

Embryonic stem cells are useful to study the functional aspects of lineage commitment. In this study, we report that using the Cre/loxP system provides a useful tool for studying multifunctional proteins that are involved in stem cell differentiation, such as calreticulin. Calreticulin is a chaperone and a major calcium buffer of the endoplasmic reticulum and it functions during both adipogenesis and cardiomyogenesis. We used both a tamoxifen-inducible and cardiomyocyte-specific alpha-myosin heavy chain promoter-driven Cre/loxP system to study cardiomyogenesis, and a tamoxifen-inducible ubiquitously expressed cytomegalovirus promoter-driven Cre/loxP system to study adipogenesis. Both Cre/loxP systems mimicked the results previously observed using the calreticulin-null stem cell systems. Our results indicate that the tamoxifen-inducible Cre/loxP system is an effective and reliable tool to use for gene ablation in studies on functional aspects of stem cell biology.

Szabo, P. and J. R. Mann (1994). "Expression and methylation of imprinted genes during in vitro

differentiation of mouse parthenogenetic and androgenetic embryonic stem cell lines." Development **120**(6): 1651-60.

Messenger RNA and methylation levels of four imprinted genes, H19, Igf2r, Igf-2 and Snrpn were examined by northern and Southern blotting in mouse parthenogenetic, androgenetic and normal or wild-type embryonic stem cell lines during their differentiation in vitro as embryoid bodies. In most instances, mRNA levels in parthenogenetic and androgenetic embryoid bodies differed from wild type as expected from previously determined patterns of monoallelic expression in midgestation embryos and at later stages of development. These findings implicate aberrant mRNA levels of these genes in the abnormal development of parthenogenetic and androgenetic embryos and chimeras. Whereas complete silence of one of the parental alleles has previously been observed in vivo, we detected some mRNA in the corresponding embryonic stem cell line. This 'leakage' phenomenon could be explained by partial erasure, bypass or override of imprints, or could represent the actual activity status at very early stages of development. The mRNA levels of H19, Igf2r and Igf-2 and the degree of methylation at specific associated sequences were correlated according to previous studies in embryos, and thereby are consistent with suggestions that the methylation might play a role in controlling transcription of these genes. Paternal-specific methylation of the H19 promoter region is absent in sperm, yet we observed its presence in undifferentiated androgenetic embryonic stem cells, or before the potential expression phase of this gene in embryoid bodies. As such methylation is likely to invoke a repressive effect, this finding raises the possibility that it is part of the imprinting mechanism of H19, taking the form of a secondary imprint or postfertilization epigenetic modification necessary for repression of the paternal allele.

Taha, M. F., M. R. Valojerdi, et al. (2007). "Effect of bone morphogenetic protein-4 (BMP-4) on cardiomyocyte differentiation from mouse embryonic stem cell." Int J Cardiol **120**(1): 92-101.

The present study was designed to evaluate the effect of BMP-4 on mouse embryonic stem cells (ESCs)-derived cardiomyocyte. Cardiac differentiation of the mouse ESCs was initiated by embryoid bodies (EBs) formation in hanging drops, transfer of EBs to the suspension culture and then plating onto gelatin-coated tissue culture plates. BMP-4 was added to culture medium throughout the suspension period. Cultures were observed daily with an inverted microscope for the appearance of contracting clusters. At the early, intermediate and terminal stages of differentiation, the choronotropic responses of cardiomyocytes to cardioactive drugs were assessed, and the cardiomyocytes immunostained for cardiac troponin I, desmin, alpha-actinin and nebulin. The contracting clusters were isolated for ultrastructural evaluation, at day 14 after plating. Moreover, total RNA extracted from contracting EBs of early and terminal stages of differentiation were examined for oct-4, alpha- and beta-myosin heavy chain, myosin light chain-2V and atrial natriuretic factor expression. The BMP-4 treatment resulted in a decrease in the percent of beating EBs and the percent of developing cardiomyocytes per EBs. As a whole, the chronotropic responses of beating cardiac clusters to cardioactive drugs in control group were better than BMP-4 treated group. The cardiomyocytes of both groups were positive immunostained for applied antibodies except for nebulin. Moreover, in the BMP-4 treated group, the ultrastructural characteristics and cardiac-specific genes expression were all retarded in the terminal stage of cardiomyocytes development. In conclusion, BMP-4 had an inhibitory effect on cardiomyocyte differentiation from the mouse ESCs in terms of ultrastructural characteristics, genes expression and functional properties.

Takahashi, Y., K. Hanaoka, et al. (1988). "Embryonic stem cell-mediated transfer and correct regulation of the chicken delta-crystallin gene in developing mouse embryos." Development **102**(2): 259-69.

To study regulation of delta-crystallin expression during ontogeny, we transferred the gene from chicken into developing mouse embryos by first transforming an embryonic stem (ES) cell line of mouse and then producing chimaeric embryos by combining them with normal mouse embryos. Using this technique, genes were transferred into a variety of developing mouse tissues with high efficiency. Two delta-crystallin gene constructs were used: the wild-type gene with 2200 bp of the 5' flanking sequence, shown to be lens-specific in an assay using cultured mouse cells, and a mutant gene with 51 bp of the 5' flanking sequence, lacking the sequence required for expression in lens cells. Five independent lines carrying the former and two lines carrying the latter were employed in producing chimaeras. In the chimaeric embryos having the wild-type gene, delta-crystallin was expressed in the lens and in specific regions of the primitive central nervous system (CNS) as is seen in embryonic expression in the chicken. In adult mouse chimaeras also, expression was restricted to the lens and the CNS, in the pyramidal neurones of the piriform cortex and the hippocampus. delta-crystallin expression in these tissues is due to proper transcriptional regulation, since no expression was

observed when chimaeras were produced with the ES lines carrying the mutant gene. The experimental results reported here demonstrate the advantage of ES-cell-mediated gene transfer in the study of embryonic gene regulation, because a number of gene constructs and chromosomal sites can be analysed shortly after embryo manipulation without requiring gene transmission to the next generation.

Takano, N., T. Kawakami, et al. (2002). "Fibronectin combined with stem cell factor plays an important role in melanocyte proliferation, differentiation and migration in cultured mouse neural crest cells." Pigment Cell Res **15**(3): 192-200.

Stem cell factor (SCF) is essential to the migration and differentiation of melanocytes during embryogenesis because mutations in either the SCF gene, or its ligand, KIT, result in defects in coat pigmentation in mice. Using a neural crest cell (NCC) primary culture system from wild-type mice, we previously demonstrated that KIT-positive and/or L-3, 4-dihydroxyphenylalanine (DOPA)-positive melanocyte precursors proliferate following the addition of SCF to the culture medium. Extracellular matrix (ECM) proteins are considered to play a role in the migration and differentiation of various cells including melanocytes. We cultured mouse NCCs in the presence of SCF in individual wells coated with ECM; fibronectin (FN), collagen I (CLI), chondroitin sulphate, or dermatan sulphate. More KIT-positive cells and DOPA-positive cells were detected in the presence of SCF on ECM-coated wells than on non-coated wells. A statistically significant increase in DOPA-positive cells was evident in FN and CLI wells. In contrast, in the absence of SCF, few DOPA-positive cells and KIT-positive cells were detected on either the ECM-coated or non-coated wells. We concluded that ECM affect melanocyte proliferation and development in the presence of SCF. To determine the key site of FN function, RGDS peptides in the FN sequence, which supports spreading of NCCs, were added to the NCC culture. The number of DOPA-positive cells decreased with RGDS concentration in a dose-dependent fashion. Immunohistochemical staining revealed the presence of integrin alpha5, a receptor of RGDS, in NCCs. These results suggest the RGDS domain of FN plays a contributory role as an active site in the induction of FN function in NCCs. In addition, we examined the effect of FN with SCF on the NCC migration by measuring cluster size, and found an increase in size following treatment with FN.

Teramura, T., Y. Onodera, et al. (2009). "Mouse androgenetic embryonic stem cells differentiated to multiple cell lineages in three embryonic germ layers in vitro." J Reprod Dev **55**(3): 283-92.

The embryos of some rodents and primates can precede early development without the process of fertilization; however, they cease to develop after implantation because of restricted expressions of imprinting genes. Asexually developed embryos are classified into parthenote/gynogenote and androgenote by their genomic origins. Embryonic stem cells (ESCs) derived from asexual origins have also been reported. To date, ESCs derived from parthenogenetic embryos (PgESCs) have been established in some species, including humans, and the possibility to be alternative sources for autologous cell transplantation in regenerative medicine has been proposed. However, some developmental characteristics, which might be important for therapeutic applications, such as multiple differentiation capacity and transplantability of the ESCs of androgenetic origin (AgESCs) are uncertain. Here, we induced differentiation of mouse AgESCs and observed derivation of neural cells, cardiomyocytes and hepatocytes in vitro. Following differentiated embryoid body (EB) transplantation in various mouse strains including the strain of origin, we found that the EBs could engraft in theoretically MHC-matched strains. Our results indicate that AgESCs possess at least two important characteristics, multiple differentiation properties in vitro and transplantability after differentiation, and suggest that they can also serve as a source of histocompatible tissues for transplantation.

Tesar, P. J. (2005). "Derivation of germ-line-competent embryonic stem cell lines from preblastocyst mouse embryos." Proc Natl Acad Sci U S A **102**(23): 8239-44.

The first differentiation event of the mammalian embryo is thought to occur during blastulation and results in two populations of cells, the inner cell mass (ICM) and the trophectoderm. Most embryonic stem (ES) cell lines have been derived from the ICM or a further subset of ICM cells known as the epiblast. There appears to be a limited period of embryonic development during which pluripotent ES cells can be adapted from the cells of the blastocyst to culture. A method is presented here that allows ES cell lines to be isolated from preblastocyst mouse embryos. These lines were derived from 129S2/SvHsd mouse morulae and earlier cleavage stages with high efficiency. The lines expressed genes and antigens characteristic of pluripotent ES cells. XY cell lines remained karyotypically stable through extensive passaging and produced germ-line-competent chimeras upon blastocyst injection. These results suggest that true ES cells can be derived from embryos explanted at any stage of preimplantation

development in the mouse. This finding raises the interesting question of whether ES cell lines derived from embryos at different stages of preimplantation development possess the same potential.

Tesar, P. J., J. G. Chenoweth, et al. (2007). "New cell lines from mouse epiblast share defining features with human embryonic stem cells." Nature **448**(7150): 196-9.

The application of human embryonic stem (ES) cells in medicine and biology has an inherent reliance on understanding the starting cell population. Human ES cells differ from mouse ES cells and the specific embryonic origin of both cell types is unclear. Previous work suggested that mouse ES cells could only be obtained from the embryo before implantation in the uterus. Here we show that cell lines can be derived from the epiblast, a tissue of the post-implantation embryo that generates the embryo proper. These cells, which we refer to as EpiSCs (post-implantation epiblast-derived stem cells), express transcription factors known to regulate pluripotency, maintain their genomic integrity, and robustly differentiate into the major somatic cell types as well as primordial germ cells. The EpiSC lines are distinct from mouse ES cells in their epigenetic state and the signals controlling their differentiation. Furthermore, EpiSC and human ES cells share patterns of gene expression and signalling responses that normally function in the epiblast. These results show that epiblast cells can be maintained as stable cell lines and interrogated to understand how pluripotent cells generate distinct fates during early development.

Thorsteinsdottir, S., B. A. Roelen, et al. (1999). "Expression of the alpha 6A integrin splice variant in developing mouse embryonic stem cell aggregates and correlation with cardiac muscle differentiation." Differentiation **64**(3): 173-84.

Mouse embryonic stem (ES) cells grown in aggregates give rise to several different cell types, including cardiac muscle. Given the lack of cardiac muscle cell lines, ES cells can be a useful tool in the study of cardiac muscle differentiation. The laminin-binding integrin alpha 6 beta 1 exists in two different splice variant forms of the alpha chain (alpha 6A and alpha 6B), the alpha 6A form having been implicated as possibly playing a role in cardiac muscle development, based on its distribution pattern [4, 53]. In this study we characterise the ES cell model system in terms of the expression of the two different alpha 6 splice variants. We correlate their expression with that of muscle markers and the transcription factor GATA-4, using the reverse transcription-polymerase chain reaction (RT-PCR). We confirm that alpha 6B is constitutively expressed by ES cells. In contrast, alpha 6A expression appears later and overlaps in time with a period when the muscle marker myosin light chain-2V (MLC-2V) is expressed, but no MyoD is present, which indicates the presence of cardiac muscle cells in the aggregates. We further show that GATA-4 is present at the same time. Culturing the aggregates under conditions that stimulate (transforming growth factor beta 1 supplement) or inhibit (TGF beta 1 plus 10(-9) M retinoic acid supplement) cardiac muscle differentiation does not lead to any qualitative differences in the timing of expression of these genes, but quantitative changes cannot be excluded. The TGF beta 1 supplement does, however, lead to a relatively greater expression of alpha 6A compared to alpha 6B than the TGF beta 1 plus 10(-9) M RA supplement after 6 days in culture, suggesting that alpha 6A expression is favoured under conditions that stimulate cardiac muscle differentiation. The switch towards alpha 6A expression in ES cell aggregates is paralleled by expression of the binding receptor for TGF beta (T beta RII). Stable expression of a mutated (dominant negative) T beta RII in ES cells, however, still resulted in (TGF beta-independent) upregulation of alpha 6A, demonstrating that these events were not causally related and that parallel or alternative regulatory pathways exist. The initial characterisation of differentiating ES cell aggregates in terms of alpha 6A integrin subunit expression suggests that this model system could be a valuable tool in the study of the role of the alpha 6A beta 1 integrin in cardiac muscle differentiation.

Tiede, B. J., L. A. Owens, et al. (2009). "A novel mouse model for non-invasive single marker tracking of mammary stem cells in vivo reveals stem cell dynamics throughout pregnancy." PLoS One **4**(11): e8035.

Mammary stem cells (MaSCs) play essential roles for the development of the mammary gland and its remodeling during pregnancy. However, the precise localization of MaSCs in the mammary gland and their regulation during pregnancy is unknown. Here we report a transgenic mouse model for luciferase-based single marker detection of MaSCs in vivo that we used to address these issues. Single transgene expressing mammary epithelial cells were shown to reconstitute mammary glands in vivo while immunohistochemical staining identified MaSCs in basal and luminal locations, with preponderance towards the basal position. By quantifying luciferase expression using bioluminescent imaging, we were able to track MaSCs non-invasively in individual mice over time. Using this model to monitor MaSC dynamics throughout pregnancy, we found that MaSCs expand in both total number and percentage

during pregnancy and then drop down to or below baseline levels after weaning. However, in a second round of pregnancy, this expansion was not as extensive. These findings validate a powerful system for the analysis of MaSC dynamics in vivo, which will facilitate future characterization of MaSCs during mammary gland development and breast cancer.

Tielens, S., B. Verhasselt, et al. (2006). "Generation of embryonic stem cell lines from mouse blastocysts developed in vivo and in vitro: relation to Oct-4 expression." Reproduction **132**(1): 59-66.

Embryonic stem (ES) cells are the source of all embryonic germ layer tissues. Oct-4 is essential for their pluripotency. Since in vitro culture may influence Oct-4 expression, we investigated to what extent blastocysts cultured in vitro from the zygote stage are capable of expressing Oct-4 and generating ES cell lines. We compared in vivo with in vitro derived blastocysts from B6D2 mice with regard to Oct-4 expression in inner cell mass (ICM) outgrowths and blastocysts. ES cells were characterized by immunostaining for alkaline phosphatase (ALP), stage-specific embryonic antigen-1 (SSEA-1) and Oct-4. Embryoid bodies were made to evaluate the ES cells' differentiation potential. ICM outgrowths were immunostained for Oct-4 after 6 days in culture. A quantitative real-time PCR assay was performed on individual blastocysts. Of the in vitro derived blastocysts, 17% gave rise to ES cells vs 38% of the in vivo blastocysts. Six-day old outgrowths from in vivo developed blastocysts expressed Oct-4 in 55% of the cases vs 31% of the in vitro derived blastocysts. The amount of Oct-4 mRNA was significantly higher for freshly collected in vivo blastocysts compared to in vitro cultured blastocysts. In vitro cultured mouse blastocysts retain the capacity to express Oct-4 and to generate ES cells, be it to a lower level than in vivo blastocysts.

Tingley, W. G., L. Pawlikowska, et al. (2007). "Gene-trapped mouse embryonic stem cell-derived cardiac myocytes and human genetics implicate AKAP10 in heart rhythm regulation." Proc Natl Acad Sci U S A **104**(20): 8461-6.

Sudden cardiac death due to abnormal heart rhythm kills 400,000-460,000 Americans each year. To identify genes that regulate heart rhythm, we are developing a screen that uses mouse embryonic stem cells (mESCs) with gene disruptions that can be differentiated into cardiac cells for phenotyping. Here, we show that the heterozygous disruption of the Akap10 (D-AKAP2) gene that disrupts the final 51 aa increases the contractile response of cultured cardiac cells to cholinergic signals. In both heterozygous and homozygous mutant mice derived from these mESCs, the same Akap10 disruption increases the cardiac response to cholinergic signals, suggesting a dominant interfering effect of the Akap10 mutant allele. The mutant mice have cardiac arrhythmias and die prematurely. We also found that a common variant of AKAP10 in humans (646V, 40% of alleles) was associated with increased basal heart rate and decreased heart rate variability (markers of low cholinergic/vagus nerve sensitivity). These markers predict an increased risk of sudden cardiac death. Although the molecular mechanism remains unknown, our findings in mutant mESCs, mice, and a common human AKAP10 SNP all suggest a role for AKAP10 in heart rhythm control. Our stem cell-based screen may provide a means of identifying other genes that control heart rhythm.

Tokuzawa, Y., E. Kaiho, et al. (2003). "Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development." Mol Cell Biol **23**(8): 2699-708.

Embryonic stem (ES) cells are immortal and pluripotent cells derived from early mammalian embryos. Transcription factor Oct3/4 is essential for self-renewal of ES cells and early mouse development. However, only a few Oct3/4 target genes have been identified. In this study, we found that F-box-containing protein Fbx15 was expressed predominantly in mouse undifferentiated ES cells. Inactivation of Oct3/4 in ES cells led to rapid extinction of Fbx15 expression. Reporter gene analyses demonstrated that this ES cell-specific expression required an 18-bp enhancer element located approximately 500 nucleotides upstream from the transcription initiation site. The enhancer contained an octamer-like motif and an adjacent Sox-binding motif. Deletion or point mutation of either motif abolished the enhancer activity. The 18-bp fragment became active in NIH 3T3 cells when Oct3/4 and Sox2 were coexpressed. A gel mobility shift assay demonstrated cooperative binding of Oct3/4 and Sox2 to the enhancer sequence. In mice having a beta-galactosidase gene knocked into the Fbx15 locus, 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside staining was detected in ES cells, early embryos (two-cell to blastocyst stages), and testis tissue. Despite such specific expression of Fbx15, homozygous mutant mice showed no gross developmental defects and were fertile. Fbx15-null ES cells were normal in morphology, proliferation, and differentiation. These data demonstrate that Fbx15 is a novel target of Oct3/4 but is dispensable for ES cell self-renewal, development, and fertility.

Triel, C., M. E. Vestergaard, et al. (2004). "Side population cells in human and mouse epidermis lack stem cell characteristics." Exp Cell Res **295**(1): 79-90.

Cells that exclude Hoechst 33342 have been found in many tissues, and common for these cells is a characteristic profile when analysed by flow fluorimetry (sp, side population). Since sp cells in some cases function as multipotent stem cells, we investigated whether the epidermis contains sp cells (Esp cells) and whether these cells were epidermal stem cells. We show that mouse and human epidermis contain sp cells, and, to identify the origin of these cells, we tested the expression of several marker genes. We find that Esp cells constitute a subpopulation of the alpha6 integrin-positive basal cells of the mouse epidermis. They are positive for sca-1 and negative for MHC class II and Flk1. They are not identical to the label-retaining population but are cycling cells in the mouse epidermis. Keratinocytes positive for sca-1 are located outside the stem cell containing bulge area of the mouse hair follicle. Forty-four human skin samples were analysed, and Esp cells were found at frequencies ranging from 0.01% to 5.39%, independently of age and body site. Human Esp cells did not express particular high levels of beta1 integrin. However, they expressed the half transporter ABCG2 and we identified high expression of this marker in the secretory duct epithelium of the sweat glands whereas low expression was found in the basal layer of the epidermis.

Tsai, M., S. Y. Tam, et al. (1993). "Distinct patterns of early response gene expression and proliferation in mouse mast cells stimulated by stem cell factor, interleukin-3, or IgE and antigen." Eur J Immunol **23**(4): 867-72.

Stem cell factor (SCF) is encoded at the Sl locus of the mouse and is the ligand for the c-kit receptor. Recombinant rat SCF164 (rrSCF164) induces proliferation and promotes maturation of mouse mast cells in vitro and in vivo and can also induce c-kit receptor-dependent mouse mast cell degranulation. We now report that in both quiescent and non-quiescent mouse bone marrow-derived cultured mast cells (BMCMC) rrSCF164 induces increased mRNA levels for the "early response genes" c-fos, c-jun and junB but has only slight effects on the expression of junD. Recombinant mouse interleukin-3 (IL-3) also promotes proliferation of both quiescent and non-quiescent BMCMC. However, IL-3 induces increased expression of c-fos and junB only in quiescent BMCMC. Cross-linking of Fc epsilon receptor type I (Fc epsilon RI) on BMCMC by IgE and specific antigen induces a pattern of early gene expression very similar to that induced by rrSCF164. However, BMCMC stimulated through the Fc epsilon RI did not proliferate and, in comparison to control BMCMC, exhibited significantly decreased proliferation in response to rrSCF164 or IL-3. These results indicate that stimulation of BMCMC proliferation by IL-3 or rrSCF164 induces distinct patterns of early response gene expression and suggest that the proliferative effects of these growth factors may be mediated through distinct signal transduction pathways. Our data also point to previously unappreciated similarities between the effects of signaling through the c-kit receptor or the Fc epsilon RI on mast cell expression of fos and jun genes.

Tsuda, H., C. E. Maynard-Currie, et al. (1997). "Inactivation of the mouse HPRT locus by a 203-bp retroposon insertion and a 55-kb gene-targeted deletion: establishment of new HPRT-deficient mouse embryonic stem cell lines." Genomics **42**(3): 413-21.

To obtain useful hypoxanthine phosphoribosyl-transferase (HPRT)-deficient mouse ES cell lines, two different methods were employed: (i) selection of spontaneous 6-TG-resistant mutants and (ii) gene targeting of the HPRT locus. The first approach resulted in the establishment of E14.1TG3B1, a spontaneous HPRT-deficient cell line with an insertional mutation of 203 bp in the third exon of the HPRT gene. The insert is highly homologous to the B2 mouse repetitive element and has all the expected retroposon characteristics, thus providing an example of gene inactivation by retroposon insertion. This clone exhibited stable 6-TG resistance and high germ-line transmission frequency. Thus E14.1TG3B1 is a useful ES cell line for modifying the mouse genome using the HPRT gene as a selection marker and for transmission at a high frequency into the mouse germ line. The second approach resulted in a 55-kb deletion of the mouse HPRT locus, demonstrating the feasibility of replacement-targeting vectors to generate large genomic DNA deletions.

Tsujimura, A., K. Fujita, et al. (2007). "Prostatic stem cell marker identified by cDNA microarray in mouse." J Urol **178**(2): 686-91.

PURPOSE: Identifying prostatic stem cells is important to elucidate the mechanisms by which the prostate develops and control prostate cancer. We recently reported that the proximal region of the mouse prostate contains a population of stem cells. However, to our knowledge the specific marker of stem cells in the proximal region remains unknown. MATERIALS AND METHODS: We performed cDNA microarray analysis of cells obtained from the proximal region and from the remaining regions in dorsal prostates to identify several candidate stem cell

markers. After we focused on 1 candidate among them we confirmed the expression of this candidate gene by reverse transcriptase-polymerase chain reaction analysis and immunohistochemistry. We also investigated the relation between positive cells for this marker and those for telomerase reverse transcriptase. Finally, we investigated the functional potential of prominin positive cells in 3-dimensional culture. RESULTS: Seven of 4,800 genes analyzed showed proximal/remaining ratios greater than 20. Of these genes we focused on prominin because it is a cell surface marker widely used to identify and isolate stem cells from various organs. We found a prominin positive cell population enriched in the basal cell layer in the proximal region, and the coincidence of prominin and telomerase reverse transcriptase immunostaining. We also found that prominin positive cells gave rise to numerous and large-branched ducts, whereas prominin negative cells formed far fewer such structures in 3-dimensional culture. CONCLUSIONS: A small population of prominin positive cells in the mouse prostate basal layer of the proximal region represents a stem cell population.

Turturici, G., F. Geraci, et al. (2009). "Hsp70 is required for optimal cell proliferation in mouse A6 mesoangioblast stem cells." Biochem J **421**(2): 193-200.

Mouse Hsp70 (70 kDa heat shock protein) is preferentially induced by heat or stress stimuli. We previously found that Hsp70 is constitutively expressed in A6 mouse mesoangioblast stem cells, but its possible role in these cells and the control of its basal transcription remained unexplored. Here we report that in the absence of stress, Ku factor is able to bind the HSE (heat shock element) consensus sequence in vitro, and in vivo it is bound to the proximal hsp70 promoter. In addition, we show that constitutive hsp70 transcription depends on the co-operative interaction of different factors such as Sp1 (specificity protein 1) and GAGA-binding protein with Ku factor, which binds the HSE consensus sequence. We used mRNA interference assays to select knockdown cell clones. These cells were able to respond to heat stress by producing a large amount of Hsp70, and produced the same amount of Hsp70 as that synthesized by stressed A6 cells. However, severe Hsp70 knockdown cells had a longer duplication time, suggesting that constitutive Hsp70 expression has an effect on the rate of proliferation.

Uy, G. D., K. M. Downs, et al. (2002). "Inhibition of trophoblast stem cell potential in chorionic ectoderm coincides with occlusion of the ectoplacental cavity in the mouse." Development **129**(16): 3913-24.

At the blastocyst stage of pre-implantation mouse development, close contact of polar trophectoderm with the inner cell mass (ICM) promotes proliferation of undifferentiated diploid trophoblast. However, ICM/polar trophectoderm intimacy is not maintained during post-implantation development, raising the question of how growth of undifferentiated trophoblast is controlled during this time. The search for the cellular basis of trophoblast proliferation in post-implantation development was addressed with an in vitro spatial and temporal analysis of fibroblast growth factor 4-dependent trophoblast stem cell potential. Two post-implantation derivatives of the polar trophectoderm - early-streak extra-embryonic ectoderm and late-streak chorionic ectoderm - were microdissected into fractions along their proximodistal axis and thoroughly dissociated for trophoblast stem cell culture. Results indicated that cells with trophoblast stem cell potential were distributed throughout the extra-embryonic/chorionic ectoderm, an observation that is probably attributable to non-coherent growth patterns exhibited by single extra-embryonic ectoderm cells at the onset of gastrulation. Furthermore, the frequency of cells with trophoblast stem cell potential increased steadily in extra-embryonic/chorionic ectoderm until the first somite pairs formed, decreasing thereafter in a manner independent of proximity to the allantois. Coincident with occlusion of the ectoplacental cavity via union between chorionic ectoderm and the ectoplacental cone, a decline in the frequency of mitotic chorionic ectoderm cells in vivo, and of trophoblast stem cell potential in vitro, was observed. These findings suggest that the ectoplacental cavity may participate in maintaining proliferation throughout the developing chorionic ectoderm and, thus, in supporting its stem cell potential. Together with previous observations, we discuss the possibility that fluid-filled cavities may play a general role in the development of tissues that border them.

van Eekelen, J. A., C. K. Bradley, et al. (2003). "Expression pattern of the stem cell leukaemia gene in the CNS of the embryonic and adult mouse." Neuroscience **122**(2): 421-36.

The basic helix-loop-helix (bHLH) transcription factor stem cell leukaemia (SCL) is a 'master regulator' of haematopoiesis, where SCL is pivotal in cell fate determination and differentiation. SCL has also been detected in CNS, where other members of the bHLH-family have been shown to be indispensable for neuronal development; however, no detailed expression pattern of SCL has so far been described. We have generated a map of SCL expression in the embryonic and adult mouse brain based on histochemical analysis of LacZ reporter gene

expression in sequential sections of brain tissue derived from SCL-LacZ knockin mice. The expression of LacZ was confirmed to reflect SCL expression by in situ hybridisation. LacZ expression was found in a range of different diencephalic, mesencephalic and metencephalic brain nuclei in adult CNS. Co-localisation of LacZ with the neuronal marker NeuN indicated expression in post-mitotic neurons in adulthood. LacZ expression by neurons was confirmed in tissue culture analysis. The nature of the pretectal, midbrain and hindbrain regions expressing LacZ suggest that SCL in adult CNS is potentially involved in processing of visual, auditory and pain related information. During embryogenesis, LacZ expression was similarly confined to thalamus, midbrain and hindbrain. LacZ staining was also evident in parts of the intermediate and marginal zone of the aqueduct and ventricular zone of the fourth ventricle at E12.5 and E14. These cells may represent progenitor stages of differentiating neural cells. Given the presence of SCL in both the developing brain and in post-mitotic neurons, it seems likely that the function of SCL in neuronal differentiation may differ from its function in maintaining the differentiated state of the mature neuron.

Vercelli, A., O. M. Mereuta, et al. (2008). "Human mesenchymal stem cell transplantation extends survival, improves motor performance and decreases neuroinflammation in mouse model of amyotrophic lateral sclerosis." Neurobiol Dis **31**(3): 395-405.

Amyotrophic lateral sclerosis (ALS) is a lethal disease affecting motoneurons. In familial ALS, patients bear mutations in the superoxide dismutase gene (SOD1). We transplanted human bone marrow mesenchymal stem cells (hMSCs) into the lumbar spinal cord of asymptomatic SOD1(G93A) mice, an experimental model of ALS. hMSCs were found in the spinal cord 10 weeks after, sometimes close to motoneurons and were rarely GFAP- or MAP2-positive. In females, where progression is slower than in males, astrogliosis and microglial activation were reduced and motoneuron counts with the optical fractionator were higher following transplantation. Motor tests (Rotarod, Paw Grip Endurance, neurological examination) were significantly improved in transplanted males. Therefore hMSCs are a good candidate for ALS cell therapy: they can survive and migrate after transplantation in the lumbar spinal cord, where they prevent astrogliosis and microglial activation and delay ALS-related decrease in the number of motoneurons, thus resulting in amelioration of the motor performance.

Wakayama, S., S. Kishigami, 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.

Walter, K., C. Bonifer, et al. (2008). "Stem cell-specific epigenetic priming and B cell-specific transcriptional activation at the mouse Cd19 locus." Blood **112**(5): 1673-82.

Low-level expression of multiple lineage-specific genes is a hallmark of hematopoietic stem cells (HSCs). HSCs predominantly express genes specific for the myeloid or megakaryocytic-erythroid lineages, whereas the transcription of lymphoid specific genes appears to begin after lymphoid specification. It has been demonstrated for a number of genes that epigenetic priming occurs before gene expression and lineage specification; however, little is known about how epigenetic priming of lymphoid genes is regulated. To address the question of how B cell-restricted expression is established, we studied activation of the Cd19 gene during hematopoietic development. We identified a B cell-specific upstream enhancer and showed that the developmental regulation of Cd19 expression involves precisely coordinated alterations in transcription factor binding and chromatin remodeling at Cd19 cis-regulatory elements. In multipotent progenitor cells, Cd19 chromatin is first remodeled at the upstream enhancer, and this remodeling is associated with binding of E2A. This is followed by the binding of EBF and PAX5 during B-cell differentiation. The Cd19 promoter is transcriptionally activated only after PAX5 binding. Our experiments give important

mechanistic insights into how widely expressed and B lineage-specific transcription factors cooperate to mediate the developmental regulation of lymphoid genes during hematopoiesis.

Wang, C., P. W. Faloon, et al. (2007). "Mouse lysocardiolipin acyltransferase controls the development of hematopoietic and endothelial lineages during in vitro embryonic stem-cell differentiation." Blood **110**(10): 3601-9.

The blast colony-forming cell (BL-CFC) was identified as an equivalent to the hemangioblast during in vitro embryonic stem (ES) cell differentiation. However, the molecular mechanisms underlying the generation of the BL-CFC remain largely unknown. Here we report the isolation of mouse lysocardiolipin acyltransferase (Lycat) based on homology to zebrafish lycat, a candidate gene for the cloche locus. Mouse Lycat is expressed in hematopoietic organs and is enriched in the Lin(-)C-Kit(+)Sca-1(+) hematopoietic stem cells in bone marrow and in the Flk1(+)/hCD4(+)(Scl(+)) hemangioblast population in embryoid bodies. The forced Lycat transgene leads to increased messenger RNA expression of hematopoietic and endothelial genes as well as increased blast colonies and their progenies, endothelial and hematopoietic lineages. The Lycat small interfering RNA transgene leads to a decrease expression of hematopoietic and endothelial genes. An unbiased genomewide microarray analysis further substantiates that the forced Lycat transgene specifically up-regulates a set of genes related to hemangioblasts and hematopoietic and endothelial lineages. Therefore, mouse Lycat plays an important role in the early specification of hematopoietic and endothelial cells, probably acting at the level of the hemangioblast.

Wang, F., S. Thirumangalathu, et al. (2006). "Establishment of new mouse embryonic stem cell lines is improved by physiological glucose and oxygen." Cloning Stem Cells **8**(2): 108-16.

Embryonic stem cell lines are routinely selected and cultured in glucose and oxygen concentrations that are well above those of the intrauterine environment. Supraphysiological glucose and hyperoxia each increase oxidative stress, which could be detrimental to survival in vitro by inhibiting proliferation and/or inducing cell death. The aim of this study was to test whether isolation of new embryonic stem cell lines from murine blastocysts is improved by culture in physiological (5%) oxygen instead of approximately 20%, the concentration of oxygen in room air, or in media containing physiological (100 mg/dL) instead of 450 mg/dL glucose. We found that culturing in either physiological oxygen or physiological glucose improved the success of establishing new murine embryonic stem cell lines, and that culture when concentrations of both oxygen and glucose were physiological improved the success of establishing new lines more than culture in either alone. Physiological oxygen and glucose reduce oxidative stress, as determined by 2',7'-dichloro-dihydrofluorescein fluorescence. BrdU incorporation suggests that physiological oxygen and glucose increase the pool of proliferating cells. Cells isolated in physiological oxygen and glucose are capable of self-renewal and differentiation into all three germ layers in vitro. However, none of the culture conditions prevents cytogenetic instability with prolonged passage. These results suggest that culture of cells derived from murine blastocysts in physiological oxygen and glucose reduces oxidant stress, which increases the success of establishing new embryonic stem cell lines.

Wang, Y., C. Mulligan, et al. (2009). "Quantitative proteomics characterization of a mouse embryonic stem cell model of Down syndrome." Mol Cell Proteomics **8**(4): 585-95.

Down syndrome, caused by the trisomy of chromosome 21, is a complex condition characterized by a number of phenotypic features, including reduced neuron number and synaptic plasticity, early Alzheimer disease-like neurodegeneration, craniofacial dysmorphia, heart development defects, increased incidence of childhood leukemia, and powerful suppression of the incidence of most solid tumors. Mouse models replicate a number of these phenotypes. The Tc1 Down syndrome model was constructed by introducing a single supernumerary human chromosome 21 into a mouse embryonic stem cell, and it reproduces a large number of Down syndrome phenotypes including heart development defects. However, little is still known about the developmental onset of the trisomy 21-induced mechanisms behind these phenotypes or the proteins that are responsible for them. This study determined the proteomic differences that are present in undifferentiated embryonic stem cells and are caused by an additional human chromosome 21. A total of 1661 proteins were identified using two-dimensional liquid chromatography followed by tandem mass spectrometry from whole embryonic stem cell lysates. Using isobaric tags for relative and absolute quantification, we found 52 proteins that differed in expression by greater than two standard deviations from the mean when an extra human chromosome 21 was present. Of these, at least 11 have a possible functional association with a Down syndrome phenotype or a human chromosome 21-encoded gene.

This study also showed that quantitative protein expression differences in embryonic stem cells can persist to adult mouse as well as reproduce in human Down syndrome fetal tissue. This indicates that changes that are determined in embryonic stem cells of Down syndrome could potentially identify proteins that are involved in phenotypes of Down syndrome, and it shows that these cell lines can be used for the purpose of studying these pathomechanisms.

Wang, Z., G. Li, et al. (2009). "Conditional deletion of STAT5 in adult mouse hematopoietic stem cells causes loss of quiescence and permits efficient nonablative stem cell replacement." Blood **113**(20): 4856-65.

Currently, there is a major need in hematopoietic stem cell (HSC) transplantation to develop reduced-intensity regimens that do not cause DNA damage and associated toxicities and that allow a wider range of patients to receive therapy. Cytokine receptor signals through c-Kit and c-Mpl can modulate HSC quiescence and engraftment, but the intracellular signals and transcription factors that mediate these effects during transplantation have not been defined. Here we show that loss of one allele of signal transducer and activator of transcription 5 (STAT5) in nonablated adult mutant mice permitted engraftment with wild-type HSC. Conditional deletion of STAT5 using Mx1-Cre caused maximal reduction in STAT5 mRNA (> 97%) and rapidly decreased quiescence-associated c-Mpl downstream targets (Tie-2, p57), increased HSC cycling, and gradually reduced survival and depleted the long-term HSC pool. Host deletion of STAT5 was persistent and permitted efficient donor long-term HSC engraftment in primary and secondary hosts in the absence of ablative conditioning. Overall, these studies establish proof of principle for targeting of STAT5 as novel transplantation conditioning and demonstrate, for the first time, that STAT5, a mitogenic factor in most cell types, including hematopoietic progenitors, is a key transcriptional regulator that maintains quiescence of HSC during steady-state hematopoiesis.

Watson, D. J., R. M. Walton, et al. (2006). "Structure-specific patterns of neural stem cell engraftment after transplantation in the adult mouse brain." Hum Gene Ther **17**(7): 693-704.

Transplantation of neural stem cells (NSCs) may be useful for delivering exogenous gene products to the diseased CNS. When NSCs are transplanted into the developing mouse brain, they can migrate extensively and differentiate into cells appropriate to the sites of engraftment, in response to the normal signals directing endogenous cells to their appropriate fates. Much of the prior work on NSC migration in the adult brain has examined directed migration within or toward focal areas of injury such as ischemia, brain tumors, or 6-hydroxydopamine (6-OHDA) lesions. However, treatment of many genetic disorders that affect the CNS will require widespread dissemination of the donor cells in the postnatal brain, because the lesions are typically distributed globally. We therefore tested the ability of NSCs to migrate in the unlesioned adult mouse brain after stereotaxic transplantation into several structures including the cortex and hippocampus. NSC engraftment was monitored in live animals by magnetic resonance imaging (MRI) after superparamagnetic iron oxide (SPIO) labeling of cells. Histological studies demonstrated that the cells engrafted in significantly different patterns within different regions of the brain. In the cerebral cortex, donor cells migrated in all directions from the injection site. The cells maintained an immature phenotype and cortical migration was enhanced by trypsin treatment of the cells, indicating a role for cell surface proteins. In the hippocampus, overall cell survival and migration were lower but there was evidence of neuronal differentiation. In the thalamus, the transplanted cells remained in a consolidated mass at the site of injection. These variations in pattern of engraftment should be taken into account when designing treatment approaches in nonlesion models of neurologic disease.

Wermann, K., S. Fruehauf, et al. (1996). "Human-mouse xenografts in stem cell research." J Hematother **5**(4): 379-90.

New progenitor cell transplantation strategies that change the composition of the graft, such as CD34+ cell selection, ex vivo expansion, and gene marking, are budding. The efficiency and safety of most techniques are evaluated by in vitro assays using human progenitor cells and murine intraspecies transplantation studies before clinical introduction. However, proliferation potential in culture and engraftment capability can be discrepant. Furthermore, some CD34 epitopes and cytokines are unique to humans, thus rendering clinical inferences from experimental results difficult. Therapeutic studies with malignant human hematopoietic cells also require appropriate models that take into account pharmacokinetics. Human-mouse interspecies progenitor cell grafts may allow us to bridge this gap. For engraftment of human cells, recipients need to be immunodeficient. The highest long-term engraftment rate of up to 96% was obtained following transplantation of peripheral blood progenitor cells into non-obese diabetic/severe combined immunodeficiency mice. Data obtained from several human-mouse xenograft transplantation models are presented and discussed.

Winger, Q. A., J. Guttormsen, et al. (2007). "Heat shock protein 1 and the mitogen-activated protein kinase 14 pathway are important for mouse trophoblast stem cell differentiation." Biol Reprod **76**(5): 884-91.

Differentiation of trophoblast cells is a critical process for the proper establishment of the placenta and is, therefore, necessary to maintain embryonic development. Trophoblast stem (TS) cells grown in culture can differentiate into different trophoblast subtypes in vitro mimicking normal trophoblast cell differentiation. Therefore, TS cells are a valuable model system that can be used to elucidate genetic factors that regulate trophoblast cell differentiation. Several transcription factors, when analyzed by targeted gene mutation in mice, have resulted in embryonic lethality due to placental defects and, more specifically, defects of the trophoblast lineages. These studies have helped improve our knowledge about trophoblast cell differentiation, but much is still unknown about the specific mechanisms involved. This study uses TS cell culture to detect proteins with differential expression in proliferating and differentiating TS cells in order to identify proteins with potential roles in the differentiation process. We identified four proteins with differential expression: dimethylarginine dimethylaminohydrolase1 (DDAH1), keratin 8, keratin 18, and HSPB1 (also known as heat shock protein 25, HSP25). Further investigation confirmed the presence of HSPB1 protein during in vitro TS cell differentiation. In addition, we confirmed that phosphorylation of HSPB1 and MAP kinase-activated protein kinase 2 (MAPKAPK2) increased in TS cells during differentiation. Inhibition of MAPK14 (also known as p38 MAPK) resulted in a reduction of HSPB1 phosphorylation and an increase in cell death during TS cell differentiation. These results suggest that HSPB1 and the MAPK14 pathway are important during TS cell differentiation.

Woldbaek, P. R., I. B. Hoen, et al. (2002). "Gene expression of colony-stimulating factors and stem cell factor after myocardial infarction in the mouse." Acta Physiol Scand **175**(3): 173-81.

Recent studies have suggested that cytokines such as macrophage colony-stimulating factor (M-CSF) might be involved in the pathogenesis of ischaemic heart disease. Macrophage colony-stimulating factor, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), stem cell factor (SCF), interleukin-3 (IL-3) and interleukin-7 (IL-7) are potent cytokines belonging to the same structual class that may affect function, growth and apoptosis both in the heart and other organs. The aims of the present study were to characterize a post-infarction model in the mouse and to examine mRNA expression of M-CSF, GM-CSF, SCF, IL-3 and IL-7 during the development of heart failure. Myocardial infarction (MI) was induced in mice by ligation of the left coronary artery. Average infarct size was 40% and the mice developed myocardial hypertrophy and pulmonary oedema. Ribonuclease (RNAase) protection assays showed abundant cardiac expression of M-CSF and SCF. After MI, we measured down-regulation of cytokine mRNA expression in the heart (M-CSF, SCF), lung (M-CSF), liver (M-CSF) and spleen (M-CSF) compared with sham. Cardiac G-CSF, GM-CSF and IL-7 mRNAs were not detected. In conclusion, abundant cardiac gene expression of M-CSF and SCF was found. In our mouse model of MI, M-CSF and SCF were down-regulated in the heart and several other organs suggesting specific roles for these cytokines during development of ischaemic heart failure.

Woltjen, K., G. Bain, et al. (2000). "Retro-recombination screening of a mouse embryonic stem cell genomic library." Nucleic Acids Res **28**(9): E41.

Targeted gene disruption is an important tool in molecular medicine, allowing for the generation of animal models of human disease. Conventional methods of targeting vector (TV) construction are difficult and represent a rate limiting step in any targeting experiment. We previously demonstrated that bacteriophage are capable of acting as TVs directly, obviating the requirement for 'rolling out' plasmids from primary phage clones and thus eliminating an additional, time consuming step. We have also developed methods which facilitate the construction of TVs using recombination. In this approach, modification cassettes and point mutations are shuttled to specific sites in phage TVs using phage-plasmid recombination. Here, we report a further improvement in TV generation using a recombination screening-based approach deemed 'retro-recombination screening' (RRS). We demonstrate that phage vectors containing specific genomic clones can be genetically isolated from a lambdaTK embryonic stem cell genomic library using a cycle of integrative recombination and condensation. By introducing the gam gene of bacteriophage lambda into the probe plasmid it is possible to select for positive clones which have excised the plasmid, thus returning to their native conformation following purification from the library. Rapid clone isolation using the RRS protocol provides another method by which the time required for TV construction may be further reduced.

Yamazaki, F., H. Okamoto, et al. (2005). "Development of a new mouse model (xeroderma pigmentosum a-deficient, stem cell factor-transgenic) of ultraviolet B-induced melanoma." J Invest Dermatol **125**(3): 521-5.

It is well established that exposure to sunlight or ultraviolet radiation (UVR) is the major environmental risk factor for the development of skin neoplasms. To date, however, there have been few appropriate mouse models available for studying the role of UVR in melanoma carcinogenesis, mainly because of the murine lack of the epidermal melanocyte, which is a major source of origin of human melanoma. In this study, we established xeroderma pigmentosum group A gene-deficient, stem cell factor-transgenic mice, which are defective in the repair of damaged DNA and do have epidermal melanocytes. The mice were exposed to UVR three times a week for 10 wk. More than 30% of the irradiated mice developed tumors of melanocyte origin that metastasized to the lymph nodes. Histologically, proliferated cells exhibited lentigo maligna melanoma or nodular melanoma. Immunohistochemistry confirmed that the tumor cells were characteristic of melanoma. Non-irradiated mice did not develop skin tumors spontaneously. The newly generated model mouse might be useful for studying the photobiological aspects of human melanoma, because the mice developed melanoma from epidermal melanocytes only after UVR exposures.

Yamazaki, J., T. Mizukami, et al. (2009). "Identification of cancer stem cells in a Tax-transgenic (Tax-Tg) mouse model of adult T-cell leukemia/lymphoma." Blood **114**(13): 2709-20.

Adult T-cell leukemia/lymphoma (ATL) is a malignant lymphoproliferative disorder caused by HTLV-I infection. In ATL, chemotherapeutic responses are generally poor, which has suggested the existence of chemotherapy-resistant cancer stem cells (CSCs). To identify CSC candidates in ATL, we have focused on a Tax transgenic mouse (Tax-Tg) model, which reproduces ATL-like disease both in Tax-Tg animals and also after transfer of Tax-Tg splenic lymphomatous cells (SLCs) to nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Using a limiting dilution transplantation, it was estimated that one CSC existed per 10(4) SLCs (0.01%). In agreement with this, we have successfully identified candidate CSCs in a side population (0.06%), which overlapped with a minor population of CD38(-)/CD71(-)/CD117(+) cells (0.03%). Whereas lymphoma did not develop after transplantation of 10(2) SLCs, 10(2) CSCs could consistently regenerate the original lymphoma. In addition, lymphoma and CSCs could also be demonstrated in the bone marrow and CD117(+) CSCs were observed in both osteoblastic and vascular niches. In the CSCs, Tax, Notch1, and Bmi1 expression was down-regulated, suggesting that the CSCs were derived from Pro-T cells or early hematopoietic progenitor cells. Taken together, our data demonstrate that CSCs certainly exist and have the potential to regenerate lymphoma in our mouse model.

Yang, D., G. E. Holt, et al. (2001). "Murine six-transmembrane epithelial antigen of the prostate, prostate stem cell antigen, and prostate-specific membrane antigen: prostate-specific cell-surface antigens highly expressed in prostate cancer of transgenic adenocarcinoma mouse prostate mice." Cancer Res **61**(15): 5857-60.

To identify genes that are differentially up-regulated in prostate cancer of transgenic adenocarcinoma mouse prostate (TRAMP) mice, we subtracted cDNA isolated from mouse kidney and spleen from cDNA isolated from TRAMP-C1 cells, a prostate tumor cell line derived from a TRAMP mouse. Using this strategy, cDNA clones that were homologous to human six-transmembrane epithelial antigen of the prostate (STEAP) and prostate stem cell antigen (PSCA) were isolated. Mouse STEAP (mSteap) is 80% homologous to human STEAP at both the nucleotide and amino acid levels and contains six potential membrane-spanning regions similar to human STEAP. Mouse PSCA (mPsca) shares 65% homology with human PSCA at the nucleotide and amino acid levels. mRNA expression of mSteap and mPsca is largely prostate-specific and highly detected in primary prostate tumors and metastases of TRAMP mice. Both mSteap and mPsca map to chromosome 5. Another known gene coding for mouse prostate-specific membrane antigen (mPsma) is also highly expressed in both primary and metastatic lesions of TRAMP mice. These results indicate that the TRAMP mouse model can be used to effectively identify genes homologous to human prostate-specific genes, thereby allowing for the investigation of their functional roles in prostate cancer. mSteap, mPsca, and mPsma constitute new tools for preventative and/or therapeutic vaccine construction and immune monitoring in the TRAMP mouse model that may provide insights into the treatment of human prostate cancer.

Yang, H., Y. Zhang, et al. (2008). "Mouse embryonic stem cell-derived cardiomyocytes express functional adrenoceptors." Biochem Biophys Res Commun **368**(4): 887-92.

The cardiogenic capacity of embryonic stem (ES) cells has been well-investigated. However, little

is known about the development of adrenoceptor (AR) systems during the process of ES cell differentiation, which are critically important in cardiac physiology and pharmacology. In this present study, we investigated the expression profile of adrenoceptor subtypes, beta-adrenergic modulation of muscarinic receptors and adrenoceptor-related signaling in cardiomyocytes derived from ES cells (ESCMs). Reverse transcription-polymerase chain reaction revealed that undifferentiated mouse ES cells expressed alpha(1A)-, alpha(1B)-, alpha(1D)- and beta(2)-AR mRNA. However, beta(1)-AR was only expressed after vitamin C induction. The expressions of alpha(1A)-, alpha(1D)- and beta(1)-ARs increased significantly while alpha(1B)- and beta(2)-ARs showed no significant change during the differentiation process. Furthermore, we detected the expression of tyrosine hydroxylase. Both alpha(1)-AR and beta-AR could activate extracellular responsive kinase in ESCMs. Isoprenaline could inhibit the expression of M(2) muscarinic receptor protein. CGP20712A, a beta(1)-AR antagonist, up-regulated the expression of M(2) muscarinic receptor while ICI118551, a beta(2)-AR antagonist, showed no effect. These results indicated that functional adrenoceptors and tyrosine hydroxylase, a critical enzyme in catecholamine biosynthesis, were differentially expressed in ESCMs. Adrenoceptor-related signaling pathways and beta-adrenergic modulation of muscarinic receptors were established during differentiation.

Yang, Y., K. Iwanaga, et al. (2008). "Phosphatidylinositol 3-kinase mediates bronchioalveolar stem cell expansion in mouse models of oncogenic K-ras-induced lung cancer." PLoS One **3**(5): e2220.

BACKGROUND: Non-small cell lung cancer (NSCLC) is the most common cause of cancer-related death in Western countries. Developing more effective NSCLC therapeutics will require the elucidation of the genetic and biochemical bases for this disease. Bronchioalveolar stem cells (BASCs) are a putative cancer stem cell population in mouse models of oncogenic K-ras-induced lung adenocarcinoma, an histologic subtype of NSCLC. The signals activated by oncogenic K-ras that mediate BASC expansion have not been fully defined. METHODOLOGY/PRINCIPAL FINDINGS: We used genetic and pharmacologic approaches to modulate the activity of phosphatidylinositol 3-kinase (PI3K), a key mediator of oncogenic K-ras, in two genetic mouse models of lung adenocarcinoma. Oncogenic K-ras-induced BASC accumulation and tumor growth were blocked by treatment with a small molecule PI3K inhibitor and enhanced by inactivation of phosphatase and tensin homologue deleted from chromosome 10, a negative regulator of PI3K. CONCLUSIONS/SIGNIFICANCE: We conclude that PI3K is a critical regulator of BASC expansion, supporting treatment strategies to target PI3K in NSCLC patients.

Yap, D. Y., D. K. Smith, et al. (2007). "Using biomarker signature patterns for an mRNA molecular diagnostic of mouse embryonic stem cell differentiation state." BMC Genomics **8**: 210.

BACKGROUND: The pluripotency and self-renewal capabilities, which define the "stemness" state, of mouse embryonic stem (ES) cells, are usually investigated by functional assays or quantitative measurements of the expression levels of known ES cell markers. Strong correlations between these expression levels and functional assays, particularly at the early stage of cell differentiation, have usually not been observed. An effective molecular diagnostic to properly identify the differentiation state of mouse ES cells, prior to further experimentation, is needed. RESULTS: A novel molecular pattern recognition procedure has been developed to diagnose the differentiation state of ES cells. This is based on mRNA transcript levels of genes differentially expressed between ES cells and their differentiating progeny. Large publicly available ES cell data sets from various platforms were used to develop and test the diagnostic model. Signature patterns consisting of five gene expression levels achieved high accuracy at determining the cell state (sensitivity and specificity > 97%). CONCLUSION: The effective ES cell state diagnostic scheme described here can be implemented easily to assist researchers in identifying the differentiation state of their cultures. It also provides a step towards standardization of experiments relying on cells being in the stem cell or differentiating state.

Yi, F., L. Pereira, et al. (2008). "Tcf3 functions as a steady-state limiter of transcriptional programs of mouse embryonic stem cell self-renewal." Stem Cells **26**(8): 1951-60.

Elucidating the underlying transcriptional control of pluripotent cells is necessary for the development of new methods of inducing and maintaining pluripotent cells in vitro. Three transcription factors, Nanog, Oct4, and Sox2, have been reported to form a feedforward circuit promoting pluripotent cell self-renewal in embryonic stem cells (ESC). Previously, we found that a transcriptional repressor activity of Tcf3, a DNA-binding effector of Wnt signaling, reduced Nanog promoter activity and Nanog levels in mouse embryonic stem cells (mESC). The objective of this study was to determine the scope of Tcf3 effects on gene expression and self-renewal

beyond the regulation of Nanog levels. We show that Tcf3 acts broadly on a genome-wide scale to reduce the levels of several promoters of self-renewal (Nanog, Tcl1, Tbx3, Esrrb) while not affecting other ESC genes (Oct4, Sox2, Fgf4). Comparing effects of Tcf3 ablation with Oct4 or Nanog knockdown revealed that Tcf3 counteracted effects of both Nanog and Oct4. Interestingly, the effects of Tcf3 were more strongly correlated with Oct4 than with Nanog, despite the normal levels of Oct4 in TCF3-/- mESC. The deranged gene expression allowed TCF3-/- mESC self-renewal even in the absence of leukemia inhibitory factor and delayed differentiation in embryoid bodies. These findings identify Tcf3 as a cell-intrinsic inhibitor of pluripotent cell self-renewal that functions by limiting steady-state levels of self-renewal factors. Disclosure of potential conflicts of interest is found at the end of this article.

Youn, Y. H., J. Feng, et al. (2003). "Neural crest stem cell and cardiac endothelium defects in the TrkC null mouse." Mol Cell Neurosci **24**(1): 160-70.

TrkC null mice have multiple cardiac malformations. Since neural crest cells participate in cardiac outflow tract septation, the aim of this study was to determine at the cellular level the putative neural crest defect. We have identified three types of progenitor cells: stem cells that undergo self-renewal and can generate many cell types, cells that are restricted in their developmental potentials, and cells that are committed to the smooth muscle cell lineage. In TrkC null mice, there is a greater than 50% decrease in stem cell numbers and an equivalent increase in fate-restricted cells. The outflow tract wall is thickened and the endothelial tube is disorganized. We conclude that deletion of the TrkC gene causes precocious fate restrictions of the neural crest stem cell and a defect of the outflow tract endothelium, both of which may contribute to the outflow tract malformations that occur in TrkC null mice.

Zampetaki, A., Q. Xiao, et al. (2006). "TLR4 expression in mouse embryonic stem cells and in stem cell-derived vascular cells is regulated by epigenetic modifications." Biochem Biophys Res Commun **347**(1): 89-99.

Embryonic stem (ES) cells and ES cell-derived differentiated cells can be used in tissue regeneration approaches. However, inflammation may pose a major hurdle. To define the inflammatory response of ES and ES cell-derived vascular cells, we exposed these cells to LPS. With the exception of MIF no significant cytokine mRNA levels were observed either at baseline or after stimulation. Further experiments revealed that these cells do not express TLR4. Analysis of the DNA methylation status of the TLR4 upstream region showed increased methylation. Moreover, in vitro methylation suppressed TLR4 promoter activity in reporter gene assays. ChIP assays showed that in this region histones H3 and H4 are hypoacetylated in ES cells. Interestingly, 5-aza-dC or TSA partially relieves this gene repression. Finally, the increased levels of TLR4 observed in ES cells after treatment with 5-aza-dC or TSA confer responsiveness to LPS, as induction of IL-6 and TNFalpha mRNA was detected in endotoxin stimulated ES cells.

Zhong, X. and Y. Jin (2009). "Critical roles of coactivator p300 in mouse embryonic stem cell differentiation and Nanog expression." J Biol Chem **284**(14): 9168-75.

p300 is a well known histone acetyltransferase and coactivator that plays pivotal roles in many physiological processes. Despite extensive research for the functions of p300 in embryogenesis and transcription regulation, its roles in regulating embryonic stem (ES) cell pluripotency are poorly understood. To address this issue, we investigated the self-renewal ability and early differentiation process in both wild-type mouse ES cells and ES cells derived from p300 knock-out (p300(-/-)) mice. We found that p300 ablation did not affect self-renewal capacity overtly when ES cells were maintained under undifferentiated conditions. Exogenous expression of Nanog rescued abnormal expression of extra-embryonic endoderm marker partially but not mesoderm and ectoderm markers. Furthermore, we demonstrate that p300 was directly involved in modulating Nanog expression. Importantly, epigenetic modification of histone acetylation at the distal regulatory region of Nanog was found to be dependent on the presence of p300, which could contribute to the mechanism of regulating Nanog expression by p300. Collectively, our results show that p300 plays an important role in the differentiation process of ES cells and provide the first evidence for the involvement of p300 in regulating Nanog expression during differentiation, probably through epigenetic modification of histone on Nanog.

Zhou, S., Y. Liu, et al. (2005). "Expression profiles of mouse dendritic cell sarcoma are similar to those of hematopoietic stem cells or progenitors by clustering and principal component analyses." Biochem Biophys Res Commun **331**(1): 194-202.

We isolated and screened two tumor cell clones DD1 and DG6 with different capacity of metastasis from the same parent cell line, a mouse dendritic cell (DC) sarcoma, using limited dilution method. The genome-wide expressions of DD1 and DG6 cells were detected by Affymetrix's MOE-430A

microarray. The expression profiles related with mouse DC development were downloaded from GEO at NCBI and ArrayExpress at EBI database. In order to compare the expression of DC sarcoma and DC developmental arrays which was performed by MG-U74av2, we had screened the best matched probesets between MOE-430A and MG-U74av2 according to the probe identities from Affymetrix technical annotation. After the normalization of 11 housekeeping genes across the 34 arrays (2 DC sarcoma and 32 DC developmental arrays), all these expression profiles were analyzed by the methods of hierarchical clustering, principal component analysis, nearest-neighborhood, and self-organizing maps. The results indicate that expression profiles of DC sarcoma are closer to those of the DC progenitors and hematopoietic stem cells from bone marrow compared with the sorted DCs from spleen. The results support the hypothesis that cancers (tumors or sarcomas) arise from stem cells. It is suggested that the DC sarcomas are more similar to the DC progenitors and hematopoietic stem cells than the relative mature DCs in gene expressions on the large-scale.

Zhu, D. Y., Y. Du, et al. (2008). "MAPEG expression in mouse embryonic stem cell-derived hepatic tissue system." Stem Cells Dev **17**(4): 775-83.

The expressions of membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG), a superfamily involved in both inflammation and cell protection, were investigated in an in vitro system of mouse embryonic stem (ES) cell-derived hepatic tissue. Gene expressions of all MAPEG members were demonstrated in a developmental-dependent manner in the derived hepatic tissue. The protein expression of microsomal glutathione S-transferase 1 (MGST1) was not detected until differentiating day 14. It gradually increased by maturation of hepatic tissue. The microsomes of ES cell-derived hepatic tissue possessed the MGST1-like catalytic activity. However, MGST1 from the microsome preparation could not form dimers as usual when exposed to reactive nitrogen species ONOO. Among the other members in MAPEG, weak expressions of leukotriene C(4) synthase (LTC(4)S) and microsomal prostaglandin E synthase 1 (mPGES-1) were observed. A stable expression of 5-Lipoxygenase activating protein (FLAP) appeared during the entire course of differentiation. MGST2 and MGST3 failed to express in the derived hepatic tissue, although mRNA of them do existed. In conclusion, ES cell-derived hepatic tissue possess MAPEG gene expression features, but not all protein expression could be detected, which helps to understand not only the nature of the tissue derived, but also the fate of bioartificial liver system, and may as well provide a valuable in vitro model for research in both inflammation process and toxic events in hepatological fields.

Zou, G. M., W. Y. Hu, et al. (2007). "TNF family molecule LIGHT regulates chemokine CCL27 expression on mouse embryonic stem cell-derived dendritic cells through NF-kappaB activation." Cell Signal **19**(1): 87-92.

Cytokine LIGHT is a type II transmembrane protein belonging to the TNF family that was originally identified as a weak inducer of apoptosis. It plays a role in inducing maturation of dendritic cells, such as upregulating CD80, CD86 expression on dendritic cells. However, whether LIGHT induces CC chemokine expression in DC and promotes their migration remains unknown. In this study, we found that esDC express CCR7 and CCR10 (the receptor of CCL27) upon the LIGHT stimulation. LIGHT also upregulates CCL27, but not CCL19 and CCL21 expression in esDC. The esDC migration potential has been increased in LIGHT activated DCs compared with control cells. LIGHT activated DCs autocrine CCL27 which regulate their migration as Blockage of CCL27 on esDC using neutralizing antibody reduces migration potential. In signaling study, we identified that LIGHT activated NF-kappaB in esDC and inhibition of NF-kappaB activation by specific inhibitor can partly attenuate the effect of LIGHT in regulation of CCL27 expression. Moreover, Shp-2 is required in LIGHT activated NF-kappaB because Knockdown of Shp-2 affects the NF-kappaB activation induced by LIGHT and consequently influences LIGHT mediated CCL27 expression. TRAF6 is critical in DC maturation in recent reports; however, knockdown of TRAF6 expression using siRNA did not alter CCL27 expression in LIGHT matured DCs. Our study demonstrates that LIGHT stimulation enhances CCL27 expression through activation of NF-kappaB in DCs.

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