Stem Cell Researches Using Animal Mouse

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Abstract: 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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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.

In 2004, Ahn et al made the analysis in mouse gene expression during differentiation of neural stem cells (NSCs) that was performed by using in-house microarrays composed of 10,368 genes. The changes in mRNA level were measured during differentiation day 1, 2, 3, 6, 12, and 15. Out of 10,368 genes analyzed, 259 genes were up-regulated or down-regulated by 2-fold or more at least at one time-point during differentiation, and were classified into six clusters based on their expression patterns by K-means clustering. Clusters characterized by gradual increase have large numbers of genes involved in transport and cell adhesion; those which showed gradual decrease have much of genes in nucleic acid metabolism, cell cycle, transcription factor, and RNA processing. In situ hybridization (ISH) validated microarray data and it also showed that Fox M1, cyclin D2, and CDK4 were highly expressed in CNS germinal zones and ectonucleotide pyrophosphatase/phosphodiesterase 2 (Enpp2) was highly expressed in choroid plexus where stem/progenitor cells are possibly located. Together, this clustering analysis of expression patterns of functionally classified genes may give insight into understanding of CNS development and mechanisms of NSCs proliferation and differentiation [Ahn, J. 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].

In 2009, Aiba et al we showed 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. They 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 [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].

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. Anderson et al 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 [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].

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

Baharvand et al describe that 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. 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].

Baker et al 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 [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].

Benveniste et al described that 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 [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].

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 [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].

Using reverse transcription-polymerase chain reaction (RT-PCR) Bourne et al 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 [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].

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), Campbell et al 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 [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].

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

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 [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].

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 [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].

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 [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].

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 [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].

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 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].

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 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].

Francini et al 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 [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].

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 [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].

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 [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].

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

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 [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].

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 Trl), 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 [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].

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 [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].

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 [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].

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 [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].

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 [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 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 [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 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, 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].

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 [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].

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. We tested the ability of hESC and their most commonly used feeder cells, mouse embryonic fibroblasts (MEF), to produce MV. Kubikova et al 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. 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. 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 [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].

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. Kutsuzawa et al 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 [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].

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 [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].

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, 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].

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 [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].

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 [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].

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 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].

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 [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].

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. Mathur et al 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. 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 [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].

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.

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 [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].

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 [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].

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. 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. Differentiated CK19(+) cells reflect an endocrine precursor cell type of ductal origin, potentially suitable for insulin replacement therapy in diabetes [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].

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

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 [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].

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 [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].

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 [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].

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

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 [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].

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 [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].

Soncin et al showed 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. Soncin et al 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 [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].

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, 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].

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 [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].

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 [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].

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