Totipotent of 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 situuition 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 tipical and important topic of life science. This material collects some literatures on totipotent of stem cell.
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In the biology field, the totipotency is the ability that a cell can divide and produce a new organism. This means that a single cell has an integrate genes to differentiate to a whole body (Baud, 2005). Totipotent cells can be any cell in a body (Cantley, 2005). Normally, the differentiation is one direction where an undifferentiated cell (especially stem cell) can differentiate into differentiated cells but differentiated cells cannot reverse to the immatured cells. However, under certain condition, this direction can reverse, especially in plant and low class of the life species. A plant cutting or callus can grow to an entire plant and this appears everywhere in the earth. Many plants reproduce for next generation through schedule and it is widely used in the agriculture. In the animal field, this reverse differentiation normally is not exist, but and only case of reverse differentiation happens in the jellyfish Turritopsis nutricula through the transdifferentiation (Ma, et al, 2011).

The zygote is totipotent. A human begins a zygote that a sperm fertilizes an egg and creates the single totipotent cell. In the beginning hours after fertilization, this cell divides into identical totipotent cells, which can later develop into any of the 3 germ layers of a human (endoderm, mesoderm, or ectoderm) and into cells of the cytotrophoblast layer or syncytiotrophoblast layer of the placenta. After reaching the 16-cell stage, the totipotent cells of the morula differentiate into cells. The differentiated cells will become either the blastocyst's inner cell mass or the outer trophoblasts. Approximately 4 days after the fertilization and a few cell divisions, these totipotent cells will be specialized.

Totipotent is the most important point for the stem cells that initiates the whole creature body. In creature development, the egg cell in a lady and the sperm from a man fuses jointly to become a free chamber zygote. The zygote divides many times and forms cells that are the precursors to the trillions of cells as the bricks of the creature body.

Totipotent stem cell can differentiate into all the human body's cells (about 200 types). In most animals, the only true totipotent stem cell is the fertilized egg and its immediate descendants. A totipotent stem cell can potentially generate a complete organism. In some cases, cells can regain totipotency. A plant cutting or callus can be used to grow an entire plant.

Differentiation results from differential gene expression. In order to clone an animal, such as a sheep, udder cells are removed from a ewe and starved for one week to cause G0 arrest. Nuclei from arrested ewe udder cells are fused with enucleated eggs from a ewe, and then stimulated to re-enter the cell cycle. After a few rounds of cell division, the embryo is transplanted into a surrogate sheep mother. The sheep that is born is genetically identical to the ewe from that the nucleus obtains.

Cellular determination results from the asymmetric segregation of cellular determinants. However, in most cases, determination is the result of inductive signaling between cells. Asymmetric segregation of cellular determinants is caused from the asymmetric localization of cytoplasmic molecules within the cell before dividing. During cell division, one daughter cell receives more localized molecules and the other daughter cell may receive less of these molecules, which results in two different daughter cells taking on different cell fates based on differences in gene expression. The localized cytoplasmic determinants are often transcription factors or mRNAs encoding by the transcription factors.

The field of stem cell biology has undergone tremendous expansion over the past two decades.

Scientific investigation has continued to expand our understanding of these complex cells at a rapidly increasing rate. This understanding has produced a vast array of potential clinical applications (Hemmat, Lieberman et al. 2010).

The direct induction of adventitious buds and somatic embryos from explants is a morphogenetic process that is under the influence of exogenous plant growth regulators and its interactions with endogenous phytohormones (de Almeida, de Almeida et al. 2012).

The ontogeny is also related to de-differentiated mesophyll cells that acquire totipotency and form the majority of embryos (Wang, Nolan et al. 2011).

Somatic cell nuclear transfer (SCNT) is a technically and biologically challenging procedure during which a differentiated committed nucleus undergoes rapid reprogramming into the totipotent state in a few hours (Shufaro and Reubinoff 2011).

Primordial germ cells (PGCs), the precursors of sperm and eggs, are the route to totipotency and require establishment of a unique epigenome in this lineage. The genetic program for PGC specification in the mouse also initiates epigenetic reprogramming that continues when PGCs migrate into the developing gonads. Among these later events is active and genome-wide DNA demethylation, which is linked to extensive chromatin remodeling (Surani and Hajkova 2010).

In many tissues, mammalian aging is associated with a decline in the replicative and functional capacity of somatic stem cells and other self-renewing compartments. Understanding the basis of this decline is a major goal of aging research (Sharpless 2010).

The octamer-binding transcription factor 4 (Oct-4) plays important role in totipotent cells differentiation. Oct-4 expressed in totipotent embryonic cells and germ cells. As totipotent cells differentiate somatic cells, Oct-4 gene is downregulated. Oct-4 expression is maintained after postgastrulation in primordial germ cells. Oct-4 is necessary for embryonic cells to keep the totipotent status keep from their differentiation to somatic cells. (Pesce M, Schöler HR., 2000).

Example of the totipotent protocols - Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability (Christopher, 1992):

Virus. Recombinant Tkneol9 virus at a titer of 1 x 10^6 per ml is generated from a T-2 producer line maintained in 10% calf serum/Dulbecco's modified Eagle's medium. Marrow cells from adult male (C57BL/6J x C3H/HeJ) F1 (B6C3F1) mice injected i.v. 4 days earlier with 5-fluorouracil (5-FU, 150 mg/kg of body weight) are infected with Tkneol9 virus using a supernatant infection protocol in which 3-5 x 10^6 marrow cells are cultured for 24 hr in 100-mm Petri dishes in 10 ml of virus supernatant containing Polybrene at 4 jug/ml, 5% pokeweed mitogen-stimulated spleen cell-conditioned medium, and 10%o agar-stimulated human leukocyte-conditioned medium as described. Cells are then recovered by gentle agitation and scraping of dishes with a rubber policeman, essential Eagle medium and resuspended in LTC medium [a medium/10% horse serum/10% fetal calf serum/10^{-6} M sodium hydrocortisone hemisuccinate/10^{-4} M 2-mercaptoethanol]. Aliquots of 3 x 10^6 cells are overlaid on previously established 3-wk-old long-term B6C3F1 female marrow adherent layers that had been irradiated with 15 Gy [250 kilovolt peak (kVp) x-rays] to inactivate persisting hematopoietic cells (Fraser, 1990). LTC are maintained by weekly removal of half of the medium and nonadherent cells and restoration of the volume with fresh LTC medium. To assay cells in LTC for repopulating cells, adherent layers are removed with a rubber policeman, suspended by passage through a 21-gauge needle and then combined with the nonadherent cells. Cells are washed once in 2% fetal calf serum/a-minimal essential medium, and aliquots from individual culture flasks are then injected i.v. into irradiated (8 Gy, 250 kVp x-rays) female recipients. In some cases, 2 x 10^5 female B6C3F1 marrow cells that had been previously subjected to two cycles of transplantation and regeneration are also injected to allow quantitative measurements of CRU in the retrovirally marked test cells to be obtained. For cultures that are used to assess recovery of repopulating cells in the nonadherent fraction over time, all of the medium and nonadherent cells are removed weekly and replaced either with fresh medium alone or with LTC medium containing 25 units per ml of recombinant mouse interleukin-3 (IL-3). The nonadherent cells removed after 3, 5, 6, and 7 weeks of culture are then injected into irradiated recipients. Hematopoietic Tissue Analysis. Recipients are sacrificed either 5 wk or between 5 and 7 months after transplantation of cultured cells. DNA is routinely extracted from marrow, spleen, and thymus. In some cases, DNA is also extracted from lymph nodes and from various subpopulations of marrow and spleen. Highly enriched (>90%) mast cell populations are generated by culturing marrow or spleen cells for 3 wk in WEHI-3B-supplemented medium (as a source of IL-3), and highly enriched (>95% Mac-i-positive) macrophage populations are generated by culturing marrow or spleen cells for 48 hr in medium supplemented with 10% WEHI-3B-conditioned medium and then for 7-10 days in medium supplemented with 35% human leukocyte-conditioned

medium. Highly enriched (>90% Thy-i-positive) T-cell populations are obtained by elution of nonadherent cells after loading of a half-spleen equivalent onto a 3-ml nylon wool column and incubating it for 1 hr at 37°C. Nylon wool adherent cells are then released by gentle agitation for 2-3 min in phosphate- buffered saline/10 mM EDTA and a highly enriched (>90% B220-positive) population of B lymphocytes subsequently isolated from this fraction by panning for 1 hr at 37°C in dishes precoated with unpurified rabbit anti-mouse immunoglobulin ($<10^8$ cells per dish) and selective removal of the adherent cells. Southern Blot Analysis. High-molecular-weight DNA is digested with HindIII or EcoRI, which cuts once in the proviral genome and releases a fragment unique to the integration site. Ten micrograms of digested DNA (5 g for male control lanes) is electrophoresed and analyzed by Southern blotting as described with a 32P-labeled probe specific for the neor gene sequence in the provirus from plasmid pMC1. HindIII-digested blots are stripped and reprobed with a Y chromosome-specific fragment from plasmid pY2 (Thomas, 1987).

Literatures

Abbott, D. E., L. M. Postovit, et al. (2007). "Exploiting the convergence of embryonic and tumorigenic signaling pathways to develop new therapeutic targets." Stem Cell Rev **3**(1): 68-78.

As our understanding of embryonic stem cell biology becomes more sophisticated, the similarities between multipotent cancer cells and these totipotent precursors are increasingly striking. Both multipotent cancer cells and embryonic stem cells possess the ability to self-renew, epigenetically alter their neighboring cellular architecture, and populate a tissue mass with a phenotypically heterogeneous composition of cells. While the molecular signature of these cell types continues to be elucidated, new insights are emerging related to the convergence of embryonic and tumorigenic signaling pathways. Understanding the molecular underpinnings of these two stem cell phenotypes may lead to new therapeutic targets for the elusive cancer cell.

Adams, I. R. and A. McLaren (2004). "Identification and characterisation of mRif1: a mouse telomere-associated protein highly expressed in germ cells and embryo-derived pluripotent stem cells." Dev Dyn **229**(4): 733-44.

We have identified a mouse ortholog of the yeast Rif1 family of telomere-associated proteins on the basis of its high expression in primordial germ cells and embryo-derived pluripotent stem cell lines. mRif1 is also highly expressed in totipotent and pluripotent cells during early mouse development, and in male and female germ cells in adult mice. mRif1 expression is induced during derivation of embryonic stem cells and is rapidly down-regulated upon differentiation of embryonic stem cells in vitro. Furthermore, we show that mRif1 physically interacts with the telomere-associated protein mTrf2 and can be cross-linked to telomeric repeat DNA in mouse embryonic stem cells. mRif1 may be involved in the maintenance of telomere length or pluripotency in the germline and during early mouse development.

Agata, K., T. Tanaka, et al. (2003). "Intercalary regeneration in planarians." Dev Dyn **226**(2): 308-16.

How can a planarian regenerate its entire body from a small portion of its body? Neoblasts, the totipotent stem cells of planarian, are assumed to be able to produce all missing cell types. However, we do not know how the cell fate of these cells is controlled during regeneration. Our recent studies with molecular markers suggest that intercalary regeneration is the fundamental principle in planarian regeneration. Here, we introduce the intercalation induced by ectopic grafting along the anteroposterior (A-P), dorsoventral (D-V), and left-right (L-R) axes. Blastema formation is evoked by ectopic D-V interactions after wound closure. Intercalation between the blastema and stump induces rearrangement of the positional identities along the A-P axis. Consequently, totipotent stem cells change their differentiation patterns according to the newly rearranged positional identities along the A-P, D-V, and L-R axes. According to the classic view, the blastema is regarded as the place where undifferentiated cells accumulate and regenerative events occur.

Aiba, K., A. A. Sharov, et al. (2006). "Defining a developmental path to neural fate by global expression profiling of mouse embryonic stem cells and adult neural stem/progenitor cells." Stem Cells **24**(4): 889-95.

To understand global features of gene expression changes during in vitro neural differentiation, we carried out the microarray analysis of embryonic stem cells (ESCs), embryonal carcinoma cells, and adult neural stem/progenitor (NS) cells. Expression profiling of ESCs during differentiation in monolayer culture revealed three distinct phases: undifferentiated ESCs, primitive ectoderm-like cells, and neural progenitor cells. Principal component (PC) analysis revealed that these cells were aligned on PC1 over the course of 6 days. This PC1 represents approximately 4,000 genes, the expression of which increased with neural commitment/differentiation. Furthermore, NS cells derived from adult brain and their differentiated cells were positioned along this PC axis further away from undifferentiated ESCs than

embryonic stem-derived neural progenitors. We suggest that this PC1 defines a path to neural fate, providing a scale for the degree of commitment/differentiation.

Ajjappala, B. S., M. S. Kim, et al. (2009). "Protein chip analysis of pluripotency-associated proteins in NIH3T3 fibroblast." Proteomics **9**(16): 3968-78.

Specific transcription factors regulate the totipotent and pluripotent capability of embryonic stem cells. Amongst these regulatory transcription factors in embryonic stem cells, Oct4 and Nanog are master factors that also have unique characteristic ability of cell-specific pluripotency and self-renewal. The expression of Nanog in fibroblasts confirms increased cell proliferation and transformation of foci-forming phenotype indicative of its oncogenic potential. The expression of Oct4, interestingly, leads to transformation of non-tumorgenic mouse into tumorigenic mouse. Our current investigation ascertains that the resultant increase in DNA synthesis and cell proliferation is the consequence of transforming the phenotype into foci formation. We used a manually curetted ProteoChip to carry out the signaling protein microarray analysis, which revealed up-regulated expression of various proteins including FAK1, MEK1 and Raf1. Some of the proteins explain the mechanism by which Oct4 and Nanog transform the phenotype. In NIH3T3 cells expressed with mouse Oct4 (mOct4), mouse Nanog (mNanog) separately as well as together, the specific knockdown of mFAK1 inhibited morphological transformation of the cells, and their invasion activity. The mFAK1 overexpression leads to morphological transformation as shown with mOct4 and mNanog.

Aladjem, M. I., L. W. Rodewald, et al. (2002). "Replication initiation patterns in the beta-globin loci of totipotent and differentiated murine cells: evidence for multiple initiation regions." Mol Cell Biol **22**(2): 442-52.

The replication initiation pattern of the murine beta-globin locus was analyzed in totipotent embryonic stem cells and in differentiated cell lines. Initiation events in the murine beta-globin locus were detected in a region extending from the embryonic Ey gene to the adult betaminor gene, unlike the restricted initiation observed in the human locus. Totipotent and differentiated cells exhibited similar initiation patterns. Deletion of the region between the adult globin genes did not prevent initiation in the remainder of the locus, suggesting that the potential to initiate DNA replication was not contained exclusively within the primary sequence of the deleted region. In addition, a deletion encompassing the six identified 5' hypersensitive sites in the mouse locus control region had no effect on initiation from within the locus. As this deletion also did not affect the chromatin structure of the locus, we propose that the sequences determining both chromatin structure and replication initiation lie outside the hypersensitive sites removed by the deletion.

Alimena, G., G. De Rossi, et al. (1980). "B cell markers in Ph1-positive acute lymphoblastic leukemia." Nouv Rev Fr Hematol **22**(3): 275-80.

A case of acute lymphoblastic leukemia (ALL) where the blast cells had B cell markers and displayed the presence of a typical Ph1 chromosome, originated by a standard t (9;22) translocation, is reported. Cytological and clinical aspects during the entire course of the disease were consistent with the diagnosis of ALL. Evidence of differentiation along a well-defined lymphoid cell line in a Ph1-positive cell confirms the presence of the Ph1 chromosome in conditions other than chronic granulocytic leukemia and shows that it possibly does not occur in an exclusively undifferentiated totipotent stem cell.

Almeida-Porada, G., C. Porada, et al. (2001). "Adult stem cell plasticity and methods of detection." Rev Clin Exp Hematol **5**(1): 26-41.

The ability to selectively produce one or more differentiated cell types at will from totipotent stem cells would be of profound clinical importance, as it would enable the specific replacement of damaged/dysfunctional cell types within the body, potentially curing numerous diseases. Until recently, it was thought that the only cells that possessed sufficient immaturity to be capable of giving rise to more than one tissue type in vitro and in vivo were the embryonic stem cells. However, recent studies have now provided compelling evidence that the adult bone marrow, brain and skeletal muscle contain stem cells that possess the remarkable ability to trans-differentiate and give rise to progeny of alternate embryologic derivations. These recent findings have shattered the existing dogma that the stages of embryologic development are irreversible. In this review, we present a brief summary of the most significant findings in the field of stem cell plasticity, emphasizing studies involving the hematopoietic system, discussing the models used thus far, and finishing with our findings on human stem cell plasticity using the fetal sheep model.

Anderson, R., K. Schaible, et al. (1999). "Expression of the homophilic adhesion molecule, Ep-CAM, in the mammalian germ line." J Reprod Fertil **116**(2): 379-84.

During normal embryonic development, mammalian germ cells use both cell migration and aggregation to form the primitive sex cords. Germ

cells must be able to interact with their environment and each other to accomplish this; however, the molecular basis of early germ cell adhesion is not well characterized. Differential adhesion is also thought to occur in the adult seminiferous tubules, since germ cells move from the periphery to the lumen as they differentiate. In a screen for additional adhesion molecules expressed by the germ line, expression of the homophilic adhesion molecule, Ep-CAM, was identified in embryonic, neonatal and adult germ cells using immunocytochemistry and flow cytometry with an Ep-CAM-specific monoclonal antibody. At embryonic stages, germ cells were found to express Ep-CAM during migration at embryonic day 10.5 and early gonad assembly at embryonic day 12.5. Expression of Ep-CAM was also found on neonatal male and female germ cells. In the adult testis, Ep-CAM was detected only on spermatogonia, and was absent from more differentiated cells. Finally, embryonic stem cells were shown to express this receptor. It is proposed that Ep-CAM plays a role in the development of the germ line and the behaviour of totipotent cells.

Ariad, S., D. Dajee, et al. (1993). "Lack of involvement of T-lymphocytes in the leukaemic population during prolonged chronic phase of Philadelphia chromosome positive chronic myeloid leukaemia." Leuk Lymphoma **10**(3): 217-21.

Nine patients with prolonged (> 2 years) chronic phase chronic myeloid leukaemia (CML) were investigated for the presence of T-cell involvement in the leukemic clone. Pure populations of peripheral blood T-cell populations were obtained by culturing separated mononuclear cells in the presence of pokeweed mitogen and IL2, until cultures showed > 99% pure T-cells. Purified T-cells and bone marrow and peripheral blood hematopoietic precursors were analysed for the presence of bcr-abl mRNA transcripts following RNA extraction and message amplification using polymerase chain reaction. In none of the 9 patients was bcr-abl mRNA found in T-lymphocytes while in all cases such transcripts were found in bone marrow and peripheral blood hematopoietic cells. Failure to detect T-cell involvement in patients with prolonged chronic phase CML using techniques designed to enhance even low level involvement of these cell populations supports the view that acquisition of the Ph chromosome abnormality does not occur in the totipotent stem cells but in more committed precursor cell/s with multilineage capacity but which only rarely retain the capacity for T-cell differentiation.

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.

Balconi, G., R. Spagnuolo, et al. (2000). "Development of endothelial cell lines from embryonic stem cells: A tool for studying genetically manipulated endothelial cells in vitro." Arterioscler Thromb Vasc Biol **20**(6): 1443-51.

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

embryo proper, or the heart microcirculation of the adult. We propose that this approach may be useful for obtaining endothelial cells carrying gene mutations that are lethal at very early stages of development.

Barker, J. E., J. Greer, et al. (1991). "Temporal replacement of donor erythrocytes and leukocytes in nonanemic W44J/W44J and severely anemic W/Wv mice." Blood **78**(6): 1432-7.

The dominant white spotting, W, locus in the mouse encodes Kit, a receptor molecule with cytosolic tyrosine kinase activity. Mutations in Kit deplete hematopoietic cells by an as yet unknown mechanism, but one that presumably affects the early progenitors of all cell lineages. To examine cell lineage-specific changes caused by different W mutations, we injected genetically marked normal marrow cells into mutant mice and monitored repopulation kinetics. In the present report, we compare repopulation of the various peripheral blood cells in nonanemic W44J/W44J and severely anemic W/Wv mice administered increasing increments of donor cells. At all doses of cells tested, donor erythrocyte repopulation precedes leukocyte repopulation regardless of the recipient phenotype. There is, in fact, little difference in the rate or extent of nonerythroid repopulation in W44J/W44J mice injected with between 6 x 10(6) and 2 x 10(7) donor cells. The fact that donor cells rapidly replace erythrocytes, even in the nonanemic W44J/W44J host, while other cell lineages become donor type more slowly provides further evidence that mutations at the W locus are especially damaging to erythrocyte progenitors.

Barker, J. E., J. H. Wolfe, et al. (1993). "Advantages of gradient vs. 5-fluorouracil enrichment of stem cells for retroviral-mediated gene transfer." Exp Hematol **21**(1): 47-54.

Retrovirally mediated gene transfer into murine totipotent hematopoietic stem cells (THSC) may be more efficient when the donor stem cells are enriched. We have used a rapid, nontoxic density gradient separation of mouse marrow to enrich stem cells. By characterizing the cell types in various fractions of the gradient, we found the majority of the THSC, spleen colony forming stem cells (CFU-S), erythroid burst forming cells (BFU-E) and dividing cells were in the same fraction. The gradient enrichment technique was then compared with one requiring 5-fluorouracil (5-FU) treatment of donor mice prior to marrow harvest. Cells enriched by both methods were tested for their ability to mediate retroviral gene transfer into normal mice. Gradient enrichment provided only one third as many nucleated cells as 5-FU treatment from the same number of donors. During the subsequent 4-day in vitro exposure to the retrovirus and growth factors, however, the number of gradient enriched cells increased 1.6-fold while the number of 5-FU treated cells decreased 3-fold. In lethally irradiated recipients, there was no difference between gradient and 5-FU enriched donor cells in the proportion of cells that generated CFU-S nor in the percentage of CFU-S that were infected. Secondary hosts did show differences.

Bernstein, A. and M. Breitman (1989). "Genetic ablation in transgenic mice." Mol Biol Med **6**(6): 523-30.

The study of mammalian development has very quickly moved from a largely descriptive endeavour to one in which very precise mechanistic questions can now be formulated and answered. Undoubtedly, advances in this area have been the result of a strong foundation in experimental embryology, the application of molecular genetic techniques to the isolation and analysis of genes of developmental interest, and the ability to manipulate genetically the embryo through transgenic mouse technology. Perhaps the most dramatic illustration of the power of these new technologies is the potential ability to generate mice either that carry mutations in virtually any gene in the germ line through gene targeting in totipotent embryonic stem (ES) cells or that lack specific cell types through the genetic ablation technology reviewed here. Together, these two approaches have made it possible to knock out either a specific gene or a specific cell type in an intact animal and thus offer almost unlimited possibilities for addressing questions concerning the molecular and cellular biology of development. As well, animal models for various human diseases such as dwarfism, immunodeficiencies and demyelination can now be generated. It is clear that further refinements in both gene targeting and genetic ablation technologies are necessary before the full potential of either approach will be realized.

Best, J. B. and M. Morita (1982). "Planarians as a model system for in vitro teratogenesis studies." Teratog Carcinog Mutagen **2**(3-4): 277-91.

Free-living flatworms such as planarians are inexpensive to culture, maintain, and use for toxicologic testing in the laboratory. A considerable number of basic studies by ourselves and others indicate that, in simplified miniature, they possess many features of biochemical and physiologic organization similar to higher animals such as mammals. These include a well-developed brain with a varied behavioral repertoire including complex maneuvers of prey capture and learning, with a number of the same neurotransmitters used in mammalian brain. They are sensitive to a variety of

the same toxicants. Undifferentiated totipotent stem cells, i.e., "neoblasts," which are capable of mitosis and differentiation into any of the various specialized cell types, permit regeneration of complete planarians from fragments. They also provide new cells to replace those lost in the normal cellular turnover of nonregenerating planarians.

Bidaut, G. and C. J. Stoeckert, Jr. (2009). "Large scale transcriptome data integration across multiple tissues to decipher stem cell signatures." Methods Enzymol **467**: 229-45.

A wide variety of stem cells has been reported to exist and renew several adult tissues, raising the question of the existence of a stemness signature-that is, a common molecular program of differentiation. To detect such a signature, we applied a data integration algorithm on several DNA microarray datasets generated by the Stem Cell Genome Anatomy Project (SCGAP) Consortium on several mouse and human tissues, to generate a cross-organism compendium that we submitted to a single layer artificial neural network (ANN) trained to attribute differentiation labels-from totipotent stem cells to differentiated ones (five labels in total were used). The inherent architecture of the system allowed studing the biology behind stem cells differentiation stages and the ANN isolated a 63 gene stemness signature. This chapter presents technological details on DNA microarray integration, ANN training through leave-one-out cross-validation, and independent testing on uncharacterized adult tissues by automated detection of differentiation capabilities on human prostate and mouse stomach progenitors. All scripts of the Stem Cell Analysis and characterization by Neural Networks (SCANN) project are available on the SourceForge Web site: http://scann.sourceforge.net.

Blaese, R. M. and K. W. Culver (1992). "Gene therapy for primary immunodeficiency disease." Immunodefic Rev **3**(4): 329-49.

Gene therapy offers the potential for developing innovative new treatments for both inherited monogenic diseases as well as polygenic and acquired disorders. For most potential clinical applications, the technology has not yet progressed to the stage where it might be reasonably tested. Problems to be solved include the isolation and characterization of the genes involved, the development of gene delivery systems that will permit efficient gene insertion in the affected cells and tissues, and the development of mechanisms to control or appropriately regulate expression of the introduced genes. The primary immunodeficiency diseases as a group actually lend themselves to the development of gene therapy strategies with current technology more readily than almost any other class of disease. Theoretically any genetic disease that can be successfully treated by allogeneic bone marrow transplantation is a potential candidate for gene therapy directed at correcting the patient's own totipotent bone marrow stem cells. In addition, some disorders lend themselves to genetic correction of more mature cells, although gene transfer in this treatment strategy might have to be repeated periodically. The rationale and preliminary results of the first gene therapy protocol for ADA deficiency SCID are described and strategies for developing somatic cell gene therapy for the other primary immunodeficiency diseases are discussed.

Blobel, G. A., M. C. Simon, et al. (1995). "Rescue of GATA-1-deficient embryonic stem cells by heterologous GATA-binding proteins." Mol Cell Biol **15**(2): 626-33.

Totipotent murine embryonic stem (ES) cells can be differentiated in vitro to form embryoid bodies (EBs) containing hematopoietic cells of multiple lineages, including erythroid cells. In vitro erythroid development parallels that which is observed in vivo. ES cells in which the gene for the erythroid transcription factor GATA-1 has been disrupted fail to produce mature erythroid cells either in vivo or in vitro. With the EB in vitro differentiation assay, constructs expressing heterologous GATA-binding proteins were tested for their abilities to correct the developmental defect of GATA-1-deficient ES cells. The results presented here show that the highly divergent chicken GATA-1 can rescue GATA-1 deficiency to an extent similar to that of murine GATA-1 (mGATA-1), as determined by size and morphology of EBs, presence of red cells, and globin gene expression. Furthermore, GATA-3 and GATA-4, which are normally expressed in different tissues, and a protein consisting of the zinc fingers of GATA-1 fused to the herpes simplex virus VP16 transcription activation domain were able to compensate for the GATA-1 defect.

Boiani, M. and H. R. Scholer (2005). "Regulatory networks in embryo-derived pluripotent stem cells." Nat Rev Mol Cell Biol **6**(11): 872-84.

Mammalian development requires the specification of over 200 cell types from a single totipotent cell. Investigation of the regulatory networks that are responsible for pluripotency in embryo-derived stem cells is fundamental to understanding mammalian development and realizing therapeutic potential. Extracellular signals and second messengers modulate cell-autonomous regulators such as OCT4, SOX2 and Nanog in a combinatorial

complexity. Knowledge of this circuitry might reveal how to achieve phenotypic changes without the genetic manipulation of Oct4, Nanog and other toti/pluripotency-associated genes.

Bollner, T., S. Howalt, et al. (1995). "Regeneration and post-metamorphic development of the central nervous system in the protochordate Ciona intestinalis: a study with monoclonal antibodies." Cell Tissue Res **279**(2): 421-32.

In this study, we use three monoclonal antibodies that recognise antigens present in the central nervous system of the ascidian Ciona intestinalis to study regeneration and post-metamorphic development of the neural ganglion. We have also used bromodeoxyuridine labelling to study generation of the neuronal precursor cells. The first antibody, CiN 1, recognises all neurones in the ganglion, whereas the second, CiN 2, recognises only a subpopulation of the large cortical neurones. Western blotting studies show that CiN 2 recognises two membrane-bound glycoproteins of apparent Mr 129 and 100 kDa. CiN 1 is not reactive on Western blots. Immunocytochemical studies with these antibodies show that CiN 1-immunoreactive neurone-like cells are present at the site of regeneration as early as 5-7 days post-ablation, a sub-population of CiN 2-immunoreactive cells being detected by 9-12 days post-ablation. The third antibody, ECM 1, stains extracellular matrix components and recognises two diffuse bands on Western blots of whole-body and ganglion homogenates. The temporal and spatial pattern of appearance of CiN 1 and CiN 2 immunoreactivity both during post-metamorphic development and in regeneration occurs in the same sequence in both processes. Studies with bromodeoxyuridine show labelled nuclei in some neurones in the regenerating ganglion. Plausibly these originate from the dorsal strand, an epithelial tube that reforms by cell proliferation during the initial phases of regeneration.

Bongso, A., C. Y. Fong, et al. (1994). "Isolation and culture of inner cell mass cells from human blastocysts." Hum Reprod **9**(11): 2110-7.

Totipotent non-committed inner cell mass (ICM) cells from human blastocyts, if demonstrated to be capable of proliferating in vitro without differentiation, will have several beneficial uses, not only in the treatment of neurodegenerative and genetic disorders, but also as a model in studying the events involved in embryogenesis and genomic manipulation. Nine patients admitted to an in-vitro fertilization programme donated 21 spare embryos for this study. All 21 embryos were grown from the 2-pronuclear until blastocyst stages on a human tubal epithelial monolayer in commercial Earle's medium (Medicult, Denmark) supplemented with 10% human serum. The medium was changed after blastocyst formation to Chang's medium supplemented with 1000 units/ml of human leukaemia inhibitory factor (HLIF) and the embryos left undisturbed for 72 h to allow the hatched ICM and trophoblast to attach to the feeder monolayer. Nineteen of the 21 embryos from nine patients produced healthy ICM lumps which could be separated and grown in vitro. Two of the lumps differentiated into fibroblasts while the remaining 17 (eight patients) produced cells with typical stem cell-like morphology, were alkaline phosphatase positive and could be maintained for two passages. It was possible to retain the stem cell-like morphology, alkaline phosphatase positiveness and normal karyotype through the two passages in all of them using repeated doses of HLIF every 48 to 72 h. This is the first report on the successful isolation of human ICM cells and their continued culture for at least two passages in vitro.

Bradley, A., R. Ramirez-Solis, et al. (1992). "Genetic manipulation of the mouse via gene targeting in embryonic stem cells." Ciba Found Symp **165**: 256-69; discussion 269-76.

Gene targeting applied to totipotent embryonic stem (ES) cells is a very powerful means of creating highly specific mutations of genes in the mouse. The successful application of this technology is however constrained by both the types of mutations that can be generated at a target locus and the ability to reconstruct a germline chimera from the manipulated cells. We have developed two cell lines that can be routinely transmitted through the germline of chimeras after cloning and prolonged selection in tissue culture. We have also established a variety of methods for generating non-selected mutations at the X-linked hprt locus in ES cells. Our observations at this locus have enabled us to generate successfully a subtle mutation at the non-selectable Hox-2.6 locus.

Brehm, A., K. Ohbo, et al. (1997). "The carboxy-terminal transactivation domain of Oct-4 acquires cell specificity through the POU domain." Mol Cell Biol **17**(1): 154-62.

The POU transcription factor Oct-4 is expressed in totipotent and pluripotent cells of the early mouse embryo and the germ cell lineage. Transactivation capacities of regions flanking the DNA binding domain of Oct-4 were analyzed in undifferentiated and differentiated cell lines. The amino- and carboxy-terminal regions (N domain and C domain) fused to the Gal4 DNA binding domain both functioned as transactivation domains in all cell lines tested. However, the C domain failed to activate

transcription in some cell lines in the context of the native protein. The underlying regulatory mechanism appears to involve the POU domain of Oct-4 and can discriminate between different POU domains, since constructs in which the C domain was instead fused to the POU domain of Pit-1 were again equally active in all cell lines. These results indicate that the C domain is subject to cell-type-specific regulation mediated by the Oct-4 POU domain. Phosphopeptide analysis revealed that the cell-type-specific difference of C-domain activity correlates with a difference in Oct-4 phosphorylation status. Since Oct-4 is expressed in a variety of distinct cell types during murine embryogenesis, these results suggest an additional regulatory mechanism for determining Oct-4 function in rapidly changing cell types during development.

Brehm, A., C. E. Ovitt, et al. (1998). "Oct-4: more than just a POUerful marker of the mammalian germline?" Apmis **106**(1): 114-24; discussion 124-6.

Mammals lack visible cytoplasmic components in the oocyte that could account for 'germline determinants' as identified in various non-mammalian species. Actually, mammals might not define the germline autonomously by localized 'germline determinants' but conditionally depending on the position of cells within the embryo. The Oct-4 gene encodes a transcription factor that is specifically expressed in the toti- and pluripotential stem cells of the mouse embryo and so far has only been found in mammalian species. Oct-4-expressing embryonal cell retain the capacity to differentiate along multiple lineages and they have been suggested to be part of a 'totipotent germline cycle' that links one generation to the next.

Brinster, R. L. (1993). "Stem cells and transgenic mice in the study of development." Int J Dev Biol **37**(1): 89-99.

In recent years, totipotent stem cells and transgenic mice have been widely used to understand the complex changes that occur during development, and these approaches underlie much of the dynamic growth in this field. The work of Barry Pierce in defining the multipotential characteristics of teratocarcinoma or embryonal carcinoma stem cells in the 1960s was an important milestone for the field and was instrumental in our choice of these cells for transfer into blastocysts in the first experiments designed to colonize a mouse with foreign totipotent cells. Following the development of transgenic techniques, the stem cell approach has become even more powerful, and during the past five years the combination of the two techniques has made possible the experimental creation of virtually any genetic change in mice, and ultimately in other species. In this review, the work in our laboratory over the past 30 years is summarized, and it reflects only a small part of the exciting array of experiments that have contributed to the explosive evolution of developmental biology during this period.

Brown, D. G., M. A. Willington, et al. (1992). "Criteria that optimize the potential of murine embryonic stem cells for in vitro and in vivo developmental studies." In Vitro Cell Dev Biol **28A**(11-12): 773-8.

Cultured mouse embryonic stem (ES) cells are used for both in vitro and in vivo studies. The uncommitted pluripotent cells provide a model system with which to study cellular differentiation and development; they can also be used as vectors to carry specific mutations into the mouse genome by homologous recombination. To ensure successful integration into the germ line, competent totipotent diploid ES cell lines are selected using a cell injection bioassay that is both time consuming and technically demanding. The prolonged in vitro culture of rapidly dividing ES cells can lead to accumulated changes and chromosomal abnormalities that will compromise the biological function and abrogate germ line transmission of chimeric mice carrying novel genetic mutations. Such in vitro conditions will vary between individual laboratories; for example, differences in the serums used for maintenance. Using a number of different criteria we attempt in this paper to define the parameters that we found to be key factors for optimization of the biological potential of established ES cell lines. The successful integration into the germ line is dependant on acquiring or deriving a competent totipotent mouse ES diploid cell line. In this paper parameters and criteria are defined which we found to be key factors for the optimization of the biological potential of established ES cell lines.

Bryja, V., S. Bonilla, et al. (2006). "An efficient method for the derivation of mouse embryonic stem cells." Stem Cells **24**(4): 844-9.

Mouse embryonic stem cells (mESCs) represent a unique tool for many researchers; however, the process of ESC derivation is often very inefficient and requires high specialization, training, and expertise. To circumvent these limitations, we aimed to develop a simple and efficient protocol based on the use of commercially available products. Here, we present an optimized protocol that we successfully applied to derive ESCs from several knockout mouse strains (Wnt-1, Wnt-5a, Lrp6, and parkin) with 50%-75% efficiency. The methodology is based on the use of mouse embryonic fibroblast feeders, knockout serum replacement (SR), and minimal handling of the blastocyst. In this protocol, all centrifugation steps (as

well as the use of trypsin inhibitor) were avoided and replaced by an ESC medium containing fetal calf serum (FCS) after the trypsinizations. We define the potential advantages and disadvantages of using SR and FCS in individual steps of the protocol. We also characterize the ESCs for the expression of ESC markers by immunohistochemistry, Western blot, and a stem cell focused microarray. In summary, we provide a simplified and improved protocol to derive mESCs that can be useful for laboratories aiming to isolate transgenic mESCs for the first time.

Bueno, D., J. Baguna, et al. (1997). "Cell-, tissue-, and position-specific monoclonal antibodies against the planarian Dugesia (Girardia) tigrina." Histochem Cell Biol **107**(2): 139-49.

To obtain specific immunological probes for studying molecular mechanisms involved in cell renewal, cell differentiation, and pattern formation in intact and regenerating planarians, we have produced a hybridoma library specific for the asexual race of the fresh-water planarian Dugesia (Girardia) tigrina. Among the 276 monoclonal antibodies showing tissue-, cell-, cell subtype-, subcellular- and position-specific staining, we have found monoclonal antibodies against all tissues and cell types with the exception of neoblasts, the undifferentiated totipotent stem-cells in planarians. We have also detected position-specific antigens that label anterior, central, and posterior regions. Patterns of expression uncovered an unexpected heterogeneity among previously thought single cell types, as well as interesting cross-reactivities that deserve further study. Characterization of some of these monoclonal antibodies suggests they may be extremely useful as molecular markers for studying cell renewal and cell differentiation in the intact and regenerating organism, tracing the origin, lineage, and differentiation of blastema cells, and characterizing the stages and mechanisms of early pattern formation. Moreover, two position-specific monoclonals, the first ones isolated in planarians, will be instrumental in describing in molecular terms how the new pattern unfolds during regeneration and in devising the pattern formation model that best fits classical data on regeneration in planarians.

Bugos, O., M. Bhide, et al. (2009). "Beyond the rat models of human neurodegenerative disorders." Cell Mol Neurobiol **29**(6-7): 859-69.

The rat is a model of choice in biomedical research for over a century. Currently, the rat presents the best "functionally" characterized mammalian model system. Despite this fact, the transgenic rats have lagged behind the transgenic mice as an experimental model of human neurodegenerative disorders. The number of transgenic rat models recapitulating key pathological hallmarks of Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, or human tauopathies is still limited. The reason is that the transgenic rats remain more difficult to produce than transgenic mice. The gene targeting technology is not yet established in rats due to the lack of truly totipotent embryonic stem cells and cloning technology. This extremely powerful technique has given the mouse a clear advantage over the rat in generation of new transgenic models. Despite these limitations, transgenic rats have greatly expanded the range of potential experimental approaches. The large size of rats permits intrathecal administration of drugs, stem cell transplantation, serial sampling of the cerebrospinal fluid, microsurgical techniques, in vivo nerve recordings, and neuroimaging procedures. Moreover, the rat is routinely employed to demonstrate therapeutic efficacy and to assess toxicity of novel therapeutic compounds in drug development. Here we suggest that the rat constitutes a slightly underestimated but perspective animal model well-suited for understanding the mechanisms and pathways underlying the human neurodegenerative disorders.

Bukovsky, A. (2007). "Cell commitment by asymmetric division and immune system involvement." Prog Mol Subcell Biol **45**: 179-204.

Asymmetric division is a fundamental means of generating cell diversity and may involve extrinsic or intrinsic factors. Here we review observations on symmetric and asymmetric expression of estrogen receptor alpha (ERA) and beta (ERB) during regeneration of trophoblast cells in human placenta and possibly other estrogen-responsive cell types. This is a type of differentiation from committed progenitor cells. Asymmetric segregation of ERA in dividing villous cytotrophoblast cells, accompanied by appearance of ERB in differentiating daughter cells and resulting syncytiotrophoblast, suggests a unique role of estrogen receptors in asymmetric division of estrogen responsive cells. We also review observations on asymmetric division of ovarian surface epithelium (OSE) stem cells resulting in formation of germ cells differentiating into oocytes in fetal and adult human ovaries. Besides germ cells, the OSE stem cells also give rise to primitive ovarian granulosa (follicular) cells, which are required for the formation of new primary follicles and preservation and differentiation of oocytes. This dual potential of OSE stem cells (germ or granulosa cells) is a type of differentiation from uncommitted and possibly totipotent adult stem cells. A possible role of immune system related cells (monocyte-derived cells and T lymphocytes-cellular signaling) and hormones in the

stimulation of OSE differentiation toward germ cells by asymmetric division, and in the continuation of ovarian follicular renewal during prime reproductive period in human females is also reviewed. Follicular renewal ceases after prime reproductive period, possibly due to the diminution of cellular signaling required for asymmetric division of OSE stem cells into the germ cells. The primary follicles persisting in premenopausal ovaries appear to accumulate genetic alterations, a cause of exponentially growing chromosomal abnormalities in the progeny of mothers between 38 years of age and menopause.

Bukovsky, A., M. R. Caudle, et al. (2009). "Immune physiology and oogenesis in fetal and adult humans, ovarian infertility, and totipotency of adult ovarian stem cells." Birth Defects Res C Embryo Today **87**(1): 64-89.

It is still widely believed that while oocytes in invertebrates and lower vertebrates are periodically renewed throughout life, oocytes in humans and higher vertebrates are formed only during the fetal/perinatal period. However, this dogma is questioned, and clashes with Darwinian evolutionary theory. Studies of oogenesis and follicular renewal from ovarian stem cells (OSCs) in adult human ovaries, and of the role of third-party bone marrow-derived cells (monocyte-derived tissue macrophages and T lymphocytes) could help provide a better understanding of the causes of ovarian infertility, its prevention, and potential treatment. We have reported differentiation of distinct cell types from OSC and the production of new eggs in cultures derived from premenopausal and postmenopausal human ovaries. OSCs are also capable of producing neural/neuronal cells in vitro after sequential stimulation with sex steroid combinations. Hence, OSC represent a unique type of totipotent adult stem cells, which could be utilized for autologous treatment of premature ovarian failure and also for autologous stem cell therapy of neurodegenerative diseases without use of allogeneic embryonic stem cells or somatic cell nuclear transfer. The in vivo application of sex steroid combinations may augment the proliferation of existing neural stem cells and their differentiation into mature neuronal cells (systemic regenerative therapy). Such treatment may also stimulate the transdifferentiation of autologous neural stem cell precursors into neural stem cells useful for topical or systemic regenerative treatment.

Bukovsky, A., P. Copas, et al. (2006). "Potential new strategies for the treatment of ovarian infertility and degenerative diseases with autologous ovarian stem cells." Expert Opin Biol Ther **6**(4): 341-65.

The 50-year-old and currently prevailing view that all oocytes in adult human ovaries persist from the fetal period of life is controversial as it clashes with Darwinian evolutionary theory. Studies of oogenesis and follicular renewal in adult human ovaries, and of the role of hormonal signals and third-party cells (tissue macrophages and T cells), could all be helpful in providing better understanding of the causes of ovarian infertility, its prevention and potential therapy. In addition, the authors recently reported differentiation of distinct cell types and the production of new eggs in cultures derived from premenopausal and postmenopausal human ovaries. It is possible that fertilisation of such eggs will open up new opportunities for providing genetically related children to infertile women for whom conventional in vitro fertilisation has failed. As ovarian stem cells appear to represent a new type of totipotent adult stem cell, they could also be utilised for autologous stem cell therapy of degenerative diseases, without any involvement of allogeneic embryonic stem cells and somatic cell nuclear transfer.

Bukovsky, A., M. Svetlikova, et al. (2005). "Oogenesis in cultures derived from adult human ovaries." Reprod Biol Endocrinol **3**: 17.

Ten years ago, we reported that in adult human females the ovarian surface epithelium (OSE) is a source of germ cells. Recently, we also demonstrated that new primary follicles are formed by assembly of oocytes with nests of primitive granulosa cells in the ovarian cortex. The components of the new primary follicles, primitive granulosa and germ cells, differentiated sequentially from the OSE, which arises from cytokeratin positive mesenchymal progenitor cells residing in the ovarian tunica albuginea. In the present study, we investigated the possibility that the oocytes and granulosa cells may differentiate in cultures derived from adult human ovaries. Cells were scrapped from the surface of ovaries and cultured for 5 to 6 days, in the presence or absence of estrogenic stimuli [phenol red (PhR)]. The OSE cells cultured in the medium without PhR differentiated into small (15 micron) cells of granulosa phenotype, and epithelial, neural, and mesenchymal type cells. In contrast, OSE cells cultured in the presence of PhR differentiated directly into large (180 micron) cells of the oocyte phenotype. Such cells exhibited germinal vesicle breakdown, expulsion of the polar body, and surface expression of zona pellucida proteins, i.e. characteristics of secondary oocytes. These in vitro studies confirm our in vivo observations that in adult human ovaries, the OSE is a bipotent source of oocytes and granulosa cells. Development of numerous mature oocytes from adult ovarian stem cells in vitro offers new strategies for the egg

preservation, IVF utilization, and treatment of female infertility. In addition, other clinical applications aiming to utilize stem cells, and basic stem cell research as well, may employ totipotent embryonic stem cells developing from fertilized oocytes.

Bukovsky, A., I. Virant-Klun, et al. (2006). "Ovarian germ cells." Methods Enzymol **419**: 208-58.

Surface cells in adult ovaries represent germ line-competent embryonic stem cells. They are a novel type of totipotent progenitors for distinct cell types including female germ cells/oocytes, with the potential for use in the autologous treatment of ovarian infertility and stem cell therapy. Ovarian infertility and stem cell therapy are complex scientific, therapeutic, and socioeconomic issues, which are accompanied by legal restrictions in many developed countries. We have described the differentiation of distinct cell types and the production of new eggs in cultures derived from adult human ovaries. The possibility of producing new eggs from ovarian surface epithelium representing totipotent stem cells supports new opportunities for the treatment of premature ovarian failure, whether idiopathic or after cytostatic chemotherapy treatment, as well as infertility associated with aged primary follicles, and infertility after natural menopause. The stem cells derived from adult human ovaries can also be used for stem cell research and to direct autologous stem cell therapy. This chapter describes general considerations regarding the egg origin from somatic progenitor cells, oogenesis and follicle formation in fetal and adult human ovaries (follicular renewal), including the promotional role of the immune system-related cells in vivo, and possible causes of ovarian infertility. It then provides detailed protocols for the separation and cultivation of adult ovarian stem cells.

Burt, R. K., L. Verda, et al. (2004). "Embryonic stem cells as an alternate marrow donor source: engraftment without graft-versus-host disease." J Exp Med **199**(7): 895-904.

A single embryonic stem cell (ESC) line can be repetitively cryopreserved, thawed, expanded, and differentiated into various cellular components serving as a potentially renewable and well-characterized stem cell source. Therefore, we determined whether ESCs could be used to reconstitute marrow and blood in major histocompatibility complex (MHC)-mismatched mice. To induce differentiation toward hematopoietic stem cells (HSCs) in vitro, ESCs were cultured in methylcellulose with stem cell factor, interleukin (IL)-3, and IL-6. ESC-derived, cytokine-induced HSCs (c-kit+/CD45+) were isolated by flow cytometry and injected either intra bone marrow or intravenously into lethally irradiated MHC-mismatched recipient mice. From 2 wk to 6 mo after injection, the peripheral blood demonstrated increasing ESC-derived mononuclear cells that included donor-derived T and B lymphocytes, monocytes, and granulocytes without clinical or histologic evidence of graft-versus-host disease (GVHD). Mixed lymphocyte culture assays demonstrated T cell tolerance to both recipient and donor but intact third party proliferative responses and interferon gamma production. ESCs might be used as a renewable alternate marrow donor source that reconstitutes hematopoiesis with intact immune responsiveness without GVHD despite crossing MHC barriers.

Cabibi, D., A. Martorana, et al. (2006). "Carcinosarcoma of monoclonal origin arising in a dermoid cyst of ovary: a case report." BMC Cancer **6**: 47.

BACKGROUND: Transformation of a cystic benign teratoma of the ovary into a "carcinosarcoma" has very rarely been reported and its histogenetic origin is still debated. CASE PRESENTATION: A case of carcinosarcoma arising from a dermoid cyst is reported. The tumor showed cystic areas delimited by normal squamous epithelium, with transitional areas through dysplastic epithelium to "in situ" and infiltrating squamous cell carcinoma (SCC). The sarcomatous component showed compact tissue composed of round cells concentrically arranged around small vessels, spindle, and pleomorphic cells with a high nuclear-cytoplasmic ratio. Positive staining for vimentin, alpha smooth muscle actin and CD10, as well as P53 and P63, was found in the sarcomatous component and in some atypical basal cells of the squamous epithelium, which also showed the usual epithelial markers. CONCLUSION: To the best of our knowledge, this is the first case of carcinosarcoma arising from a dermoid cyst in which a histogenetic origin from totipotent stem cells, located in the basal squamous layer, is supported by immunohistochemical findings.

Capel, B., R. Hawley, et al. (1989). "Clonal contributions of small numbers of retrovirally marked hematopoietic stem cells engrafted in unirradiated neonatal W/Wv mice." Proc Natl Acad Sci U S A **86**(12): 4564-8.

Mice were repopulated with small numbers of retrovirally marked hematopoietic cells operationally definable as totipotent hematopoietic stem cells, without engraftment of cells at later stages of hematopoiesis, in order to facilitate analysis of stem cell clonal histories. This result depended upon the use of unirradiated W/Wv newborn recipients. Before transplantation, viral integration markers were introduced during cocultivation of fetal liver or bone

marrow cells with helper cell lines exporting defective recombinant murine retroviruses of the HHAM series. Omission of selection in culture [although the vector contained the bacterial neomycin-resistance (neo) gene] also limited the proportion of stem cells that were virally labeled. Under these conditions, engraftment was restricted to a small population of marked and unmarked normal donor stem cells, due to their competitive advantage over the corresponding defective cells of the mutant hosts. A relatively simple and coherent pattern emerged, of one or a few virally marked clones, in contrast to previous studies. In order to establish the totipotent hematopoietic stem cell identity of the engrafted cells, tissues were sampled for viral and inbred-strain markers for periods close to one year after transplantation. The virally labeled clones were characterized as stem cell clones by their extensive self-renewal and by formation of the wide range of myeloid and lymphoid lineages tested. Results clearly documented concurrent contributions of cohorts of stem cells to hematopoiesis. A given stem cell can increase or decrease its proliferative activity, become completely inactive or lost, or become active after a long latent period. The contribution of a single clone present in a particular lineage was usually between 5% and 20%.

Capel, B., R. G. Hawley, et al. (1990). "Long- and short-lived murine hematopoietic stem cell clones individually identified with retroviral integration markers." Blood **75**(12): 2267-70.

The proliferative longevity of totipotent hematopoietic stem cells (THSC) is a limiting factor in normal hematopoiesis and in therapy by cell- or gene-replacement, but has not yet been ascertained. We have followed the long-term fate of individual clones of mouse THSC from fetal liver or adult bone marrow, after labeling in culture, followed by engraftment and serial transplantation in unirradiated W/Wv-C57BL/6 hosts. The ancestor cell of each clone and its mitotic progeny were uniquely identifiable retrospectively by the DNA integration pattern experimentally produced by replication-incompetent recombinant murine retroviruses. These viruses provided physiologically neutral markers. The marked clones proved to be derived from THSC, based on their contributions to a wide array of myeloid and lymphoid blood lineages in the hosts. The label also identified the target cells as the population displaying clonal succession. The various labeled stem cell clones proliferated for substantially different periods of time. The longest observed clone endured, after the original cell was marked, for at least 2 1/2 years--the equivalent of a mouse's lifetime. However, the results suggest that THSC clones are not all long-lived and that even the longest-lived ones may not be potentially immortal. Thus, the unpredictable lifespan of any given THSC clone indicates the desirability of introducing multiple clones in therapeutic transplants.

Carroll, P., Y. Renoncourt, et al. (2001). "Sorting nexin-14, a gene expressed in motoneurons trapped by an in vitro preselection method." Dev Dyn **221**(4): 431-42.

A gene-trap strategy was set up in embryonic stem (ES) cells with the aim of trapping genes expressed in restricted neuronal lineages. The vector used trap genes irrespective of their activity in undifferentiated totipotent ES cells. Clones were subjected individually to differentiation in a system in which ES cells differentiated into neurons. Two ES clones in which the trapped gene was expressed in ES-derived neurons were studied in detail. The corresponding cDNAs were cloned, sequenced, and analysed by in situ hybridisation on wild-type embryo sections. Both genes are expressed in the nervous system. One gene, YR-23, encodes a large intracellular protein of unknown function. The second clone, YR-14, represents a sorting nexin (SNX14) gene whose expression in vivo coincides with that of LIM-homeodomain Islet-1 in several tissues. Sorting nexins are proteins associated with the endoplasmic reticulum (ER) and may play a role in receptor trafficking. Gene trapping followed by screening based on in vitro preselection of differentiated ES recombinant clones, therefore, has the potential to identify integration events in subsets of genes before generation of mouse mutants.

Caruana, G. and A. Bernstein (2001). "Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene." Mol Cell Biol **21**(5): 1475-83.

The discs large (Dlg) protein, or synapse-associated protein 97 (SAP97), is a member of the membrane-associated guanylate kinase family of multidomain scaffolding proteins which recruits transmembrane and signaling molecules to localized plasma membrane sites. Murine dlg is the homologue of the Drosophila dlg tumor suppressor gene. The loss of dlg function in Drosophila disrupts cellular growth control, apicobasal polarity, and cell adhesion of imaginal disc epithelial cells, resulting in embryonic lethality. In this study, we isolated a mutational insertion in the murine dlg locus by gene trapping in totipotent embryonic stem cells. This insertion results in a truncated protein product that contains the N-terminal three PSD-95/DLG/ZO-1 domains of Dlg fused to the LacZ reporter and subsequently lacks the src homology 3 (SH3), protein 4.1 binding, and guanylate kinase (GUK)-like domains. The Dlg-LacZ fusion protein is expressed in epithelial, mesenchymal,

neuronal, endothelial, and hematopoietic cells during embryogenesis. Mice homozygous for the dlg mutation exhibit growth retardation in utero, have hypoplasia of the premaxilla and mandible, have a cleft secondary palate, and die perinatally. Consistent with this phenotype, Dlg-LacZ is expressed in mesenchymal and epithelial cells throughout palatal development. Our genetic and phenotypic analysis of dlg mutant mice suggests that protein-protein interactions involving the SH3, protein 4.1 binding, and/or GUK-like domains are essential to the normal function of murine Dlg within craniofacial and palatal morphogenesis.

Cauffman, G., M. De Rycke, et al. (2009). "Markers that define stemness in ESC are unable to identify the totipotent cells in human preimplantation embryos." Hum Reprod **24**(1): 63-70.

BACKGROUND: During human preimplantation development, early blastomeres are believed to be totipotent. It is likely, however, that blastomeres are allocated to a specific lineage prior to any morphological differentiation. NANOG, SOX2 and SALL4 are transcription factors that play a key role in controlling stemness in embryonic stem cells (ESC) and are therefore candidate markers for developmental triggers in early embryos. KRT18, a trophoblast-determining gene, may mark early differentiation. Examining the expression pattern of these genes may inform us about when and in which cells totipotency is lost during early human development. METHODS: Thirtheen oocytes, 124 preimplantation embryos and 7 human embryonic stem cell (hESC) lines were examined for the presence of NANOG, SOX2, SALL4 or KRT18 proteins using immunostaining and confocal microscopy. RESULTS: All stemness markers were expressed in the hESC, but none of them was specific for totipotent cells during human preimplantation development, and none of them seemed to mark cells allocated to the inner cell mass (ICM) or trophectoderm. After lineage specification, only the nuclear expression of NANOG and SOX2 became restricted to the ICM, at least to some cells because only a subpopulation expressed NANOG. KRT18 expression was seen for the first time during compaction in some outer cells. KRT18 was not expressed in hESC. CONCLUSION: We conclude that the protein expression patterns of markers that define stemness in ESC do not identify the totipotent cells in human preimplantation embryos. Assessing the presence of KRT18 proteins implied that the outer cells of compacting embryos have probably lost their totipotent competence prior to any visible differentiation.

Cauffman, G., I. Liebaers, et al. (2006). "POU5F1 isoforms show different expression patterns in human embryonic stem cells and preimplantation embryos." Stem Cells **24**(12): 2685-91.

The contribution of the POU domain, class 5, transcription factor-1 (POU5F1) in maintaining totipotency in human embryonic stem cells (hESCs) has been repeatedly proven. In humans, two isoforms are encoded: POU5F1_iA and POU5F1_iB. So far, no discrimination has been made between the isoforms in POU5F1 studies, and it is unknown which isoform contributes to the undifferentiated phenotype. Using immunocytochemistry, expression of POU5F1_iA and POU5F1_iB was examined in hESCs and all stages of human preimplantation development to look for differences in expression, biological activity, and relation to totipotency. POU5F1_iA and POU5F1_iB displayed different temporal and spatial expression patterns. During human preimplantation development, a significant POU5F1_iA expression was seen in all nuclei of compacted embryos and blastocysts and a clear POU5F1_iB expression was detected from the four-cell stage onwards in the cytoplasm of all cells. The cytoplasmic localization might imply no or other biological functions beyond transcription activation for POU5F1_iB. The stemness properties of POU5F1 can be assigned to POU5F1_iA because hESCs expressed POU5F1_iA but not POU5F1_iB. However, POU5F1_iA is not the appropriate marker to identify totipotent cells, because POU5F1_iA was also expressed in the nontotipotent trophectoderm and was not expressed in zygotes and early cleavage stage embryos, which are assumed to be totipotent. The expression pattern of POU5F1_iA may suggest that POU5F1_iA alone cannot sustain totipotency and that coexpression with other stemness factors might be the key to totipotency.

Cazillis, M., A. F. Bringuier, et al. (2004). "Disruption of MKK4 signaling reveals its tumor-suppressor role in embryonic stem cells." Oncogene **23**(27): 4735-44.

The dual Ser/Thr kinase MKK4 and its downstream targets JNK and p38 regulate critical cellular functions during embryogenesis and development. MKK4 has been identified as a putative tumor-suppressor gene in human solid tumors of breast, prostate and pancreas. To clarify the mechanisms underlying the transforming potential of molecular defects targeting MKK4, we have generated totipotent embryonic stem (ES) cells expressing the dominant-negative mutant DN-MKK4(Ala), S257A/T261A. Stably transfected DN-MKK4-ES cells exhibit a transformed fibroblast-like morphology, reduced proliferation rate, were no more submitted to cell contact inhibition, were growing in soft agar, and were much more tumorigenic than parental ES cells in

athymic nude mice. These phenotypic changes: (i) are consistent with the protection of DN-MKK4-transfected ES cells from spontaneous, cell density-dependent, and stress-induced apoptosis (DAPI staining and poly (ADP-ribose) polymerase (PARP) cleavage) and (ii) correlated with alterations in JNK, p38, and Erk-1/-2 MAPK/SAPK signaling. Taken together, our data provide a new mechanism linking the MKK4 signaling pathways to cancer progression and identify MKK4 as a tumor-suppressor gene implicated in several transforming functions.

Chen, S. K., S. Kurdyukov, et al. (2009). "The association of homeobox gene expression with stem cell formation and morphogenesis in cultured Medicago truncatula." Planta **230**(4): 827-40.

Somatic embryogenesis (SE) is induced in vitro in Medicago truncatula 2HA by auxin and cytokinin but rarely in wild type Jemalong. The putative WUSCHEL (MtWUS), CLAVATA3 (MtCLV3) and the WUSCHEL-related homeobox gene WOX5 (MtWOX5) were investigated in M. truncatula (Mt) and identified by the similarity to Arabidopsis WUS, CLV3 and WOX5 in amino acid sequence, phylogeny and in planta and in vitro expression patterns. MtWUS was induced throughout embryogenic cultures by cytokinin after 24-48 h and maximum expression occurred after 1 week, which coincides with the induction of totipotent stem cells. During this period there was no MtCLV3 expression to suppress MtWUS. MtWUS expression, as illustrated by promoter-GUS studies, subsequently localised to the embryo, and there was then the onset of MtCLV3 expression. This suggests that the expression of the putative MtCLV3 coincides with the WUS-CLAVATA feedback loop becoming operational. RNAi studies showed that MtWUS expression is essential for callus and somatic embryo production. Based on the presence of MtWUS promoter binding sites, MtWUS may be required for the induction of MtSERF1, postulated to have a key role in the signalling required for SE induced in 2HA. MtWOX5 expressed in auxin-induced root primordia and root meristems and appears to be involved in pluripotent stem cell induction. The evidence is discussed that the homeobox genes MtWUS and MtWOX5 are "hijacked" for stem cell induction, which is key to somatic embryo and de novo root induction. In relation to SE, a role for WUS in the signalling involved in induction is discussed.

Chervenick, P. A. (1985). "Methods for measuring suppression of hematopoiesis." Exp Hematol **13 Suppl 16**: 8-15.

Mature blood cells have a finite life span and therefore continued production is required to maintain a constant level in tissues. Continuous replenishment is achieved by a constant feed-in from normal functioning hematopoietic stem cell compartments. The hematopoietic stem cell (HSC) system is characterized as follows: A totipotent hematopoietic stem cell (THSC) gives rise to all hematopoietic cells. Separate cells exist that are more differentiated progeny of THSC and are pluripotent for the myeloid system (PMSC: CFU-S, CFU-GEMM) and for the lymphoid system (PLSC). The PMSC gives rise to still more differentiated progenitor cells committed to erythrocytes (BFU-E, CFU-E), neutrophil-macrophages (CFU-NM) and megakaryocytes (CFU-MEG). One class of PMSC (CFU-S) is assayed in vivo. A second class of PMSC (CFU-GEMM), and most other types of progenitors (CFU-E, CFU-NM, CFU-MEG, etc.), are assayed in vitro. The mouse is the usual vehicle for the in vivo study of the CFU-S (colony-forming unit-spleen). Bone marrow cells are infused into lethally irradiated recipient mice, lodge in the spleen, and proliferate to form macroscopic colonies on the surface. There is no similar assay available in man, but the ability to clone mixed colonies (CFU-GEMM) in vitro allows one to study human pluripotent stem cells. In the presence of appropriate stimuli, CFU-GEMM form colonies in soft gel that contain granulocytes, erythroid cells, macrophages, and megakaryocytes. In addition to this class of PMSC, the differentiated progenitors that are committed to produce erythroid cells, neutrophils, megakaryocytes, or monocytes-macrophages also form colonies in vitro. A third method of determining the effect of antineoplastic agents on marrow cells is by use of the diffusion chamber (DC) culture technique. Marrow cells are inoculated into a diffusion chamber that is then implanted into the peritoneum of a mouse. After various time periods, chambers are removed and the number and differentiated cell types are determined. Modifications of the DC chamber technique include suspending marrow cells in a plasma clot or in agar within the chambers, which permits the growth of colonies within the chamber. A fourth method of assessing toxicity is by the use of the continuous long-term in vitro culture system. In this system, proliferation of marrow cells is supported by an adherent layer of marrow stromal cells.(ABSTRACT TRUNCATED AT 400 WORDS)

Clipsham, R., K. Niakan, et al. (2004). "Nr0b1 and its network partners are expressed early in murine embryos prior to steroidogenic axis organogenesis." Gene Expr Patterns **4**(1): 3-14.

Ahch is an orphan nuclear receptor encoded by Nr0b1 on the murine X chromosome and is the ortholog of human DAX1. Nr0b1/NR0B1 expression at appropriate dosages is required for normal

steroidogenic axis development: mutation of the human ortholog, NR0B1, results in adrenal hypoplasia congenita and hypogonadotropic hypogonadism; and duplication or transgenic overexpression in humans or mice, respectively, results in XY phenotypic females, a phenotype known as dosage sensitive sex-reversal. Complete loss of Nr0b1 by targeted deletion has been hypothesized to be lethal in embryonic stem (ES) cells and preliminary evidence suggested that ES cells might express Nr0b1. These investigations examined Nr0b1 expression and its network partners in both cultured ES cells and preimplantation embryos. We cultured ES cells in the absence or presence of differentiation agents and analyzed expression of Nr0b1 and associated network partners by northern blot hybridization and reverse transcriptase-polymerase chain reaction. Nrob1 was highly expressed by totipotent ES cells with reduced expression following induction toward individual germ layer fates. Nr5a1/Sf1, Wt1 and other genes that encode proteins known to interact with Nr0b1 were also expressed. Immunohistochemical analysis of preimplantation embryos for Ahch and key partners confirmed in vivo expression of network components. These findings are consistent with the existence of a potentially functional network of transcription factors, including Ahch, very early in embryonic development. These results validate ES cells as a developmentally dynamic model for mechanistic investigations into this regulatory network early in embryogenesis preceding organogenesis.

Cogle, C. R., S. M. Guthrie, et al. (2003). "An overview of stem cell research and regulatory issues." Mayo Clin Proc **78**(8): 993-1003.

Stem cells are noted for their ability to self-renew and differentiate into a variety of cell types. Some stem cells, described as totipotent cells, have tremendous capacity to self-renew and differentiate. Embryonic stem cells have pluripotent capacity, able to form tissues of all 3 germ layers but unable to form an entire live being. Research with embryonic stem cells has enabled investigators to make substantial gains in developmental biology, therapeutic tissue engineering, and reproductive cloning. However, with these remarkable opportunities many ethical challenges arise, which are largely based on concerns for safety, efficacy, resource allocation, and methods of harvesting stem cells. Discussing the moral and legal status of the human embryo is critical to the debate on stem cell ethics. Religious perspectives and political events leading to regulation of stem cell research are presented and discussed, with special attention directed toward the use of embryonic stem cells for therapeutic and reproductive cloning. Adult stem cells were previously thought to have a restricted capacity to differentiate; however, several reports have described their plasticity potential. Furthermore, there have been close ties between the behavior of stem cells and cancer cells. True eradication of cancer will require a deeper understanding of stem cell biology. This article was written to inform medical scientists and practicing clinicians across the spectrum of medical education about the research and regulatory issues affecting the future of stem cell therapy.

Colombo, E., S. G. Giannelli, et al. (2006). "Embryonic stem-derived versus somatic neural stem cells: a comparative analysis of their developmental potential and molecular phenotype." Stem Cells **24**(4): 825-34.

Reliable procedures to induce neural commitment of totipotent undifferentiated embryonic stem (ES) cells have provided new tools for investigating the molecular mechanisms underlying cell fate choices. We extensively characterized the developmental potential of ES-induced neural cells obtained using an adaptation of the multistep induction protocol. We provided evidence that ES-derived neural proliferating cells are endowed with stem cell properties such as extensive self-renewal capacity and single-cell multipotency. In differentiating conditions, cells matured exclusively into neurons, astrocytes, and oligodendrocytes. All these features have been previously described in only somatic neural stem cells (NSCs). Therefore, we consider it more appropriate to rename our cells ES-derived NSCs. These similarities between the two NSC populations induced us to carefully compare their proliferation ability and differentiation potential. Although they were very similar in overall behavior, we scored specific differences. For instance, ES-derived NSCs proliferated at higher rate and consistently generated a higher number of neurons compared with somatic NSCs. To further investigate their relationships, we carried out a molecular analysis comparing their transcriptional profiles during proliferation. We observed a large fraction of shared expressed transcripts, including genes previously described to be critical in defining somatic NSC traits. Among the genes differently expressed, candidate genes possibly responsible for divergences between the two cell types were selected and further investigated. In particular, we showed that an enhanced MAPK (mitogen-activated protein kinase) signaling is acting in ES-induced NSCs, probably triggered by insulin-like growth factor-II. This may contribute to the high proliferation rate exhibited by these cells in culture.

Cooper, H. M., R. N. Tamura, et al. (1991). "The major laminin receptor of mouse embryonic stem cells is a novel isoform of the alpha 6 beta 1 integrin." J Cell Biol **115**(3): 843-50.

Laminin is the first extracellular matrix protein expressed in the developing mouse embryo. It is known to influence morphogenesis and affect cell migration and polarization. Several laminin receptors are included in the integrin family of extracellular matrix receptors. Ligand binding by integrin heterodimers results in signal transduction events controlling cell motility. We report that the major laminin receptor on murine embryonic stem (ES) cells is the integrin heterodimer alpha 6 beta 1, an important receptor for laminin in neurons, lymphocytes, macrophages, fibroblasts, platelets and other cell types. However, the cytoplasmic domain of the ES cell alpha 6 (alpha 6 B) differs totally from the reported cytoplasmic domain amino acid sequence of alpha 6 (alpha 6 A). Comparisons of alpha 6 cDNAs from ES cells and other cells suggest that the alpha 6 A and alpha 6 B cytoplasmic domains derive from alternative mRNA splicing. Anti-peptide antibodies to alpha 6 A are unreactive with ES cells, but react with mouse melanoma cells and embryonic fibroblasts. When ES cells are cultured under conditions that permit their differentiation, they become positive for alpha 6 A, concurrent with the morphologic appearance of differentiated cell types. Thus, expression of the alpha 6 B beta 1 laminin receptor may be favored in undifferentiated, totipotent cells, while the expression of alpha 6 A beta 1 receptor occurs in committed lineages. While the functions of integrin alpha chain cytoplasmic domains are not understood, it is possible that they contribute to transferring signals to the cell interior, e.g., by delivering cytoskeleton organizing signals in response to integrin engagement with extracellular matrix ligands. It is therefore reasonable to propose that the cellular responses to laminin may vary, according to what alpha subunit isoform (alpha 6 A or alpha 6 B) is expressed as part of the alpha 6 beta 1 laminin receptor. The switch from alpha 6 B to alpha 6 A, if confirmed in early embryos, could then be of striking potential relevance to the developmental role of laminin.

Dani, C., A. G. Smith, et al. (1997). "Differentiation of embryonic stem cells into adipocytes in vitro." J Cell Sci **110 (Pt 11)**: 1279-85.

Embryonic stem cells, derived from the inner cell mass of murine blastocysts, can be maintained in a totipotent state in vitro. In appropriate conditions embryonic stem cells have been shown to differentiate in vitro into various derivatives of all three primary germ layers. We describe in this paper conditions to induce differentiation of embryonic stem cells reliably and at high efficiency into adipocytes. A prerequisite is to treat early developing embryonic stem cell-derived embryoid bodies with retinoic acid for a precise period of time. Retinoic acid could not be substituted by adipogenic hormones nor by potent activators of peroxisome proliferator-activated receptors. Treatment with retinoic acid resulted in the subsequent appearance of large clusters of mature adipocytes in embryoid body outgrowths. Lipogenic and lipolytic activities as well as high level expression of adipocyte specific genes could be detected in these cultures. Analysis of expression of potential adipogenic genes, such as peroxisome proliferator-activated receptors gamma and delta and CCAAT/enhancer binding protein beta, during differentiation of retinoic acid-treated embryoid bodies has been performed. The temporal pattern of expression of genes encoding these nuclear factors resembled that found during mouse embryogenesis. The differentiation of embryonic stem cells into adipocytes will provide an invaluable model for the characterisation of the role of genes expressed during the adipocyte development programme and for the identification of new adipogenic regulatory genes.

Dattena, M., S. Pilichi, et al. (2009). "Sheep embryonic stem-like cells transplanted in full-thickness cartilage defects." J Tissue Eng Regen Med **3**(3): 175-87.

Articular cartilage regeneration is limited. Embryonic stem (ES) cell lines provide a source of totipotent cells for regenerating cartilage. Anatomical, biomechanical, physiological and immunological similarities between humans and sheep make this animal an optimal experimental model. This study examines the repair process of articular cartilage in sheep after transplantation of ES-like cells isolated from inner cell masses (ICMs) derived from in vitro-produced (IVP) vitrified embryos. Thirty-five ES-like colonies from 40 IVP embryos, positive for stage-specific embryonic antigens (SSEAs), were pooled in groups of two or three, embedded in fibrin glue and transplanted into osteochondral defects in the medial femoral condyles of 14 ewes. Empty defect (ED) and cell-free glue (G) in the controlateral stifle joint served as controls. The Y gene sequence was used to detect ES-like cells in the repair tissue by in situ hybridization (ISH). Two ewes were euthanized at 1 month post-operatively, three each at 2 and 6 months and four at 12 months. Repairing tissue was examined by biomechanical, macroscopic, histological, immunohistochemical (collagen type II) and ISH assays. Scores of all treatments showed no statistical significant differences among treatment groups at a given time period, although ES-like grafts showed a

tendency toward a better healing process. ISH was positive in all ES-like specimens. This study demonstrates that ES-like cells transplanted into cartilage defects stimulate the repair process to promote better organization and tissue bulk. However, the small number of cells applied and the short interval between surgery and euthanasia might have negatively affected the results.

Davidson, A. J., S. A. Freeman, et al. (1997). "Expression of murine interleukin 11 and its receptor alpha-chain in adult and embryonic tissues." Stem Cells **15**(2): 119-24.

Interleukin 11 (IL-11) is a multifunctional cytokine that has diverse effects on blood cells and their precursors and on a number of cell types outside of the hematopoietic system. The cDNAs encoding murine IL-11 and its receptor alpha-chain (IL-11R alpha) have recently been isolated. We have used the RNase protection assay to examine the expression of murine IL-11 and IL-11R alpha in a range of adult mouse tissues, in embryos, and during development of embryonic stem (ES) cells into cystic embryoid bodies in vitro. The testis showed a high level of IL-11 gene expression while a much lower level of expression was detected in the lung, stomach, small intestine, and large intestine. Expression of IL-11 was not detected between day 10.5 and day 18.5 post coitum of embryonic development or in differentiating ES cells in vitro. In contrast, the IL-11R alpha was found to be expressed in all adult tissues examined, during embryonic development, and in totipotent and differentiating ES cells.

de Kretser, D. (2007). "Totipotent, pluripotent or unipotent stem cells: a complex regulatory enigma and fascinating biology." J Law Med **15**(2): 212-8.

The search for sources of human stem cells has become a controversial topic from an ethical point of view primarily as it has required the destruction of human embryos. The development of alternative techniques that enable the generation of pluripotent stem cells from adult cells has opened new avenues of research but the generation of such cells has again been controversial since it requires the use of human eggs, using a technique called somatic cell nuclear transfer. Since the cells so generated have a very small potential to generate an "embryo" and since the production of the cell lines requires destruction of that "embryo", a further ethical issue arises. This article discusses these issues and suggests a framework that may assist their consideration. Finally, the article reviews some recent developments that have the potential to remove the need for the use of eggs or embryos in the generation of stem cell lines and highlights the danger of developing legislation on only our current knowledge.

De Mulder, K., D. Pfister, et al. (2009). "Stem cells are differentially regulated during development, regeneration and homeostasis in flatworms." Dev Biol **334**(1): 198-212.

The flatworm stem cell system is exceptional within the animal kingdom, as totipotent stem cells (neoblasts) are the only dividing cells within the organism. In contrast to most organisms, piwi-like gene expression in flatworms is extended from germ cells to somatic stem cells. We describe the isolation and characterization of the piwi homologue macpiwi in the flatworm Macrostomum lignano. We use in situ hybridization, antibody staining and RNA interference to study macpiwi expression and function in adults, during postembryonic development, regeneration and upon starvation. We found novelties regarding piwi function and observed differences to current piwi functions in flatworms. First, macpiwi was essential for the maintenance of somatic stem cells in adult animals. A knock-down of macpiwi led to a complete elimination of stem cells and death of the animals. Second, the regulation of stem cells was different in adults and regenerates compared to postembryonic development. Third, sexual reproduction of M. lignano allowed to follow germline formation during postembryonic development, regeneration, and starvation. Fourth, piwi expression in hatchlings further supports an embryonic formation of the germline in M. lignano. Our findings address new questions in flatworm stem cell research and provide a basis for comparison with higher organisms.

De Sousa, P. A., S. J. da Silva, et al. (2004). "Neurotrophin signaling in oocyte survival and developmental competence: a paradigm for cellular toti-potency." Cloning Stem Cells **6**(4): 375-85.

While not fulfilling the criterion of a "stem cell" in being capable of self-renewal, mature and fertilized oocytes are the original "toti-potent" cells, whose capacity for expansion and differentiation can only be approximated by stem cells of embryonic or adult origin in vitro. As such, the mechanisms by which oocytes acquire and manifest competence to support embryo development is of fundamental interest to efforts to control and re-specify somatic cell fate and toti-potency. This is underscored by the unparalleled capacity of oocyte cytoplasm to successfully re-specify the genetic program of animal development following cell nuclear replacement (i.e., cloning). Thus, the knowledge gained by understanding the acquisition of oocyte developmental competence could ultimately facilitate the creation of adult stem cells in vitro from terminally differentiated

cells, ex ovo. In this paper, we review the concept of oocyte developmental competence, and focus on our own research and that of others implicating a role for neurotrophins in this process, and that of oocyte cell survival. Lastly we propose a role for neurotrophin signalling in embryo stem cell survival.

de Vries, W. N., A. V. Evsikov, et al. (2008). "Reprogramming and differentiation in mammals: motifs and mechanisms." Cold Spring Harb Symp Quant Biol **73**: 33-8.

The natural reprogramming of the mammalian egg and sperm genomes is an efficient process that takes place in less than 24 hours and gives rise to a totipotent zygote. Transfer of somatic nuclei to mammalian oocytes also leads to their reprogramming and formation of totipotent embryos, albeit very inefficiently and requiring an activation step. Reprogramming of differentiated cells to induced pluripotent stem (iPS) cells takes place during a period of time substantially longer than reprogramming of the egg and sperm nuclei and is significantly less efficient. The stochastic expression of endogenous proteins during this process would imply that controlled expression of specific proteins is crucial for reprogramming to take place. The fact that OCT4, NANOG, and SOX2 form the core components of the pluripotency circuitry would imply that control at the transcriptional level is important for reprogramming to iPS cells. In contradistinction, the much more efficient reprogramming of the mammalian egg and sperm genomes implies that other levels of control are necessary, such as chromatin remodeling, translational regulation, and efficient degradation of no longer needed proteins and RNAs.

Deacon, T., J. Dinsmore, et al. (1998). "Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation." Exp Neurol **149**(1): 28-41.

In order to assess the potential of embryonic stem cells to undergo neuronal differentiation in vivo, totipotent stem cells from mouse blastocysts (D3 and E14TG2a; previously expanded in the presence of leukemia inhibitory factor) were transplanted, with or without retinoic acid pretreatment, into adult mouse brain, adult lesioned rat brain, and into the mouse kidney capsule. Intracerebral grafts survived in 61% of cyclosporine immunosuppressed rats and 100% of mouse hosts, exhibited variable size and morphology, and both intracerebral and kidney capsule grafts developed large numbers of cells exhibiting neuronal morphology and immunoreactivity for neurofilament, neuron-specific enolase, tyrosine hydroxylase (TH), 5-hydroxytryptamine (5-HT), and cells immunoreactive for glial fibrillary acidic protein. Though graft size and histology were variable, typical grafts of 5-10 mm3 contained 10-20,000 TH+ neurons, whereas dopamine-beta-hydroxylase+ cells were rare. Most grafts also included nonneuronal regions. In intracerebral grafts, large numbers of astrocytes immunoreactive for glial fibrillary acidic protein were present. Both TH+ and 5-HT+ axons from intracerebral grafts grew into regions of the dopamine-lesioned host striatum. TH+ axons grew preferentially into striatal gray matter, while 5-HT+ axons showed no white/gray matter preference. These findings demonstrate that transplantation to the brain or kidney capsule can induce a significant fraction of totipotent embryonic stem cells to become putative dopaminergic or serotonergic neurons and that when transplanted to the brain these neurons are capable of innervating the adult host striatum.

Dean, W., F. Santos, et al. (2003). "Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer." Semin Cell Dev Biol **14**(1): 93-100.

Epigenetic modifications of the genome play a significant role in the elaboration of the genetic code as established at fertilisation. These modifications affect early growth and development through their influence on gene expression especially on imprinted genes. Genome-wide epigenetic reprogramming in germ cells is essential in order to reset the parent-of-origin specific marking of imprinted genes, but may have a more general role in the restoration of totipotency in the early embryo. In a similar way, on somatic nuclear cloning, a differentiated cell must become 'reprogrammed' restoring totipotency in order to undergo development. Here we discuss the dynamic epigenetic reprogramming that takes place during normal development and highlight those areas with relevance to somatic nuclear cloning and the possibility of improving the efficiency of this process. We propose the concept of 'epigenetic checkpoints' for normal progression of development and the loss of totipotency.

Del Vecchio, F., A. Filareto, et al. (2005). "Cellular genetic therapy." Transplant Proc **37**(6): 2657-61.

Cellular genetic therapy is the ultimate frontier for those pathologies that are consequent to a specific nonfunctional cellular type. A viable cure for there kinds of diseases is the replacement of sick cells with healthy ones, which can be obtained from the same patient or a different donor. In fact, structures can be corrected and strengthened with the introduction of undifferentiated cells within specific target tissues, where they will specialize into the desired cellular types. Furthermore, consequent to the recent results obtained with the transdifferentiation

experiments, a process that allows the in vitro differentiation of embryonic and adult stem cells, it has also became clear that many advantages may be obtained from the use of stem cells to produce drugs, vaccines, and therapeutic molecules. Since stem cells can sustain lineage potentials, the capacity for differentiation, and better tolerance for the introduction of exogenous genes, they are also considered as feasible therapeutic vehicles for gene therapy. In fact, it is strongly believed that the combination of cellular genetic and gene therapy approaches will definitely allow the development of new therapeutic strategies as well as the production of totipotent cell lines to be used as experimental models for the cure of genetic disorders.

Denker, H. W. (2006). "Potentiality of embryonic stem cells: an ethical problem even with alternative stem cell sources." J Med Ethics **32**(11): 665-71.

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

Dewey, M. J., D. W. Martin, Jr., et al. (1977). "Mosaic mice with teratocarcinoma-derived mutant cells deficient in hypoxanthine phosphoribosyltransferase." Proc Natl Acad Sci U S A **74**(12): 5564-8.

Mutagenized stem cells of a cultured mouse teratocarcinoma cell line were selected for resistance to the purine base analog 6-thioguanine. Cells of a resistant clone were completely deficient in activity of the enzyme hypoxanthine phosphoribosyltransferase (HPRT, IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8), the same X-linked lesion as occurs in human Lesch-Nyhan disease. After microinjection into blastocysts of another genetic strain, the previously malignant cells successfully participated in normal embryogenesis and tumor-free, viable mosaic mice were obtained. Cells of tumor lineage were identified by strain markers in virtually all tissues of some individuals. Mature function of those cells was evident from their tissue-specific products (e.g., melanins, liver proteins). These mutagenized teratocarcinoma cells are therefore developmentally totipotent. Retention of the severe HPRT deficiency in the differentiated state was documented in extracts of mosaic tissues by depressed specific activity of the enzyme, and also by presence of unlabeled clones in autoradiographs of explanted cells incubated in [(3)H]hypoxanthine. Some mosaic individuals had mutant-strain cells in only one or a few tissues. Such animals may provide unique opportunities to identify the tissue sources of particular aspects of the complex disease syndrome. The tissue distribution of HPRT-deficient cells suggests that selection against them is particularly strong in blood of the mosaic mice, as is already known to be the case in human heterozygotes. This phenotypic parallelism supports the expectation that afflicted F(1) male mice that might be obtained from mutant germ cells can serve as a model of the human disease.

Do, J. T., D. W. Han, et al. (2006). "Reprogramming somatic gene activity by fusion with pluripotent cells." Stem Cell Rev **2**(4): 257-64.

Fertilized eggs and early blastomeres, that have the potential to develop to fetuses when placed into a uterus, are totipotent. Those cells in the embryo, that can give rise to all cell types of an organism, but not to an organism itself, are pluripotent. Embryonic stem (ES), embryonic carcinoma (EC), and embryonic germ (EG) cells are powerful in vitro artifacts derived from different embryonic stages and are pluripotent. Totipotent and pluripotent cells have the potential to greatly benefit biological research and medicine. One powerful feature is that the genetic program of somatic cells can be converted into that of totipotent or pluripotent cells, as shown by nuclear transfer or cell fusion experiments. During reprogramming by cell fusion various features of pluripotent cells are acquired. These include the typical morphology of the respective pluripotent fusion partner, a specific epigenetic state, a specific gene profile, inactivation of tissue-specific genes expressed in the somatic fusion partner, and the developmental as well as differentiation potential of pluripotent cells. In this review, we will discuss what is known about the reprogramming process mediated by cell fusion and the potential use of fusion-induced reprogramming for therapeutic applications.

Dominguez-Bendala, J., H. Priddle, et al. (2003). "Elevated expression of exogenous Rad51 leads to identical increases in gene-targeting frequency in murine embryonic stem (ES) cells with both functional and dysfunctional p53 genes." Exp Cell Res **286**(2): 298-307.

The Rad51 gene is the mammalian homologue of the bacterial RecA gene and catalyses homologous recombination in mammalian cells. In some cell types Rad51 has been shown to interact with p53, leading to inhibition of Rad51 activity. Here, we show a two- to four-fold increase in gene-targeting frequency at the HPRT locus using murine ES clones preengineered to overexpress Rad51, and a twofold increase in targeting frequency when a Rad51 expression cassette was cointroduced to wild-type ES cells with the targeting construct. In addition to its effect on homologous recombination, we show that Rad51 may down-regulate illegitimate recombination. We investigated the dependence of these phenomena upon p53 and found no evidence that the Rad 51-mediated increase is affected by the functional status of p53, a conclusion supported by the observed cytoplasmic localisation of p53 in ES cells following electroporation. Furthermore, in the absence of additional Rad51, p53-deficient ES cells do not have elevated rates of homologous recombination with extrachromosomal DNA. These findings demonstrate that Rad51 levels modify both homologous and illegitimate recombination, but that these phenomena are independent of p53 status.

Drab, M., H. Haller, et al. (1997). "From totipotent embryonic stem cells to spontaneously contracting smooth muscle cells: a retinoic acid and db-cAMP in vitro differentiation model." Faseb J **11**(11): 905-15.

Vascular smooth muscle cell (VSMC) differentiation is important in understanding vascular disease; however, no in vitro model is available. Totipotent mouse embryonic stem (ES) cells were used to establish such a model. To test whether the ES cell-derived smooth muscle cells expressed VSMC-specific properties, the differentiated cells were characterized by 1) morphological analysis, 2) gene expression, 3) immunostaining for VSMC-specific proteins, 4) expression of characteristic VSMC ion channels, and 5) formation of [Ca2+]i transients in response to VSMC-specific agonists. Treatment of embryonic stem cell-derived embryoid bodies with retinoic acid and dibutyryl-cyclic adenosine monophosphate (db-cAMP) induced differentiation of spontaneously contracting cell clusters in 67% of embryoid bodies compared with 10% of untreated controls. The highest differentiation rate was observed when retinoic acid and db-cAMP were applied to the embryoid bodies between days 7 and 11 in combination with frequent changes of culture medium. Other protocols with retinoic acid and db-cAMP, as well as single or combined treatment with VEGF, ECGF, bFGF, aFGF, fibronectin, matrigel, or hypoxia did not influence the differentiation rate. Single-cell RT-PCR and sequencing of the PCR products identified myosin heavy chain (MHC) splice variants distinguishing between gut and VSMC isoforms. RT-PCR with VSMC-specific MHC primers and immunostaining confirmed the presence of VSMC transcripts and MHC protein. Furthermore, VSMC expressing MHC had typical ion channels and responded to specific agonists with an increased [Ca2+]i. Here we present a retinoic acid + db-cAMP-inducible embryonic stem cell model of in vitro vasculogenesis. ES cell-derived cells expressing VSMC-specific MHC and functional VSMC properties may be a suitable system to study mechanisms of VSMC differentiation.

Dupin, E., F. Sextier-Sainte-Claire Deville, et al. (1993). "The ontogeny of the neural crest." C R Acad Sci III **316**(9): 1062-81.

The neural crest is part of a larger embryonic structure, the neural folds, belonging to the neural primordium of the Vertebrate embryo. The neural fold is formed by the anterior and lateral ridges of the neural anlage, which fuse mediodorsally when the neural tube closes. Anteriorly, the epithelium of the neural fold does not convert into mesenchymal cells and yields Rathke's pouch, the olfactory organ and the epithelium of the mouth roof, of the upper lip and of the frontal region of the head. From the level of the diencephalon (at the level of the epiphysis) downwards the neural fold epithelium undergoes the epitheliomesenchymal transition and yields the neural crest cells which become later on highly diversified and form various structures and tissues throughout the body. A large amount of data have shown that the environmental cues exerted on crest cells both during their migration and when they have reached their target sites are critical in determining their fate. In order to understand the mechanisms through which environmental factors influence crest cell differentiation, the developmental capacities of single neural crest cells were investigated at different time points of their ontogeny. Single cell cultures of crest cells have revealed that already at the migratory stage the neural crest is made up of cells at different states of determination. In particular, the analysis of clones obtained from single cell cultures of cephalic migratory crest cells has shown that, although many clonogenic cells are multipotent to varying degrees, others are committed to give rise to one single derivative. Totipotent progenitors able to generate

representatives of virtually all the phenotypes (neuronal, glial, melanocytic and mesectodermal) encountered in cephalic neural crest derivatives were also found. We proposed that they represent stem cells analogous to those which in the hemopoietic system generate the various types of blood cells. The neural crest stem cell gives rise to diverse progenitors that become progressively restricted in their potentialities according to an essentially stochastic mechanism while dividing during and after completion of the migration process. Similar cloning experiments of crest cells that have already reached their target organs, i. e. sensory ganglia or enteric plexuses, showed that the phenotypic repertoire expressed by crest-derived cells decreases with increasing embryonic age. Efforts are made to elucidate the nature of the factors which influence either the survival and/or the differentiation of neural crest cells in the various types of environments in which they evolve.(ABSTRACT TRUNCATED AT 400 WORDS)

Dutra, H. S., M. I. Rossi, et al. (1997). "Haematopoietic capacity of colony-forming cells mobilized in hepatic inflammatory reactions as compared to that of normal bone marrow cells." Res Immunol **148**(7): 437-44.

Chronic inflammatory periovular granulomatous reactions elicited in liver by schistosomal infection are a site of active myelopoiesis. We quantified the colony-forming cells (CFCs) in granulomas and found that the whole liver contains a number of CFCs roughly equivalent to 50% of a femur. Clonogenic analysis showed the presence of committed as well as pluripotent and totipotent CFCs. Long-term Dexter-type cultures showed that the granuloma-derived totipotent CFCs do not have self-renewal capacity. Hence, they did not correspond functionally to haematopoietic stem cells, despite the fact that the stroma established by adherent cells harvested from granulomas had the capacity to sustain long-term proliferation of bone-marrow-derived haematopoietic stem cells. We conclude that myelopoietic cytokines produced by inflammatory reactions in schistosomiasis elicit mobilization of bone marrow CFCs into the circulation, which can settle in hepatic granulomas. This environment may induce their proliferation and differentiation, but not their self-renewal, sustaining temporary production of myeloid cell lineages which nevertheless depends upon cell renewal from the bone marrow pool of haematopoietic precursors.

Eaves, C., P. Zandstra, et al. (1998). "Changes in the cytokine regulation of stem cell self-renewal during ontogeny." Stem Cells **16 Suppl 1**: 177-84.

The last 10 years have seen the development of a quantitative assay that is specific for transplantable totipotent murine hematopoietic cells with durable in vivo blood-forming ability. Recently, this assay has been successfully adapted to allow the detection and enumeration of an analogous population of human hematopoietic stem cells using myelosuppressed immunodeficient (nonobese diabetic/severe-combined immunodeficiency) mice as recipients. Characterization of the cells detected by this assay indicates their close relationship in both mice and humans with cells detected in vitro as long-term culture-initiating cells (LTC-IC). Culture conditions have now been identified that support a significant net expansion of these cells from both species. More detailed analyses of the cytokine requirements for this response indicate that the viability, mitogenesis and maintenance of LTC-IC function by human CD34+ CD38- cells can be independently regulated by exogenous factors. Superimposed on this uncoupling of hematopoietic stem cell "self-renewal" and proliferation control is a change during ontogeny in the particular cytokines that regulate their responses. These findings unite stochastic and deterministic models of hematopoietic stem cell control through the concept of a molecular mechanism that actively blocks stem cell differentiation and must be maintained when these cells are stimulated to divide by exposure to certain types and concentrations of cytokines.

Eaves, C. J., J. D. Cashman, et al. (1991). "Molecular analysis of primitive hematopoietic cell proliferation control mechanisms." Ann N Y Acad Sci **628**: 298-306.

Cells at two distinct early stages in the development of mature human blood cells from primitive totipotent hematopoietic stem cells can now be defined and quantitated by separate in vitro assays. Current evidence suggests that most, if not all, colony-forming cells--that is, cells that give rise to colonies of mature progeny within one to three weeks in semisolid culture systems, represent an intermediate stage of hematopoietic progenitor. These cells are not self-sustaining; if they are used to initiate hematopoiesis on competent marrow stromal layers, they rapidly disappear as they differentiate or die. However, clonogenic cells can be generated in such cultures from another cell type over a period of four to eight weeks. We have, therefore, assigned the term long-term culture initiating cell (LTC-IC) to this latter type of clonogenic precursor cell. The production and differentiation of cells in both of these compartments in LTC are dependent on, and regulated by, nonhematopoietic "stromal" cells that form a heterogeneous adherent layer in which close-range

interactions with hematopoietic cells take place. The use of separate endpoints to monitor the maintenance, differentiation, and reversible activation or arrest of cycling of these cells has recently revealed different molecular mechanisms regulating their respective functions. However, an important common feature appears to be the relative local concentration of positive and negative regulators to which the target hematopoietic cell is exposed. Both gene expression and growth factor release measurements as well as results obtained using genetically engineered stroma and repeated soluble growth factor addition implicate G-CSF as an endogenous positive regulator of primitive hematopoietic cells. Similarly, gene expression, factor production, factor addition, and neutralizing antibody experiments implicate TGF-beta as an endogenous inhibitor of primitive hematopoietic cells.

Eaves, C. J., H. J. Sutherland, et al. (1992). "The human hematopoietic stem cell in vitro and in vivo." Blood Cells **18**(2): 301-7.

A quantitative assay for a primitive human hematopoietic cell has been developed. The cell identified has been assigned the operational designation of long-term culture (LTC)-initiating cell based on its ability when cultured on supportive fibroblast monolayers to give rise to daughter cell(s) detectable by standard in vitro colony assays. Three lines of evidence support the view that the LTC-initiating cell assay may allow the relatively specific enumeration of totipotent cells with in vivo reconstituting potential. These involve the demonstration: (1) that conditions in analogous murine long-term cultures stimulate the extensive amplification (self-renewal) of some totipotent long-term repopulating cells, (2) that most of the LTC-initiating cells in normal human bone marrow are phenotypically different from most of the colony-forming cells present in the same cell suspensions in their possession of a number of characteristics specifically associated with transplantable stem cells; and (3) that cultured marrow cells from patients with chronic myeloid leukemia which, after maintenance under LTC conditions for 10 days contain some normal LTC-initiating cells but no detectable leukemic LTC-initiating cells, can after autografting reconstitute the hematopoietic system with normal cells.

Eggan, K., K. Baldwin, et al. (2004). "Mice cloned from olfactory sensory neurons." Nature **428**(6978): 44-9.

Cloning by nuclear transplantation has been successfully carried out in various mammals, including mice. Until now mice have not been cloned from post-mitotic cells such as neurons. Here, we have generated fertile mouse clones derived by transferring the nuclei of post-mitotic, olfactory sensory neurons into oocytes. These results indicate that the genome of a post-mitotic, terminally differentiated neuron can re-enter the cell cycle and be reprogrammed to a state of totipotency after nuclear transfer. Moreover, the pattern of odorant receptor gene expression and the organization of odorant receptor genes in cloned mice was indistinguishable from wild-type animals, indicating that irreversible changes to the DNA of olfactory neurons do not accompany receptor gene choice.

Egger, B., P. Ladurner, et al. (2006). "The regeneration capacity of the flatworm Macrostomum lignano--on repeated regeneration, rejuvenation, and the minimal size needed for regeneration." Dev Genes Evol **216**(10): 565-77.

The lion's share of studies on regeneration in Plathelminthes (flatworms) has been so far carried out on a derived taxon of rhabditophorans, the freshwater planarians (Tricladida), and has shown this group's outstanding regeneration capabilities in detail. Sharing a likely totipotent stem cell system, many other flatworm taxa are capable of regeneration as well. In this paper, we present the regeneration capacity of Macrostomum lignano, a representative of the Macrostomorpha, the basal-most taxon of rhabditophoran flatworms and one of the most basal extant bilaterian protostomes. Amputated or incised transversally, obliquely, and longitudinally at various cutting levels, M. lignano is able to regenerate the anterior-most body part (the rostrum) and any part posterior of the pharynx, but cannot regenerate a head. Repeated regeneration was observed for 29 successive amputations over a period of almost 12 months. Besides adults, also first-day hatchlings and older juveniles were shown to regenerate after transversal cutting. The minimum number of cells required for regeneration in adults (with a total of 25,000 cells) is 4,000, including 160 neoblasts. In hatchlings only 1,500 cells, including 50 neoblasts, are needed for regeneration. The life span of untreated M. lignano was determined to be about 10 months.

Enright, B. P., L. Y. Sung, et al. (2005). "Methylation and acetylation characteristics of cloned bovine embryos from donor cells treated with 5-aza-2'-deoxycytidine." Biol Reprod **72**(4): 944-8.

Differentiated somatic cells and embryos cloned from somatic cells by nuclear transfer (NT) have higher levels of DNA methylation than gametes and early embryos produced in vivo. Reducing DNA methylation in donor cells before NT by treating them with chemicals such as the DNA methyl-transferase

inhibitor (5-aza-2'-deoxycytidine; 5-aza-dC) may improve cloning efficiency of NT embryos by providing donor cells with similar epigenetic characteristics as in vivo embryos. Previously, high levels of this reagent were used to treat donor cells, and decreased development of cloned embryos was observed. In this study, we tested a lower range (0.005 to 0.08 microM) of this drug and used cell cycle distribution changes as an indicator of changes in the characteristics of donor cells. We found that at 0.01 microM 5-aza-dC induced changes in the cycle stage distribution of donor cells, increased the fusion rate of NT embryos, and had no deleterious effect on the percentage of blastocyst development. Levels of 5-aza-dC greater than 0.01 microM significantly decreased embryo development. Embryos cloned from donor cells treated with a low dose of 5-aza-dC had higher levels of DNA methylation than embryos produced by in vitro fertilization, but they also had higher levels of histone acetylation. Although 5-aza-dC at 0.04 microM or higher reduced DNA methylation and histone acetylation levels to those of in vitro-fertilized embryos, development to blastocyst was reduced, suggesting that this concentration of the drug was detrimental. In summary, 5-aza-dC at 0.01 microM altered donor cell characteristics while showing no deleterious effects on embryos cloned from treated cells.

Estrov, Z. (2009). "Stem cells and somatic cells: reprogramming and plasticity." Clin Lymphoma Myeloma **9 Suppl 3**: S319-28.

Recent seminal discoveries have significantly advanced the field of stem cell research and received worldwide attention. Improvements in somatic cell nuclear transfer (SCNT) technology, enabling the cloning of Dolly the sheep, and the derivation and differentiation of human embryonic stem cells raised hopes that normal cells could be generated to replace diseased or injured tissue. At the same time, in vitro and in vivo studies demonstrated that somatic cells of one tissue are capable of generating cells of another tissue. It was theorized that any cell might be reprogrammed, by exposure to a new environment, to become another cell type. This concept contradicts two established hypotheses: (1) that only specific tissues are generated from the endoderm, mesoderm, and ectoderm and (2) that tissue cells arise from a rare population of tissue-specific stem cells in a hierarchical fashion. SCNT, cell fusion experiments, and most recent gene transfer studies also contradict these hypotheses, as they demonstrate that mature somatic cells can be reprogrammed to regain pluripotent (or even totipotent) stem cell capacity. On the basis of the stem cell theory, hierarchical cancer stem cell differentiation models have been proposed. Cancer cell plasticity is an established phenomenon that supports the notion that cellular phenotype and function might be altered. Therefore, mechanisms of cellular plasticity should be exploited and the clinical significance of the cancer stem cell theory cautiously assessed.

Ferri, A. L., M. Cavallaro, et al. (2004). "Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain." Development **131**(15): 3805-19.

In many species, the Sox2 transcription factor is a marker of the nervous system from the beginning of its development, and we have previously shown that Sox2 is expressed in embryonic neural stem cells. It is also expressed in, and is essential for, totipotent inner cell mass stem cells and other multipotent cell lineages, and its ablation causes early embryonic lethality. To investigate the role of Sox2 in the nervous system, we generated different mouse mutant alleles: a null allele (Sox2beta-geo 'knock-in'), and a regulatory mutant allele (Sox2DeltaENH), in which a neural cell-specific enhancer is deleted. Sox2 is expressed in embryonic early neural precursors of the ventricular zone and, in the adult, in ependyma (a descendant of the ventricular zone). It is also expressed in the vast majority of dividing precursors in the neurogenic regions, and in a small proportion of differentiated neurones, particularly in the thalamus, striatum and septum. Compound Sox2(beta-geo/DeltaENH) heterozygotes show important cerebral malformations, with parenchymal loss and ventricle enlargement, and L-dopa-rescuable circling behaviour and epilepsy. We observed striking abnormalities in neurones; degeneration and cytoplasmic protein aggregates, a feature common to diverse human neurodegenerative diseases, are observed in thalamus, striatum and septum. Furthermore, ependymal cells show ciliary loss and pathological lipid inclusions. Finally, precursor cell proliferation and the generation of new neurones in adult neurogenic regions are greatly decreased, and GFAP/nestin-positive hippocampal cells, which include the earliest neurogenic precursors, are strikingly diminished. These findings highlight a crucial and unexpected role for Sox2 in the maintenance of neurones in selected brain areas, and suggest a contribution of neural cell proliferative defects to the pathological phenotype.

Finch, K. A., G. Fonseka, et al. (2008). "Nuclear organisation in totipotent human nuclei and its relationship to chromosomal abnormality." J Cell Sci **121**(Pt 5): 655-63.

Studies of nuclear organisation, most commonly determining the nuclear location of

chromosome territories and individual loci, have furthered our understanding of nuclear function, differentiation and disease. In this study, by examining eight loci on different chromosomes, we tested hypotheses that: (1) totipotent human blastomeres adopt a nuclear organisation akin to that of committed cells; (2) nuclear organisation is different in chromosomally abnormal blastomeres; and (3) human blastomeres adopt a ;chromocentre' pattern. Analysis of in vitro fertilisation (IVF) conceptuses permits valuable insight into the cell biology of totipotent human nuclei. Here, extrapolations from images of preimplantation genetic screening (PGS) cases were used to make comparisons between totipotent blastomeres and several committed cells, showing some differences and similarities. Comparisons between chromosomally abnormal nuclei and those with no detected abnormality (NDA) suggest that the former display a significant non-random pattern for all autosomal loci, but there is a less distinct, possibly random, pattern in 'NDA' nuclei. No evidence was found that the presence of an extra chromosome is accompanied by an altered nuclear location for that chromosome. Centromeric loci on chromosomes 15 and 16 normally seen at the nuclear periphery were mostly centrally located in aneuploid cells, providing some evidence of a 'chromocentre'; however, the chromosome-18 centromere was more peripheral, similar to committed cells. Our results provide clues to the nature of totipotency in human cells and might have future applications for preimplantation diagnosis and nuclear transfer.

First, N. L., M. M. Sims, et al. (1994). "Systems for production of calves from cultured bovine embryonic cells." Reprod Fertil Dev **6**(5): 553-62.

The development of totipotent bovine embryonic cell cultures has great value in cattle breeding. They provide: (1) a mechanism for making large numbers of clonal offspring by nuclear transfer; (2) an efficient gene transfer system through the use of selectable markers to select transgenic cells; and (3) a mechanism for site-specific gene transfer or deletion by homologous DNA sequence recombination. Bovine embryonic cell cultures have been established from blastocyst inner cell mass (ICM) cells, morulae and the precompaction 16-20-cell stage. All have exhibited similar morphology to mouse embryonic stem (ES) cells, pluripotency on differentiation and proliferation in culture. Culture systems have consisted of microdrop loose suspension short-term cultures or long-term cultures on bovine or murine fibroblast feeder layers, in either a microdrop or a culture dish. The relative merit of culture systems or media requirements for mitosis and prevention of differentiation have not been determined. At present, totipotency is also unknown for cultured cells of the 16-20-cell stage. For cultured ICM cells, totipotency was demonstrated by the birth of four calves from ICM cells cultured 27 days or less in a loose suspension microdrop. Advanced pluripotency and perhaps totipotency was demonstrated in one fetus in a recently reported study where morulae cells cultured in vitro were chimaerized with non-cultured cells. DNA fingerprinting to associate cell lines with offspring and karyotyping to ascertain chromatin normalcy is important in ES cell research. Data pertaining to the use of each are presented.

Fleischman, R. A., R. P. Custer, et al. (1982). "Totipotent hematopoietic stem cells: normal self-renewal and differentiation after transplantation between mouse fetuses." Cell **30**(2): 351-9.

Successful engraftment of mouse fetal liver cells in early fetal recipients, after microinjection via the placental circulation, is attributable to seeding of the recipient's liver by a cell type that is ancestral to both the myeloid and lymphoid definitive lineages and is capable of sustained self-renewal and differentiation for more than 2 years. This primitive cell is therefore the normal totipotent hematopoietic stem cell (THSC). The use of a large series of mutant anemic recipients with decreasing severity of an endogenous stem-cell defect (W/W, Wv/Wv, Wf/Wf, Wv/+), and therefore of graded selective advantage to normal donor cells, has revealed that engraftment entails marginal numbers of cells--probably individual ones--in the least afflicted hosts. Thus the observed progressive and coordinate shift toward donor-strain erythrocytes, granulocytes and B and T lymphocytes, over time, indicates THSC expansion to form a larger stem-cell pool and normally regulated differentiation of cells from the pool. This transplant system allows allogeneic combinations with impunity and therefore provides many novel experimental possibilities for investigating THSC normal development, genetic abnormalities or neoplastic potential in relation to the intact developmental succession of hematopoietic tissue environments in vivo.

Fleischman, R. A. and B. Mintz (1984). "Development of adult bone marrow stem cells in H-2-compatible and -incompatible mouse fetuses." J Exp Med **159**(3): 731-45.

Bone marrow of normal adult mice was found, after transplacental inoculation, to contain cells still able to seed the livers of early fetuses. The recipients' own hematopoietic stem cells, with a W-mutant defect, were at a selective disadvantage. Progression of donor strain cells to the bone marrow, long-term self-renewal, and differentiation into

myeloid and lymphoid derivatives was consistent with the engraftment of totipotent hematopoietic stem cells (THSC) comparable to precursors previously identified (4) in normal fetal liver. More limited stem cells, specific for the myeloid or lymphoid cell lineages, were not detected in adult bone marrow. The bone marrow THSC, however, had a generally lower capacity for self-renewal than did fetal liver THSC. They had also embarked upon irreversible changes in gene expression, including partial histocompatibility restriction. While completely allogeneic fetal liver THSC were readily accepted by fetuses, H-2 incompatibility only occasionally resulted in engraftment of adult bone marrow cells and, in these cases, was often associated with sudden death at 3-5 mo. On the other hand, H-2 compatibility, even with histocompatibility differences at other loci, was sufficient to ensure long-term success as often as with fetal liver THSC.

Flohr, T. R., H. Bonatti, Jr., et al. (2009). "The use of stem cells in liver disease." Curr Opin Organ Transplant **14**(1): 64-71.

PURPOSE OF REVIEW: Cell transplantation to restore liver function as an alternative to whole liver transplantation has thus far not been successful in humans. RECENT FINDINGS: Adult mature hepatocytes and various populations of liver progenitors and stem cells are being studied for their regenerative capabilities. Hepatocyte transplantation to treat metabolic deficiencies has shown promising early improvement in liver function; however, long-term success has not been achieved. Liver progenitor cells can now be identified and were shown to be capable to differentiate into a hepatocyte-like phenotype. Despite evidence of mesenchymal stem cell fusion in animal models of liver regeneration, encouraging results were seen in a small group of patients receiving autologous transplantation of CD133 mesenchymal stem cells to repopulate the liver after extensive hepatectomy for liver masses. Ethical issues, availability, potential rejection and limited understanding of the totipotent capabilities of embryonic stem cells are the limitations that prevent their use for restoration of liver function. The effectiveness of embryonic stem cells to support liver function has been proven with their application in the bioartificial liver model in rodents. SUMMARY: There is ongoing research to restore liver function in cell biology, animal models and clinical trials using mature hepatocytes, liver progenitor cells, mesenchymal stem cells and embryonic stem cells.

Fraser, C. C., C. J. Eaves, et al. (1990). "Expansion in vitro of retrovirally marked totipotent hematopoietic stem cells." Blood **76**(6): 1071-6.

A large number of biologic, technological, and clinical studies await the development of procedures that will allow totipotent hematopoietic stem cells to be expanded in vitro. Previous work has suggested that hematopoiesis can be reconstituted using transplants of cells from long-term marrow cultures. We have used retrovirus mediated gene transfer to demonstrate that marked totipotent hematopoietic stem cells are both maintained and can be amplified in such cultures, and then subsequently regenerate and sustain lympho-myeloid hematopoiesis in irradiated recipients. Marrow cells from 5-fluorouracil-treated male mice were infected with a recombinant virus carrying the neomycin resistence gene and seeded onto irradiated adherent layers of pre-established, long-term marrow cultures of female origin. At 4 weeks, cells from individual cultures were transplanted into single or multiple female recipients. Southern blot analysis of hematopoietic tissue 45 days posttransplantation showed retrovirally marked clones common to lymphoid and myeloid tissues in 14 of 23 mice examined. Strikingly, for 3 of 4 long-term cultures, multiple recipients of cells from a single flask showed marrow and thymus repopulation with the same unique retrovirally marked clone. These results establish the feasibility of retroviral-marking techniques to demonstrate the maintenance of totipotent lympho-myeloid stem cells for at least 4 weeks in the long-term marrow culture system and provide the first evidence of their proliferation in vitro. Therefore, such cultures may serve as a starting point for identifying factors that stimulate totipotent hematopoietic stem cell expansion.

Fraser, C. C., S. J. Szilvassy, et al. (1992). "Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability." Proc Natl Acad Sci U S A **89**(5): 1968-72.

Marrow cells from male mice pretreated with 5-fluorouracil were infected with helper-free neomycin-resistant (neor) recombinant retrovirus and then used to initiate long-term cultures (LTC) on irradiated adherent marrow feeder layers. Four weeks later LTC cells were harvested and injected into lethally irradiated female recipients either alone or together with 2 x 10(5) female marrow cells with selectively compromised long-term repopulating potential to assay for totipotent and competitive repopulating units (CRU), respectively. A total of 46 unique clones were detected in recipients 5 wk to 7 mo after transplant. Half of these clones (22 of 46) included both lymphoid and myeloid progeny. Eight of the 22 lympho-myeloid clones were represented in multiple recipients, in some cases after injection of limiting numbers of CRU, thus indicating

repopulation from sibling totipotent stem cells generated during the initial 4-wk period in LTC. Serial analysis of cells released into the nonadherent fraction of LTC for up to 7 wk provided additional evidence of the continuing proliferation in LTC of totipotent stem cells with long-term repopulating potential. The frequency of CRU determined from limiting-dilution analyses of LTC-derived cells was the same for recipients analyzed at 5 wk or 7 mo after transplantation and was also the same whether marrow or thymus repopulation was assessed. These assays showed that concurrent with the expansion of some totipotent cells revealed by retroviral marking, there was a slow but net 6.5-fold decrease in total CRU numbers after 4 wk in LTC. These results show the capacity of some totipotent hematopoietic stem cells to be maintained and amplified over extensive time periods in vitro without diminution of their long-term in vivo repopulating potential. These results also set the stage for analogous studies of human stem cell selection and expansion in vitro, which may be important for future gene therapy protocols.

Friedlander, M. R., C. Adamidi, et al. (2009). "High-resolution profiling and discovery of planarian small RNAs." Proc Natl Acad Sci U S A **106**(28): 11546-51.

Freshwater planarian flatworms possess uncanny regenerative capacities mediated by abundant and collectively totipotent adult stem cells. Key functions of these cells during regeneration and tissue homeostasis have been shown to depend on PIWI, a molecule required for Piwi-interacting RNA (piRNA) expression in planarians. Nevertheless, the full complement of piRNAs and microRNAs (miRNAs) in this organism has yet to be defined. Here we report on the large-scale cloning and sequencing of small RNAs from the planarian Schmidtea mediterranea, yielding altogether millions of sequenced, unique small RNAs. We show that piRNAs are in part organized in genomic clusters and that they share characteristic features with mammalian and fly piRNAs. We further identify 61 novel miRNA genes and thus double the number of known planarian miRNAs. Sequencing, as well as quantitative PCR of small RNAs, uncovered 10 miRNAs enriched in planarian stem cells. These miRNAs are down-regulated in animals in which stem cells have been abrogated by irradiation, and thus constitute miRNAs likely associated with specific stem-cell functions. Altogether, we present the first comprehensive small RNA analysis in animals belonging to the third animal superphylum, the Lophotrochozoa, and single out a number of miRNAs that may function in regeneration. Several of these miRNAs are deeply conserved in animals.

Fulka, H., J. C. St John, et al. (2008). "Chromatin in early mammalian embryos: achieving the pluripotent state." Differentiation **76**(1): 3-14.

Gametes of both sexes (sperm and oocyte) are highly specialized and differentiated but within a very short time period post-fertilization the embryonic genome, produced by the combination of the two highly specialized parental genomes, is completely converted into a totipotent state. As a result, the one-cell-stage embryo can give rise to all cell types of all three embryonic layers, including the gametes. Thus, it is evident that extensive and efficient reprogramming steps occur soon after fertilization and also probably during early embryogenesis to reverse completely the differentiated state of the gamete and to achieve toti- or later on pluripotency of embryonic cells. However, after the embryo reaches the blastocyst stage, the first two distinct cell lineages can be clearly distinguished--the trophectoderm and the inner cells mass. The de-differentiation of gametes after fertilization, as well as the differentiation that is associated with the formation of blastocysts, are accompanied by changes in the state and properties of chromatin in individual embryonic nuclei at both the whole genome level as well as at the level of individual genes. In this contribution, we focus mainly on those events that take place soon after fertilization and during early embryogenesis in mammals. We will discuss the changes in DNA methylation and covalent histone modifications that were shown to be highly dynamic during this period; moreover, it has also been documented that abnormalities in these processes have a devastating impact on the developmental ability of embryos. Special attention will be paid to somatic cell nuclear transfer as it has been shown that the aberrant and inefficient reprogramming may be responsible for compromised development of cloned embryos.

Furusawa, C. and K. Kaneko (2002). "Origin of multicellular organisms as an inevitable consequence of dynamical systems." Anat Rec **268**(3): 327-42.

The origin of multicellular organisms is studied by considering a cell system that satisfies minimal conditions, that is, a system of interacting cells with intracellular biochemical dynamics, and potentiality in reproduction. Three basic features in multicellular organisms-cellular diversification, robust developmental process, and emergence of germ-line cells-are found to be general properties of such a system. Irrespective of the details of the model, such features appear when there are complex oscillatory dynamics of intracellular chemical concentrations. Cells differentiate from totipotent stem cells into other cell types due to instability in the intracellular dynamics with cell-cell interactions, as explained by our isologous diversification theory (Furusawa and

Kaneko, 1998a; Kaneko and Yomo, 1997). This developmental process is shown to be stable with respect to perturbations, such as molecular fluctuations and removal of some cells. By further imposing an adequate cell-type-dependent adhesion force, some cells are released, from which the next generation cell colony is formed, and a multicellular organism life-cycle emerges without any finely tuned mechanisms. This recursive production of multicellular units is stabilized if released cells are few in number, implying the separation of germ cell lines. Furthermore, such an organism with a variety of cellular states and robust development is found to maintain a larger growth speed as an ensemble by achieving a cooperative use of resources, compared to simple cells without differentiation. Our results suggest that the emergence of multicellular organisms is not a "difficult problem" in evolution, but rather is a natural consequence of a cell colony that can grow continuously.

Gabutti, V., F. Timeus, et al. (1993). "Expansion of cord blood progenitors and use for hemopoietic reconstitution." Stem Cells **11 Suppl 2**: 105-12.

A high number of stem cells migrate in fetal blood and, at birth, the number of progenitors in cord blood equals or exceeds that of adult bone marrow. Recently hemopoiesis has been successfully reconstituted with the infusion of cord blood cells. It is important to clearly define the quantity and quality of cord blood totipotent and multilineage progenitors to evaluate the possibility of their utilization in transplants. Our first aim was to study the growth characteristics of cord blood progenitors. We have evaluated the number of cycling cells with the thymidine suicide technique and the production, by phytohemagglutinin (PHA) stimulated cord blood mononuclear cells, of some cytokines involved in the proliferation of progenitor cells, such as granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin 6 (IL-6) and leukemia inhibitory factor (LIF). We have also studied by flow cytometry the CD34+CD33-, CD34+CD33+ cell subsets and the presence of the c-kit receptor in order to quantitate the number of earlier progenitors. Our second aim was to elucidate whether the cord blood totipotent stem cell population or the committed progenitors could be expanded in vitro. Our results showed that in cord blood the number of early progenitors, as evaluated by the number of mixed lineage colony forming units (CFU-Mix), by the CD34+CD33- subsets and the expression of the c-kit, is higher than in bone marrow. We have also demonstrated the possibility in vitro of increasing the number of progenitors by more than 30-fold by utilizing stem cell factor (SCF) in association with other cytokines.(ABSTRACT TRUNCATED AT 250 WORDS)

Gaziova, I. and K. M. Bhat (2007). "Generating asymmetry: with and without self-renewal." Prog Mol Subcell Biol **45**: 143-78.

At some point during the history of organismal evolution, unicellular, unipotent and mitotically active cells acquired an ability to undergo a special type of cell division called asymmetric division. By this special type of cell division, these cells could divide to generate two different progeny or to self-renew and at the same time generate a progeny that is committed to become a cell different from the mother cell. This type of cell division, which forms the basis for the functioning of totipotent or multipotent stem cells, underlies the fundamental basis for the developmental evolution of organisms. It is not clear if the asymmetric division without self-renewal preceded the asymmetric division with self-renewal. It is reasonable to assume that the asymmetric division without self-renewal preceded the asymmetric division with self-renewal. In this review we explore the genetic regulation of these two types of asymmetric divisions using the Drosophila central nervous system (CNS) as a model system. The results from recent studies argue that for cells to undergo a self-renewing asymmetric division, certain "stem cell" proteins must be maintained or up-regulated, while genes encoding proteins responsible for differentiation must be repressed or down-regulated. As long as a balance between these two classes of proteins is maintained via asymmetric segregation and activation/repression, the progeny that receives stem cell proteins/maintains stem cell competence will have the potential to undergo self-renewing asymmetric division. The other progeny will commit to differentiate. In non-self-renewing asymmetric division, down-regulation of stem cell proteins/competence combined with asymmetric segregation of cell identity specifying factors (either cell-autonomous or a combination of cell autonomous and non-cell autonomous signals) cause the two progeny to assume different differentiated identities. Identification of mutations that confer a stem cell type of division to nonstem cell precursors, or mutations that eliminate asymmetric division, has led the way in elucidating the molecular basis for these divisions. Given that there is a considerable degree of conservation of genes and their function, these studies should provide clear insight into how the self-renewing asymmetric division of stem cells in neural and other lineages is regulated not only in Drosophila but also in vertebrates including humans.

Geier, F., J. U. Lohmann, et al. (2008). "A quantitative and dynamic model for plant stem cell regulation." PLoS One **3**(10): e3553.

Plants maintain pools of totipotent stem cells throughout their entire life. These stem cells are embedded within specialized tissues called meristems, which form the growing points of the organism. The shoot apical meristem of the reference plant Arabidopsis thaliana is subdivided into several distinct domains, which execute diverse biological functions, such as tissue organization, cell-proliferation and differentiation. The number of cells required for growth and organ formation changes over the course of a plants life, while the structure of the meristem remains remarkably constant. Thus, regulatory systems must be in place, which allow for an adaptation of cell proliferation within the shoot apical meristem, while maintaining the organization at the tissue level. To advance our understanding of this dynamic tissue behavior, we measured domain sizes as well as cell division rates of the shoot apical meristem under various environmental conditions, which cause adaptations in meristem size. Based on our results we developed a mathematical model to explain the observed changes by a cell pool size dependent regulation of cell proliferation and differentiation, which is able to correctly predict CLV3 and WUS over-expression phenotypes. While the model shows stem cell homeostasis under constant growth conditions, it predicts a variation in stem cell number under changing conditions. Consistent with our experimental data this behavior is correlated with variations in cell proliferation. Therefore, we investigate different signaling mechanisms, which could stabilize stem cell number despite variations in cell proliferation. Our results shed light onto the dynamic constraints of stem cell pool maintenance in the shoot apical meristem of Arabidopsis in different environmental conditions and developmental states.

Gokhan, S. and M. F. Mehler (2001). "Basic and clinical neuroscience applications of embryonic stem cells." Anat Rec **265**(3): 142-56.

There have been recent dramatic advances in our understanding of the molecular mechanisms governing the elaboration of mature tissue-specific cellular subpopulations from embryonic stem (ES) cells. These investigations have generated a range of new biological and potential therapeutic reagents to allow us to dissect specific stages of mammalian development that were previously experimentally inaccessible. Ultimately, we will be able to reconstitute seminal signaling pathways to promote regeneration of the nervous system. Totipotent ES cells possess an unlimited proliferative capacity that make them attractive candidates for use in a series of innovative transplantation paradigms. Elucidation of the molecular and physiologic properties of ES cells also has important implications for our understanding of the integrative cellular processes underlying neural induction, patterning of the neural tube, neural lineage restriction and commitment, neuronal differentiation, regional neuronal subtype specification, and the specific pathological consequences of alterations in discrete components of these fundamental neurodevelopmental pathways. In addition, recent experimental observations suggest that neurodegenerative disease pathology may involve alterations in a range of progressive neural inductive and neurodevelopmental events through novel biological mechanisms that result in sublethal impairments in cellular homeostasis within evolving regional neuronal precursor populations containing the mutant proteins, culminating in increased vulnerability of their differentiated neuronal progeny to late-onset apoptosis. Future discoveries in ES cell research will offer unique conceptual and therapeutic perspectives that representing an alternative to neural stem cell therapeutic strategies for ameliorating the pathologic consequences of a broad range of genetic and acquired insults to the developing, adult, and aging brain. Evolving regenerative strategies for both neurodevelopmental and neurodegenerative diseases will likely involve the targeting of vulnerable regional neural precursor populations during "presymptomatic" clinicopathological stages prior to the occurrence of irrevocable neural cell injury and cell death.

Gonzalez-Estevez, C., T. Momose, et al. (2003). "Transgenic planarian lines obtained by electroporation using transposon-derived vectors and an eye-specific GFP marker." Proc Natl Acad Sci U S A **100**(24): 14046-51.

To generate transgenic planarians we used a set of versatile vectors for animal transgenesis based on the promiscuous transposons, mariner, Hermes and piggyBac, and a universal enhanced GFP (EGFP) marker system with three Pax6 dimeric binding sites, the 3xP3-EGFP developed by Berghammer et al. [Berghammer, A. J., Klinger, M. & Wimmer, E. A. (1999) Nature 402, 370-371]. This marker is expressed specifically in the eyes of various arthropod taxa. Upon microinjection into the parenchyma of adult planarians and subsequent electroporation, these vectors transpose efficiently into the planarian genome. One of the cell types transformed are the totipotent "neoblast" stem cells present in the adults, representing 30% of total cells. The neoblast represents a unique cell type with the capacity to proliferate and to differentiate into all somatic cell types as well as into germ cells. All three transposon vectors have high transformation efficiency, but only

Hermes and piggyBac show stable integration. The mariner vector is frequently lost presumably because of the presence of active mariner-type transposons in the genome of the Girardia tigrina. Transformed animals are mosaics containing both transformed and untransformed neoblasts. These differentiate to form EGFP-positive and -negative photoreceptor cells. Such mosaicism is maintained through several cycles of regeneration induced by decapitation or asexual reproduction. Transformed neoblasts also contribute to the germ line, and can give rise to pure transgenic planarian lines in which EGFP is expressed in all photoreceptor cells after sexual reproduction. The presence of the transgenes was confirmed by PCR, plasmid rescue assay, inverse PCR, and Southern blotting. Our results with the 3xP3-EGFP marker confirm the presence of Pax6 activity in the differentiated photoreceptor cells of planarian eyes. Transgenesis will be an important tool to dissect developmental molecular mechanisms in planarian regeneration, development and stem cell biology, and may also be an entry point to analyze the biology of parasitic Platyhelminthes.

Grabarek, J. B., F. Wianny, et al. (2003). "RNA interference by production of short hairpin dsRNA in ES cells, their differentiated derivatives, and in somatic cell lines." Biotechniques **34**(4): 734-6, 739-44.

dsRNA of several hundred nucleotides in length is effective at interfering with gene expression in mouse oocytes, pre-implantation embryos, and embryonic stem (ES) cells but is not as efficient in differentiated cell lines. Here we describe a method to achieve RNA interference in totipotent and differentiated ES cells together with a wide range of other mammalian cell types that is both simple and efficient. It utilizes a linearized plasmid that directs the expression of a hairpin RNA with a 22-nucleotide-paired region. This molecule has a 13-nucleotide 5' overhang that would be subject to capping on its 5' phosphoryl group and thus differs from the ideal structure suggested for effective small interfering RNAs. Thus, it appears either that the structure of small inhibitory RNA molecules may not need to be as precise as previously thought or that such a transcript is efficiently processed to a form that is effective in interfering with gene expression.

Gregori, N., C. Proschel, et al. (2002). "The tripotential glial-restricted precursor (GRP) cell and glial development in the spinal cord: generation of bipotential oligodendrocyte-type-2 astrocyte progenitor cells and dorsal-ventral differences in GRP cell function." J Neurosci **22**(1): 248-56.

We have found that the tripotential glial-restricted precursor (GRP) cell of the embryonic rat spinal cord can give rise in vitro to bipotential cells that express defining characteristics of oligodendrocyte-type-2 astrocyte progenitor cells (O2A/OPCs). Generation of O2A/OPCs is regulated by environmental signals and is promoted by platelet-derived growth factor (PDGF), thyroid hormone (TH) and astrocyte-conditioned medium. In contrast to multiple observations indicating that oligodendrocyte precursor cells in the embryonic day 14 (E14) spinal cord are ventrally restricted, GRP cells are already present in both the dorsal and ventral spinal cord at E13.5. Ventral-derived GRP cells, however, were more likely to generate O2A/OPCs and/or oligodendrocytes than were their dorsal counterparts when exposed to TH, PDGF, or even bone morphogenetic protein-4. The simplest explanation of our results is that oligodendrocyte generation occurs as a result of generation of GRP cells from totipotent neuroepithelial stem cells, of O2A/OPCs from GRP cells and, finally, of oligodendrocytes from O2A/OPCs. In this respect, the responsiveness of GRP cells to modulators of this process may represent a central control point in the initiation of this critical developmental sequence. Our findings provide an integration between the earliest known glial precursors and the well-studied O2A/OPCs while opening up new questions concerning the intricate spatial and temporal regulation of precursor cell differentiation in the CNS.

Gschwentner, R., P. Ladurner, et al. (2001). "Stem cells in a basal bilaterian. S-phase and mitotic cells in Convolutriloba longifissura (Acoela, Platyhelminthes)." Cell Tissue Res **304**(3): 401-8.

In Platyhelminthes, totipotent stem cells (neoblasts) are supposed to be the only dividing cells. They are responsible for the renewal of all cell types during development, growth, and regeneration, a unique situation in the animal kingdom. In order to further characterize these cells, we have applied two immunocytochemical markers to detect neoblasts in different stages of the cell cycle in the acoel flatworm Convolutriloba longifissura: (1) the thymidine analog 5'-bromo-2'-deoxyuridine (BrdU) to identify cells in S-phase, and (2) an antibody to phosphorylated histone H3 to locate mitosis. BrdU pulse-chase experiments were carried out to follow differentiation of neoblasts. We demonstrate the differentation into four labeled, differentiated cell types. S-phase cells and mitotic cells showed a homogenous distribution pattern throughout the body of C. longifissura. Two different types of S-phase cells could be distinguished immunocytochemically by their pattern of incorporated BrdU in the nuclei. Transmission

electron microscopy was used to study ultrastructural characters of neoblasts and revealed two different stages in maturation of neoblasts, each with a characteristic organization of heterochromatin. The stem-cell pool of C. longifissura is an important prerequisite for the extraordinary mode of asexual reproduction and the high capacity of regeneration. A comparison of the stem-cell pool in Acoela and higher platyhelminth species can provide evidence for the phylogenetic relationships of these taxa.

Guan, K., J. Rohwedel, et al. (1999). "Embryonic stem cell differentiation models: cardiogenesis, myogenesis, neurogenesis, epithelial and vascular smooth muscle cell differentiation in vitro." Cytotechnology **30**(1-3): 211-26.

Embryonic stem cells, totipotent cells of the early mouse embryo, were established as permanent cell lines of undifferentiated cells. ES cells provide an important cellular system in developmental biology for the manipulation of preselected genes in mice by using the gene targeting technology. Embryonic stem cells, when cultivated as embryo-like aggregates, so-called 'embryoid bodies', are able to differentiate in vitro into derivatives of all three primary germ layers, the endoderm, ectoderm and mesoderm. We established differentiation protocols for the in vitro development of undifferentiated embryonic stem cells into differentiated cardiomyocytes, skeletal muscle, neuronal, epithelial and vascular smooth muscle cells. During differentiation, tissue-specific genes, proteins, ion channels, receptors and action potentials were expressed in a developmentally controlled pattern. This pattern closely recapitulates the developmental pattern during embryogenesis in the living organism. In vitro, the controlled developmental pattern was found to be influenced by differentiation and growth factor molecules or by xenobiotics. Furthermore, the differentiation system has been used for genetic analyses by 'gain of function' and 'loss of function' approaches in vitro.

Guo, J., A. Jauch, et al. (2005). "Multicolor karyotype analyses of mouse embryonic stem cells." In Vitro Cell Dev Biol Anim **41**(8-9): 278-83.

The manipulation of embryonic stem (ES) cells to introduce directional genetic changes into the genome of mice has become an important tool in biomedical research. Monitoring of cell morphology before and after DNA manipulation and special culture conditions are a prerequisite to preserve the pluripotent properties of ES cells and thus their ability to generate chimera and effective germline transmission (GLT). It has been reported that prolonged cell culturing may affect the diploid chromosomal composition of cells and therefore the percentage of chimerism and GLT. Herein, we report multicolor-fluorescence in situ hybridization (M-FISH) analysis of four different ES cell lines/clones. Although the morphology of all four ES cell lines/clones appeared normal and all four expressed the early markers Oct-3/4 and Nanog, two cell lines presented consistent numerical and structural chromosome aberrations. We demonstrate that M-FISH is a sensitive and accurate method for a comprehensive karyotype analysis of ES cells and may minimize time, costs, and disappointments due to inadequate ES cell sources.

Guo, X., W. Ying, et al. (2001). "Proteomic characterization of early-stage differentiation of mouse embryonic stem cells into neural cells induced by all-trans retinoic acid in vitro." Electrophoresis **22**(14): 3067-75.

Embryonic stem (ES) cells are totipotent stem cells, which can differentiate into various kinds of cell types, including neurons. They are widely used as a model system for investigating mechanisms of differentiation events during early mouse development. In this study, proteomic techniques were used to approach the protein profile associated with the early-stage differentiation of ES cells into neuronal cells induced by all-trans retinoic acid (ATRA) in vitro. In comparison of the protein profile of parent ES cells with that of ES-derived neural-committed cells, which was induced by ATRA for four days, 24 differentially displayed protein spots were selected from two-dimensional electrophoresis (2-DE) gels for further protein identification by pepide mass fingerprinting (PMF). Nine proteins were known to being involved in the process of neural differentiation and/or neural survival. Of those, alpha-3/alpha-7 tubulin and vimentin were down-regulated, while cytokeratin 8, cytokeratin 18, G1/S-special cyclin D2, follistatin-related protein, NEL protein, platelet-activating factor acetylhydrolase IB alpha-subunit, and thioredoxin peroxidase 2 were upregulated during differentiation of ES cells to neural cells. Additionally, other 12 protein (five upregulated and seven downregulated) spots associated with ES cell differentiation into neuronal cells were not matched to known proteins so far, implicating that they might be novel proteins. The results above indicated that the molecular mechanisms of differentiation of ES cells to neural cells in vitro might be similar to those of other neural systems in vitro and identified that proteomic analysis is an effective strategy to comprehensively unravel the regulatory network of differentiation.

Guo, Y., R. Costa, et al. (2002). "The embryonic stem cell transcription factors Oct-4 and FoxD3 interact to

regulate endodermal-specific promoter expression." Proc Natl Acad Sci U S A **99**(6): 3663-7.

The POU homeodomain protein Oct-4 and the Forkhead Box protein FoxD3 (previously Genesis) are transcriptional regulators expressed in embryonic stem cells. Down-regulation of Oct-4 during gastrulation is essential for proper endoderm development. After gastrulation, FoxD3 is generally down-regulated during early endoderm formation, although it specifically remains expressed in the embryonic neural crest. In these studies, we have found that Oct-4 and FoxD3 can bind to identical regulatory DNA sequences. In addition, Oct-4 physically interacted with the FoxD3 DNA-binding domain. Cotransfection of Oct-4 and FoxD3 expression vectors activated the osteopontin enhancer, which is expressed in totipotent embryonic stem cells. FoxA1 and FoxA2 (previously HNF-3alpha and HNF-3beta) are Forkhead Box transcription factors that participate in liver and lung formation from foregut endoderm. Although FoxD3 activated the FoxA1 and FoxA2 promoters, Oct-4 inhibited FoxD3 activation of the FoxA1 and FoxA2 endodermal promoters. These data indicate that Oct-4 functions as a corepressor of FoxD3 to provide embryonic lineage-specific transcriptional regulatory activity to maintain appropriate developmental timing.

Guo, Y., B. Graham-Evans, et al. (2006). "Murine embryonic stem cells secrete cytokines/growth modulators that enhance cell survival/anti-apoptosis and stimulate colony formation of murine hematopoietic progenitor cells." Stem Cells **24**(4): 850-6.

Stromal cell-derived factor (SDF)-1/CXCL12, released by murine embryonic stem (ES) cells, enhances survival, chemotaxis, and hematopoietic differentiation of murine ES cells. Conditioned medium (CM) from murine ES cells growing in the presence of leukemia inhibitory factor (LIF) was generated while the ES cells were in an undifferentiated Oct-4 expressing state. ES cell-CM enhanced survival of normal murine bone marrow myeloid progenitors (CFU-GM) subjected to delayed growth factor addition in vitro and decreased apoptosis of murine bone marrow c-kit(+)lin- cells. ES CM contained interleukin (IL)-1alpha, IL-10, IL-11, macrophage-colony stimulating factor (CSF), oncostatin M, stem cell factor, vascular endothelial growth factor, as well as a number of chemokines and other proteins, some of which are known to enhance survival/anti-apoptosis of progenitors. Irradiation of ES cells enhanced release of some proteins and decreased release of others. IL-6, FGF-9, and TNF-alpha, not detected prior to irradiation was found after ES cells were irradiated. ES cell CM also stimulated CFU-GM colony formation. Thus, undifferentiated murine ES cells growing in the presence of LIF produce/release a number of biologically active interleukins, CSFs, chemokines, and other growth modulatory proteins, results which may be of physiological and/or practical significance.

Haegel, H., A. Dierich, et al. (1994). "CD44 in differentiated embryonic stem cells: surface expression and transcripts encoding multiple variants." Dev Immunol **3**(4): 239-46.

Expression of the surface-adhesion molecule CD44 was investigated during the in vitro differentiation of the embryonic stem (ES) cell line D3. By immunofluorescence analysis, totipotent, undifferentiated ES cells did not show surface expression of CD44, although two transcripts of approximately 1.6 and 3.3 kb were detected on Northern blots. Following 1 week of differentiation in either suspension or substrate-attached cultures, CD44 appeared on the surface of some D3 cells, and synthesis of an additional 4.5 kb mRNA species was detected on Northern blots. At this stage, at least three distinct transcripts encoding CD44 variants were induced within the cultures, resulting from alternative splicing of additional exons in the variable domains of CD44. From PCR analysis, they all appeared to contain the variable exon v10, and two of them in addition contained v6. Taken together, these results suggest that CD44 may play a role in cell migration and adhesion in the early development of the mouse embryo.

Hall, B., A. Limaye, et al. (2009). "Overview: generation of gene knockout mice." Curr Protoc Cell Biol **Chapter 19**: Unit 19 12 19 12 1-17.

The technique of gene targeting allows for the introduction of engineered genetic mutations into a mouse at a determined genomic locus. The process of generating mouse models with targeted mutations was developed through both the discovery of homologous recombination and the isolation of murine embryonic stem cells (ES cells). Homologous recombination is a DNA repair mechanism that is employed in gene targeting to insert a designed mutation into the homologous genetic locus. Targeted homologous recombination can be performed in murine ES cells through electroporation of a targeting construct. These ES cells are totipotent and, when injected into a mouse blastocyst, they can differentiate into all cell types of a chimeric mouse. A chimeric mouse harboring cells derived from the targeted ES cell clone can then generate a whole mouse containing the desired targeted mutation. The initial step for the generation of a mouse with a targeted mutation is the

construction of an efficient targeting vector that will be introduced into the ES cells.

Handberg-Thorsager, M. and E. Salo (2007). "The planarian nanos-like gene Smednos is expressed in germline and eye precursor cells during development and regeneration." Dev Genes Evol **217**(5): 403-11.

Planarians are highly regenerative organisms with the ability to remake all their cell types, including the germ cells. The germ cells have been suggested to arise from totipotent neoblasts through epigenetic mechanisms. Nanos is a zinc-finger protein with a widely conserved role in the maintenance of germ cell identity. In this work, we describe the expression of a planarian nanos-like gene Smednos in two kinds of precursor cells namely, primordial germ cells and eye precursor cells, during both development and regeneration of the planarian Schmidtea mediterranea. In sexual planarians, Smednos is expressed in presumptive male primordial germ cells of embryos from stage 8 of embryogenesis and throughout development of the male gonads and in the female primordial germ cells of the ovary. Thus, upon hatching, juvenile planarians do possess primordial germ cells. In the asexual strain, Smednos is expressed in presumptive male and female primordial germ cells. During regeneration, Smednos expression is maintained in the primordial germ cells, and new clusters of Smednos-positive cells appear in the regenerated tissue. Remarkably, during the final stages of development (stage 8 of embryogenesis) and during regeneration of the planarian eye, Smednos is expressed in cells surrounding the differentiating eye cells, possibly corresponding to eye precursor cells. Our results suggest that similar genetic mechanisms might be used to control the differentiation of precursor cells during development and regeneration in planarians.

Hansis, C. (2006). "Totipotency, cell differentiation and reprogramming in humans." Reprod Biomed Online **13**(4): 551-7.

Understanding the molecular mechanisms defining totipotency and cell differentiation in humans is a promising strategy in order to expand knowledge about reprogramming. Totipotency and the very first steps of cell differentiation can be studied well in early human embryos. Based on analysis of marker genes such as Oct-4 and -HCG, blastomeres seem to differ in their potency and can be regarded as lineage-specific stem cells as early as the 4-cell stage. The allocation of these stem cells to specific fates might hereby follow a pattern reminiscent of animal and vegetal poles. On the opposite end of the developmental spectrum, differentiated human cells can be used as a means of studying nuclear reprogramming. Intact human 293T kidney cells and primary leukocytes were reprogrammed towards a more undifferentiated state by Xenopus laevis egg extract. Molecular screens identified the chromatin-remodelling ATPase BRG1 as a factor required for this process. Based on these results, more efficient reprogramming protocols allowing for the generation of fully differentiated or undifferentiated human cells for clinical application may be developed.

Hawley, R. G., T. S. Hawley, et al. (1996). "Thrombopoietic potential and serial repopulating ability of murine hematopoietic stem cells constitutively expressing interleukin 11." Proc Natl Acad Sci U S A **93**(19): 10297-302.

Based on transplantation studies with bone marrow cultured under various conditions, a role of interleukin 11 (IL-11) in the self-renewal and/or the differentiation commitment of hematopoietic stem cells has been indicated. To better evaluate the in vivo effects of IL-11 on stem/progenitor cell biology, lethally irradiated mice were serially transplanted with bone marrow cells transduced with a defective retrovirus, termed MSCV-mIL-11, carrying the murine IL-11 (mIL-11) cDNA and the bacterial neomycin phosphotransferase (neo) gene. High serum levels (i.e., > 1 ng/ml) of mIL-11 in all (20/20) primary and 86% (12/14) of secondary long-term reconstituted mice, as well as 86% (12/14) of tertiary recipients examined at 6 weeks posttransplant, demonstrated persistence of vector expression subsequent to transduction of bone marrow precursors functionally definable as totipotent hematopoietic stem cells. In agreement with results obtained with human IL-11 in other myeloablation models, ectopic mIL-11 expression accelerated recovery of platelets, neutrophils, and, to some extent, total leukocytes while preferentially increasing peripheral platelet counts in fully reconstituted mice. When analyzed 5 months posttransplant, tertiary MSCV-mIL-11 recipients had a significantly greater percentage of G418-resistant colony-forming cells in their bone marrow compared with control MSCV animals. Collectively, these data show that persistent stimulation of platelet production by IL-11 is not detrimental to stem cell repopulating ability; rather, they suggest that IL-11 expression in vivo may have resulted in enhanced maintenance of the most primitive hematopoietic stem cell compartment. The prolonged expression achieved by the MSCV retroviral vector, despite the presence of a selectable marker, contrasts with the frequent transcriptional extinction observed with other retroviral vectors carrying two genes. These findings have potentially important implications for clinical bone marrow

transplantation and gene therapy of the hematopoietic system.

Hayashi, S., T. Yamane, et al. (1998). "Commitment and differentiation of stem cells to the osteoclast lineage." Biochem Cell Biol **76**(6): 911-22.

Osteoclasts are hematopoietic cells which play important roles in bone remodeling and resorption. They have phenotypic characteristics of the monocyte/macrophage lineages. In this review we first describe the phylogeny of osteoclasts. Osteoclast generation is closely linked to the presence of bone tissues. The formation of bone cavities in aquatic animals is underdeveloped, even though they have cells which have the potential to differentiate into osteoclasts. Next we describe recent advances in our understanding of osteoclastogenesis that have resulted from the identification of critical molecules and mutated genes of osteopetrotic mice. Reports that transcriptional factors PU.1 and c-Fos are essential for commitment and (or) differentiation into the osteoclast lineage and novel culture systems, which have clarified some characteristics of osteoclast precursors, are also described. We are now able to induce mature osteoclasts from hematopoietic stem cells and even from totipotent embryonic stem cells. Cell lines that differentiate into osteoclasts are also available.

Hays, E. F. and N. Margaretten (1985). "Long-term oral cadmium produces bone marrow hypoplasia in mice." Exp Hematol **13**(3): 229-34.

Marrow hypoplasia is described in CBA/H mice that drank water containing 300 mg/liter cadmium chloride for 12 months. This was characterized by a significant reduction of the totipotent stem cells (CFU-s), granulocyte-monocyte progenitor cells (GM-CFUc), and erythroid progenitor cells (CFU-e). The bone marrow cellularity and the proliferative capacity of GM-CFUc in vitro were decreased. The animals reflected these marrow alterations by demonstrating an anemia with reticulocytopenia and neutropenia. They did not show increased mortality or increased susceptibility to infections; however, their body weight was significantly reduced. In addition, iron deficiency was demonstrated in the cadmium-treated mice. The animals had a hypochromia of the peripheral red cells and diminished marrow iron stores. Thus, the anemia of cadmium toxicity is probably the combined result of bone marrow hypoplasia and iron deficiency.

Hillmen, P., S. M. Lewis, et al. (1995). "Natural history of paroxysmal nocturnal hemoglobinuria." N Engl J Med **333**(19): 1253-8.

BACKGROUND: Paroxysmal nocturnal hemoglobinuria (PNH), which is characterized by intravascular hemolysis and venous thrombosis, is an acquired clonal disorder associated with a somatic mutation in a totipotent hematopoietic stem cell. An understanding of the natural history of PNH is essential to improve therapy. METHODS: We have followed a group of 80 consecutive patients with PNH who were referred to Hammersmith Hospital, London, between 1940 and 1970. They were treated with supportive measures, such as oral anticoagulant therapy after established thromboses, and transfusions. RESULTS: The median age of the patients at the time of diagnosis was 42 years (range, 16 to 75), and the median survival after diagnosis was 10 years, with 22 patients (28 percent) surviving for 25 years. Sixty patients have died; 28 of the 48 patients for whom the cause of death is known died from either venous thrombosis or hemorrhage. Thirty-one patients (39 percent) had one or more episodes of venous thrombosis during their illness. Of the 35 patients who survived for 10 years or more, 12 had a spontaneous clinical recovery. No PNH-affected cells were found among the erythrocytes or neutrophils of the patients in prolonged remission, but a few PNH-affected lymphocytes were detectable in three of the four patients tested. Leukemia did not develop in any of the patients.

Hilton, D. J., N. A. Nicola, et al. (1991). "Distribution and comparison of receptors for leukemia inhibitory factor on murine hemopoietic and hepatic cells." J Cell Physiol **146**(2): 207-15.

Leukemia inhibitory factor (LIF) is a glycoprotein that induces the differentiation of the monocytic leukemia cell line M1 but suppresses the differentiation of totipotent embryonic stem cells. In an attempt to define the normal cellular targets for LIF, the distribution of LIF receptors within hemopoietic and hepatic tissue was analyzed by binding cells with radioiodinated LIF (125I-LIF) and subsequently carrying out autoradiography. Autoradiography demonstrated that in each hemopoietic tissue examined cells of monocyte/macrophage lineage were the primary cell type labeled with 125I-LIF. Moreover, both fetal and adult parenchymal hepatocytes displayed higher levels of labeling than either monocytes or macrophages. The number of receptors per positive cell varied from 150 for bone marrow monocytes to 2,000 for adult hepatocytes. In each case, however, binding was of high affinity, with an apparent KD of 34-100 pM, and binding was specific, since labeling was competed for by unlabeled LIF but not a range of other structurally unrelated growth and differentiation factors. It is suggested that LIF may play a role in regulating macrophage function and hepatic acute phase protein synthesis in response to infection.

Ho, L., J. L. Ronan, et al. (2009). "An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency." Proc Natl Acad Sci U S A **106**(13): 5181-6.

Mammalian SWI/SNF [also called BAF (Brg/Brahma-associated factors)] ATP-dependent chromatin remodeling complexes are essential for formation of the totipotent and pluripotent cells of the early embryo. In addition, subunits of this complex have been recovered in screens for genes required for nuclear reprogramming in Xenopus and mouse embryonic stem cell (ES) morphology. However, the mechanism underlying the roles of these complexes is unclear. Here, we show that BAF complexes are required for the self-renewal and pluripotency of mouse ES cells but not for the proliferation of fibroblasts or other cells. Proteomic studies reveal that ES cells express distinctive complexes (esBAF) defined by the presence of Brg (Brahma-related gene), BAF155, and BAF60A, and the absence of Brm (Brahma), BAF170, and BAF60C. We show that this specialized subunit composition is required for ES cell maintenance and pluripotency. Our proteomic analysis also reveals that esBAF complexes interact directly with key regulators of pluripotency, suggesting that esBAF complexes are specialized to interact with ES cell-specific regulators, providing a potential explanation for the requirement of BAF complexes in pluripotency.

Hochedlinger, K. and R. Jaenisch (2002). "Nuclear transplantation: lessons from frogs and mice." Curr Opin Cell Biol **14**(6): 741-8.

Nuclear transplantation was developed 50 years ago in frogs to test whether nuclei from differentiated cells remain genetically equivalent to zygotic nuclei. Results from cloning experiments in frogs and mice indicate that nuclei gradually lose potency during development from embryonic to adult cells. However, even though adult mature lymphocytes were recently shown to remain genetically totipotent, no evidence exists to show that surviving clones originate from the nuclei of terminally differentiated cells. Thus, it is equally possible that many cloned animals are in fact derived from the nuclei of less differentiated adult cells such as adult stem cells.

Hochedlinger, K., W. M. Rideout, et al. (2004). "Nuclear transplantation, embryonic stem cells and the potential for cell therapy." Hematol J **5 Suppl 3**: S114-7.

Nuclear transfer experiments in mammals have shown that the nucleus of an adult cell has the ability to direct the development of an entire organism, id est its genome is totipotent. However, these experiments did not conclusively demonstrate that the nuclei of terminally differentiated adult cells remain totipotent. It is possible that rare adult stem cells served as donors for the few surviving clones. To address this question, we have generated monoclonal mice from terminally differentiated lymphocytes that carry a single antigen receptor rearrangement in all tissues. Nuclear transfer technology may provide a powerful method for obtaining autologous cells for replacement therapy. We have demonstrated the feasibility of this concept by combining nuclear transfer with gene and cell therapy to treat the immune deficiency of Rag2 mutant mice, thus establishing a paradigm for 'therapeutic cloning'. Moreover, we will discuss the potential use of nuclear transfer to study the role of reversible genomic (epigenetic) modifications during tumorigenesis.

Hoei-Hansen, C. E., A. Sehested, et al. (2006). "New evidence for the origin of intracranial germ cell tumours from primordial germ cells: expression of pluripotency and cell differentiation markers." J Pathol **209**(1): 25-33.

Primary intracranial germ cell tumours are rare neoplasms that occur in children and adolescents. This study examined both the biology and the origin of these tumours, as it has been hypothesized that they originate from a totipotent primordial germ cell. We applied recent knowledge from gonadal germ cell tumours and analysed expression of a wide panel of stem cell-related proteins (C-KIT, OCT-3/4 (POU5F1), AP-2gamma (TFAP2C), and NANOG) and developmentally regulated germ cell-specific proteins (including MAGE-A4, NY-ESO-1, and TSPY). Expression at the protein level was analysed in 21 children and young adults with intracranial germinomas and non-germinomas, contributing to a careful description of these unusual tumours and adding to the understanding of pathogenesis. Stem cell related proteins were highly expressed in intracranial germ cell tumours, and many similarities were detected with their gonadal equivalents, including a close similarity with primordial germ cells. A notable difference was the sex-specific expression of TSPY, a gene previously implicated in the origin of gonadoblastoma. TSPY was only detected in germ cell tumours in the central nervous system (CNS) from males, suggesting that it is not required for the initiation of malignant germ cell transformation. The expression of genes associated with embryonic stem cell pluripotency in CNS germ cell tumours strongly suggests that these tumours are derived from cells that retain, at least partially, an embryonic stem cell-like

phenotype, which is a hallmark of primordial germ cells.

Honecker, F., H. Stoop, et al. (2006). "Germ cell lineage differentiation in non-seminomatous germ cell tumours." J Pathol **208**(3): 395-400.

Human germ cell tumours (GCTs) have long fascinated investigators for a number of reasons. Being pluripotential tumours, they can differentiate into both extra-embryonic and embryonic (somatic) tissues. However, it has never been shown convincingly that, in humans, these tumours are truly totipotent and can also give rise to the germ lineage, the third major differentiation lineage occurring early during embryonic life. Using a number of newly available, distinct, immunohistochemical markers, such as OCT3/4, VASA and TSPY, the occurrence of germ cells was investigated in a number of germ cell tumours. Development of germ cells was identified in three independent non-seminomas, including two pure yolk sac tumours and one mixed tumour composed of yolk sac tumour and immature teratoma. Our finding indicates a previously unknown totipotent potential of human GCTs and raises the question of whether, under certain culture conditions, primordial germ cells could be derived from human GCT cell lines.

Hong, Y. and M. Schartl (2006). "Isolation and differentiation of medaka embryonic stem cells." Methods Mol Biol **329**: 3-16.

Medaka is a small laboratory fish that daily produces eggs easily controllable by light cycles. This fish represents a unique lower vertebrate compared to mammals, in which embryonic stem (ES) cell lines can be derived from midblastula embryos (MBEs). Like mouse ES cells, medaka ES cells most resemble the totipotent embryonic cells at the blastula stage. Medaka ES cells retain a diploid karyotype, pluripotency in vitro, and chimera competence in vivo. They give rise to high efficiencies of transient and stable gene transfer and maintain their pluripotency after long-term drug selection for transgene integration. They can also be directed to differentiate into particular cell types. Medaka is the most distantly related vertebrate to mammals, and its ES cell lines provide an ideal reference to mammalian ES cells for the molecular analysis of stemness. More important, medaka ES cell lines on their own offer an excellent tool for studying stem cell biology in vitro and in vivo because production and observation of ES-derived chimeras as well as phenotypic analyses are very easy because of its external, transparent, and temperature-adjustable embryology.

Hong, Y., C. Winkler, et al. (2004). "Activation of the mouse Oct4 promoter in medaka embryonic stem cells and its use for ablation of spontaneous differentiation." Mech Dev **121**(7-8): 933-43.

The determination and maintenance of the cell fate is ultimately due to differential gene activity. In the mouse, expression of the transcription factor Oct4 is high in totipotent inner cell mass, germ cells and undifferentiated embryonic stem (ES) cells, but dramatically reduced or extinct upon differentiation. Here, we show that medaka blastula embryos and cells of the ES cell line MES1 are able to activate the Oct4 promoter. Ectopic expression of a fusion gene for beta-galactosidase and neomycin resistance from the Oct4 promoter conferred resistance to G418. G418 selection led to a homogeneous population of undifferentiated ES cells which were able to undergo induced or directed differentiation into various cell types including neuron-like cells and melanocytes. Furthermore, GFP-labeled GOF18geo-MES1 cells after differentiation ablation were able to contribute to a wide variety of organ systems derived from all the three germ layers.

Horii, T., Y. Nagao, et al. (2003). "Serum-free culture of murine primordial germ cells and embryonic germ cells." Theriogenology **59**(5-6): 1257-64.

Fetal calf serum (FCS) has usually been used for culture of embryonic stem (ES) cell as a component of the culture medium. However, FCS contains undefined factors, which promote cell proliferation and occasionally stimulate differentiation of ES cells. Recently, a chemically-defined serum replacement, Knockout Serum Replacement (KSR), was developed to maintain ES cells in an undifferentiated state. In this experiment, we examined the effects of KSR on the growth and differentiation of primordial germ cells (PGCs) and embryonic germ (EG) cells. PGCs were collected 8.5 days postcoitum (dpc) from B6D2F1 (C57BL/6JxDBA/2J) female mice mated with B6D2F1 males. Most of the PGCs that were cultured in FCS-supplemented medium (FCS medium) had alkaline phosphatase (AP) activity and acquired a fibroblast cell shape. In contrast, PGCs in KSR-supplemented medium (KSR medium) proliferated, maintaining round and stem cell-like morphology. In addition, EG cells were established more easily from PGCs cultured in KSR medium than from PGCs cultured in FCS medium.

Houldsworth, J., J. E. Korkola, et al. (2006). "Biology and genetics of adult male germ cell tumors." J Clin Oncol **24**(35): 5512-8.

Adult male germ cell tumors (GCTs) arise by transformation of totipotent germ cells. They have the unique potential to activate molecular pathways, in part mimicking those occurring during gametogenesis

and normal human development, as evidenced by the array of histopathologies observed in vivo. Recent expression profiling studies of GCTs along with advances in embryonic stem-cell research have contributed to our understanding of the underlying biology of the disease. Gain of the short arm of chromosome 12 detected in almost all adult GCTs appears to be multifunctional in germ cell tumorigenesis on the basis of the observed overexpression of genes mapped to this region involved in maintenance of pluripotency and oncogenesis. Expression signatures associated with the different histopathologies have yielded clues as to the functional mechanisms involved in GCT invasion, loss of pluripotency, and lineage differentiation. Genomic and epigenomic abnormalities that contribute to or cause these events have been identified by traditional genome analyses and continue to be revealed as genome-scanning technologies develop.

Hubner, K., G. Fuhrmann, et al. (2003). "Derivation of oocytes from mouse embryonic stem cells." Science **300**(5623): 1251-6.

Continuation of mammalian species requires the formation and development of the sexually dimorphic germ cells. Cultured embryonic stem cells are generally considered pluripotent rather than totipotent because of the failure to detect germline cells under differentiating conditions. Here we show that mouse embryonic stem cells in culture can develop into oogonia that enter meiosis, recruit adjacent cells to form follicle-like structures, and later develop into blastocysts. Oogenesis in culture should contribute to various areas, including nuclear transfer and manipulation of the germ line, and advance studies on fertility treatment and germ and somatic cell interaction and differentiation.

Hug, K. (2005). "Sources of human embryos for stem cell research: ethical problems and their possible solutions." Medicina (Kaunas) **41**(12): 1002-10.

Using different sources of human embryonic stem cells for research raises different ethical problems. Experimenting on embryos created for in vitro fertilization but left unused, or embryos, created specially for research raise ethical questions. In the first case--whether using "spare" human embryos for research means a lack of respect for the beginning of human life, and in the second--whether creation of embryos for research is morally worse than experimentation on already created, but unused human embryos. The possibility of therapeutic cloning also raises a question whether it is ethical to create human embryos for therapeutic purposes. When balancing the possible benefit of embryonic stem cell research inventing new therapies, and the ethical problems, raised by this research, a question is posed whether there are any equally effective alternatives to research on viable human embryos that could avoid or at least decrease these problems. The aim of this literature review is to present the main arguments for and against using different sources of human embryonic stem cells and to acquaint with possible alternatives to human embryo research. METHODS: The literature review of the last five years. CONCLUSIONS: The currently used sources of human embryonic stem cells and research methods raise ethical objections in certain sectors of society, based on the arguments for the need of respect for the human embryo.

Huntriss, J., M. Hinkins, et al. (2004). "Expression of mRNAs for DNA methyltransferases and methyl-CpG-binding proteins in the human female germ line, preimplantation embryos, and embryonic stem cells." Mol Reprod Dev **67**(3): 323-36.

Recent evidence indicates that mammalian gametogenesis and preimplantation development may be adversely affected by both assisted reproductive and stem cell technologies. Thus, a better understanding of the developmental regulation of the underlying epigenetic processes that include DNA methylation is required. We have, therefore, monitored the expression, by PCR, of the mRNAs of DNA methyltransferases (DNMTs), methyl-CpG-binding domain proteins (MBDs), and CpG binding protein (CGBP) in a developmental series of amplified cDNA samples derived from staged human ovarian follicles, oocytes, preimplantation embryos, human embryonic stem (hES) cells and in similar murine cDNA samples.

Huss, R. (2000). "Perspectives on the morphology and biology of CD34-negative stem cells." J Hematother Stem Cell Res **9**(6): 783-93.

The CD34 antigen is the classical indicator molecule of pluripotent hematopoietic stem cells. But there is more and more evidence that progenitors of a yet uncommitted stem cell population do not express this surrogate marker. The bone marrow and other sites of hematopoiesis consist also of fibroblast-like stromal cells, quiescent hematopoietic stem cells, and mesenchymal stem cells. Depending on their stage of differentiation, CD34- stem cells cannot only generate hematopoietic progenitors, but also more specified mesenchymal precursors, such as osteoblasts, chondrocytes, myocytes, adipocyts, and others. The stromal cell compartment produces not only matrix proteins, such as collagens, fibronectin and others, but also the essential growth factors, which initiate and support the differentiation of primary quiescent, but eventually activated CD34- stem cells into CD34+

hematopoietic progenitors. In vivo studies have shown that long-term hematopoietic and mesenchymal reconstitution can be achieved with CD34- stem cell lines, isolated from various sources, although the frequency of CD34- stem cells seams to be quite low among the progenitor population. Some authors deny the reconstitution ability of CD34- cells. The majority of CD34- stem cells are quiescent fibroblast-like cells, which can be identified in the bone marrow biopsy as "bone lining cells". Some of those bone lining cells show protein synthesis and contain secretory vesicles.

Jaisser, F. and A. T. Beggah (1998). "Transgenic models in renal tubular physiology." Exp Nephrol **6**(5): 438-46.

Animal transgenesis has proven to be useful for physiological as well as physiopathological studies. Besides the classical approach based on the random integration of a DNA construct in the mouse genome, gene targeting can be achieved using totipotent embryonic stem (ES) cells for targeted transgenesis. Transgenic mice are then derived from the transgenic ES cells. This allows the introduction of null mutations in the genome (so-called knock-out) or the control of the transgene expression by the endogenous regulatory sequences of the gene of interest (so-called knock-in). Development of these transgenic animals leads to a better understanding of the cellular function of many genes or to the generation of animal models for human diseases. The purpose of this short review is to describe animal models in renal tubular physiopathology. Recent progresses will allow the generation of animal models with conditional expression of the transgene of interest or with a conditional gene mutation. This permits spatial and temporal control of the expression of the transgene or of the mutation. This should allow the generation of models suitable for physiological analysis or closer to disease state.

Jeong, D., D. J. McLean, et al. (2003). "Long-term culture and transplantation of murine testicular germ cells." J Androl **24**(5): 661-9.

The objectives of this study were to develop an in vitro culture system to optimize germ cell proliferation and to measure the potential of the cultured germ cells to produce mature spermatozoa after transplantation into a recipient. Donor germ cells isolated from ROSA26 male mice were cultured with a STO feeder cell layer in Dulbecco's minimal essential medium (DMEM) supplemented with fetal bovine serum (FBS), stem cell factor, leukemia inhibitory factor, basic fibroblast growth factor, insulin-like growth factor 1, interleukin-11, L-glutamine, sodium pyruvate, 2-mercaptoethanol, murine oncostatin M, and platelet-derived growth factor. Donor germ cells formed colonies in the primary cultures after 8-21 days. These cultured colonies were maintained for 4 weeks or longer without subculture and proliferated for up to 8 passages over a period of 3 months. These colonies had alkaline phosphatase activity and incorporated 5-bromo-2'-deoxyuridine. These colonies were positive partially when screened with antibody for germ cell nuclear antigen and c-kit. Germ cells cultured with this supplemented medium showed enhanced colonization vs controls cultured with DMEM and FBS. Cultured germ cells from Rosa26 donors were transplanted into testes and were identified by X-gal staining and histological screening. The cells cultured in the supplemented medium colonized the tubules and initiated spermatogenesis in the recipient mice. This is an improved method for culturing germ cells and may be useful in gene therapy and the production of transgenic animals.

Jordan, C. T. and I. R. Lemischka (1990). "Clonal and systemic analysis of long-term hematopoiesis in the mouse." Genes Dev **4**(2): 220-32.

We have analyzed the temporal in vivo fate of 142 individual stem cell clones in 63 reconstituted mice. Long-term sequential analyses of the four major peripheral blood lineages, obtained from animals engrafted with genetically marked stem cells, indicate that developmental behavior is primarily a function of time. As such, the first 4-6 months post-engraftment is characterized by frequent fluctuations in stem cell proliferation and differentiation behavior. Gradually, a stable hematopoietic system emerges, dominated by a small number of totipotent clones. We demonstrate that single stem cell clones are sufficient to maintain hematopoiesis over the lifetime of an animal and suggest that mono- or oligoclonality may be a hallmark of long-term reconstituted systems. A model is proposed, wherein lineage-restricted differentiation and dramatic clonal flux are consequences of mechanisms acting on an expanding pool of totipotent cells and are not indicative of intrinsically distinct stem cell classes.

Jordan, C. T., J. P. McKearn, et al. (1990). "Cellular and developmental properties of fetal hematopoietic stem cells." Cell **61**(6): 953-63.

We have characterized the fetal totipotent hematopoietic stem cell using a novel strategy that integrates physical analysis of cell properties and genetic analysis of in vivo developmental behavior. This approach allows the simultaneous isolation and in vivo characterization of any stem cell population. Using this procedure we demonstrate that a cell surface marker, recognized by monoclonal antibody AA4.1, defines 0.5%-1.0% of fetal liver tissue that

contains the entire hierarchy of primitive hematopoietic cells. The AA4.1+ subpopulation includes multipotential in vitro progenitors, CFU-S cells, and lymphoid-myeloid stem cells that function to yield permanent and oligoclonal blood systems. Further fractionation of these cells by analysis of density, fibronectin binding, and surface antigen distribution has defined 0.1%-0.2% of fetal liver that contains the totipotent stem cell.

Kaeffer, B. (2002). "Mammalian intestinal epithelial cells in primary culture: a mini-review." In Vitro Cell Dev Biol Anim **38**(3): 123-34.

Epithelial cells lining the digestive tract represent a highly organized system built up by multipotent stem cells. A process of asymmetric mitosis produces a population of proliferative cells that are rapidly renewed and migrate along the crypt-villus axis, differentiating into functional mature cells before dying and exfoliating into the intestinal lumen. Isolated crypts or epithelial cells retaining high viability can be prepared within a few h after tissue sampling. After cells are cultured in serum-free media, short-term studies (16-48 h) can be conducted for endocrinology, energy metabolism, or programmed cell death. However, long-term primary culture of intestinal cells (up to 10 d) is still difficult despite progress in isolation methodologies and manipulation of the cell microenvironment. The main problem in developing primary culture is the lack of structural markers specific to the stem cell compartment. The design of a microscopic multidimensional analytic system to record the expression profiles of biomarkers all along the living intestinal crypt should improve basic knowledge of the survival and growth of adult crypt stem cells, and the selection of totipotent embryonic stem cells capable of differentiating into intestinal tissues should facilitate studies of the genomic basis of endodermal tissue differentiation.

Kanatsu, M. and S. I. Nishikawa (1996). "In vitro analysis of epiblast tissue potency for hematopoietic cell differentiation." Development **122**(3): 823-30.

In murine embryogenesis, all cells that will constitute the embryonic structures originate from the epiblast (primitive ectoderm) tissue, the epithelial cell sheet of the gastrulating embryo. The cells of this tissue are totipotent at the beginning of gastrulation, but at the end of this period are specified to particular cell lineages. Thus, it is likely that during murine gastrulation, the potency of epiblast cells that were originally totipotent becomes restricted as development progresses. However, the mechanisms of this process are unknown. We have investigated this process in vitro, focusing on the hematopoietic cell lineage. To detect the hematogenic potency of the epiblast tissue, we established an in vitro culture system in which the hematopoietic cell differentiation of the epiblast tissue was supported by a stromal cell layer. With this culture system, we investigated the process by which this potency becomes spatially and temporally restricted during gastrulation. The results showed that hematogenic potency resides in the entire epiblast of the early- to mid-gastrulating embryo, but becomes restricted to the posterior half of the epiblast at the headfold stage. Furthermore, we showed that this process is altered by exogenous bone morphogenetic protein-4 (BMP-4) or activin A, which may be mesoderm inducers in Xenopus embryogenesis.

Kang, H. and A. Sanchez Alvarado (2009). "Flow cytometry methods for the study of cell-cycle parameters of planarian stem cells." Dev Dyn **238**(5): 1111-7.

Due to their characteristic inaccessibility and low numbers, little is known about the cell-cycle dynamics of most stem cells in vivo. A powerful, established methodology to study cell-cycle dynamics is flow cytometry, which is used routinely to study the cell-cycle dynamics of proliferating cells in vitro. Its use in heterogeneous mixtures of cells obtained from whole animals, however, is complicated by the relatively low abundance of cycling to non-cycling cells. We report on flow cytometric methods that take advantage of the abundance of proliferating stem cells in the planarian Schmidtea mediterranea. The optimized protocols allow us to measure cell-cycle dynamics and follow BrdU-labeled cells specifically in complex mixtures of cells. These methods expand on the growing toolkit being developed to study stem cell biology in planarians, and open the door to detailed cytometric studies of a collectively totipotent population of adult stem cells in vivo.

Karp, J. M., L. S. Ferreira, et al. (2006). "Cultivation of human embryonic stem cells without the embryoid body step enhances osteogenesis in vitro." Stem Cells **24**(4): 835-43.

Osteogenic cultures of embryonic stem cells (ESCs) are predominately derived from three-dimensional cell spheroids called embryoid bodies (EBs). An alternative method that has been attempted and merits further attention avoids EBs through the immediate separation of ESC colonies into single cells. However, this method has not been well characterized and the effect of omitting the EB step is unknown. Herein, we report that culturing human embryonic stem cells (hESCs) without the EB stage leads to a sevenfold greater number of osteogenic cells and to spontaneous bone nodule formation after 10-12 days. In contrast, when hESCs were differentiated as EBs

for 5 days followed by plating of single cells, bone nodules formed after 4 weeks only in the presence of dexamethasone. Furthermore, regardless of the inclusion of EBs, bone matrix formed, including cement line matrix and mineralized collagen, which displayed apatitic mineral (PO4) with calcium-to-phosphorous ratios similar to those of hydroxyapatite and human bone. Together these results demonstrate that culturing hESCs without an EB step can be used to derive large quantities of functional osteogenic cells for bone tissue engineering.

Kato, Y., H. Imabayashi, et al. (2004). "Nuclear transfer of adult bone marrow mesenchymal stem cells: developmental totipotency of tissue-specific stem cells from an adult mammal." Biol Reprod **70**(2): 415-8.

Recent studies have demonstrated that somatic stem cells have a flexible potential greater than previously expected when they are transplanted into different tissues. On the other hand, recent studies also have revealed that these potentials might occur because of spontaneous cell fusion with recipient cells. The nuclei of somatic cells could have been reprogrammed when they were artificially or spontaneously fused with mouse embryonic stem (ES) cells. The resultant hybrid cells acquired a developmental pluripotency that the original somatic cells did not have but that ES cells did. LaBarge and Blau (Cell 2002; 111:589-601) demonstrated that adult bone marrow-derived cells contributed to muscle tissue in a stepwise biological progression. This means that bone marrow-derived cells became satellite cells of mononucleate muscle stem cells after the first irradiation-induced damage to the mouse, and after the second irradiation-induced damage, multinucleate myofibers appeared from the bone marrow-derived cells. Considered together, the differentiation potential of the somatic stem cell nucleus itself remains unclear. Although the pluripotency of somatic stem cell populations has been evaluated, the developmental totipotency of the nuclei of somatic stem cells, whether or not they fused with other cells, has not been shown, except in only one study concerning fetal neural cells (never in adult stem cells). Here, we showed the developmental totipotency of adult bovine mesenchymal stem cells by nuclear transfer.

Katoh, Y. and M. Katoh (2007). "Comparative integromics on JMJD2A, JMJD2B and JMJD2C: preferential expression of JMJD2C in undifferentiated ES cells." Int J Mol Med **20**(2): 269-73.

Fertilized egg or totipotent zygote undergoes cleavage divisions to form a blastocyst, consisting of outer trophoectoderm cells and inner cell mass with pluripotent primitive ectoderm cells. Epigenetic reprogramming, erasure and maintenance of epigenetic modification, occurs during early embryogenesis. In 2004, we identified and characterized JMJD2A/JHDM3A, JMJD2B, JMJD2C, JMJD2D, JMJD2E and JMJD2F. JMJD2A, JMJD2B and JMJD2C share the common domain architecture with JmjN, JmjC, two PHD, and two TUDOR domains. In 2006, other groups characterized JMJD2 family members as the H3K9 and/or H3K36 histone demethylases. Here, comparative integromics analyses on JMJD2A, JMJD2B and JMJD2C were carried out. Mouse Jmjd2a was expressed in fertilized egg and 2-cell embryos, while human JMJD2A was expressed in undifferentiated and differentiated ES cells. AP1-binding site and six bHLH-binding sites within intron 13 of human JMJD2A gene were conserved in mouse Jmjd2a gene. Mouse Jmjd2b was expressed in 8-cell embryos and undifferentiated ES cells, while human JMJD2B was expressed in undifferentiated and differentiated ES cells. Two GATA-binding sites within intron 6 of human JMJD2B gene were conserved in mouse Jmjd2b gene. Mouse Jmjd2c and human JMJD2C were preferentially expressed in undifferentiated ES cells. Four NANOG-binding sites, one TCF/ LEF-binding site, and one bHLH-binding site were located within evolutionary conserved region at the 3'-flanking region of human JMJD2C gene. NANOG- TCF/LEF-, and bHLH-binding sites within the 3'-flanking region of human JMJD2C gene were conserved in chimpanzee, cow, mouse and rat JMJD2C othologs. Together these facts indicate that JMJD2C is the evolutionarily conserved target of Homeo-domain transcription factor NANOG, and that JMJD2C is the histone demethylase implicated in the epigenetic reprogramming during the early embryogenesis.

Kiessling, A. A., R. Bletsa, et al. (2009). "Evidence that human blastomere cleavage is under unique cell cycle control." J Assist Reprod Genet **26**(4): 187-95.

PURPOSE: To understand the molecular pathways that control early human embryo development. METHODS: Improved methods of linear amplification of mRNAs and whole human genome microarray analyses were utilized to characterize gene expression in normal appearing 8-Cell human embryos, in comparison with published microarrays of human fibroblasts and pluripotent stem cells. RESULTS: Many genes involved in circadian rhythm and cell division were over-expressed in the 8-Cells. The cell cycle checkpoints, RB and WEE1, were silent on the 8-Cell arrays, whereas the recently described tumor suppressor, UHRF2, was up-regulated >10-fold, and the proto-oncogene, MYC, and the core element of circadian rhythm, CLOCK, were elevated up to >50-fold on the 8-Cell arrays. CONCLUSIONS: The canonical G1 and G2 cell cycle

checkpoints are not active in totipotent human blastomeres, perhaps replaced by UHRF2, MYC, and intracellular circadian pathways, which may play important roles in early human development.

Kim, J. M., M. Yamada, et al. (2003). "Functions of mammalian Cdc7 kinase in initiation/monitoring of DNA replication and development." Mutat Res **532**(1-2): 29-40.

Cdc7 kinase plays an essential role in firing of replication origins by phosphorylating components of the replication complexes. Cdc7 kinase has also been implicated in S phase checkpoint signaling downstream of the ATR and Chk1 kinases. Inactivation of Cdc7 in yeast results in arrest of cell growth with 1C DNA content after completion of the ongoing DNA replication. In contrast, conditional inactivation of Cdc7 in undifferentiated mouse embryonic stem (ES) cells leads to growth arrest with rapid cessation of DNA synthesis, suggesting requirement of Cdc7 functions for continuation of ongoing DNA synthesis. Furthermore, loss of Cdc7 function induces recombinational repair (nuclear Rad51 foci) and G2/M checkpoint responses (inhibition of Cdc2 kinase). Eventually, p53 becomes highly activated and the cells undergo massive p53-dependent apoptosis. Thus, defective origin activation in mammalian cells can generate DNA replication checkpoint signals. Efficient removal of those cells in which replication has been perturbed, through cell death, may be beneficial to maintain the highest level of genetic integrity in totipotent stem cells. Partial, rather than total, loss of Cdc7 kinase expression results in retarded growth at both cellular and whole body levels, with especially profound impairment of germ cell development.

Kinoshita, T., M. E. Medof, et al. (1985). "Distribution of decay-accelerating factor in the peripheral blood of normal individuals and patients with paroxysmal nocturnal hemoglobinuria." J Exp Med **162**(1): 75-92.

Decay-accelerating factor (DAF) is a 70,000 Mr protein that has been isolated from the membrane of red cells. The function of DAF is to inhibit the assembly of amplifying enzymes of the complement cascade on the cell surface, thereby protecting them from damage by autologous complement. We raised monoclonal antibodies to DAF and used them to study its distribution in cells from the peripheral blood of normal individuals and of patients with paroxysmal nocturnal hemoglobinuria (PNH), a disease characterized by the unusual susceptibility of red cells to the hemolytic activity of complement. The results of immunoradiometric assays and of fluorescence-activated cell sorter analysis showed that DAF was present not only on red cells but was widely distributed on the surface membrane of platelets, neutrophils, monocytes, and B and T lymphocytes. By Western blotting, we observed small but consistent differences in the Mr of DAF from the membranes of various cell types. Quantitative studies showed that phagocytes and B lymphocytes, which presumably enter more frequently in contact with immune complexes and other potential activators of complement, had the highest DAF levels. As previously reported by others, the red cells from PNH patients were DAF deficient. When the patients' red cells were incubated in acidified serum (Ham test), only the DAF-deficient cells were lysed. In addition, we detected defects in DAF expression on platelets and all types of leukocytes. The observed patterns of DAF deficiency in these patients were consistent with the concept that the PNH cells were of monoclonal origin. In one patient, abnormal and normal cells were found only in the erythroid, myeloid, and megakaryocytic lineages. In two other patients, the lymphocytes were also DAF deficient, suggesting that a mutation occurred in a totipotent stem cell. It appears, therefore, that the lesion leading to PNH can occur at various stages in the differentiation of hematopoietic cells.

Kirby, S. L., D. N. Cook, et al. (1996). "Proliferation of multipotent hematopoietic cells controlled by a truncated erythropoietin receptor transgene." Proc Natl Acad Sci U S A **93**(18): 9402-7.

The long-term efficacy of gene therapy using bone marrow transplantation requires the engraftment of genetically altered totipotent hematopoietic stem cells (THSCs). Ex vivo expansion of corrected THSCs is one way to increase the efficiency of the procedure. Similarly, selective in vivo expansion of the therapeutic THSCs rather than the endogenous THSCs could favor the transplant. To test whether a conferred proliferative advantage gene can facilitate the in vitro and in vivo expansion of hematopoietic stem cells, we have generated transgenic mice expressing a truncated receptor for the growth factor erythropoietin. These mice are phenotypically normal, but when treated in vivo with exogenous erythropoietin they exhibit a marked increase in multipotent, clonogenic hematopoietic cells [colony-forming units in the spleen (CFU-S) and CFUs that give rise to granulocytes, erythroid cells, macrophages, and megakaryocytes within the same colony (CFU-GEMM)] in comparison with the wild-type mice. In addition, long-term in vitro culture of tEpoR transgenic bone marrow in the presence of erythropoietin induces exponential expansion of trilineage hematopoietic stem cells not seen with wild-type bone marrow. Thus, the truncated erythropoietin

receptor gene shows promise as a means for obtaining cytokine-inducible hematopoietic stem cell proliferation to facilitate the direct targeting of THSCs and to provide a competitive repopulation advantage for transplanted therapeutic stem cells.

Kobayashi, N., J. D. Rivas-Carrillo, et al. (2005). "Gene delivery to embryonic stem cells." Birth Defects Res C Embryo Today **75**(1): 10-8.

Since the establishment of embryonic stem (ES) cells and the identification of tissue-specific stem cells, researchers have made great strides in the analysis of the natural biology of such stem cells for the development of therapeutic applications. Specifically, ES cells are capable of differentiating into all of the cell types that constitute the whole body. Thus, ES cell research promises new type of treatments and possible cures for a variety of debilitating diseases and injuries. The potential medical benefits obtained from stem cell technology are compelling and stem cell research sees a bright future. Control of the growth and differentiation of stem cells is a critical tool in the fields of regenerative medicine, tissue engineering, drug discovery, and toxicity testing. Toward such a goal, we present here an overview of gene delivery in ES cells, covering the following topics: significance of gene delivery in ES cells, stable versus transient gene delivery, cytotoxicity, suspension versus adherent cells, expertise, time, cost, viral vectors for gene transduction (lentiviruses, adenoviruses, and adeno-associated viruses, chemical methods for gene delivery, and mechanical or physical gene delivery methods (electroporation, nucleofection, microinjection, and nuclear transfer).

Krichevsky, A. M., K. C. Sonntag, et al. (2006). "Specific microRNAs modulate embryonic stem cell-derived neurogenesis." Stem Cells **24**(4): 857-64.

MicroRNAs (miRNAs) are recently discovered small non-coding transcripts with a broad spectrum of functions described mostly in invertebrates. As post-transcriptional regulators of gene expression, miRNAs trigger target mRNA degradation or translational repression. Although hundreds of miRNAs have been cloned from a variety of mammalian tissues and cells and multiple mRNA targets have been predicted, little is known about their functions. So far, a role of miRNA has only been described in hematopoietic, adipocytic, and muscle differentiation; regulation of insulin secretion; and potentially regulation of cancer growth. Here, we describe miRNA expression profiling in mouse embryonic stem (ES) cell- derived neurogenesis in vitro and show that a number of miRNAs are simultaneously co-induced during differentiation of neural progenitor cells to neurons and astrocytes. There was a clear correlation between miRNA expression profiles in ES cell-derived neurogenesis in vitro and in embryonal neurogenesis in vivo. Using both gain-of-function and loss-of-function approaches, we demonstrate that brain-specific miR-124a and miR-9 molecules affect neural lineage differentiation in the ES cell-derived cultures. In addition, we provide evidence that signal transducer and activator of transcription (STAT) 3, a member of the STAT family pathway, is involved in the function of these miRNAs. We conclude that distinct miRNAs play a functional role in the determination of neural fates in ES cell differentiation.

Kucia, M., W. Wu, et al. (2007). "Bone marrow-derived very small embryonic-like stem cells: their developmental origin and biological significance." Dev Dyn **236**(12): 3309-20.

Data from our and other laboratories provide evidence that bone marrow (BM) contains a population of stem cells that expresses early developmental markers such as (1) stage-specific embryonic antigen (SSEA) and (2) transcription factors Oct-4 and Nanog. These are the markers characteristic for embryonic stem cells, epiblast stem cells, and primordial germ cells (PGC). The presence of these stem cells in adult BM supports the concept that this organ contains some population of pluripotent stem cells that is deposited in embryogenesis during early gastrulation. We hypothesize that these cells could be direct descendants of the germ lineage that, to pass genes on to the next generations, has to create soma and, thus, becomes a "mother lineage" for all somatic cell lineages present in the adult body. Germ potential is established after conception in totipotent zygotes and retained in blastomeres of morula, cells from the inner cell mass of blastocyst, epiblast, and population of PGC. We will present a concept that SSEA(+) Oct-4(+) Nanog(+) cells identified in BM could be descendants of epiblast cells as well as some rare migrating astray PGC.

Kucia, M., E. K. Zuba-Surma, et al. (2007). "Adult marrow-derived very small embryonic-like stem cells and tissue engineering." Expert Opin Biol Ther **7**(10): 1499-514.

A population of CXCR4(+) lin(-) CD45(-) cells that express SSEA, Oct-4 and Nanog has been identified in adult bone marrow. These cells are very small and display several features typical for primary embryonic stem cells such as: i) a large nuclei surrounded by a narrow rim of cytoplasm; ii) open-type chromatin (euchromatin); and iii) high telomerase activity. These cells were named very small embryonic-like stem cells (VSEL-SC). The authors

hypothesized that they are direct descendants of the germ lineage. Germ lineage, in order to pass genes on to the next generation, has to create soma and thus becomes a 'mother lineage' for all somatic cell lineages present in the adult body. Germ potential is established after conception in a totipotent zygote and retained subsequently during development in blastomers of morula, cells form the inner cell mass of blastocyst, epiblast and population of primordial germ cells. The authors envision that VSEL-SC are epiblast-derived pluripotent stem cells and could potentially become a less-controversial source of stem cells for regeneration.

Ladurner, P., R. Rieger, et al. (2000). "Spatial distribution and differentiation potential of stem cells in hatchlings and adults in the marine platyhelminth macrostomum sp.: a bromodeoxyuridine analysis." Dev Biol **226**(2): 231-41.

Stem cells (neoblasts) in Platyhelminthes are pluripotent, and likely totipotent, undifferentiated cells which retain throughout adult life the capacity to proliferate and from which all somatic cells as well as the germ cells derive. However, basic data on the pool and heterogeneity of neoblasts, their rates of differentiation into sets and subsets of differentiated cells, and their migration to different body regions are still lacking. To fill this gap, S-phase cells in the macrostomid Macrostomum sp. were labeled with the thymidine analog 5-bromo-2'-deoxyuridine (BrdU). S-phase cells were found to be neoblasts and to be distributed in two bands along the lateral sides of the body leaving unlabeled the median axis of the body and the region anterior to the eyes. This distribution is parallel to that of mitotic cells demonstrated using an antibody to phosphorylated histone H3. At different chase times, clusters of BrdU-labeled cells appear, labeled cells migrate to formerly unlabeled areas, and they differentiate into several somatic cell types and into germ cells. Finally, continuous exposure to BrdU shows an extensive renewal of the epithelial cells. Altogether, these results strengthen the idea of platyhelminth neoblasts as an unparalleled stem-cell system within the Animal Kingdom calling for further investigation.

Lang, D., M. M. Lu, et al. (2005). "Pax3 functions at a nodal point in melanocyte stem cell differentiation." Nature **433**(7028): 884-7.

Most stem cells are not totipotent. Instead, they are partially committed but remain undifferentiated. Upon appropriate stimulation they are capable of regenerating mature cell types. Little is known about the genetic programmes that maintain the undifferentiated phenotype of lineage-restricted stem cells. Here we describe the molecular details of a nodal point in adult melanocyte stem cell differentiation in which Pax3 simultaneously functions to initiate a melanogenic cascade while acting downstream to prevent terminal differentiation. Pax3 activates expression of Mitf, a transcription factor critical for melanogenesis, while at the same time it competes with Mitf for occupancy of an enhancer required for expression of dopachrome tautomerase, an enzyme that functions in melanin synthesis. Pax3-expressing melanoblasts are thus committed but undifferentiated until Pax3-mediated repression is relieved by activated beta-catenin. Thus, a stem cell transcription factor can both determine cell fate and simultaneously maintain an undifferentiated state, leaving a cell poised to differentiate in response to external stimuli.

Le Douarin, N. M., E. Dupin, et al. (1994). "Genetic and epigenetic control in neural crest development." Curr Opin Genet Dev **4**(5): 685-95.

The neural crest is a fascinating structure of the vertebrate embryo; its ontogeny includes a transient period during which its component cells undergo an epithelio-mesenchymal transition and become migratory. This phase was shown recently to be controlled by the 'Slug' gene which belongs to the 'Snail' family of Drosophila transcription factors. After homing to specific sites in the embryo, the crest-derived cells produce a large variety of phenotypes. Recent advances have shown that during migration most crest cells exhibit various degrees of pluripotentiality, some being already committed to a single and definite fate. Moreover, several lines of evidence point to the existence of totipotent stem cells in the neural crest, the progeny of which become progressively diversified through a combination of intrinsic and extrinsic influences. The latter have been documented by the disruption of several neurotrophin genes, which results in severe deficiencies of selected subsets of neural crest derivatives. The neural crest has also been shown to play an important role in the development of the vertebrate head and hypobranchial region. The genetic control of this process depends on the activity of developmental genes, among which the vertebrate Hox genes are essential, particularly at the rhombencephalic level.

Lee, J., B. K. Rhee, et al. (2005). "Stimulation of Oct-4 activity by Ewing's sarcoma protein." Stem Cells **23**(6): 738-51.

The Oct-4 gene encodes a transcription factor that is expressed in embryonic stem (ES) cells and germ cells. Oct-4 is known to function as a transcriptional activator of genes involved in maintaining an undifferentiated totipotent state and possibly in preventing expression of genes activated

during differentiation. In addition, it is a putative proto-oncogene and a critical player in the genesis of human testicular germ cell tumors. Although much effort has gone toward characterizing Oct-4, there is still little known about the molecular mechanisms and the proteins that regulate Oct-4 function. To identify cofactors that control Oct-4 function in vivo, we used a recently developed bacterial two-hybrid screening system and isolated a novel ES cell-derived cDNA encoding Ewing's sarcoma protein (EWS). EWS is a proto-oncogene and putative RNA-binding protein involved in human cancers. By using glutathione-S-transferase (GST) pull-down assays, we were able to confirm the interaction between Oct-4 and EWS in vitro, and moreover, coimmunoprecipitation and colocalization studies have shown that these proteins also associate in vivo. We have mapped the EWS-interacting region to the POU domain of Oct-4. In addition, three independent sites on EWS are involved in binding to Oct-4. In this study, we report that Oct-4 and EWS are coexpressed in the pluripotent mouse and human ES cells. Consistent with its ability to bind to and colocalize with Oct-4, ectopic expression of EWS enhances the transactivation ability of Oct-4. Moreover, a chimeric protein generated by fusion of EWS (1-295) to the GAL4 DNA-binding domain significantly increases promoter activity of a reporter containing GAL4 DNA-binding sites, suggesting the presence of a strong activation domain within EWS. Taken together, our results suggest that Oct-4-mediated transactivation is stimulated by EWS.

Leibfried, A., J. P. To, et al. (2005). "WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators." Nature **438**(7071): 1172-5.

Plants continuously maintain pools of totipotent stem cells in their apical meristems from which elaborate root and shoot systems are produced. In Arabidopsis thaliana, stem cell fate in the shoot apical meristem is controlled by a regulatory network that includes the CLAVATA (CLV) ligand-receptor system and the homeodomain protein WUSCHEL (WUS). Phytohormones such as auxin and cytokinin are also important for meristem regulation. Here we show a mechanistic link between the CLV/WUS network and hormonal control. WUS, a positive regulator of stem cells, directly represses the transcription of several two-component ARABIDOPSIS RESPONSE REGULATOR genes (ARR5, ARR6, ARR7 and ARR15), which act in the negative-feedback loop of cytokinin signalling. These data indicate that ARR genes might negatively influence meristem size and that their repression by WUS might be necessary for proper meristem function. Consistent with this hypothesis is our observation that a mutant ARR7 allele, which mimics the active, phosphorylated form, causes the formation of aberrant shoot apical meristems. Conversely, a loss-of-function mutation in a maize ARR homologue was recently shown to cause enlarged meristems.

Lelias, J. M., C. N. Adra, et al. (1993). "cDNA cloning of a human mRNA preferentially expressed in hematopoietic cells and with homology to a GDP-dissociation inhibitor for the rho GTP-binding proteins." Proc Natl Acad Sci U S A **90**(4): 1479-83.

We have identified the mRNA for a human gene, denoted D4, which is expressed at very high levels in hematopoietic cell lines and in normal cells of lymphoid and myeloid origin. The 1.5-kb transcript is absent or detectable only at low levels in nonhematopoietic tissues. D4 encodes a 201-amino acid protein with homology to rhoGDI, an inhibitor of GDP dissociation for the ras-homologous protein rho. D4 might function also as a regulator of guanine nucleotide exchange for small GTP-binding proteins. A homologous transcript of similar size is also preferentially expressed in murine hematopoietic tissues. When totipotent murine embryonic stem cells develop in vitro into hematopoietic cells, the gene is activated with the onset of hematopoiesis. When hematopoietic cell lines are induced to differentiate, the expression of D4 is modulated. Thus, D4 appears to be a developmentally regulated gene. Its preferential expression in hematopoietic cells indicates that D4 likely plays some significant role in the growth and differentiation processes of hematopoietic cells. This significance is underscored by increasing evidence for the involvement of regulators of G proteins in clinical diseases.

Lemez, P. (1990). "Significance of lineage specific differentiation markers for complex classification of acute leukemias. I. Acute myeloid leukemias." Neoplasma **37**(3): 253-66.

Acute leukemias are clonal malignant neoplastic diseases which do not originate from the transformation of totipotent hematopoietic stem cells but of progenitors committed to the myeloid, T-lymphatic or B-lymphatic differentiation lineage. The transforming event seems to be associated with a nonrandom aberrant DNA rearrangement. Although a leukemic population is clonal, originating from a single cell, it exhibits phenotypic, and sometimes even karyotypic, heterogeneity. Leukemic cells are allocated to a particular differentiation cell lineage on the basis of a positive finding of the lineage specific differentiation marker (LSDM) in the presented classification of acute leukemias. Criteria for common types of acute myeloid leukemias are described and

the possible existence of several other types is discussed.

Lemieux, M. E., V. I. Rebel, et al. (1995). "Characterization and purification of a primitive hematopoietic cell type in adult mouse marrow capable of lymphomyeloid differentiation in long-term marrow "switch" cultures." Blood **86**(4): 1339-47.

In this report, we describe a modification of the assay for long-term culture-initiating cells (LTC-IC) that allows a subset of murine LTC-IC (designated as LTC-ICML) to express both their myeloid (M) and lymphoid (L) differentiative potentials in vitro. The modified assay involves culturing test cells at limiting dilutions on irradiated mouse marrow feeder layers for an initial 4 weeks under conditions that support myelopoiesis and then for an additional week under conditions permissive for B-lymphopoiesis. All of the clonogenic pre-B progenitors (colony-forming unit [CFU] pre-B) detected in such postswitch LTC appear to be the progeny of uncommitted cells present in the original cell suspension because exposure of lymphoid-restricted progenitors to myeloid LTC conditions for > or = 7 days was found to irreversibly terminate CFU-pre-B production and, in cultures initiated with limiting numbers of input cells (no progenitors of any type detected in > 70% of cultures 1 week after the switch), the presence of CFU-pre-B was tightly associated with the presence of myeloid clonogenic cells, regardless of the purity of the input population. Limiting dilution analysis of the proportion of negative cultures measured for different numbers of input cells showed the frequency of LTC-ICML in normal adult mouse marrow to be 1 per 5 x 10(5) cells with an enrichment of approximately 500-fold in the Sca-1+ Lin-WGA+ fraction, as was also found for competitive in vivo repopulating units (CRU) and conventionally defined LTC-IC. LTC-ICML also exhibited the same resistance to treatment in vivo with 5-fluorouracil (5-FU) as CRU and LTC-IC, thereby distinguishing these three populations from the great majority of both in vitro clonogenic cells and day 12 CFU-S. The ability to quantitate cells with dual lymphoid and myeloid differentiation potentials in vitro, without the need for their prior purification, should facilitate studies of totipotent hematopoietic stem cell regulation.

Lemischka, I. R. (1991). "Clonal, in vivo behavior of the totipotent hematopoietic stem cell." Semin Immunol **3**(6): 349-55.

Classical and more recent studies have provided a description of the in vivo behavior of the totipotent hematopoietic stem cell and its clonal progeny. These reconstitution experiments, employing clonotypic markers have shown that single or few engrafted lymphoid-myeloid stem cells are both necessary and sufficient for long-term, stable hematopoiesis in a reconstituted mouse. This underscores the remarkable developmental capacity of individual stem cell clones. Furthermore, the long-term and retransplantation studies have provided an indication of stem cell self-renewal ability. Taken together, the long-term analyses have also shed light on the dynamic behavior of engrafted stem cell clones and of the entire reconstituted hematopoietic system. A model is presented where the developmental and proliferative behavior of totipotent stem cells is a function of time. In this model commitment versus self-renewal decisions may be governed by stochastic mechanisms. However, the actual contribution by stem cells to particular mature cell populations may be more a function of lineage specific demands as they change over post-engraftment time.

Lemischka, I. R. (1992). "The haematopoietic stem cell and its clonal progeny: mechanisms regulating the hierarchy of primitive haematopoietic cells." Cancer Surv **15**: 3-18.

Transplantation and marking studies have provided an accurate description of various aspects of developmental and proliferative behaviour of totipotent haematopoietic stem cells. In particular, the remarkable ability of different clones to contribute to all lineages in a continuous, stable and long term manner is a hallmark of stem cell behaviour. Taken together, in vivo reconstitution experiments also provide the basis for a model of stem cell regulation that incorporates stochastic and non-stochastic components. More recent approaches provide optimism that many descriptive aspects of stem cell biology will soon be supported by elucidation of molecular mechanisms.

Li, L. and K. Akashi (2003). "Unraveling the molecular components and genetic blueprints of stem cells." Biotechniques **35**(6): 1233-9.

Remarkable progress in stem cell biology research over the past few years has provoked a promise for the future of tissue regeneration and gene therapies; so much so, that the use of stem cells in clinical therapy seemed to be just around the corner. However, we now realize there is still a huge task before us to improve our understanding of the nature of stem cells before utilizing them to benefit human health. Stem cell behavior is determined by specific gene products; thus, unraveling the molecular components and genetic blueprints of stem cells will provide important insight into understanding stem cell properties. Here we summarize the research of various groups using microarray technology and other approaches to determine the gene expression profiles

in stem cells, particularly in hematopoietic stem cells (HSCs). These works have, to a certain degree, helped to narrow down the candidate genes predominantly expressed in HSCs, revealed a list of stemness genes, and indirectly demonstrated the wide-open chromatin state of stem cells and, with it, the molecular basis of the multipotentiality of stem cells.

Li, S. and P. D. Yurchenco (2006). "Matrix assembly, cell polarization, and cell survival: analysis of peri-implantation development with cultured embryonic stem cells." Methods Mol Biol **329**: 113-25.

A variety of mutations, including those affecting laminin expression and basement membrane, cause early embryonic lethality in the peri-implantation period. However, low cell numbers and inaccessibility of these small embryos make it difficult to study the molecular mechanisms that underlie these defects. Embryoid bodies cultured as suspended spherical cell aggregates derived from normal and defective embryonic stem cells provide a tractable experimental system with which the early developmental processes can be recapitulated under defined conditions. Thus, endoderm formation and maturation, basement membrane assembly and its signaling consequences, epiblast polarization, apoptosis, and cavitation can be studied using a combination of genetic, biochemical, cell, and molecular biology approaches.

Liu, J., J. F. Schiltz, et al. (2003). "Hmga1 is required for normal sperm development." Mol Reprod Dev **66**(1): 81-9.

The Hmgi protein family of chromosomal architectural factors is extensively studied for its roles in embryogenesis and its association with benign mesenchymal tumors. Although the biochemical function of Hmga1 has been studied in vitro, to provide in vivo insight into its biological function, a targeted disruption of Hmga1 was initiated. Chimeric founder mice were derived from embryonic stem (ES) cells harboring a targeted mutation in a single Hmga1 allele. These 14 different chimeric founders produced 494 black progeny. Since none of these 494 progeny were agouti, none of them were derived from ES cells. Control injections of the wild-type ES cell lines resulted in ES cell derived agouti mice, indicating that the ES cells were totipotent. Therefore, our results indicate that one intact Hmga1 allele was not sufficient for germ-line transmission of the ES cells. Seven chimeric founder mice that were examined histologically demonstrated aberrant regions in their reproductive organs. Aberrant regions of seminiferous tubules were reduced in diameter, demonstrated vacuolated Sertoli cells, and had an absolute deficiency of sperm. While the Hmga1(+/-) ES cells were shown to contribute to the formation of the epididymides, they did not significantly contribute to the testes of chimeric founder mice. No sperm isolated from any of the Hmga1(+/-) chimeric mice were shown to arise from the ES cells, as none of them contained the targeted disruption of the Hmga1 gene. Our results suggest that both alleles of Hmga1 are required for normal sperm production in the mouse.

Liu, L., E. Czerwiec, et al. (2004). "Effect of ploidy and parental genome composition on expression of Oct-4 protein in mouse embryos." Gene Expr Patterns **4**(4): 433-41.

The transcription factor Oct-4 is expressed in germ cells and also is considered as a marker for pluripotency of stem cells. We first examined dynamics of Oct-4 protein expression during preimplantation development using both Western blot analysis, and immunofluorescence staining. We show that intact Oct-4 protein is not detected in either ovulated mature oocytes, or in zygotes and 2-4-cell embryos, which are the only known totipotent cell types in mammals. This finding is unexpected, since Oct-4 has been proposed to play a role in the control of totipotency. The results suggest that Oct-4 is not indispensable for fertilization and early cleavage. Rather, expression of Oct-4 protein is first detected in the nuclei of 8-16 cell morula, increases in early blastocysts, and declines in late blastocysts, in which most Oct-4 protein is confined to the inner cell mass (ICM) region, consistent with previous findings. We further compared Oct-4 protein expression in diploid and tetraploid blastocysts derived from normal fertilization or parthenogenesis, as well as expression in diploid androgenetic blastocysts. Expression levels and localization of Oct-4 protein are similar in both diploid and tetraploid early blastocysts, regardless of whether blastocysts are derived from fertilization or parthenogenesis. Androgenetic diploid blastocysts also express similar levels of Oct-4. Late blastocysts generated by both fertilization and parthenogenesis show a similar pattern of Oct-4 expression, suggesting that paternal genome activation is not required for Oct-4 expression. Expression of Oct-4 protein does not differ between diploid and tetraploid embryos, indicating that tetraploidy does not influence Oct-4 expression. Thus, expression of Oct-4 protein is initiated at morula stage in preimplantation embryos and completely controlled by a mechanism activated in oocytes. Downregulation of Oct-4 expression coincides with differentiation of trophectoderm. Similar profiles of Oct-4 expression observed in embryos with different ploidy and genome composition, are suggestive of Oct-4 being necessary but not sufficient for developmental potency.

Loring, J. F., J. G. Porter, et al. (2001). "A gene expression profile of embryonic stem cells and embryonic stem cell-derived neurons." Restor Neurol Neurosci **18**(2-3): 81-8.

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

Louie, S. G. and B. Jung (1993). "Clinical effects of biologic response modifiers." Am J Hosp Pharm **50**(7 Suppl 3): S10-8.

The clinical use of the biologic response modifiers filgrastim, sargramostim, and regramostim is reviewed. All circulating blood cells are derived from totipotent hematopoietic stem cells. Various biologic response modifiers, including lymphokines and colony-stimulating factors, regulate and activate the lymphoid and myeloid cells of the blood. One of the more important types of blood cell for fighting infection is the neutrophil. Patients with low neutrophil concentrations are at high risk of developing neutropenic fevers and infections. The colony-stimulating factors filgrastim, sargramostim, and regramostim increase the production of circulating neutrophils, and this action is clinically useful in patients undergoing myelosuppressive antineoplastic therapy or bone marrow transplantation and in patients with the acquired immunodeficiency syndrome. Clinical studies of these agents in comparison with antimicrobial prophylaxis or placebo have shown a decreased rate of neutropenic-associated hospitalizations and infections. These agents are also under study for dose intensification of antineoplastics in patients with various solid tumors and for augmenting patient responses to antimicrobial therapy in situations where there is high risk of morbidity and mortality. Sargramostim and regramostim are both granulocyte-macrophage colony-stimulating factors that differ in their degree of glycosylation and source of production, and at high doses they can cause life-threatening adverse effects because they stimulate the production of a broad range of leukocytes. Filgrastim, which stimulates only the production of neutrophils, has been better tolerated, especially at higher doses. Biologic response modifiers hold much promise for improving therapy of certain clinical conditions by decreasing myelosuppressive complications and enhancing responses to other drugs.(ABSTRACT TRUNCATED AT 250 WORDS)

Lu, W. G., H. Chen, et al. (2007). "Regionspecific survival and differentiation of mouse embryonic stem cell-derived implants in the adult rat brain." Sheng Li Xue Bao **59**(1): 51-7.

Totipotent and regionally non-specified embryonic stem (ES) cells provide a powerful tool to understand mechanisms controlling stem cell differentiation in different regions of the adult brain. As the development capacity of ES cells in the adult brain is still largely unknown, we grafted small amounts of mouse ES (mES) cells into adult rat brains to explore the survival and differentiation of implanted mES cells in different rat brain regions. We transplanted the green fluorescent protein (GFP)-positive mES cells into the hippocampus, septal area, cortex and caudate nucleus in rat brains. Then the rats were sacrificed 5, 14 and 28 d later. Of all the brain regions, the survival rate of the transplanted cells and their progeny were the highest in the hippocampus and the lowest in the septal area ($P<0.01$). The grafted ES cells could differentiate into nestin-positive neural stem cells. Nestin-positive/GFP-positive cells were observed in all brain regions with the highest frequency of nestin-positive cells in the hippocampus and the lowest in the medial septal area ($P<0.01$). mES cells differentiated into end cells such as neurons and glial cells in all transplantation sites in recipient brains. In the hippocampus, the ES cells differentiated into neurons in large amounts. These results demonstrate that only some brain regions permit survival of mES cells and their progeny, and form instructive environments for neuronal differentiation of mES cells. Thus, because of region specific presence of microenvironmental cues and their

environmental fields, the characteristics of the recipient tissue were considerably important in formulating cell replacement strategies for neural disorders.

Luo, Y., C. Schwartz, et al. (2006). "A focused microarray to assess dopaminergic and glial cell differentiation from fetal tissue or embryonic stem cells." Stem Cells **24**(4): 865-75.

We designed oligonucleotide gene-specific probes to develop a focused array that can be used to discriminate between neural phenotypes, identify biomarkers, and provide an overview of the process of dopaminergic neuron and glial differentiation. We have arrayed approximately 100 genes expressed in dopaminergic neurons, oligodendrocytes, and astrocytes, an additional 200 known cytokines, chemokines, and their respective receptors, as well as markers for pluripotent and progenitor cells. The gene-specific 60-mer 3' biased oligonucleotides for these 281 genes were arrayed in a 25 x 12 format based on function. Using human adult brain substantia nigra, human embryonic stem cells (ESCs), and the differentiated progeny of pluripotent cells, we showed that this array was capable of distinguishing dopaminergic neurons, glial cells, and pluripotent cells by their gene expression profiles in a concentration-dependent manner. Using linear correlation coefficients of input RNA with output intensity, we identified a list of genes that can serve as reporting genes for detecting dopaminergic neurons, glial cells, and contaminating ESCs and progenitors. Finally, we monitored NTera2 differentiation toward dopaminergic neurons and have shown the ability of this array to distinguish stages of differentiation and provide important clues to factors regulating differentiation, the degree of contaminating populations, and stage of cell maturity. We suggest that this focused array will serve as a useful complement to other large-scale arrays in routine assessment of cell properties prior to their therapeutic use.

MacKinney, A. A., Jr., S. S. Clark, et al. (1993). "Simultaneous demonstration of the Philadelphia chromosome in T, B, and myeloid cells." Am J Hematol **44**(1): 48-52.

A patient presented with lymphoblastic lymphoma in lymph-nodes and chronic myelogenous leukemia (CML) in narrow and peripheral blood. All marrow and unstimulated peripheral blood cells contained the Philadelphia chromosome[t(9:22)]. Lymphoma cells were analyzed by flow cytometry and were identified as T cells (CD2+CD5+CD7+CD34+). All fresh lymphoma cells contained the t(9:22) translocation. Cultures of purified peripheral blood T and B cells and specifically stimulated NK cells revealed that 59% of the B cells, 10% of the NK cells, and none of the normal T cells contained the translocation. The lack of translocation in normal peripheral T cells is attributed to their long lifespan. No rearrangement of immunoglobulin or T cell receptor beta or gamma genes was found in either the leukemia or lymphoma cells. Analysis of the DNA from cryopreserved lymphoma biopsy showed clonal rearrangement within the common breakpoint cluster region of the bcr gene identical to the bcr rearrangement in DNA from leukemia blood cells. The data support the concept that T and B cells originate in the patient's totipotent stem cell from which the CML is also derived.

Magyar, J. P., M. Nemir, et al. (2001). "Mass production of embryoid bodies in microbeads." Ann N Y Acad Sci **944**: 135-43.

Embryonic stem cells (ESC) are totipotent cells that can differentiate into a large number of different cell types. Stem cell-derived, differentiated cells are of increasing importance as a potential source for non-proliferating cells (e.g., cardiomyocytes or neurons) for future tissue engineering applications. Differentiation of ESC is initiated by the formation of embryoid bodies (EB). Current protocols for the generation of EB are either of limited productivity or deliver EB with a large variation in size and differentiation state. To establish an efficient and robust EB production process, we encapsulated mouse ESC into alginate microbeads using various microencapsulation technologies. Microencapsulation and culturing of ESC in 1.1% alginate microbeads gives rise to discoid colonies, which further differentiate within the beads to cystic EB and later to EB containing spontaneously beating areas. However, if ESC are encapsulated into 1.6% alginate microbeads, differentiation is inhibited at the morula-like stage, so that no cystic EB can be formed within the beads. ESC colonies, which are released from 1.6% alginate microbeads, can further differentiate to cystic EB with beating cardiomyocytes. Extended supplementation of the growth medium with retinoic acid promotes differentiation to smooth muscle cells.

Mannini, L., L. Rossi, et al. (2004). "Djeyes absent (Djeya) controls prototypic planarian eye regeneration by cooperating with the transcription factor Djsix-1." Dev Biol **269**(2): 346-59.

A conserved network of nuclear proteins is crucial to eye formation in both vertebrates and invertebrates. The finding that freshwater planarians can regenerate eyes without the contribution of Pax6 suggests that alternative combinations of regulatory

elements may control the morphogenesis of the prototypic planarian eye. To further dissect the molecular events controlling eye regeneration in planarians, we investigated the role of eyes absent (Djeya) and six-1 (Djsix-1) genes in Dugesia japonica. These genes are expressed in both regenerating eyes and in differentiated photoreceptors of intact adults. Through RNAi studies, we show that Djsix-1 and Djeya are both critical for the regeneration of normal eyes in planarians and genetically cooperate in vivo to establish correct eye cell differentiation. We further demonstrate that the genetic interaction is mediated by physical interaction between the evolutionarily conserved domains of these two proteins. These data indicate that planarians use cooperatively Djsix-1 and Djeya for the proper specification of photoreceptors, implicating that the mechanism involving their evolutionarily conserved domains can be very ancient. Finally, both Djsix-1 and Djeya double-stranded RNA are substantially more effective at producing no-eye phenotypes in the second round of regeneration. This is probably due to the significant plasticity of the planarian model system, based on the presence of a stable population of totipotent stem cells, which ensure the rapid cell turnover of all differentiated cell types.

Mardanpour, P., K. Guan, et al. (2008). "Potency of germ cells and its relevance for regenerative medicine." J Anat **213**(1): 26-9.

Germline stem cells, which can self-renew and generate gametes, are unique stem cells in that they are solely dedicated to transmit genetic information from generation to generation. The germ cells have a special place in the life cycle because they must be able to retain the ability to recreate the organism, a property known as developmental totipotency. Several lines of evidence have suggested the extensive proliferation activity and pluripotency of prenatal, neonatal and adult germline stem cells. We showed that adult male germline stem cells, spermatogonial stem cells, can be converted into embryonic stem cell-like cells, which can differentiate into the somatic stem cells of three germ layers. Different cell types such as vascular, heart, liver, pancreatic and blood cells could also be obtained from these stem cells. Understanding how spermatogonial stem cells can give rise to pluripotent stem cells and how somatic stem cells differentiate into germ cells could give significant insight into the regulation of developmental totipotency as well as having important implications for male fertility and regenerative medicine.

Marguerie, G., V. Roullot, et al. (1996). "Dissecting megakaryocytopoiesis in vivo with toxigenes." Stem Cells **14 Suppl 1**: 200-5.

The genetic programs that regulate the commitment of a totipotent stem cell to the megakaryocytic lineage remain poorly defined and require appropriate in vivo models. Using a cell-specific obliteration technique, a transgenic mouse model was produced where perturbations of megakaryocytopoiesis and platelet production may be induced on demand. This was achieved by targeting the expression of the herpes virus thymidine kinase (HSV-tk) to megakaryocytes using the regulatory regions of the gene coding for the alphaIIb gene, an early marker of megakaryocytopoiesis, which encodes the alpha subunit of the platelet integrin alphaIIb beta3. The HSV-tk gene is not toxic by itself, but sensitizes the target cell to the effect of ganciclovir (GCV), leading to the inhibition of DNA synthesis in dividing cells. The programmed eradication of the megakaryocytic lineage was induced by treating transgenic mice bearing the hybrid construct (alphaIIb-tk) with GCV. After 10 days of treatment, the platelet number was reduced by greater than 96.5% and megakaryocytes were not detectable in the bone marrow (BM). After discontinuing GCV, BM was repopulated with megakaryocytes, and the platelet count was restored within seven days. The recovery was accelerated by the administration of interleukin 11. Prolonged GCV treatment induced erythropenia in the transgenic mice. Assays of myeloid progenitor cells in vitro demonstrated that the transgene was expressed in early erythro-megakaryocytic bipotent progenitor cells. The reversibility and facility of this system provide a powerful model to determine both the critical events in megakaryocytic and erythroid lineage development, and for evaluating the precise role that platelets play in the pathogenesis of a number of vascular occlusive disorders.

Marguerie, G. and D. Tronik-Le Roux (1998). "Analysis of hematopoietic stem cell reprogramming with toxigenicity." Stem Cells **16 Suppl 2**: 85-9.

The molecular mechanisms by which a stem cell is committed to individual lineage are largely unknown. Two different models, though not mutually exclusive, are currently debated. The first describes the temporal and hierarchical coordination of lineage-specific transcriptional programs. The second suggests that multilineage genes are expressed in a self-renewing and undifferentiated cell prior to lineage commitment. To challenge these two models in in vivo-appropriate conditions, the expression of an exogenous toxigene was used to create transgenic animals in which an inducible, reversible cell knock-out at a specific stage of differentiation could be

achieved. Both additional transgenesis using the megakaryocyte specific alphaIIb promoter and targeted transgenesis were used to express the herpes virus thymidine kinase (tk) gene in the megakaryocytic lineage. When the tk gene was targeted to the locus of the megakaryocyte-specific alphaIIb gene, a typical Glanzman thrombasthenic syndrome was created. Despite this bleeding disorder, the lack of expression of the alphaIIb gene did not affect the development of the mice. In both transgenic and targeted animals, all progenitor cells were sensitive to the effect of the gancyclovir (GCV), both in vivo and ex vivo. Long-term bone marrow cell cultures on stromal layers indicated that most of the very early progenitor cells expressed the enzyme. All the results obtained with this inducible toxic phenotype indicated that genetic programs that are in control of the expression of lineage-specific genes are operative in a totipotent stem cell prior to lineage commitment and strongly support the concept that stem cells express a multilineage transcriptome.

Matin, A., G. B. Collin, et al. (1998). "Testicular teratocarcinogenesis in mice--a review." Apmis **106**(1): 174-82.

Spontaneous testicular germ cell tumours in humans and mice are remarkable for their diverse composition. These tumours are usually composed of an extraordinary variety of cell and tissue types including muscle, skin, bone, cartilage, and neuroepithelia. Their diverse composition reflects their origin from totipotent primordial germ cells at about Day 12 of fetal development. Although much is known about the development of these tumours, remarkably little is known about the genetics of the mammalian primordial germ cell lineage or about the genes that control susceptibility to spontaneous testicular germ cell tumours in humans or mice. Conventional genetic analysis of susceptible 129/Sv mice is difficult because of the large number of susceptibility genes and their low penetrance. We are taking advantage of the Ter mutation to simplify the genetic analysis. Various evidence suggests that Ter is neither necessary nor sufficient for tumourigenesis. Instead, Ter acts as a modifier, dramatically increasing tumour incidence from approximately 1% in +/+ males, to approximately 17% in Ter/+ males and approximately 94% in Ter/Ter males. Segregation analysis suggests that Ter increases tumour incidence by requiring some, but perhaps not all, of the 129/Sv-derived susceptibility genes. With standard crosses that segregate for the Ter mutation, identification not only of Ter but also of these 129/Sv-derived susceptibility genes should be possible. In this paper, we review the genetics and development of germ cell tumours in 129/Sv mice, summarize the status of Ter mapping, and provide evidence that different genetic pathways lead to unilateral and bilateral tumours.

Metcalf, D. (2003). "The unsolved enigmas of leukemia inhibitory factor." Stem Cells **21**(1): 5-14.

Leukemia inhibitory factor (LIF) is a polyfunctional glycoprotein cytokine whose inducible production can occur in many, perhaps all, tissues. LIF acts on responding cells by binding to a heterodimeric membrane receptor composed of a low-affinity LIF-specific receptor and the gp130 receptor chain also used as the receptor for interleukin-6, oncostatin M, cardiotrophin-1, and ciliary neurotrophic factor. LIF is essential for blastocyst implantation and the normal development of hippocampal and olfactory receptor neurons. LIF is used extensively in experimental biology because of its key ability to induce embryonic stem cells to retain their totipotentiality. LIF has a wide array of actions, including acting as a stimulus for platelet formation, proliferation of some hematopoietic cells, bone formation, adipocyte lipid transport, adrenocorticotropic hormone production, neuronal survival and formation, muscle satellite cell proliferation, and acute phase production by hepatocytes. Unwanted actions of LIF can be minimized by circulating soluble LIF receptors and by intracellular suppression by suppressors of cytokine-signaling family members. However, the outstanding problems remain of how the induction of LIF is mediated in response to demands from such a heterogeneity of target tissues and why it makes design sense to use LIF in the regulation of such a diverse and unrelated series of biological processes.

Metzger, J. M., W. I. Lin, et al. (1995). "Myosin heavy chain expression in contracting myocytes isolated during embryonic stem cell cardiogenesis." Circ Res **76**(5): 710-9.

Mouse embryonic stem (ES) cells are totipotent cells derived from the inner cell mass of the preimplantation blastocyst and are capable of differentiating in vitro into cardiac myocytes. Attached cultures of differentiating ES cells were established to document the timing of contractile development by microscopic observation and to permit the microdissection of cardiac myocytes from culture. The onset of spontaneous contraction varied markedly in differentiation culture, with contraction being maintained on average for 9 days (range, 1 to 75 days). Indirect immunofluorescence in microscopy showed that myosin expression was localized to the contracting cardiac myocytes in culture. Myosin heavy chain (MHC) isoform expression in microdissected ES cell-derived cardiac myocytes was determined by means of sodium dodecyl sulfate-

polyacryl-amide gel electrophoresis. The distribution of MHC isoform expression in isolated ES cell cardiac myocytes was as follows: 27% expressed the beta-MHC isoform, 33% expressed both the alpha- and beta-MHC isoforms, and 40% expressed the alpha-MHC isoform. MHC phenotype was correlated to the duration of continuous contractile activity of the myocytes. Myocytes that had just initiated spontaneous contractile activity predominantly expressed the beta-MHC (average days of contraction before isolation, 2.5 +/- 0.7). The alpha-MHC isoform was detected after mouse prolonged contractile activity in vitro (1 to 5 weeks). A strong correlation was obtained between MHC phenotype and days of contraction of the cardiac myocyte preparations isolated from ES cell cultures (r = .93). The apparent transition in MHC isoform expression during ES cell differentiation parallels the beta- to alpha-MHC isoform transition characteristic of murine cardiac development in vivo. These findings are evidence that ES cell cardiac myocyte differentiation follows the normal developmental program of murine cardiogenesis.

Miller, C. L., V. I. Rebel, et al. (1996). "Studies of W mutant mice provide evidence for alternate mechanisms capable of activating hematopoietic stem cells." Exp Hematol **24**(2): 185-94.

Previous studies have suggested that Steel factor (SF) can influence the behavior of many types of hematopoietic progenitor cells both in vivo and in vitro, although whether these may include the most primitive populations of totipotent repopulating cells remains controversial. To approach this question, we measured the number of Sca1+Lin-WGA+ cells, the number of cells with demonstrable myeloid (long-term culture-initiating cell [LTC-IC]) or both myeloid and lymphoid (LTC-IC(ML)) potential in 4- to 5-week-old long-term cultures containing irradiated primary marrow feeder layers, and the number of multilineage long-term in vivo repopulating cells (competitive repopulating unit [CRU]) present in the marrow of W42/+ or W41/W41 mice compared to +/+ controls. There was no significant effect of either of these W mutations on the number of Sca1+Lin-WGA+ cells and, in W41/W41 mice, neither LTC-IC nor LTC-IC(ML) populations appeared to be affected. On the other hand, although W41/W41 and W42/+ cells could both be detected in the in vivo CRU assay, their numbers were markedly reduced (17- and seven-fold, respectively) in spite of the fact that both of these W mutant genotypes contained near normal numbers of day-9 and -12 colony-forming units-spleen (CFU-S). In vitro quantitation of erythroid (burst-forming units-erythroid [BFU-E]), granulopoietic (CFU-granulocyte/macrophage [CFU-GM]), multilineage (CFU-granulocyte/erythrocyte/monocyte/macrophage [CFU-GEMM]), and pre-B clonogenic progenitors (CFU-pre-B) also revealed no differences in the numbers (or proliferative potential) of any of these cells when W41/W41 or W42/+ and normal mice were compared, although day 3 BFU-E from both types of W mutant mice showed no response to the typical enhancing effect exerted by SF on their +/+ counterparts. Taken together, these findings are consistent with the view that SF activation of c-kit receptor-induced signaling events is not a rate-limiting mechanism controlling red blood cell production during normal development until hematopoietic cells differentiate beyond the day-3 BFU-E stage. Nevertheless, normal hematopoietic stem cells do appear to be responsive to SF, since their W mutant counterparts display a disadvantage in the in vivo setting which is exaggerated under conditions of hematopoietic regeneration. On the other hand, alternative mechanisms also appear to contribute to the regulation of hematopoietic stem cell numbers in vivo and to their detection as LTC-IC in vitro.

Mintz, B., K. Anthony, et al. (1984). "Monoclonal derivation of mouse myeloid and lymphoid lineages from totipotent hematopoietic stem cells experimentally engrafted in fetal hosts." Proc Natl Acad Sci U S A **81**(24): 7835-9.

Mutant mouse fetuses with a hematopoietic stem cell defect were injected with a mixture of two normal strains of fetal liver cells to test the possibility of seeding with single stem cells and of deriving all hematopoietic lineages clonally. Recipients were either Wf/Wf, with a mild endogenous defect offering only marginal selective advantage to a normal donor cell, or W/W, with a severe defect. Among 11 Wf/Wf animals with long-term grafts, 8 had only one or the other of the donor strains. Some of these individuals must have been seeded by only a single donor cell (P = 0.1); the frequency of this event was at least 20% (90% confidence) and most likely 50% of the cases. Cell-specific strain markers in myeloid and lymphoid lineages reinforced the likelihood that renewal and differentiation had occurred from a totipotent hematopoietic stem cell. In a smaller W/W group, some hosts were seeded by at most two cells (P = 0.1), and single-cell seeding could not be ruled out. The experiment allows stem cell pedigrees to be examined during the normal developmental progression. In both groups observed here, some mice displayed a regular and complementary rise and fall in proportions of cells of different genotypes, thereby suggesting clonal succession in a hierarchy of stem cell compartments. This transplant system also offers advantages for future experiments on regulated expression in vivo of

genes transferred (in vitro) into totipotent hematopoietic stem cells.

Mintz, B. and C. Cronmiller (1981). "METT-1: a karyotypically normal in vitro line of developmentally totipotent mouse teratocarcinoma cells." Somatic Cell Genet **7**(4): 489-505.

A karyotypically normal, chromosomally female (X/X) in vitro line of mouse teratocarcinoma stem cells was established from a malignant mouse teratocarcinoma of the 129/Sv Sl C P inbred strain. The tumor of origin was experimentally induced by ectopic transplantation of a 6-day embryo. The normal number of chromosomes was observed in 92% of metaphases of the cultured cells. This high frequency of euploidy, as well as karyotypic normalcy, were maintained during numerous passages in culture without a feeder-cell layer and after freezing and thawing of the cells. The line has been designated METT-1 (Mouse Euploid Totipotent Teratocarcinoma), signifying that it is the first such in vitro line that has proved (in tests by T. Stewart and B. Mintz, manuscript in preparation) to be developmentally totipotent, i.e., capable of both somatic and germinal differentiation when injected into blastocysts, even after freezing and thawing and prolonged culture. This unique ensemble of properties renders the cell line suitable for selection of specific mutant genes and for gene-transfer experiments in culture, for the purpose of producing from the mutant cells new strains of mice with predetermined genetic changes.

Mintz, B., C. Cronmiller, et al. (1978). "Somatic cell origin of teratocarcinomas." Proc Natl Acad Sci U S A **75**(6): 2834-8.

Malignant teratocarcinomas arise from developmentally totipotent normal stem cells. Whether the targets are embryonal somatic cells or germinal cells has long been a matter of controversy. Past experiments on teratocarcinoma induction by ectopic grafting of early rodent embryos or fetal germinal ridges have remained ambiguous because embryos ordinarily soon form germ cells, and parthenogenetic germ cells form "embryos." In order to interrupt the developmental cycle at its most telling point, day 6 (egg-cylinder stage) mouse embryos of genetically sterile types were grafted; in such grafts, only a terminal residue of totipotent embryonal somatic ("ectoderm") cells is available, and subsequent germ cell development is severely impaired. One graft series, from S1(J)/+ matings, comprised 25% S1(J)/S1(J) presumptive sterile embryos; these grafts formed tumors containing embryonal carcinoma cells as often (47%) as did control +/+ grafts (41%) on the same genetic background. In another series, from W/+ matings, tumors of the sterile W/W genotype were individually identified by means of a closely linked marker, phosphoglucomutase (PGM, EC 2.7.5.1; Pgm-1 locus), coding for electrophoretic enzyme variants and incorporated into the stock. Four tumors were obtained (out of 16) that had the PGM-1D phenotype diagnostic for W/W, and that also contained embryonal carcinoma cells. Therefore, the malignancy arises here in susceptible somatic embryonal stem cells at the terminal stage of their capacity for totipotency. Other teratocarcinomas-whether induced or spontaneous-of ostensible germ-cell origin by parthenogenesis may also depend upon development of the same somatic target cells before neoplastic conversion can occur. A general model based on these experiments is proposed for all malignancies: Malignant transformation of a particular kind of normal stem cell may be possible only when that stem cell has progressed to the threshold of further differentiation.

Mintz, B. and K. Illmensee (1975). "Normal genetically mosaic mice produced from malignant teratocarcinoma cells." Proc Natl Acad Sci U S A **72**(9): 3585-9.

Malignant mouse teratocarcinoma (or embryonal carcinoma) cells with a normal modal chromosome number were taken from the "cores" of embryoid bodies grown only in vivo as an ascites tumor for 8 years, and were injected into blastocysts bearing many genetic markers, in order to test the developmental capacities, genetic constitution, and reversibility of malignancy of the core cells. Ninety-three live normal pre- and postnatal animals were obtained. Of 14 thus far analyzed, three were cellular genetic mosaics with substantial contributions of tumor-derived cells in many developmentally unrelated tissues, including some never seen in the solid tumors that form in transplant hosts. The tissues functioned normally and synthesized their specific products (e.g., immunoglobulins, adult hemoglobin, liver proteins) coded for by strain-type alleles at known loci. In addition, a tumor-contributed color gene, steel, not previously known to be present in the carcinoma cells, was detected from the coat phenotype. Cells derived from the carcinoma, which is of X/Y sex chromosome constitution, also contributed to the germ line and formed reproductively functional sperms, some of which transmitted the steel gene to the progeny. Thus, after almost 200 transplant generations as a highly malignant tumor, embryoid body core cells appear to be developmentally totipotent and able to express, in an orderly sequence in differentiation of somatic and germ-line tissues, many genes hitherto silent in the tumor of origin. This experimental system

of "cycling" teratocarcinoma core cells through mice, in conjunction with experimental mutagenesis of those cells, may therefore provide a new and useful tool for biochemical, developmental, and genetic analyses of mammalian differentiation. The results also furnish an unequivocal example in animals of a non-mutational basis for transformation to malignancy and of reversal to normalcy. The origin of this tumor from a disorganized embryo suggests that malignancies of some other, more specialized, stem cells might arise comparably through tissue disorganization, leading to developmental aberrations of gene expression rather than changes in gene structure.

Mitalipov, S. and D. Wolf (2009). "Totipotency, pluripotency and nuclear reprogramming." Adv Biochem Eng Biotechnol **114**: 185-99.

Mammalian development commences with the totipotent zygote which is capable of developing into all the specialized cells that make up the adult animal. As development unfolds, cells of the early embryo proliferate and differentiate into the first two lineages, the pluripotent inner cell mass and the trophectoderm. Pluripotent cells can be isolated, adapted and propagated indefinitely in vitro in an undifferentiated state as embryonic stem cells (ESCs). ESCs retain their ability to differentiate into cells representing the three major germ layers: endoderm, mesoderm or ectoderm or any of the 200+ cell types present in the adult body. Since many human diseases result from defects in a single cell type, pluripotent human ESCs represent an unlimited source of any cell or tissue type for replacement therapy thus providing a possible cure for many devastating conditions. Pluripotent cells resembling ESCs can also be derived experimentally by the nuclear reprogramming of somatic cells. Reprogrammed somatic cells may have an even more important role in cell replacement therapies since the patient's own somatic cells can be used for reprogramming thereby eliminating immune based rejection of transplanted cells. In this review, we summarize two major approaches to reprogramming: (1) somatic cell nuclear transfer and (2) direct reprogramming using genetic manipulations.

Mizuno, S., Y. Sono, et al. (2006). "Expression and subcellular localization of GSE protein in germ cells and preimplantation embryos." J Reprod Dev **52**(3): 429-38.

We previously identified a novel gonad-specific expression gene (Gse) and investigated its expression during gametogenesis in the mouse testis and ovary. In this study, we generated a polyclonal antibody to GSE protein and determined the profiles of the protein's expression in germ cells and preimplantation embryos in detail using immunocytochemical and immunofluorescence staining. In a Western blot analysis, the anti-GSE antibody recognized long and short isoforms (approximately 27.6 kDa and 23.1 kDa) of the protein in the mouse testis and the long isoform in the ovary. In the mouse testis, GSE protein was expressed in spermatocytes I in the pachytene stage, round spermatids, and elongated spermatids. In the mouse ovary, the protein was located in the cytoplasm and nucleus of all oocytes regardless of the stage of the ovarian follicles. In preimplantation embryos from the pronuclear to blastocyst stage, however, GSE protein was mainly detected in the nuclei of cells. At the blastocyst stage, the protein was confirmed to have accumulated in the inner cell mass (ICM), whereas it had mostly disappeared from the trophectoderm (TE). These findings suggest that GSE protein may play a role in the establishment of nuclear totipotency and may be associated with early lineage specification.

Moller, E., G. Stenman, et al. (2008). "POU5F1, encoding a key regulator of stem cell pluripotency, is fused to EWSR1 in hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands." J Pathol **215**(1): 78-86.

The EWSR1 gene is known to play a crucial role in the development of a number of different bone and soft tissue tumours, notably Ewing's sarcoma. POU5F1 is expressed during early development to maintain the totipotent status of embryonic stem and germ cells. In the present study, we report the fusion of EWSR1 and POU5F1 in two types of epithelial tumours: hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands. This finding not only broadens considerably the spectrum of neoplasms associated with EWSR1 fusion genes but also strengthens the evidence for shared pathogenetic mechanisms in the development of adnexal and salivary gland tumours. Reminiscent of the previously reported fusion genes involving EWSR1, the identified transcript is predicted to encode a chimeric protein consisting of the EWSR1 amino-terminal domain and the POU5F1 carboxy-terminal domain. We assessed the transcriptional activation potential of the chimera compared to the wild-type proteins, as well as activation of transcription through the oct/sox composite element known to bind POU5F1. Among other POU5F1 target genes, this element is present in the promoter of NANOG and in the distal enhancer of POU5F1 itself. Our results show that although the chimera is capable of significant transcriptional activation, it may in fact convey a negative regulatory effect on target genes.

Money, N. P. (2002). "Mushroom stem cells." Bioessays **24**(10): 949-52.

Contrary to the rarity of totipotent cells in animals, almost every cell formed by a fungus can function as a "stem cell". The multicellular fruiting bodies of basidiomycete fungi consist of the same kind of filamentous hyphae that form the feeding phase, or mycelium, of the organism, and visible cellular differentiation is almost nonexistent. Mushroom primordia develop from masses of converging hyphae, and the stipe (or stem), cap, and gills are clearly demarcated within the embryonic fruiting body long before the organ expands and unfolds through water uptake and cell wall loosening. Though frequent references are made to gilled mushrooms in this article, the totipotent nature of fruiting body cells and lack of meristems is also applicable to basidiomycetes that spread their spore-producing tissues inside tubes (e.g., boletes), over spines and rippled surfaces, or form spores in cavities within the fruiting body. Even in the mature mushroom, every hypha retains its totipotency. Among animals, only sponges exhibit a similar degree of developmental flexibility, which is interesting, because these simple metazoans may be relatively close relatives of fungi.

Moore, J. C., L. W. van Laake, et al. (2005). "Human embryonic stem cells: genetic manipulation on the way to cardiac cell therapies." Reprod Toxicol **20**(3): 377-91.

Almost 7 years after their first derivation from human embryos, a pressing urgency to deliver the promises of therapies based on human embryonic stem cells (hESC) has arisen. Protocols have been developed to support long-term growth of undifferentiated cells and partially direct differentiation to specific cell lineages. The stage has almost been set for the next step: transplantation in animal models of human disease. Here, we review the state-of-the-art with respect to the transplantation of embryonic stem cell-derived heart cells in animals. One problem affecting progress in this area and functional analysis in vivo in general, is the availability of genetically marked hESC. There are only a few cell lines that express reporter genes ubiquitously, and none is associated with particular lineages; a major hurdle has been the resistance of hESC to established infection and chemical transfection methodologies to introduce ectopic genes. The methods that have been successful are reviewed. We also describe the processes for generating a new, genetically-modified hESC line that constitutively expresses GFP as well as some of its characteristics, including its ability to form cardiomyocytes with electrophysiological properties of ventricular-like cells.

Mora, J. I., J. E. Barroeta, et al. (2004). "Paneth cell carcinoma of the ampulla of Vater." Arch Pathol Lab Med **128**(8): 908-10.

We describe a Paneth cell carcinoma arising within the ampulla of Vater in a 64-year-old man. The phenotype of virtually all neoplastic cells was consistent with that of Paneth cells, based on routine morphology and their strong positive immunostaining for lysozyme. Additional widespread positive immunostaining for carcinoembryonic antigen and CA 19.9 supports a totipotential cell as the origin of such neoplastic cells. This case, therefore, represents a true Paneth cell carcinoma, as opposed to inclusion of occasional neoplastic Paneth cells into a poorly differentiated adenocarcinoma. This pattern of differentiation is rare, and predictions regarding its ultimate biological behavior and malignant potential must be guarded.

Morey, C., P. Navarro, et al. (2004). "The region 3' to Xist mediates X chromosome counting and H3 Lys-4 dimethylation within the Xist gene." Embo J **23**(3): 594-604.

A counting process senses the X chromosome/autosome ratio and ensures that X chromosome inactivation (XCI) initiates in the female (XX) but not in the male (XY) mouse embryo. Counting is regulated by the X-inactivation centre, which contains the Xist gene. Deleting 65 kb 3' to Xist in XO embryonic stem (ES) cells affects counting and results in inappropriate XCI upon differentiation. We show here that normal counting can be rescued in these deleted ES cells using cre/loxP re-insertion, and refine the location of elements controlling counting within a 20 kb bipartite domain. Furthermore, we show that the 65 kb deletion also leads to inappropriate XCI in XY differentiated ES cells, which excludes the involvement of sex-specific mechanisms in the initiation of XCI. At the chromatin level, we have found that the Xist gene corresponds to a peak of H3 Lys-4 dimethylation, which is dramatically and specifically affected by the deletion 3' to Xist. Our results raise the possibility that H3 Lys-4 dimethylation within Xist may be functionally implicated in the counting process.

Moscatelli, I., E. Pierantozzi, et al. (2009). "p75 neurotrophin receptor is involved in proliferation of undifferentiated mouse embryonic stem cells." Exp Cell Res **315**(18): 3220-32.

Neurotrophins and their receptors are known to play a role in the proliferation and survival of many different cell types of neuronal and non-neuronal lineages. In addition, there is much evidence in the literature showing that the p75 neurotrophin receptor (p75(NTR)), alone or in association with members of

the family of Trk receptors, is expressed in a wide variety of stem cells, although its role in such cells has not been completely elucidated. In the present work we have investigated the expression of p75(NTR) and Trks in totipotent and pluripotent cells, the mouse pre-implantation embryo and embryonic stem and germ cells (ES and EG cells). p75(NTR) and TrkA can be first detected in the blastocyst from which ES cell lines are derived. Mouse ES cells retain p75(NTR)/TrkA expression. Nerve growth factor is the only neurotrophin able to stimulate ES cell growth in culture, without affecting the expression of stem cell markers, alkaline phosphatase, Oct4 and Nanog. Such proliferation effect was blocked by antagonizing either p75(NTR) or TrkA. Interestingly, immunoreactivity to anti-p75(NTR) antibodies is lost upon ES cell differentiation. The expression pattern of neurotrophin receptors in murine ES cells differs from human ES cells, that only express TrkB and C, and do not respond to NGF. In this paper we also show that, while primordial germ cells (PGC) do not express p75(NTR), when they are made to revert to an ES-like phenotype, becoming EG cells, expression of p75(NTR) is turned on.

Muller, W. A., R. Teo, et al. (2004). "Totipotent migratory stem cells in a hydroid." Dev Biol **275**(1): 215-24.

Hydroids, members of the most ancient eumetazoan phylum, the Cnidaria, harbor multipotent, migratory stem cells lodged in interstitial spaces of epithelial cells and are therefore referred to as interstitial cells or i-cells. According to traditional understanding, based on studies in Hydra, these i-cells give rise to several cell types such as stinging cells, nerve cells, and germ cells, but not to ectodermal and endodermal epithelial cells; these are considered to constitute separate cell lineages. We show here that, in Hydractinia, the developmental potential of these migratory stem cells is wider than previously anticipated. We eliminated the i-cells from subcloned wild-type animals and subsequently introduced i-cells from mutant clones and vice versa. The mutant donors and the wild-type recipients differed in their sex, growth pattern, and morphology. With time, the recipient underwent a complete conversion into the phenotype and genotype of the donor. Thus, under these experimental conditions the interstitial stem cells of Hydractinia exhibit totipotency.

Muller, W. E. (2006). "The stem cell concept in sponges (Porifera): Metazoan traits." Semin Cell Dev Biol **17**(4): 481-91.

Sponges are considered the oldest living animal group and provide important insights into the earliest evolutionary processes in the Metazoa. This paper reviews the evidence that sponge stem cells have essential roles in cellular specialization, embryogenesis and Bauplan formation. Data indicate that sponge archaeocytes not only represent germ cells but also totipotent stem cells. Marker genes have been identified which are expressed in totipotent stem cells and gemmule cells. Furthermore, genes are described for the three main cell lineages in sponge, which share a common origin from archaeocytes and result in the differentiation of skeletal, epithelial, and contractile cells.

Muller, W. E., M. Korzhev, et al. (2003). "Origin of metazoan stem cell system in sponges: first approach to establish the model (Suberites domuncula)." Biomol Eng **20**(4-6): 369-79.

It is established that Porifera (sponges) represent the earliest phylum which branched off from the common ancestor of all multicellular animals, the Urmetazoa. In the present study, the hypothesis is tested if, during this transition, pluripotent stem cells were formed which are provided-similar to the totipotent cells (archaeocytes/germ cells)-with a self-renewal capacity. As a model system, primmorphs from the sponge Suberites domuncula were used. These 3D-cell aggregates were cultivated in medium (RPMI 1640/seawater) either lacking silicate and ferric iron or in medium which was supplemented with these 'morphogenetic' factors. As molecular markers for the potential existence of stem cells in primmorphs, two genes which encode proteins found in stem cells of higher metazoan species, were cloned from S. domuncula. First, the noggin gene, which is present in the Spemann organizer of amphibians and whose translation product acts during the formation of dorsal mesoderm derivatives. The second gene encodes the mesenchymal stem cell-like protein. Both cDNAs were used to study their expression in primmorphs in dependence on the incubation conditions. It was found that noggin expression is strongly upregulated in primmorphs kept in the presence of silicate and ferric iron, while the expression of the mesenchymal stem cell-like protein was downregulated. These data are discussed with respect to the existence of stem cells in sponges.

Murphy, C. L. (2006). "Internal standards in differentiating embryonic stem cells in vitro." Methods Mol Biol **329**: 101-12.

Embryonic stem (ES) cell lines are important for use in developmental biology studies, and because these cells are totipotent, they may provide a much-needed source of differentiated cells for certain therapeutic applications. The phenotype of the ES cell in culture is often assessed by (semi)quantitative RNA analyses. In such cases, it is critical to use appropriate

internal standards to correct for experimentally induced sources of error. This is particularly true for ES cell differentiation because it is heterogeneous in nature. We describe protocols for determining the suitability of housekeeping genes to act as internal controls in differentiating ES cell cultures. Such assessment is needed for every experimental condition under investigation. The protocol focuses on polymerase chain reaction; however, the principle and experimental design are applicable to any (semi)quantitative RNA assay.

Nagy, Z. P. and C. C. Chang (2005). "Current advances in artificial gametes." Reprod Biomed Online **11**(3): 332-9.

The birth of Louise Brown, the first IVF baby, in 1978 marked a breakthrough in infertility treatment. In recent decades, several important new techniques have been introduced. One limiting factor has been the requirement to use reproductive cells (gametes) for fertilization and for embryonic development. Somatic cell nuclear transfer (cloning) has been successful in mammals, opening a potential new approach for the treatment of human infertility. In addition, nuclear transfer to achieve embryo development starting from somatic cells instead of gametes, and the creation of artificial oocytes/spermatozoa has been attempted. The present paper reviews the various alternative approaches to haploidization of somatic cells. It has been observed that chromosome segregation (of the donor somatic nucleus) may take place; however, this process is largely random, thus leading to major cytogenetic abnormalities. An alternative approach is related to stem cell technology, to be further explored in the future. Culture conditions may be adjusted so that the totipotent embryonic stem cells will differentiate to specific gametes, sperm cells or egg cells. Injecting spermatozoa produced in this manner into recipient oocytes has led to pronuclear formation and early cleavage stages in some embryos. Finally, the birth of parthenogenetic mice indicates that some of these epigenetic problems can be overcome, and that some of the embryos may survive to birth.

Nakayama, N., I. Fang, et al. (1998). "Natural killer and B-lymphoid potential in CD34+ cells derived from embryonic stem cells differentiated in the presence of vascular endothelial growth factor." Blood **91**(7): 2283-95.

Differentiation of totipotent mouse embryonic stem (ES) cells to various lymphohematopoietic cells is an in vitro model of the hematopoietic cell development during embryogenesis. To understand this process at cellular levels, differentiation intermediates were investigated. ES cells generated progeny expressing CD34, which was significantly enhanced by vascular endothelial growth factor (VEGF). The isolated CD34+ cells were enriched for myeloid colony-forming cells but not significantly for erythroid colony-forming cells. When cultured on OP9 stroma cells in the presence of interleukin-2 and interleukin-7, the CD34+ cells developed two types of B220+ CD34- lymphocytes: CD3- cytotoxic lymphocytes and CD19+ pre-B cells, and such lymphoid potential was highly enriched in the CD34+ population. Interestingly, the cytotoxic cells expressed the natural killer (NK) cell markers, such as NKR-P1, perforin, and granzymes, classified into two types, one of which showed target specificity of NK cells. Thus, ES cells have potential to generate NK-type cytotoxic lymphocytes in vitro in addition to erythro-myeloid cells and pre-B cells, and both myeloid and lymphoid cells seem to be derived from the CD34+ intermediate, on which VEGF may play an important role.

Nakayama, N., J. Lee, et al. (2000). "Vascular endothelial growth factor synergistically enhances bone morphogenetic protein-4-dependent lymphohematopoietic cell generation from embryonic stem cells in vitro." Blood **95**(7): 2275-83.

The totipotent mouse embryonic stem (ES) cell is known to differentiate into cells expressing the beta-globin gene when stimulated with bone morphogenetic protein (BMP)-4. Here, we demonstrate that BMP-4 is essential for generating both erythro-myeloid colony-forming cells (CFCs) and lymphoid (B and NK) progenitor cells from ES cells and that vascular endothelial growth factor (VEGF) synergizes with BMP-4. The CD45(+) myelomonocytic progenitors and Ter119(+) erythroid cells began to be detected with 0.5 ng/mL BMP-4, and their levels plateaued at approximately 2 ng/mL. VEGF alone weakly elevated the CD34(+) cell population though no lymphohematopoietic progenitors were induced. However, when combined with BMP-4, 2 to 20 ng/mL VEGF synergistically augmented the BMP-4-dependent generation of erythro-myeloid CFCs and lymphoid progenitors from ES cells, which were enriched in CD34(+) CD31(lo) and CD34(+) CD45(-)cell populations, respectively, in a dose-dependent manner. Furthermore, during the 7 days of in vitro differentiation, BMP-4 was required within the first 4 days, whereas VEGF was functional after the action of BMP-4 (in the last 3 days). Thus, VEGF is a synergistic enhancer for the BMP-4-dependent differentiation processes, and it seems to be achieved by the ordered action of the 2 factors.

Nakhei, H., A. Lingott, et al. (1998). "An alternative splice variant of the tissue specific transcription factor

HNF4alpha predominates in undifferentiated murine cell types." Nucleic Acids Res **26**(2): 497-504.

The transcription factor hepatocyte nuclear factor 4alpha (HNF4alpha) is a tissue specific transcription factor mainly expressed in the liver, kidney, intestine and the endocrine pancreas, but is also an essential regulator for early embryonic events. Based on its protein structure HNF4alpha is classified as an orphan member of the nuclear receptor superfamily. Comparing HNF4alpha transcription factors in the differentiated and dedifferentiated murine hepatocyte cell line MHSV-12 we identified in dedifferentiated cells the novel splice variant HNF4alpha7. This variant is characterized by an alternative first exon and has a lower transactivation potential in transient transfection assays using HNF4 dependent reporter genes. HNF4alpha7 mRNA and the corresponding protein are expressed in the undifferentiated pluripotent embryonal carcinoma cell line F9, whereas HNF4alpha1 only appears after differentiation of F9 cells to visceral endoderm. HNF4alpha7 mRNA is also found in totipotent embryonic stem cells. However, the function of HNF4alpha7 seems not to be restricted to embryonic cells as the HNF4alpha7 mRNA is also present in adult tissues, most notably the stomach. All these features suggest that the presence of distinct splice variants of HNF4alpha modulates the activity of HNF4alphain a cell type specific way.

Niemann, H., X. C. Tian, et al. (2008). "Epigenetic reprogramming in embryonic and foetal development upon somatic cell nuclear transfer cloning." Reproduction **135**(2): 151-63.

The birth of 'Dolly', the first mammal cloned from an adult donor cell, has sparked a flurry of research activities to improve cloning technology and to understand the underlying mechanism of epigenetic reprogramming of the transferred somatic cell nucleus. Especially in ruminants, somatic cell nuclear transfer (SCNT) is frequently associated with pathological changes in the foetal and placental phenotype and has significant consequences for development both before and after birth. The most critical factor is epigenetic reprogramming of the transferred somatic cell nucleus from its differentiated status into the totipotent state of the early embryo. This involves an erasure of the gene expression program of the respective donor cell and the establishment of the well-orchestrated sequence of expression of an estimated number of 10 000-12 000 genes regulating embryonic and foetal development. The following article reviews the present knowledge on the epigenetic reprogramming of the transferred somatic cell nucleus, with emphasis on DNA methylation, imprinting, X-chromosome inactivation and telomere length restoration in bovine development. Additionally, we briefly discuss other approaches towards epigenetic nuclear reprogramming, including the fusion of somatic and embryonic stem cells and the overexpression of genes crucial in the formation and maintenance of the pluripotent status. Improvements in our understanding of this dramatic epigenetic reprogramming event will be instrumental in realising the great potential of SCNT for basic biological research and for various agricultural and biomedical applications.

Nimeth, K., P. Ladurner, et al. (2002). "Cell renewal and apoptosis in macrostomum sp. [Lignano]." Cell Biol Int **26**(9): 801-15.

In platyhelminths, all cell renewal is accomplished by totipotent stem cells (neoblasts). Tissue maintenance is achieved in a balance between cell proliferation and apoptosis. It is known that in Macrostomum sp. the epidermis undergoes extensive cell renewal. Here we show that parenchymal cells also exhibit a high rate of cell turnover. We demonstrate cell renewal using continuous 5'bromo-2-deoxyuridine (BrdU) exposure. About one-third of all cells are replaced after 14 days. The high level of replacement requires an equivalent removal of cells by apoptosis. Cell death is characterized using a combination of three methods: (1). terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL), (2). specific binding of phosphatidyl-serine to fluorescent-labelled annexin V and (3). identification of apoptotic stages by ultrastructure. The number of cells observed in apoptosis is insufficient to explain the homeostasis of tissues in Macrostomum. Apoptosis-independent mechanisms may play an additional role in tissue dynamics.

Nimeth, K. T., B. Egger, et al. (2007). "Regeneration in Macrostomum lignano (Platyhelminthes): cellular dynamics in the neoblast stem cell system." Cell Tissue Res **327**(3): 637-46.

Neoblasts are potentially totipotent stem cells and the only proliferating cells in adult Platyhelminthes. We have examined the cellular dynamics of neoblasts during the posterior regeneration of Macrostomum lignano. Double-labeling of neoblasts with bromodeoxyuridine and the anti-phospho histone H3 mitosis marker has revealed a complex cellular response in the first 48 h after amputation; this response is different from that known to occur during regeneration in triclad platyhelminths and in starvation/feeding experiments in M. lignano. Mitotic activity is reduced during the first 8 h of regeneration but, at 48 h after amputation, reaches almost twice the value of control animals. The total number of S-phase cells significantly increases after 1

day of regeneration. A subpopulation of fast-cycling neoblasts surprisingly shows the same dynamics during regeneration as those in control animals. Wound healing and regeneration are accompanied by the formation of a distinct blastema. These results present new insights, at the cellular level, into the early regeneration of rhabditophoran Platyhelminthes.

Nimeth, K. T., M. Mahlknecht, et al. (2004). "Stem cell dynamics during growth, feeding, and starvation in the basal flatworm Macrostomum sp. (Platyhelminthes)." Dev Dyn **230**(1): 91-9.

Development, growth, and regeneration in Macrostomum are based--as in all Platyhelminthes--on likely totipotent stem cells (neoblasts), basic for all Bilaterians. We demonstrate dynamics and migration of neoblasts during postembryonic development, starvation, and feeding of Macrostomum sp. Double labeling of S-phase and mitotic cells revealed a fast cell turnover. Conflicting with recent results from planarians, we have some indication of slow cycling neoblasts. As in planarians, starvation dramatically reduced mitotic activity and a very basic level was maintained after 30 days of starvation. Afterward, feeding induced a dramatic immediate proliferative response probably caused by G2-arrested neoblasts. The following 12 hr showed a significant mitotic decline, caused by the depletion of the G2 neoblast pool. Neoblasts that pass through S-phase led to a maximum of mitoses after 48 hr. Our results allow deeper insight into cellular dynamics of an ancestral bilaterian stem cell system of a basal Platyhelminth.

Nishikawa, S. I., S. Nishikawa, et al. (1998). "Progressive lineage analysis by cell sorting and culture identifies FLK1+VE-cadherin+ cells at a diverging point of endothelial and hemopoietic lineages." Development **125**(9): 1747-57.

Totipotent murine ES cells have an enormous potential for the study of cell specification. Here we demonstrate that ES cells can differentiate to hemopoietic cells through the proximal lateral mesoderm, merely upon culturing in type IV collagen-coated dishes. Separation of the Flk1+ mesoderm from other cell lineages was critical for hemopoietic cell differentiation, whereas formation of the embryoid body was not. Since the two-dimensionally spreading cells can be monitored easily in real time, this culture system will greatly facilitate the study of the mechanisms involved in the cell specification to mesoderm, endothelial, and hemopoietic cells. In the culture of ES cells, however, lineages and stages of differentiating cells can only be defined by their own characteristics. We showed that a combination of monoclonal antibodies against E-cadherin, Flk1/KDR, PDGF receptor(alpha), VE-cadherin, CD45 and Ter119 was sufficient to define most intermediate stages during differentiation of ES cells to blood cells. Using this culture system and surface markers, we determined the following order for blood cell differentiation: ES cell (E-cadherin+Flk1-PDGFRalpha-), proximal lateral mesoderm (E-cadherin-Flk1+VE-cadherin-), progenitor with hemoangiogenic potential (Flk1+VE-cadherin+CD45-), hemopoietic progenitor (CD45+c-Kit+) and mature blood cells (c-Kit-CD45+ or Ter119+), though direct differentiation of blood cells from the Flk1+VE-cadherin- stage cannot be ruled out. Not only the VE-cadherin+CD45- population generated from ES cells but also those directly sorted from the yolk sac of 9.5 dpc embryos have a potential to give rise to hemopoietic cells. Progenitors with hemoangiogenic potential were identified in both the Flk1+VE-cadherin- and Flk1+VE-cadherin+ populations by the single cell deposition experiment. This line of evidence implicates Flk1+VE-cadherin+ cells as a diverging point of hemopoietic and endothelial cell lineages.

Ogawa, K., A. Wakayama, et al. (1998). "Identification of a receptor tyrosine kinase involved in germ cell differentiation in planarians." Biochem Biophys Res Commun **248**(1): 204-9.

To investigate external signals involved in germ cell differentiation from somatic stem cells, we have tried to identify protein kinases whose expression is regulated during the process of sexualization of asexual-state planarians. It is known that in planarians germ cells differentiate from totipotent somatic stem cells called "neoblasts" during sexualization. As a first step, we have isolated twelve protein kinase genes from cDNAs of sexual-state planarians, including three non-receptor tyrosine kinases, three receptor-tyrosine kinases and three non-receptor serine/threonine kinases, and then analyzed their expression patterns during sexualization. One of them, the DjPTK1 gene, is specifically expressed in germ cells of sexual-state planarians. DjPTK1-positive cells were also detected in the mesenchymal space during the process of sexualization, and it appears that these cells migrate to the dorsal side and then differentiate into spermatogonia/spermatocytes in testis. Sequence analysis indicated that the DjPTK1 gene encodes a receptor protein tyrosine kinase belonging to the FGFR/PDGF family. These results suggest that a receptor tyrosine kinase system may be involved both at an early stage of germ cell differentiation and in a step of germ cell maturation in planarians.

Oh, S. K., H. S. Kim, et al. (2005). "Derivation and characterization of new human embryonic stem cell

lines: SNUhES1, SNUhES2, and SNUhES3." Stem Cells **23**(2): 211-9.

Here we report the derivation and characterization of new human embryonic stem cell (hESC) lines, SNUhES1, SNUhES2, and SNUhES3. These cells, established from the inner cell mass using an STO feeder layer, satisfy the criteria that characterize pluripotent hESCs: The cell lines express high levels of alkaline phosphatase, cell surface markers (such as SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81), transcription factor Oct-4, and telomerase. When grafted into severe combined immunodeficient mice after prolonged proliferation, these cells maintained the developmental potentials to form derivatives of all three embryonic germ layers. The cell lines have normal karyotypes and distinct identities, revealed from DNA fingerprinting. Interestingly, analysis by electron microscopy clearly shows the morphological difference between undifferentiated and differentiated hESCs. Undifferentiated hESCs have a high ratio of nucleus to cytoplasm, prominent nucleoli, indistinct cell membranes, free ribosomes, and small mitochondria with a few crista, whereas differentiated cells retain irregular nuclear morphology, desmosomes, extensive cytoplasmic membranes, tonofilaments, and highly developed cellular organelles such as Golgi complex with secretory vesicles, endoplasmic reticulum studded with ribosomes, and large mitochondria. Existence of desmosomes and tonofilaments indicates that these cells differentiated into epithelial cells. When in vitro differentiation potentials of these cell lines into cardiomyocytes were examined, SNUhES3 was found to differentiate into cardiomyocytes most effectively.

Ohtaka, M., T. Kumasaka, et al. (2002). "Carcinosarcoma of the esophagus characterized by myoepithelial and ductal differentiations." Pathol Int **52**(10): 657-63.

We report a case of carcinosarcoma of the esophagus characterized by ductal and myoepithelial differentiation. A 61-year-old man was operated on for a polypoid tumor of the distal esophagus. Histologically, this tumor was composed of ductal structures and sarcomatous spindle cells surrounding the ducts at the central area of the tumor. The tumor was also composed of squamous cell and basaloid carcinoma in the periphery. Immunohistochemically, a few spindle cells surrounding the ductal structures showed immunopositivity for alpha-smooth muscle actin and S-100 protein. Electron microscopy revealed that the spindle cells had tonofilament and pinocytic vesicles in the cytoplasm, and basal lamina adjacent to the cytoplasmic membrane. Both of the results strongly supported the suggestion that the spindle cells may be myoepithelial cells. Basaloid carcinoma showed a gradual transition to chondrosarcomatous cells producing the matrix, which had both immunopositivities for S-100 protein and cytokeratin. Therefore, chondrosarcomatous cells may be derived from carcinoma cells. The histogenesis of this tumor may be associated with a totipotent stem cell of esophageal mucosa, which has the potential to differentiate into squamous cells, ductal cells or myoepithelial cells.

Okoye, U. C., C. C. Malbon, et al. (2008). "Wnt and Frizzled RNA expression in human mesenchymal and embryonic (H7) stem cells." J Mol Signal **3**: 16.

BACKGROUND: Wnt signals are important for embryonic stem cells renewal, growth and differentiation. Although 19 Wnt, 10 Frizzled genes have been identified in mammals, their expression patterns in stem cells were largely unknown. RESULTS: We conducted RNA expression profiling for the Wnt ligands, their cellular receptors "Frizzleds" and co-receptors LRP5/6 in human embryonic stem cells (H7), human bone marrow mesenchymal cells, as well as mouse totipotent F9 teratocarcinoma embryonal cells. Except failing to express Wnt2 gene, totipotent F9 cells expressed RNA for all other 18 Wnt genes as well as all 10 members of Frizzled gene family. H7 cells expressed RNA for each of the 19 Wnt genes. In contrast, human mesenchymal cells did not display detectable RNA expression of Wnt1, Wnt8a, Wnt8b, Wnt9b, Wnt10a, and Wnt11. Analysis of Frizzled RNAs in H7 and human mesechymal cells revealed expression of 9 members of the receptor gene family, except Frizzled8. Expression of the Frizzled co-receptor LRP5 and LRP6 genes were detected in all three cell lines. Human H7 and mouse F9 cells express nearly a full complement of both Wnts and Frizzleds genes. The human mesenchymal cells, in contrast, have lost the expression of six Wnt ligands, i.e. Wnt1, 8a, 8b, 9b, 10a and 11. CONCLUSION: Puripotent human H7 and mouse F9 embryonal cells express the genes for most of the Wnts and Frizzleds. In contrast, multipotent human mesenchymal cells are deficient in expression of Frizzled-8 and of 6 Wnt genes.

Orii, H., T. Sakurai, et al. (2005). "Distribution of the stem cells (neoblasts) in the planarian Dugesia japonica." Dev Genes Evol **215**(3): 143-57.

It has been postulated that the high regeneration ability of planarians is supported by totipotent stem cells, called neoblasts. There have been a few reports showing the distribution of neoblasts in planarians. However, the findings were not completely consistent. To determine the distribution of neoblasts, we focused on proliferating

cell nuclear antigen (PCNA), which is present in proliferative cells. We cloned and sequenced the cDNA of PCNA from the planarian Dugesia japonica and produced an antiserum recognizing the gene product. X-ray irradiation caused rapid loss of all PCNA-positive cells and loss of the neoblasts (which were morphologically defined by the presence of the chromatoid body), strongly suggesting that all PCNA-positive cells were true neoblasts. Using the antiserum, we were successful in identifying the neoblasts more clearly than any previous work. In addition to their dispersed distribution in the dorsal and ventral mesenchyme, the neoblasts were distributed as clusters along the midline and bilateral lines in the dorsal mesenchyme. We also examined the behavior of the neoblasts after decapitation. Decapitation did not seem to affect the migration of neoblasts far from the wound. We demonstrated here that DjPCNA is a powerful tool for identifying planarian neoblasts.

Pagano, J. M., B. M. Farley, et al. (2009). "RNA recognition by the embryonic cell fate determinant and germline totipotency factor MEX-3." Proc Natl Acad Sci U S A **106**(48): 20252-7.

Totipotent stem cells have the potential to differentiate into every cell type. Renewal of totipotent stem cells in the germline and cellular differentiation during early embryogenesis rely upon posttranscriptional regulatory mechanisms. The Caenorhabditis elegans RNA binding protein, MEX-3, plays a key role in both processes. MEX-3 is a maternally-supplied factor that controls the RNA metabolism of transcripts encoding critical cell fate determinants. However, the nucleotide sequence specificity and requirements of MEX-3 mRNA recognition remain unclear. Only a few candidate regulatory targets have been identified, and the full extent of the network of MEX-3 targets is not known. Here, we define the consensus sequence required for MEX-3 RNA recognition and demonstrate that this element is required for MEX-3 dependent regulation of gene expression in live worms. Based on this work, we identify several candidate MEX-3 targets that help explain its dual role in regulating germline stem cell totipotency and embryonic cell fate specification.

Parfenov, V. N., G. N. Pochukalina, et al. (2003). "Nuclear distribution of Oct-4 transcription factor in transcriptionally active and inactive mouse oocytes and its relation to RNA polymerase II and splicing factors." J Cell Biochem **89**(4): 720-32.

The intranuclear distribution of the transcription factor Oct-4, which is specifically expressed in totipotent mice stem and germ line cells, was studied in mouse oocytes using immunogold labeling/electron microscopy and immunofluorescence/confocal laser scanning microcopy. The localization of Oct-4 was studied in transcriptionally active (uni/bilaminar follicles) and inactive (antral follicles) oocytes. Additionally, the Oct-4 distribution was examined relative to that of the unphosphorylated form of RNA polymerase II (Pol II) and splicing factor (SC 35) in the intranuclear entities such as perichromatin fibrils (PFs), perichromatin granules (PGs), interchromatin granule clusters (IGCs), Cajal bodies (CBs), and nucleolus-like bodies (NLBs). It was shown that: (i) Oct-4 is localized in PFs, IGCs, and in the dense fibrillar component (DFC) of the nucleolus at the transcriptionally active stage of the oocyte nucleus; (ii) Oct-4 present in PFs and IGCs colocalizes with Pol II and SC 35 at the transcriptionally active stage; (iii) Oct-4 accumulates in NLBs, CBs, and PGs at the inert stage of the oocyte. The results confirm the previous suggestion that PFs represent the major nucleoplasmic structural domain involved in active pre-mRNA transcription/processing. The colocalization of Oct-4 with Pol II in both IGCs and PFs in active oocytes (uni/bilaminar follicles) suggests that Oct-4 is intimately associated with the Pol II holoenzyme before and during transcription. The colocalization of Oct-4, Pol II, and SC 35 with coilin-containing structures such as NLBs and CBs at the inert stage (antral follicles) suggests that the latter may represent storage sites for the transcription/splicing machinery during the decline of transcription.

Patel, P. (2006). "A natural stem cell therapy? How novel findings and biotechnology clarify the ethics of stem cell research." J Med Ethics **32**(4): 235-9.

The natural replacement of damaged cells by stem cells occurs actively and often in adult tissues, especially rapidly dividing cells such as blood cells. An exciting case in Boston, however, posits a kind of natural stem cell therapy provided to a mother by her fetus-long after the fetus is born. Because there is a profound lack of medical intervention, this therapy seems natural enough and is unlikely to be morally suspect. Nevertheless, we feel morally uncertain when we consider giving this type of therapy to patients who would not naturally receive it. Much has been written about the ethics of stem cell research and therapy; this paper will focus on how recent advances in biotechnology and biological understandings of development narrow the debate. Here, the author briefly reviews current stem cell research practices, revisits the natural stem cell therapy case for moral evaluation, and ultimately demonstrates the importance of permissible stem cell research and therapy, even absent an agreement about the definition of when embryonic life begins. Although one promising technology, blighted ovum utilisation, uses

fertilised but developmentally bankrupt eggs, it is argued that utilisation of unfertilised eggs to derive totipotent stem cells obviates the moral debate over when life begins. There are two existing technologies that fulfil this criterion: somatic cell nuclear transfer and parthenogenic stem cell derivation. Although these technologies are far from therapeutic, concerns over the morality of embryonic stem cell derivation should not hinder their advancement.

Pawliuk, R., C. Eaves, et al. (1996). "Evidence of both ontogeny and transplant dose-regulated expansion of hematopoietic stem cells in vivo." Blood **88**(8): 2852-8.

Recent assessment of the long-term repopulating activity of defined subsets of hematopoietic cells has offered new insights into the characteristics of the transplantable stem cells of this system; however, as yet, there is very little known about mechanisms that regulate their self-renewal in vivo. We have now exploited the ability to quantitate these cells using the competitive repopulating unit (CRU) assay to identify the role of both intrinsic (ontological) and extrinsic (transplanted dose-related) variables that may contribute to the regulation of CRU recovery in vivo. Ly5.1 donor cells derived from day-14.5 fetal liver (FL) or the bone marrow (BM) of adult mice injected 4 days previously with 5-fluorouracil were transplanted at doses estimated to contain 10, 100, or 1,000 long-term CRU into irradiated congenic Ly5.2 adult recipient mice. Eight to 12 months after transplantation, there was a complete recovery of BM cellularity and in vitro clonogenic progenitor numbers and a nearly full recovery of day-12 colony-forming unit-spleen numbers irrespective of the number or origin of cells initially transplanted. In contrast, regeneration of Ly5.1+ donor-derived CRU was incomplete in all cases and was dependent on both the origin and dose of the transplant, with FL being markedly superior to that of adult BM. As a result, the final recovery of the adult marrow CRU compartment ranged from 15% to 62% and from 1% to 18% of the normal value in recipients of FL and adult BM transplantation, respectively, with an accompanying maximum CRU amplification of 150-fold for recipients of FL cells and 15-fold for recipients of adult BM cells. Interestingly, the extent of CRU expansion from either source was inversely related to the number of CRU transplanted. These data suggest that recovery of mature blood cell production in vivo may activate negative feedback regulatory mechanisms to prematurely limit stem cell self-renewal ability. Proviral integration analysis of mice receiving retrovirally transduced BM cells confirmed regeneration of totipotent lymphomyeloid repopulating cells and provided evidence for a greater than 300-fold clonal amplification of a single transduced stem cell. These results highlight the differential regenerative capacities of CRU from fetal and adult sources that likely reflect intrinsic, genetically defined determinants of CRU expansion but whose contribution to the magnitude of stem cell amplification ultimately obtained in vivo is also strongly influenced by the initial number of CRU transplanted. Such findings set the stage for attempts to enhance CRU regeneration by administration of agents that may enable full expression of regenerative potential or through the expression of intracellular gene products that may alter intrinsic regenerative capacity.

Pease, S. and R. L. Williams (1990). "Formation of germ-line chimeras from embryonic stem cells maintained with recombinant leukemia inhibitory factor." Exp Cell Res **190**(2): 209-11.

Murine embryonic stem (ES) cells can be maintained as stem cells in vitro only in the presence of feeder cells or a soluble factor produced by a number of cell lines. We have previously demonstrated that leukemia inhibitory factor (LIF) is the molecule which prevents ES cell differentiation in culture. In this report we demonstrate that recombinant LIF can substitute for feeder cells in maintaining the full developmental potential of ES cells. The totipotent D3 ES cell line, previously isolated and maintained on growth-arrested primary embryo fibroblasts, was transferred to media supplemented with 1000 U/ml (10 ng/ml) recombinant LIF. In the presence of LIF the ES cells were maintained for over 2 months as undifferentiated cells in the absence of any feeder cells. When injected into blastocysts the ES cells which had been maintained in LIF-supplemented media efficiently formed germ-line chimeras.

Pei, Y., J. Ma, et al. (2003). "Serum-free culture of rhesus monkey embryonic stem cells." Arch Androl **49**(5): 331-42.

Previous studies have shown that the maintenance and proliferation of undifferentiated rhesus monkey embryonic stem (rES) cells requires medium supplemented with fetal bovine serum (FBS). Due to the uncharacterized composition and variation in serum nature, the present study aimed to replace the serum-containing medium with a serum-free medium in the rES cell culture. The results showed that after the initial 48-h culture in the routinely used serum-containing medium, rES cells can grow and proliferate for a prolonged period in the serum-free medium composed of DMEM supplemented with a cocktail of BSA, IGF-1, TGF-alpha, bFGF, aFGF, estradiol, and progesterone. rES cells cultured in the serum-free

medium maintained high level of alkaline phosphatase activity and OCT4 level. There was no indication of differentiation as judged by the marker gene expression of all three embryonic germ layers and trophoblast. In addition, serum-free culture would not affect the passage capacity and differentiation potential of rES cells. This work will facilitate the future study of induced differentiation of rES cells and other applications.

Pelus, L. M. and S. Fukuda (2008). "Chemokine-mobilized adult stem cells; defining a better hematopoietic graft." Leukemia **22**(3): 466-73.

Stem cell research is currently focused on totipotent stem cells and their therapeutic potential, however adult stem cells, while restricted to differentiation within their tissue or origin, also have therapeutic utility. Transplantation with bone marrow hematopoietic stem cells (HSC) has been used for curative therapy for decades. More recently, alternative sources of HSC, particularly those induced to exit marrow or mobilize to peripheral blood by G-CSF, have become the most widely used hematopoietic graft and show significant superiority to marrow HSC. The chemokine/chemokine receptor axis also mobilizes HSC that occurs more rapidly than with G-CSF. In mice, the HSC and progenitor cells (HPC) mobilized by the CXCR2 receptor agonist GRObeta can be harvested within minutes of administration and show significantly lower levels of apoptosis, enhanced homing to marrow, expression of more activated integrin receptors and superior repopulation kinetics and more competitive engraftment than the equivalent cells mobilized by G-CSF. These characteristics suggest that chemokine axis-mobilized HSC represent a population of adult stem cells distinct from those mobilized by G-CSF, with superior therapeutic potential. It remains to be determined if the chemokine mobilization axis can be harnessed to mobilize other populations of unique adult stem cells with clinical utility.

Penn, M. S. and N. Mal (2006). "Stem cells in cardiovascular disease: methods and protocols." Methods Mol Med **129**: 329-51.

Stem cells are cells capable of proliferation, self-renewal, and differentiation into various organ-specific cell types. Stem cells are subclassified based on their species of origin (mice, rat, human), developmental stage of the species (embryonic, fetal, or adult), tissue of origin (hematopoietic, mesenchymal, skeletal, neural), and potential to differentiate into one or more specific types of mature cells (totipotent, pluripotent, multipotent). Embryonic stem (ES) cells are totipotent, primitive cells derived from the embryo that have the potential to become all specialized cell types. Conversely, adult stem cells are undifferentiated cells found in differentiated tissue that retain the potential to renew themselves and differentiate to yield organ-specific tissues. Stem cells are attractive candidates for novel therapeutics for patients with different heart diseases, including congestive heart failure, most commonly caused by myocardial infarction. The remarkable proliferative and differentiation capacity of stem cells promises an almost unlimited supply of specific cell types including viable functioning cardiomyocytes to replace the scarred myocardium following transplantation.

Pesce, M., M. K. Gross, et al. (1998). "In line with our ancestors: Oct-4 and the mammalian germ." Bioessays **20**(9): 722-32.

The transcription factor Oct-4 is expressed specifically in the totipotent germline cycle of mice. Cells that lose Oct-4 differentiate along different paths to form embryonic and extraembryonic somatic tissue. Oct-4 may maintain the potency of stem and germline cells by preventing all other differentiation pathways. Oct-4 may also regulate the molecular differentiation of cells in the germ lineage as it progresses from the fertilized egg, through cleavage stage/morula blastomeres, blastocyst, inner cell mass, epiblast, germ cells, and gametes. The factors that regulate, and are regulated by, Oct-4 are reviewed with respect to the phenomena of cell potency and germ/soma segregation and differentiation.

Pesce, M. and H. R. Scholer (2001). "Oct-4: gatekeeper in the beginnings of mammalian development." Stem Cells **19**(4): 271-8.

The Oct-4 POU transcription factor is expressed in mouse totipotent embryonic stem and germ cells. Differentiation of totipotent cells to somatic lineages occurs at the blastocyst stage and during gastrulation, simultaneously with Oct-4 downregulation. Stem cell lines derived from the inner cell mass and the epiblast of the mouse embryo express Oct-4 only if undifferentiated. When embryonic stem cells are triggered to differentiate, Oct-4 is downregulated thus providing a model for the early events linked to somatic differentiation in the developing embryo. In vivo mutagenesis has shown that loss of Oct-4 at the blastocyst stage causes the cells of the inner cell mass to differentiate into trophectoderm cells. Recent experiments indicate that an Oct-4 expression level of roughly 50%-150% of the endogenous amount in embryonic stem cells is permissive for self-renewal and maintenance of totipotency. However, upregulation above these levels causes stem cells to express genes involved in the lineage differentiation of primitive endoderm. These

novel advances along with latest findings on Oct-4-associated factors, target genes, and dimerization ability, provide new insights into the understanding of the early steps regulating mammalian embryogenesis.

Pessina, A. and L. Gribaldo (2006). "The key role of adult stem cells: therapeutic perspectives." Curr Med Res Opin **22**(11): 2287-300.

BACKGROUND: The origin, function and physiology of totipotent embryonic cells are configured to construct organs and create cross-talk between cells for the biological and neurophysiologic development of organisms. Adult stem cells are involved in regenerating tissues for renewal and damage repair. FINDINGS: Adult stem cells have been isolated from adult tissue, umbilical cord blood and other non-embryonic sources, and can transform into many tissues and cell types in response to pathophysiological stimuli. Clinical applications of adult stem cells and progenitor cells have potential in the regeneration of blood cells, skin, bone, cartilage and heart muscle, and may have potential in degenerative diseases. Multi-pluripotent adult stem cells can change their phenotype in response to trans-differentiation or fusion and their therapeutic potential could include therapies regulated by pharmacological modulation, for example mobilising endogenous stem cells and directing them within a tissue to stimulate regeneration. Adult stem cells could also provide a vehicle for gene therapy, and genetically-engineered human adult stem cells have shown success in treatment of genetic disease. CONCLUSION: Deriving embryonic stem cells from early human embryos raises ethical, legal, religious and political questions. The potential uses of stem cells for generating human tissues are the subject of ongoing public debate. Stem cells must be used in standardised and controlled conditions in order to guarantee the best safety conditions for the patients. One critical point will be to verify the risk of tumourigenicity; this issue may be more relevant to embryonic than adult stem cells.

Petit, T., B. Raynal, et al. (1994). "Highly sensitive polymerase chain reaction methods show the frequent survival of residual recipient multipotent progenitors after non-T-cell-depleted bone marrow transplantation." Blood **84**(10): 3575-83.

Twenty-four male patients grafted for various pathologies with the marrow of a female donor and presenting a complete donor-type hematopoiesis when analyzed by polymerase chain reaction (PCR) amplification of minisatellite sequences 33.6.3 and MS51 (0.1% to 1% sensitivity) were studied by the highly sensitive technique of PCR amplification of the Y-chromosome-specific DYZ1 sequence (0.01% sensitivity). Residual recipient male cells were detected in all peripheral blood samples collected within 1 year posttransplantation. These residual cells were present in both the lymphocyte and polymorphonuclear cell fractions when such a separation was performed by Ficoll gradient centrifugation and, for samples of 13 of 15 patients, at comparable levels in both fractions. In 3 samples collected from 3 patients 4 months or more posttransplantation, residual recipient cells were detected in the polymorphonuclear cell fraction but were present at a lower level or were undetectable in the lymphocyte fraction. These cells are of hematopoietic origin because they were detected at equivalent levels in whole blood and in B and T lymphocytes sorted with antibody-coated magnetic beads. They were not detected in samples collected more than 15 months posttransplantation for 6 of 7 patients. The persistence of residual recipient cells within 1 year posttransplantation is not restricted to male patients receiving a transplant from a female donor because they were also detected in 2 female patients using an allele-specific amplification method for the thyroid peroxydase gene that also has a high sensitivity (0.01%). Our results indicate that at least residual recipient myeloid progenitors and possibly totipotent hematopoietic stem cells may survive intensive pretransplant conditioning regimen and support a transient residual hematopoiesis of the host posttransplantation.

Pinho Mde, F., S. P. Hurtado, et al. (2002). "Myelopoiesis in the omentum of normal mice and during abdominal inflammatory processes." Cell Tissue Res **308**(1): 87-96.

Coelomic cavities are relatively isolated from the systemic circulation of blood cells. Resident cell populations have a proper phenotype and kinetics, maintaining their steady-state populations and their responsiveness to local inflammatory reactions, in which the number and quality of coelomic cells can be greatly increased and modified. We have addressed the question of whether the increase in cell infiltrate in the inflamed abdominal cavity is sustained by the proliferation of myeloid cells in the omentum, and if so what are the characteristics of the progenitor cells involved and how the omentum controls their proliferation and differentiation. In the omentum under normal conditions and with inflammation due to schistosomal infection we found that pluripotent early myeloid progenitors were capable of giving rise to all the myeloid lineages in clonogenic assays, but not to the totipotent blood stem cells. Besides the major haemopoietins (GM-CSF, M-CSF, G-CSF, IL-5), the omentum stroma constitutively expressed SDF-1 alpha, the chemokine which elicits homing of

circulating early haemopoietic progenitors. While normal omentum stroma produced LIF, its expression was substituted by SCF in inflamed tissues. In the first situation a slow steady-state renewal of progenitors is potentially favoured, while their intense expansion may be predominant in the latter one.

Podolsky, D. K. (1993). "Regulation of intestinal epithelial proliferation: a few answers, many questions." Am J Physiol **264**(2 Pt 1): G179-86.

The epithelium of the gastrointestinal tract mucosa is a highly dynamic and diverse mixture of cell populations requiring exquisite integration of the processes of cellular proliferation, differentiation, and senescence. It is likely that the proliferative compartment of the intestinal epithelium encompasses a hierarchy of totipotent and pluripotent stem cells in a manner similar to that which generates diversity in hematopoietic cell populations. Identification and characterization of the stem cell and progenitor populations in the intestine has been limited by the absence of markers or culture systems to identify these cells. Regulation of the proliferative compartment may be accomplished through the combined integration of key peptide growth factors and constituents of the extracellular matrix. The relative contribution of the epithelial populations themselves and the contributions made by associated cell populations such as pericryptal fibroblasts remain unclear. Recent studies have suggested that the transforming growth factors-alpha and -beta, two structurally unrelated peptide growth factors, might serve to regulate the balanced proliferation and turnover of intestinal epithelial cells. The proproliferative effects of TGF-alpha may be counterbalanced by the proliferation-inhibiting TGF-beta.

Podsakoff, G., K. K. Wong, Jr., et al. (1994). "Efficient gene transfer into nondividing cells by adeno-associated virus-based vectors." J Virol **68**(9): 5656-66.

Gene transfer vectors based on adeno-associated virus (AAV) are emerging as highly promising for use in human gene therapy by virtue of their characteristics of wide host range, high transduction efficiencies, and lack of cytopathogenicity. To better define the biology of AAV-mediated gene transfer, we tested the ability of an AAV vector to efficiently introduce transgenes into nonproliferating cell populations. Cells were induced into a nonproliferative state by treatment with the DNA synthesis inhibitors fluorodeoxyuridine and aphidicolin or by contact inhibition induced by confluence and serum starvation. Cells in logarithmic growth or DNA synthesis arrest were transduced with vCWR:beta gal, an AAV-based vector encoding beta-galactosidase under Rous sarcoma virus long terminal repeat promoter control. Under each condition tested, vCWR:beta Gal expression in nondividing cells was at least equivalent to that in actively proliferating cells, suggesting that mechanisms for virus attachment, nuclear transport, virion uncoating, and perhaps some limited second-strand synthesis of AAV vectors were present in nondividing cells. Southern hybridization analysis of vector sequences from cells transduced while in DNA synthetic arrest and expanded after release of the block confirmed ultimate integration of the vector genome into cellular chromosomal DNA. These findings may provide the basis for the use of AAV-based vectors for gene transfer into quiescent cell populations such as totipotent hematopoietic stem cells.

Pomerantz, J. and H. M. Blau (2004). "Nuclear reprogramming: a key to stem cell function in regenerative medicine." Nat Cell Biol **6**(9): 810-6.

The goal of regenerative medicine is to restore form and function to damaged tissues. One potential therapeutic approach involves the use of autologous cells derived from the bone marrow (bone marrow-derived cells, BMDCs). Advances in nuclear transplantation, experimental heterokaryon formation and the observed plasticity of gene expression and phenotype reported in multiple phyla provide evidence for nuclear plasticity. Recent observations have extended these findings to show that endogenous cells within the bone marrow have the capacity to incorporate into defective tissues and be reprogrammed. Irrespective of the mechanism, the potential for new gene expression patterns by BMDCs in recipient tissues holds promise for developing cellular therapies for both proliferative and post-mitotic tissues.

Prakash, S. K., T. A. Cormier, et al. (2002). "Loss of holocytochrome c-type synthetase causes the male lethality of X-linked dominant microphthalmia with linear skin defects (MLS) syndrome." Hum Mol Genet **11**(25): 3237-48.

Girls with MLS syndrome have microphthalmia with linear skin defects of face and neck, sclerocornea, corpus callosum agenesis and other brain anomalies. This X-linked dominant, male-lethal condition is associated with heterozygous deletions of a critical region in Xp22.31, from the 5' untranslated region of MID1 at the telomeric boundary to the ARHGAP6 gene at the centromeric boundary. HCCS, encoding human holocytochrome c-type synthetase, is the only gene located entirely inside the critical region. Because single gene analysis is not feasible in MLS patients (all have deletions), we

generated a deletion of the equivalent region in the mouse to study the molecular basis of this syndrome. This deletion inactivates mouse Hccs, whose homologs in lower organisms (cytochrome c or c1 heme lyases) are essential for function of cytochrome c or c1 in the mitochondrial respiratory chain. Ubiquitous deletions generated in vivo lead to lethality of hemizygous, homozygous and heterozygous embryos early in development. This lethality is rescued by expression of the human HCCS gene from a transgenic BAC, resulting in viable homozygous, heterozygous and hemizygous deleted mice with no apparent phenotype. In the presence of the HCCS transgene, the deletion is easily transmitted to subsequent generations. We did obtain a single heterozygous deleted female that does not express human HCCS, which is analogous to the low prevalence of the heterozygous MLS deletion in humans. Through the study of these genetically engineered mice we demonstrate that loss of HCCS causes the male lethality of MLS syndrome.

Priddle, H., D. R. Jones, et al. (2006). "Hematopoiesis from human embryonic stem cells: overcoming the immune barrier in stem cell therapies." Stem Cells **24**(4): 815-24.

The multipotency and proliferative capacity of human embryonic stem cells (hESCs) make them a promising source of stem cells for transplant therapies and of vital importance given the shortage in organ donation. Recent studies suggest some immune privilege associated with hESC-derived tissues. However, the adaptability of the immune system makes it unlikely that fully differentiated tissues will permanently evade immune rejection. One promising solution is to induce a state of immune tolerance to a hESC line using tolerogenic hematopoietic cells derived from it. This could provide acceptance of other differentiated tissues from the same line. However, this approach will require efficient multilineage hematopoiesis from hESCs.

Psarras, S., N. Karagianni, et al. (2004). "Gene transfer and genetic modification of embryonic stem cells by Cre- and Cre-PR-expressing MESV-based retroviral vectors." J Gene Med **6**(1): 32-42.

BACKGROUND: Genetic modification of embryonic stem (ES) cells represents a powerful tool for transgenic and developmental experiments. We report that retroviral constructs based on murine embryonal stem cell virus (MESV) can efficiently deliver and express Cre recombinase or a post-translationally inducible Cre-Progesterone receptor (Cre.PR) fusion in mouse fibroblasts and ES cells. METHODS: To study the vectors a sensitive reporter cell line, 3TZ, was derived from the murine 3T6 fibroblast line that expresses beta-galactosidase only upon Cre-mediated recombination. This was used together with the ROSA26-R ES cell Cre-reporter system or unmodified mouse ES cells as targets of infection. Efficiency of gene transfer was evaluated immunohistochemically by the use of an anti-Cre polyclonal antibody, and by monitoring the expression of beta-galactosidase. Infection of the 3TZ cells with high titer 718C or 719CP virus revealed efficient gene transduction of constitutive or hormone-inducible recombinase activity, respectively. The vectors efficiently transduced murine ES cells with Cre, Cre-PR (fusion of Cre and progesterone receptor) or beta-galactosidase. Cre-mediated recombination in more than 60% of ROSA26-R ES cells was achieved when infected by a VSV-G-pseudotyped MESV retrovirus at MOI of 50. CONCLUSIONS: The MESV-based retroviral systems, when combined with hormone inducible Cre, represent efficient tools for the transfer of Cre activity in ES cells.

Ratajczak, M. Z., E. K. Zuba-Surma, et al. (2007). "Bone-marrow-derived stem cells--our key to longevity?" J Appl Genet **48**(4): 307-19.

Bone marrow (BM) was for many years primarily regarded as the source of hematopoietic stem cells. In this review we discuss current views of the BM stem cell compartment and present data showing that BM contains not only hematopoietic but also heterogeneous non-hematopoietic stem cells. It is likely that similar or overlapping populations of primitive non-hematopoietic stem cells in BM were detected by different investigators using different experimental strategies and hence were assigned different names (e.g., mesenchymal stem cells, multipotent adult progenitor cells, or marrow-isolated adult multilineage inducible cells). However, the search still continues for true pluripotent stem cells in adult BM, which would fulfill the required criteria (e.g. complementation of blastocyst development). Recently our group has identified in BM a population of very small embryonic-like stem cells (VSELs), which express several markers characteristic for pluripotent stem cells and are found during early embryogenesis in the epiblast of the cylinder-stage embryo.

Ratajczak, M. Z., E. K. Zuba-Surma, et al. (2008). "Hunt for pluripotent stem cell -- regenerative medicine search for almighty cell." J Autoimmun **30**(3): 151-62.

Regenerative medicine and tissue engineering are searching for a novel stem cell based therapeutic strategy that will allow for efficient treatment or even potential replacement of damaged organs. The pluripotent stem cell (PSC), which gives

rise to cells from all three germ lineages, seems to be the most ideal candidate for such therapies. PSC could be extracted from developing embryos. However, since this source of stem cells for potential therapeutic purposes remains controversial, stem cell researchers look for PSC that could be isolated from the adult tissues or generated from already differentiated cells. True PSC should possess both potential for multilineage differentiation in vitro and, more importantly, also be able to complement in vivo blastocyst development. This review will summarize current approaches and limitations to isolate PSC from adult tissues or, alternatively, to generate it by nuclear reprogramming from already differentiated somatic cells.

Ray, R., K. Ray, et al. (1997). "Differential alterations in metabolic pattern of the six major UsnRNAs during development." Mol Cell Biochem **177**(1-2): 79-88.

The uridylic acid rich nuclear RNAs (U1-U6 snRNAs) are involved mainly in the processing of pre-mRNA and pre-rRNA. So, any control of cell growth through pre-mRNA/pre-rRNA processing may have some regulation through altered UsnRNAs metabolism. With this idea, attempts have been made to see how the metabolism of the six major UsnRNAs' changed during the normal process of cellular proliferation associated with differentiation from pluripotent/totipotent stem cells of early embryonic stage to much more differentiated state of different cell/tissue lineages in different tissues/organs during the fetal and neonatal stages of growth. It has been seen that the levels of the six major UsnRNAs were high in day 8 embryo when the cells were mainly pluripotent/totipotent in nature, and during the progression of embryonic development the levels of these UsnRNAs gradually decreased (approximately 35-65%) up to the midgestational period (day 13) with some exception, when the organogenesis has already been started. However in the fetal life, the levels of these UsnRNAs were maximum or comparable around 18 +/- 2 days of gestation in comparison to that in day 8 embryo when the kinetics of the maturational status of the different organs were quite high. But, the levels of these UsnRNAs' became low during day 21 of fetal life or in day 0 of birth (perturation period) in all the tissues/organs except high UsnRNAs' level in spleen. In the neonatal life, around 3 +/- 1 days of birth these UsnRNAs' levels again became maximum in all the tissues/organs (except in thymus) followed by decrease up to 5/6 days, and to become steady with slight increase within one to two weeks, when the kinetics of the organ maturation reached to a steady state. In case of thymus, the levels of the U3-U6 snRNAs were high on day 0 of birth followed by decrease in their level on day 1/2 and then increased to become steady within 2-4 weeks; whereas the U1 and U2 snRNAs' levels were high on day 3 of birth and the subsequent changes were similar to that in other tissues/organs. Thus the different UsnRNAs' metabolism in the perturation period and in the early stages of neonatal life has indicated the differential cellular functions in these two stages of development. These alterations in the metabolism of these UsnRNAs might be due to the differential changes in the rate of synthesis of these UsnRNAs and/or with their differential turnover rate in the different stages of development. Also, the differential variations of these UsnRNAs' levels have been observed among the different tissues/organs at the respective stages of development indicating the differences in the UsnRNAs' metabolism among the different cell/tissue lineages.

Ray, W. J. and D. I. Gottlieb (1996). "Regulation of protein abundance in pluripotent cells undergoing commitment to the neural lineage." J Cell Physiol **168**(2): 264-75.

The P19 cell line is a widely studied model of neural differentiation When pluripotent P19 cells are cultured as aggregates in the presence of retinoic acid for 4 days, the cells commit to the neural fate, but have not yet undergone overt differentiation. Two-dimensional polyacrylamide gel electrophoresis was used to analyze cellular protein expression during this induction. Approximately 500 abundant polypeptides were analyzed. Seventeen polypeptides were upregulated during induction; several of these were significantly regulated 48 h after the addition of retinoic acid. No downregulations were observed. Fifteen of the 17 polypeptides continued to be expressed throughout terminal differentiation. The upregulation of 14 of the 17 polypeptides requires both retinoic acid and aggregation, which alone do not induce neural differentiation. Furthermore, these regulated polypeptides are expressed in neural tissue, suggesting they are associated with neural function in vivo. Embryonic stem cells, a totipotent line, also neurally differentiate in response to retinoic acid and aggregation. Comparison of embryonic stem cells to P19 cells shows that the two systems regulate a similar set of polypeptides and are thus likely to utilize a similar pathway. These studies are a step toward determining the full extent of regulation involved in the commitment of pluripotent cells to the neural fate.

Raz, E. (2002). "Primordial germ cell development in zebrafish." Semin Cell Dev Biol **13**(6): 489-95.

In sexually reproducing organisms, primordial germ cells (PGCs) give rise to gametes that are responsible for the development of a new

organism in the next generation. These cells follow a characteristic developmental path that is manifested in specialized regulation of basic cell functions and behavior making them an attractive system for studying cell fate specification, differentiation and migration. This review summarizes studies aimed at understanding the development of this cell population in zebrafish and compares these results with those obtained in other model organisms.

Rich, I. N. (1995). "Primordial germ cells are capable of producing cells of the hematopoietic system in vitro." Blood **86**(2): 463-72.

The identity of the cells giving rise to the hematopoietic system in the mouse embryo are unknown. The results presented here strongly suggest that hematopoietic cells are derived from a nonhematopoietic cell population that has been previously thought to give rise to the germ cells. These cells are called primordial germ cells (PGCs) and can be recognized as large cells showing blebbing and pseudopodial extrusions on their surface. They are alkaline phosphatase (AP) positive and possess a stage-specific embryonic antigen (SSEA-1) on their surface. They represent a small pool of cells in the extraembryonic mesoderm at the base of the allantois in late day-6 embryos. Primordial germ cells from 7.5- and 8.5-day visceral yolk sac and embryo proper form AP+ and SSEA-1+ colonies within 5 days when grown on an embryonic fibroblast feeder cell layer in the presence of leukemia inhibitory factor (LIF), stem cell factor (SCF), and interleukin-3 (IL-3). Individual colonies taken from day-5 cultures can be shown to differentiate into erythroid lineage cells in secondary methyl cellulose culture and produce secondary and tertiary PGCs in the presence of LIF, SCF, and IL-3. Cells taken from the region of the allantois and primitive streak can form colonies on hydrophilic Teflon (DuPont, Wilmington, DE) foils precoated with collagen and fibronectin. The cells from these colonies were then shown to form cobblestone areas on irradiated adult bone marrow stromal layers, indicating that the most primitive in vitro hematopoietic stem cell, the cobblestone-area forming cell (CAFC), was present. PGC colonies were grown in methyl cellulose in the presence of LIF, SCF, and IL-3 for 5 days, and the colonies were removed and passaged 3 times on pretreated extracellular matrix hydrophilic Teflon foils. After each passage, the cells were assayed for their differentiation capacity and PGC content. After the last passage, the number of CAFCs was also determined. It was found that, under these conditions, the PGC population expanded more than 400-fold and also contained CAFCs. It is postulated that the PGC represents a totipotent stem cell population capable of producing a variety of different cell types including cells of the hematopoietic system.

Rindi, G., C. Ratineau, et al. (1999). "Targeted ablation of secretin-producing cells in transgenic mice reveals a common differentiation pathway with multiple enteroendocrine cell lineages in the small intestine." Development **126**(18): 4149-56.

The four cell types of gut epithelium, enteroendocrine cells, enterocytes, Paneth cells and goblet cells, arise from a common totipotent stem cell located in the mid portion of the intestinal gland. The secretin-producing (S) cell is one of at least ten cell types belonging to the diffuse neuroendocrine system of the gut. We have examined the developmental relationship between secretin cells and other enteroendocrine cell types by conditional ablation of secretin cells in transgenic mice expressing herpes simplex virus 1 thymidine kinase (HSVTK). Ganciclovir-treated mice showed markedly increased numbers of apoptotic cells at the crypt-villus junction. Unexpectedly, ganciclovir treatment induced nearly complete ablation of enteroendocrine cells expressing cholecystokinin and peptide YY/glucagon (L cells) as well as secretin cells, suggesting a close developmental relationship between these three cell types. In addition, ganciclovir reduced the number of enteroendocrine cells producing gastric inhibitory polypeptide, substance-P, somatostatin and serotonin. During recovery from ganciclovir treatment, the enteroendocrine cells repopulated the intestine in normal numbers, suggesting that a common early endocrine progenitor was spared. Expression of BETA2, a basic helix-loop-helix protein essential for differentiation of secretin and cholecystokinin cells was examined in the proximal small intestine. BETA2 expression was seen in all enteroendocrine cells and not seen in nonendocrine cells. These results suggest that most small intestinal endocrine cells are developmentally related and that a close developmental relationship exists between secretin-producing S cells and cholecystokinin-producing and L type enteroendocrine cells. In addition, our work shows the existence of a multipotent endocrine-committed cell type and locates this hybrid multipotent cell type to a region of the intestine populated by relatively immature cells.

Rinkevich, B., Z. Shlemberg, et al. (1995). "Whole-body protochordate regeneration from totipotent blood cells." Proc Natl Acad Sci U S A **92**(17): 7695-9.

Cell differentiation, tissue formation, and organogenesis are fundamental patterns during the development of multicellular animals from the dividing cells of fertilized eggs. Hence, the complete morphogenesis of any developing organism of the

animal kingdom is based on a complex series of interactions that is always associated with the development of a blastula, a one-layered hollow sphere. Here we document an alternative pathway of differentiation, organogenesis, and morphogenesis occurring in an adult protochordate colonial organism. In this system, any minute fragment of peripheral blood vessel containing a limited number of blood cells isolated from Botrylloides, a colonial sea squirt, has the potential to give rise to a fully functional organism possessing all three embryonic layers. Regeneration probably results from a small number of totipotent stem cells circulating in the blood system. The developmental process starts from disorganized, chaotic masses of blood cells. At first an opaque cell mass is formed. Through intensive cell divisions, a hollow, blastula-like structure results, which may produce a whole organism within a short period of a week.

Rinkevich, B. and I. Yankelevich (2004). "Environmental split between germ cell parasitism and somatic cell synergism in chimeras of a colonial urochordate." J Exp Biol **207**(Pt 20): 3531-6.

Colonies of the urochordate Botryllus schlosseri may fuse upon contact if they share common alleles on the highly polymorphic fusibility/histocompatibility locus. While, in these chimeras, one of the partners is usually morphologically eliminated (resorbed), circulating totipotent cells of the inferior genotype on the resorption phenomenon may parasitize either the soma or the germ line of the winner. Here, we show an environmental split of the two stem cell lineages that may develop germ cell parasitism vs somatic cell cooperation. Each naturally formed Botryllus chimera can be a composite of component genotypes created through two unlinked parasitic germ and somatic cell lineage interactions. The germ line parasitism is inherited through a pedigree. Conversely, by using amplified fragment length polymorphism (AFLP) and microsatellite alleles as polymorphic genetic markers, and seawater temperature as the variable environmental factor, we documented that the somatic constituent of chimeric zooids was shifted from one genotype to another, in accordance with the changes in seawater temperatures. This variable somatic state of chimerism in the field may, thus, carry benefits to the chimeral entity, which presents synergistically, at any time, the best-fitted combination of its genetic components.

Robin, C., F. Pflumio, et al. (1999). "Identification of lymphomyeloid primitive progenitor cells in fresh human cord blood and in the marrow of nonobese diabetic-severe combined immunodeficient (NOD-SCID) mice transplanted with human CD34(+) cord blood cells." J Exp Med **189**(10): 1601-10.

Transplantation of genetically marked donor cells in mice have unambiguously identified individual clones with full differentiative potential in all lymphoid and myeloid pathways. Such evidence has been lacking in humans because of limitations inherent to clonal stem cell assays. In this work, we used single cell cultures to show that human cord blood (CB) contains totipotent CD34(+) cells capable of T, B, natural killer, and granulocytic cell differentiation. Single CD34(+) CD19(-)Thy1(+) (or CD38(-)) cells from fresh CB were first induced to proliferate and their progeny separately studied in mouse fetal thymic organotypic cultures (FTOCs) and cocultures on murine stromal feeder layers. 10% of the clones individually analyzed produced CD19(+), CD56(+), and CD15(+) cells in stromal cocultures and CD4(+)CD8(+) T cells in FTOCs, identifying totipotent progenitor cells. Furthermore, we showed that totipotent clones with similar lymphomyeloid potential are detected in the bone marrow of nonobese diabetic severe combined immunodeficient (NOD-SCID) mice transplanted 4 mo earlier with human CB CD34(+) cells. These results provide the first direct demonstration that human CB contains totipotent lymphomyeloid progenitors and transplantable CD34(+) cells with the ability to reconstitute, in the marrow of recipient mice, the hierarchy of hematopoietic compartments, including a compartment of functional totipotent cells. These experimental approaches can now be exploited to analyze mechanisms controlling the decisions of such primitive human progenitors and to design conditions for their ampification that can be helpful for therapeutic purposes.

Roche, E. and B. Soria (2004). "Generation of new islets from stem cells." Cell Biochem Biophys **40**(3 Suppl): 113-24.

Spain ranks number one in organ donors (35 per million per yr). Although the prevalence of diabetes is low (100,000 type 1 diabetic patients and 2 million type 2 diabetic patients), the expected number of patients receiving islet transplants should be estimated at 200 per year. Islet replacement represents a promising cure for diabetes and has been successfully applied in a limited number of type 1 diabetic patients, resulting in insulin independence for periods longer than 3 yr. However, it has been difficult to obtain sufficient numbers of islets from cadaveric donors. Interesting alternatives include acquiring renewable sources of cells using either embryonic or adult stem cells to overcome the islet scarcity problem. Stem cells are capable of extensive proliferation rates and are capable of differentiating

into other cell types of the body. In particular, totipotent stem cells are capable of differentiating into all cell types in the body, whereas pluripotent stem cells are limited to the development of a certain number of differentiated cell types. Insulin-producing cells have been obtained from both embryonic and adult stem cells using several approaches. In animal models of diabetes, the therapeutic application of bioengineered insulin-secreting cells derived from stem cells has delivered promising results. This review will summarize the different approaches that have been used to obtain insulin-producing cells from embryonic and adult stem cells and highlights the key points that will allow in vitro differentiation and subsequent transplantation in the future.

Rodrigues, P., D. Limback, et al. (2008). "Oogenesis: Prospects and challenges for the future." J Cell Physiol **216**(2): 355-65.

Oogenesis serves a singular role in the reproductive success of plants and animals. Of their remarkable differentiation pathway what stands out is the ability of oocytes to transform from a single cell into the totipotent lineages that seed the early embryo. As our understanding that commonalities between diverse organisms at the genetic, cellular and molecular levels are conserved to achieve successful reproduction, the notion that embryogenesis presupposes oogenesis has entered the day-to-day parlance of regenerative medicine and stem cell biology. With emphasis on the mammalian oocyte, this review will cover (1) current concepts regarding the birth, survival and growth of oocytes that depends on complex patterns of cell communication between germ line and soma, (2) the notion of "maternal inheritance" from a genetic and epigenetic perspective, and (3) the relative value of model systems with reference to current clinical and biotechnology applications.

Rodriguez, C. I., A. Galan, et al. (2006). "Derivation of clinical-grade human embryonic stem cells." Reprod Biomed Online **12**(1): 112-8.

Embryonic stem cells proliferate in vitro while maintaining an undifferentiated state, and are capable of differentiating into most cell types under appropriate conditions. These properties imply great potential in the treatment of various diseases and disabilities. In fact, the first clinical trials with hESC for treating spinal cord injuries will begin next year. However, therapeutic application of human embryonic stem cell derivatives is compromised by the exposure of existing lines to animal and human components, with the subsequent risk of contamination with retroviruses and other pathogens, which can be transmitted to patients. The scientific community is striving to avoid the use of xenogeneic or allogeneic components in the process of derivation new hESC lines. This review summarizes attempts that have been made to avoid these contaminants and the breakthroughs achieved in the derivation of clinical-grade hESC that could be used for therapeutic purposes.

Roelen, B. A. and S. M. Lopes (2008). "Of stem cells and gametes: similarities and differences." Curr Med Chem **15**(13): 1249-56.

Fusion of a mammalian sperm cell with an oocyte will lead to the formation of a new organism. As this new organism develops, the cells that construct the organism gradually lose developmental competence and become differentiated, a process which is in part mediated via epigenetic modifications. These mechanisms include DNA methylation, histone tail modifications and association with Polycomb and Trithorax proteins. Several cells within the organism must however maintain or regain developmental competence while they are highly specialized. These are the primordial germ cells that form the gametes; the oocytes and sperm cells. In this review different epigenetic modifying mechanisms will be discussed as they occur in developing embryos. In addition, aspects of nuclear reprogramming that are likely to occur via removal of epigenetic modifications are important, and several epigenetic removal mechanisms are indeed also active in developing germ cells. In vivo, a pluripotent cell has the capacity to form gametes, but in vitro terminal gametogenesis has proven to be difficult. Although development of pluripotent cells to cells with the characteristics of early germ cells has been unequivocally demonstrated, creating the correct culture milieu that enables further maturation of these cells has as yet been futile.

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.

Rossant, J. (2007). "Stem cells and lineage development in the mammalian blastocyst." Reprod Fertil Dev **19**(1): 111-8.

The mammalian blastocyst is the source of the most pluripotent stem cells known: embryonic stem (ES) cells. However, ES cells are not totipotent; in mouse chimeras, they do not contribute to extra-embryonic cell types of the trophectoderm (TE) and primitive endoderm (PrE) lineages. Understanding the genetic pathways that control pluripotency v. extra-embryonic lineage restriction is key to understanding not only normal embryonic development, but also how to reprogramme adult cells to pluripotency. The trophectoderm and primitive endoderm lineages also provide the first signals that drive patterned differentiation of the pluripotent epiblast cells of the embryo. My laboratory has produced permanent mouse cell lines from both the TE and the PrE, termed trophoblast stem (TS) and eXtra-embryonic ENdoderm (XEN) cells. We have used these cells to explore the genetic and molecular hierarchy of lineage restriction and identify the key factors that distinguish the ES cell v. the TS or XEN cell fate. The major molecular pathways of lineage commitment defined in mouse embryos and stem cells are probably conserved across mammalian species, but more comparative studies of lineage development in embryos of non-rodent mammals will likely yield interesting differences in terms of timing and details.

Rossi, L., A. Salvetti, et al. (2008). "Planarians, a tale of stem cells." Cell Mol Life Sci **65**(1): 16-23.

Planarians possess amazing abilities to regulate tissue homeostasis and regenerate missing body parts. These features reside on the presence of a population of pluripotent/totipotent stem cells, the neoblasts, which are considered as the only planarian cells able to proliferate in the asexual strains. Neoblast distribution has been identified by mapping the cells incorporating bromodeoxyuridine, analyzing mitotic figures and using cell proliferation markers. Recently identified molecular markers specifically label subgroups of neoblasts, revealing thus the heterogeneity of the planarian stem cell population. Therefore, the apparent totipotency of neoblasts probably reflects the composite activities of multiple stem cell types. First steps have been undertaken to understand how neoblasts and differentiated cells communicate with each other to adapt the self-renewal and differentiation rates of neoblasts to the demands of the body. Moreover, the introduction of molecular resource database on planarians now paves the way to renewed strategies to understand planarian regeneration and stem cell-related issues.

Rossi, L., A. Salvetti, et al. (2006). "DjPiwi-1, a member of the PAZ-Piwi gene family, defines a subpopulation of planarian stem cells." Dev Genes Evol **216**(6): 335-46.

Planarian regeneration, based upon totipotent stem cells, the neoblasts, provides a unique opportunity to study in vivo the molecular program that defines a stem cell. In this study, we report the identification of DjPiwi-1, a planarian homologue of Drosophila Piwi. Expression analysis showed that DjPiwi-1 transcripts are preferentially accumulated in small cells distributed along the midline of the dorsal parenchyma. DjPiwi-1 transcripts were not detectable after X-ray irradiation by whole mount in situ hybridization. Real time reverse transcriptase polymerase chain reaction analysis confirmed the significant reduction of DjPiwi-1 expression after X-ray treatment. However, the presence of residual DjPiwi-1 transcription suggests that, although the majority of DjPiwi-1-positive cells can be neoblasts, this gene is also expressed in differentiating/differentiated cells. During regeneration DjPiwi-1-positive cells reorganize along the midline of the stump and no accumulation of hybridization signal was observed either in the blastema area or in the parenchymal region beneath the blastema. DjPiwi-1-positive cells, as well as the DjMCM2-expressing neoblasts located along the midline and those spread all over the parenchyma, showed a lower tolerance to X-ray with respect to the DjMCM2-expressing neoblasts distributed along the lateral lines of the parenchyma. Taken together, these findings suggest the presence of different neoblast subpopulations in planarians.

Sahai, J. and S. G. Louie (1993). "Overview of the immune and hematopoietic systems." Am J Hosp Pharm **50**(7 Suppl 3): S4-9.

Current knowledge of the immune and hematopoietic systems is reviewed. All blood cells are derived from the totipotent stem cell, also known as the pluripotent stem cell. The differentiation of pluripotent peripheral stem cells into blood cells is controlled by a variety of biologic response modifiers, including colony-stimulating factors (CSFs) and

interleukins. Among the known CSFs are stem cell growth factor, granulocyte-macrophage CSF, multilineage CSF (interleukin-3), granulocyte CSF, macrophage CSF, and erythropoietin. CSFs are categorized as class I (those that stimulate the production of several types of blood cells; also called pluripotent) and class II (those that stimulate only one cell line; also called unipotent). Effects of CSFs can be studied using laboratory tests of colony-forming-unit activity. Pathogens entering the body through damaged skin or mucous membranes are met with both a cellular response (neutrophils, macrophages, cytotoxic T lymphocytes, and natural killer cells) and a humoral response (antibodies and complement). There is interplay between these two arms of the immune system to defend against foreign antigens. This interplay can occur by cell-to-cell contact and by cytokines. Hematopoietic and immune cells of the body are produced and destroyed under precise control of many different biologic response modifiers, including the colony-stimulating factors, interleukins, and interferons.

Salminen, M., B. I. Meyer, et al. (1998). "Efficient poly A trap approach allows the capture of genes specifically active in differentiated embryonic stem cells and in mouse embryos." Dev Dyn **212**(2): 326-33.

Special vectors have been constructed that allow the trapping of genes in mouse embryonic stem (ES) cells. These vectors generally contain the neomycin phosphotransferase (neo) gene for selection and the beta-galactosidase (beta-gal) gene as a marker. Promoterless vectors can be used to identify genes that are active in undifferentiated ES cells. To also have access to genes that are inactive in totipotent ES cells, we constructed a polyadenylation (poly A) trap vector in which the expression of a poly A less neo gene is driven by a constitutive promoter, whereas the expression of beta-gal depends on the trapped sequences. We demonstrate here that this vector integrates with a high frequency into transcription units and that it traps genes with very different expression patterns in vitro and in vivo. The vector integrates efficiently into transcription units that are inactive in undifferentiated ES cells and which can be activated through in vitro differentiation. Furthermore, in vivo expression patterns demonstrate that this vector integrates into genes that exhibit a highly specific temporal and spatial expression pattern during embryogenesis.

Salo, E. (2006). "The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes)." Bioessays **28**(5): 546-59.

The great powers of regeneration shown by freshwater planarians, capable of regenerating a complete organism from any tiny body fragment, have attracted the interest of scientists throughout history. In 1814, Dalyell concluded that planarians could "almost be called immortal under the edge of the knife". Equally impressive is the developmental plasticity of these platyhelminthes, including continuous growth and fission (asexual reproduction) in well-fed organisms, and shrinkage (degrowth) during prolonged starvation. The source of their morphological plasticity and regenerative capability is a stable population of totipotent stem cells--"neoblasts"; this is the only cell type in the adult that has mitotic activity and differentiates into all cell types. This cellular feature is unique to planarians in the Bilateria clade. Over the last fifteen years, molecular studies have begun to reveal the role of developmental genes in regeneration, although it would be premature to propose a molecular model for planarian regeneration. Genomic and proteomic data are essential in answering some of the fundamental questions concerning this remarkable morphological plasticity. Such information should also pave the way to understanding the genetic pathways associated with metazoan somatic stem-cell regulation and pattern formation.

Salvetti, A., L. Rossi, et al. (2005). "DjPum, a homologue of Drosophila Pumilio, is essential to planarian stem cell maintenance." Development **132**(8): 1863-74.

As stem cells are rare and difficult to study in vivo in adults, the use of classical models of regeneration to address fundamental aspects of the stem cell biology is emerging. Planarian regeneration, which is based upon totipotent stem cells present in the adult--the so-called neoblasts--provides a unique opportunity to study in vivo the molecular program that defines a stem cell. The choice of a stem cell to self-renew or differentiate involves regulatory molecules that also operate as translational repressors, such as members of PUF proteins. In this study, we identified a homologue of the Drosophila PUF gene Pumilio (DjPum) in the planarian Dugesia japonica, with an expression pattern preferentially restricted to neoblasts. Through RNA interference (RNAi), we demonstrate that gene silencing of DjPum dramatically reduces the number of neoblasts, thus supporting the intriguing hypothesis that stem cell maintenance may be an ancestral function of PUF proteins.

Santos, F. and W. Dean (2004). "Epigenetic reprogramming during early development in mammals." Reproduction **127**(6): 643-51.

Epigenetic modifications serve as an extension of the information content by which the

underlying genetic code may be interpreted. These modifications mark genomic regions and act as heritable and stable instructions for the specification of chromatin organisation and structure that dictate transcriptional states. In mammals, DNA methylation and the modification of histones account for the major epigenetic alterations. Two cycles of DNA methylation reprogramming have been characterised. During germ cell development, epigenetic reprogramming of DNA methylation resets parent-of-origin based genomic imprints and restores totipotency to gametes. On fertilisation, the second cycle is triggered resulting in an asymmetric difference between parental genomes. Further epigenetic asymmetry is evident in the establishment of the first two lineages at the blastocyst stage. This differentiative event sets the epigenetic characteristics of the lineages as derivatives of the inner cell mass (somatic) and trophectoderm (extra-embryonic). It is the erasure and subsequent re-tracing of the epigenetic checkpoints that pose the most serious obstacles to somatic nuclear transfer. Elaboration of the mechanisms of these interactions will be invaluable in our fundamental understanding of biological processes and in achieving substantial therapeutic advances.

Sato, H., K. Amagai, et al. (2009). "Stable generation of serum- and feeder-free embryonic stem cell-derived mice with full germline-competency by using a GSK3 specific inhibitor." Genesis **47**(6): 414-22.

C57BL/6 (B6)-derived embryonic stem (ES) cells are not widely used to generate knockout mice despite the advantage of a well-defined genetic background because of poor developmental potential. We newly established serum- and feeder-free B6 ES cells with full developmental potential by using leukemia inhibitory factor (LIF) and 6-bromoindirubin-3'-oxime (BIO), a glycogen synthase kinase-3 (GSK3) inhibitor. BIO treatment significantly increased the expression levels of 364 genes including pluripotency markers such as Nanog and Klf family. Unexpectedly, by aggregating or microinjecting those ES cells to each eight-cell-stage diploid embryo, we stably generated germline-competent ES-derived mice. Furthermore, founder mice completely derived from female XO, heterozygous, or homozygous mutant B6 ES cells were directly available for intercross breeding and phenotypic analysis. We hereby propose that serum- and feeder-free B6 ES cells stimulated with LIF plus GSK3 inhibitor are valuable for generating mouse models on B6 background.

Sauvageau, G., U. Thorsteinsdottir, et al. (1995). "Overexpression of HOXB4 in hematopoietic cells causes the selective expansion of more primitive populations in vitro and in vivo." Genes Dev **9**(14): 1753-65.

Hox genes were first recognized for their role in embryonic development and may also play important lineage-specific functions in a variety of somatic tissues including the hematopoietic system. We have recently shown that certain members of the Hox A and B clusters, such as HOXB3 and HOXB4, are preferentially expressed in subpopulations of human bone marrow that are highly enriched for the most primitive hematopoietic cell types. To assess the role these genes may play in regulating the proliferation and/or differentiation of such cells, we engineered the overexpression of HOXB4 in murine bone marrow cells by retroviral gene transfer and analyzed subsequent effects on the behavior of various hematopoietic stem and progenitor cell populations both in vitro and in vivo. Serial transplantation studies revealed a greatly enhanced ability of HOXB4-transduced bone marrow cells to regenerate the most primitive hematopoietic stem cell compartment resulting in 50-fold higher numbers of transplantable totipotent hematopoietic stem cells in primary and secondary recipients, compared with serially passaged neo-infected control cells. This heightened expansion in vivo of HOXB4-transduced hematopoietic stem cells was not accompanied by identifiable anomalies in the peripheral blood of these mice. Enhanced proliferation in vitro of day-12 CFU-S and clonogenic progenitors was also documented. These results indicate HOXB4 to be an important regulator of very early but not late hematopoietic cell proliferation and suggest a new approach to the controlled amplification of genetically modified hematopoietic stem cell populations.

Scholtz, B., D. Kelly, et al. (1995). "Cis-regulatory elements and transcription factors involved in the regulation of the transforming growth factor-beta 2 gene." Mol Reprod Dev **41**(2): 140-8.

Embryonal carcinoma (EC) cells and embryonic stem (ES) cells provide useful model systems for studying differentiation during early mammalian development. Previous studies have demonstrated that differentiation of two restricted mouse EC cell lines is accompanied by activation of the TGF-beta 2 gene. Moreover, one negative and two positive regulatory regions upstream of the transcription start site were identified, which appear to play key roles in the transcriptional regulation of the human TGF-beta 2 gene. In this report, we demonstrate that the same three regulatory regions strongly influence the activity of the TGF-beta 2 promoter in differentiated cells derived from the multipotent human EC cell line, NT2/D1, and from the murine totipotent ES cell line, CCE. We also

determined that the same three regions are active in the regulation of the TGF-beta 2 gene in the murine parietal endoderm-like cell line, PYS-2. However, an additional negative regulatory region appears to contribute to the regulation of the TGF-beta 2 gene in PYS-2 cells. Last, mutation of a CRE/ATF element located just upstream of the transcription start site of the TGF-beta 2 gene reduces significantly the activity of the TGF-beta 2 promoter in the differentiated cells. However, in contrast to our previous findings, our gel mobility shift analyses demonstrate that this CRE/ATF element is bound by similar proteins in nuclear extracts prepared from undifferentiated and differentiated mouse EC cells as well as from undifferentiated human EC cells.(ABSTRACT TRUNCATED AT 250 WORDS)

Schwartz, P. H. (2008). "Training the next generation of pluripotent stem cell researchers." J Transl Med **6**: 40.

Human pluripotent stem cells (PSCs) have the unique properties of being able to proliferate indefinitely in their undifferentiated state and of being able to differentiate into any somatic cell type. These cells are thus posited to be extremely useful for furthering our understanding of both normal and abnormal human development, providing a human cell preparation that can be used to screen for new reagents or therapeutic agents, and generating large numbers of differentiated cells that can be used for transplantation purposes. PSCs in culture have a specific morphology and they express characteristic surface antigens and nuclear transcription factors; thus, PSC culture is very specific and requires a core skill set for successful propagation of these unique cells. Specialized PSC training courses have been extremely valuable in seeding the scientific community with researchers that possess this skill set.

Sell, S. (2004). "Stem cell origin of cancer and differentiation therapy." Crit Rev Oncol Hematol **51**(1): 1-28.

Our forefathers in pathology, on observing cancer tissue under the microscope in the mid-19th century, noticed the similarity between embryonic tissue and cancer, and suggested that tumors arise from embryo-like cells [Recherches dur le Traitement du Cancer, etc. Paris. (1829); Editoral Archiv fuer pathologische Anatomie und Physiologie und fuer klinische Medizin 8 (1855) 23]. The concept that adult tissues contain embryonic remnants that generally lie dormant, but that could be activated to become cancer was later formalized by Cohnheim [Path. Anat. Physiol. Klin. Med. 40 (1867) 1-79; Virchows Arch. 65 (1875) 64] and Durante [Arch. Memori ed Osservazioni di Chirugia Practica 11 (1874) 217-226], as the "embryonal rest" theory of cancer. An updated version of the embryonal rest theory of cancer is that cancers arise from tissue stem cells in adults. Analysis of the cellular origin of carcinomas of different organs indicates that there is, in each instance, a determined stem cell required for normal tissue renewal that is the most likely cell of origin of carcinomas [Lab. Investig. 70 (1994) 6-22]. In the present review, the nature of normal stem cells (embryonal, germinal and somatic) is presented and their relationships to cancer are further expanded. Cell signaling pathways shared by embryonic cells and cancer cells suggest a possible link between embryonic cells and cancer cells. Wilm's tumors (nephroblastomas) and neuroblastomas are presented as possible tumors of embryonic rests in children. Teratocarcinoma is used as the classic example of the totipotent cancer stem cell which can be influenced by its environment to differentiate into a mature adult cell. The observation that "promotion" of an epidermal cancer may be accomplished months or even years after the initial exposure to carcinogen ("initiation"), implies that the original carcinogenic event occurs in a long-lived epithelial stem cell population. The cellular events during hepatocarcinogenesis illustrate that cancers may arise from cells at various stages of differentiation in the hepatocyte lineage. Examples of genetic mutations in epithelial and hematopoietic cancers show how specific alterations in gene expression may be manifested as maturation arrest of a cell lineage at a specific stage of differentiation. Understanding the signals that control normal development may eventually lead us to insights in treating cancer by inducing its differentiation (differentiation therapy). Retinoid acid (RA) induced differentiation therapy has acquired a therapeutic niche in treatment of acute promyelocytic leukemia and the ability of RA to prevent cancer is currently under examination.

Senju, S., S. Hirata, et al. (2003). "Generation and genetic modification of dendritic cells derived from mouse embryonic stem cells." Blood **101**(9): 3501-8.

We developed a method to generate dendritic cells (DCs) from mouse embryonic stem (ES) cells. We cultured ES cells for 10 days on feeder cell layers of OP9, in the presence of granulocyte-macrophage colony-stimulating factor in the latter 5 days. The resultant ES cell-derived cells were transferred to bacteriologic Petri dishes without feeder cells and further cultured. In about 7 days, irregularly shaped floating cells with protrusions appeared and these expressed major histocompatibility complex class II, CD11c, CD80, and CD86, with the capacity to stimulate primary mixed lymphocyte reaction (MLR) and to process and present protein antigen to T cells. We designated them ES-DCs (ES cell-derived

dendritic cells), and the functions of ES-DCs were comparable with those of DCs generated from bone marrow cells. Upon transfer to new dishes and stimulation with interleukin-4 plus tumor necrosis factor alpha, combined with anti-CD40 monoclonal antibody or lipopolysaccharide, ES-DCs completely became mature DCs, characterized by a typical morphology and higher capacity to stimulate MLR. Using an expression vector containing the internal ribosomal entry site-puromycin N-acetyltransferase gene or a Cre-lox-mediated exchangeable gene-trap system, we could efficiently generate ES cell transfectants expressing the products of introduced genes after their differentiation to DCs. ES-DCs expressing invariant chain fused to a pigeon cytochrome C epitope presented the epitope efficiently in the context of E(k). We primed ovalbumin (OVA)-specific cytotoxic T lymphocytes in vivo by injecting mice with ES-DCs expressing OVA, thus demonstrating immunization with ES-DCs genetically engineered to express antigenic protein. The methods may be applicable to immunomodulation therapy and gene-trap investigations of DCs.

Sermon, K. D. (2006). "Preimplantation genetic diagnosis." Verh K Acad Geneeskd Belg **68**(1): 5-32.

Preimplantation genetic diagnosis (PGD) is an early form of prenatal diagnosis whereby embryos obtained in vitro are tested for the presence of a certain genetic disease. Patients who are at risk to have a child with a genetic disease can thus avoid a prenatal diagnosis and a possible termination of pregnancy. At the Centres for Medical Genetics and Reproductive Medicine, we have applied PGD for monogenic diseases since 1993 and are now one of the largest in the world. In this paper, the theoretical and technical side of PGD will first be explained. Thereafter, our activity since 1993 will be described. At the end of 2004, we had carried out 713 cycles for 319 patients for 54 different indications, leading to 159 (22 %) clinical pregnancies. The ESHRE PGD Consortium, of which I am chairperson, has been collating data on PGD from PGD centres from the whole world. This has led to the publication of four reports that are considered widely as important documents, as well as a comprehensive set of guidelines. Finally, there is the aspect of the scientific research ensuing from the PGD programme. The research into the causes of instability of triplet repeats in genetic diseases such as DM1 and Huntington's disease has made significant progress. The embryonic stem cell lab was started up in 2002, and has already succeeded in deriving five new lines, as well as progressing significantly into the research in differentiation to muscle cells and the behaviour of triplet repeats in totipotent stem cells.

Shahab, N. (2007). "Extrapulmonary small cell carcinoma of the bladder." Semin Oncol **34**(1): 15-21.

Among the many sites for primary small cell cancer is the genitourinary system. The majority of cases have been observed in the bladder and prostate. Small cell carcinoma accounts for less than 1% of all bladder tumors. Definitive predisposing factors are unknown; however, small cell carcinoma of the bladder has been associated with cigarette smoking, long-standing cystitis, bladder calculus, and augmented cystoplasty. Contrary to the early theory of derivation from Kulchitsky cells, it is now believed that small cell carcinoma of the bladder originates from the totipotent stem cells present in the submucosa of the bladder wall. A number of chromosomal aberrations have been reported in small cell cancer of the bladder. There are no specific clinical features that differentiate these patients from transitional cell carcinoma of the bladder; however, some patients may have associated paraneoplastic conditions. Diagnosis is established by cystoscopic-assisted biopsy. Like small cell carcinoma of the lung, small cell carcinoma of the bladder has a propensity for early metastases. There is no standard therapy for small cell carcinoma of the bladder and the prognosis is poor; however, patients treated with cisplatin-based chemotherapy regimens seem to have a better prognosis.

Shanthly, N., M. R. Aruva, et al. (2006). "Stem cells: a regenerative pharmaceutical." Q J Nucl Med Mol Imaging **50**(3): 205-16.

Stem cells (SC), found in both adult and fetal tissues, are self-renewing elements that can generate the various cell types in the body. There are 3 classes of SC: totipotent, multipotent, and pluripotent. The SC with a significant developmental potential are the embryonic stem (ES) cells, which are derived from the early stages of mammalian embryo. SC possess regenerative properties and this offers unprecedented opportunities for developing medical therapies for debilitating diseases. Hematopoietic SC have been used successfully in bone marrow transplants for over 40 years. Pluripotent SC offer renewable source of replacement of cells and tissues to treat a myriad of diseases. However there are limiting factors. Adult SC are rare and cannot multiply as the ES. Pluripotent SC have great therapeutic potential, but face technical challenges. A serious concern is the ethical issue since they are derived from human embryos or fetal tissue. Quite often SC have been targets of mutations and risk carcinogenesis. Various markers have been identified based on the uniqueness of SC receptors and in vivo tracking studies using nanocolloids and radioactive tracers have been performed. Though 111In-oxine has

been used to image SC transplants, PET with a high spatial resolution would be ideal. Currently 2 agents are being studied, 18F-FDG and 64Cu-Pyruvaldehyde bi(N4-methylthiosemicarbazone). The following few pages bring forth the various limitations and summarize progress made in SC utilization so as to create awareness of SC research in ISORBE community and to foster strategy that ISORBE community can disseminate information and exchange knowledge on radio labeled SC.

Shi, C. Z., R. N. Dhir, et al. (1995). "Mouse embryonic stem cells express receptors of the insulin family of growth factors." Mol Reprod Dev **42**(2): 173-9.

Insulin and insulin-like growth factors (IGF-I and -II) are members of a family of growth factors which are known to be developmentally regulated during preimplantation mouse embryogenesis. The physiological actions of the insulin family of growth factors are mediated by interactions with specific cell surface receptors that are detectable on the cells of preimplantation mouse embryos. Mouse embryonic stem (ES) cells are totipotent cells derived directly from the inner cell mass of the blastocyst. ES cells have the ability to differentiate into all three germ layers and have unlimited growth potential under certain culture conditions. The great advantage of ES cells is the ability to obtain large amounts of tissue for biochemical studies as compared with preimplantation embryos. To examine in greater detail the biological actions of the insulin family of growth factors, the expression of their cognate receptors on ES cells was examined. ES cells were cultured in DMEM medium supplemented with leukemia inhibitory factor (LIF) to maintain the undifferentiated state. Receptor expression was evaluated at the mRNA level using the reverse transcription polymerase chain reaction (RT-PCR), and at the protein level by radioactive labeled ligand-receptor binding assay. Using RT-PCR, mRNAs of all three growth factor receptors were detected in ES cells. Messenger RNA from ES cells was reverse transcribed into cDNA by AMV reverse transcriptase at 42 degrees C for 1 hr. The reverse transcription reaction was amplified with Taq polymerase and specific primers for insulin, IGF-I, or IGF-II receptors by PCR. RT-PCR and the control plasmid cDNA PCR products were resolved electrophoretically on 3% agarose gels. Each amplified PCR product showed the predicted correct size.(ABSTRACT TRUNCATED AT 250 WORDS)

Shibata, N., Y. Umesono, et al. (1999). "Expression of vasa(vas)-related genes in germline cells and totipotent somatic stem cells of planarians." Dev Biol **206**(1): 73-87.

Planarians are known for their strong regenerative ability. This ability has been considered to reside in the totipotent somatic stem cell called the "neoblast." Neoblasts contain a unique cytoplasmic structure called the "chromatoid body," which has similar characteristics to the germline granules of germline cells of other animals. The chromatoid bodies decrease in number and size during cytodifferentiation and disappear in completely differentiated cells during regeneration. However, germ cells maintain the chromatoid body during their differentiation from neoblasts. These observations suggest that the chromatoid body is concerned with the totipotency of cells. To understand the molecular nature of the chromatoid body in the neoblast, we focused on vasa (vas)-related genes, since VAS and VAS-related proteins are known to be components of the germline granules in Drosophila and Caenorhabditis elegans. By PCR, two vas-related genes (Dugesia japonica vasa-like gene, DjvlgA and DjvlgB) were isolated, and they were shown to be expressed in germ cells. Interestingly, DjvlgA was also expressed in a number of somatic cells in the mesenchymal space. In regenerating planarians, accumulation of DjvlgA-expressing cells was observed in both the blastema and the blastema-proximal region. In X-ray-irradiated planarians, which had lost regenerative capacity, the number of DjvlgA-expressing cells decreased drastically. These results suggest that the product of DjvlgA may be a component of the chromatoid body and may be involved in the totipotency of the neoblast.

Shigematsu, Y., N. Yoshida, et al. (2007). "Novel embryonic stem cells expressing tdKaede protein photoconvertible from green to red fluorescence." Int J Mol Med **20**(4): 439-44.

Kaede protein is a photoconvertible tracer that emits green fluorescence after synthesis, which changes to stable red fluorescence upon irradiation with violet or UV illumination. This color-change characteristic is a very effective means of optically marking living cells of interest. We established novel embryonic stem (ES) cell lines, B6KED-1 and -2, from C57BL/6J transgenic mouse blastocysts ubiquitously expressing tandem dimeric Kaede (tdKaede) protein. Undifferentiated B6KED-1 and -2 cells showed bright green fluorescence and mRNAs of pluripotent marker genes. Photoconversion of tdKaede protein in undifferentiated and differentiated B6KED cells in vitro occurred upon short-term UV irradiation. B6KED cells completely generated ES cell-derived females on transfer into tetraploid blastomeres. All organs showed strong green emission in the females derived completely from B6KED cells. These novel ES cell lines ubiquitously expressing photoconvertible

Kaede protein, B6KED-1 and -2, are useful for basic research in developmental biology and regenerative medicine.

Shih, C. C., D. DiGiusto, et al. (2002). "Hematopoietic potential of neural stem cells: plasticity versus heterogeneity." Leuk Lymphoma **43**(12): 2263-8.

Organ-specific stem cells have been identified in a variety of mammalian tissues. These cells hold great promise for cellular therapy if they can reliably produce functional progeny of specific lineages. A central dogma in development has been that organ-specific stem cells are restricted to making the differentiated cell types of the tissue from which they are isolated. However, a substantial body of evidence exists that stem-cell populations from neural and hematopoietic tissues can generate the other cell types, suggesting that adult organ-specific stem cells may have a broader differentiation potential than originally thought. It remains unclear whether this apparent stem cell plasticity is attributable to transdifferentiation of tissue specific stem cells, the co-existence of multiple stem cells with different potentials, or resident totipotent stem cells in these tissues. Recent evidence, in fact, indicates that there may be a fourth explanation for the "apparent" plasticity of stem cells: cell fusion. Here, the authors critically examine the existing data to assess the extent of phenotypic conversion of bone marrow-to-brain and brain-to-blood and discuss some of the contentious issues surrounding these studies. We conclude that there is strong evidence for a multipotent neurohematopoietic stem-cell population in human and mouse brain, although further characterization of these cells will be required if the goal of engineering tissues for therapeutic applications is to be realized.

Shiroi, A., M. Yoshikawa, et al. (2002). "Identification of insulin-producing cells derived from embryonic stem cells by zinc-chelating dithizone." Stem Cells **20**(4): 284-92.

BACKGROUND AND AIMS: Embryonic stem (ES) cells have a pluripotent ability to differentiate into a variety of cell lineages in vitro. We have recently identified the emergence of cellular clusters within differentiated ES cell cultures by staining with dithizone (DTZ). DTZ is a zinc-chelating agent known to selectively stain pancreatic beta cells because of their high zinc content. The aim of the present study was to investigate the characteristics of DTZ-stained cellular clusters originating from ES cells. METHODS: Embryoid bodies (EBs), formed by a 5-day hanging drop culture of ES cells, were allowed to form outgrowths in the culture. The outgrowths were incubated in DTZ solution (final concentration, 100 microg/ml) for 15 minutes before being examined microscopically. The gene expression of endocrine pancreatic markers was also analyzed by reverse transcriptase-polymerase chain reaction. In addition, insulin production was examined immunohistochemically, and its secretion was examined using enzyme-linked immunosorbent assay. RESULTS: DTZ-stained cellular clusters appeared after approximately 16 days in the EB culture and became more apparent by day 23. They were found to be immunoreactive to insulin and expressed pancreatic-duodenal homeobox 1 (PDX1), proinsulin 1, proinsulin 2, glucagon, pancreatic polypeptide, glucose transporter-2 (GLUT2), and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) mRNA. They were also able to secrete detectable amounts of insulin. CONCLUSIONS: ES cell-derived DTZ-positive cellular clusters possess characteristics of the endocrine pancreas, including insulin secretion. Further, DTZ staining is a useful method for the identification of differentiated pancreatic islets developed from EBs in vitro.

Shostak, S. (2006). "(Re)defining stem cells." Bioessays **28**(3): 301-8.

Stem-cell nomenclature is in a muddle! So-called stem cells may be self-renewing or emergent, oligopotent (uni- and multipotent) or pluri- and totipotent, cells with perpetual embryonic features or cells that have changed irreversibly. Ambiguity probably seeped into stem cells from common usage, flukes in biology's history beginning with Weismann's divide between germ and soma and Haeckel's biogenic law and ending with contemporary issues over the therapeutic efficacy of adult versus embryonic cells. Confusion centers on tissue dynamics, whether stem cells are properly members of emerging or steady-state populations. Clarity might yet be achieved by codifying differences between cells in emergent populations, including embryonic stem and embryonic germ (ES and EG) cells in tissue culture as opposed to self-renewing (SR) cells in steady-state populations.

Sillaber, C., S. Walchshofer, et al. (1999). "Immunophenotypic characterization of human bone marrow endosteal cells." Tissue Antigens **53**(6): 559-68.

In order to determine the relationship between bone marrow (bm) endosteal cells (EDC) and hemopoietic progenitors, we have analyzed the immunophenotype of EDC using various antibodies (Ab) against mesenchymal antigens. The Ab were applied on paraffin sections of normal bm (iliac crest, n=17; talus, n=1; phalanx, n=1), myeloregenerative

bm (after chemotherapy), and hematologic disorders (acute myeloid leukemia (AML), n=8; chronic myeloid leukemia (CML), n=6; myelodysplastic syndromes (MDS), n=14; severe aplastic anemia (SAA), n=4; essential thrombocythemia (ET), n=2; idiopathic (primary) osteomyelo-fibrosis (IMF), n=1; polycythemia vera (PV), n=1). In normal bm, EDC were found to react with Ab against vimentin, tenascin, alpha-smooth muscle actin, osteocalcin, CD51, and CD56, but did not react with Ab against CD3, CD15, CD20, CD34, CD45, CD68, or CD117. An identical phenotype of EDC was found in AML, MDS, SAA, ET, IMF, PV, myeloregenerative bm, and peripheral bones lacking active hemopoiesis (talus, phalanx). In patients with CML, EDC reacted with Ab to CD51, but did not react with Ab to CD56. Based on their unique antigen profile, EDC were enriched from normal bm by enzyme digestion and cell sorting. However, these enriched cells (CD56+, CD45-, CD34-) did not give rise to hemopoietic cells under the culture conditions used, i.e. in the presence of the growth factors IGF-1, bFGF, SCF, IL-3, and GM-CSF Together, our data do not support the hypothesis that EDC are totipotent mesenchymal progenitors giving rise to hemopoietic cells.

Silva, J., W. Mak, et al. (2003). "Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes." Dev Cell **4**(4): 481-95.

Previous studies have implicated the Eed-Enx1 Polycomb group complex in the maintenance of imprinted X inactivation in the trophectoderm lineage in mouse. Here we show that recruitment of Eed-Enx1 to the inactive X chromosome (Xi) also occurs in random X inactivation in the embryo proper. Localization of Eed-Enx1 complexes to Xi occurs very early, at the onset of Xist expression, but then disappears as differentiation and development progress. This transient localization correlates with the presence of high levels of the complex in totipotent cells and during early differentiation stages. Functional analysis demonstrates that Eed-Enx1 is required to establish methylation of histone H3 at lysine 9 and/or lysine 27 on Xi and that this, in turn, is required to stabilize the Xi chromatin structure.

Sims, M. and N. L. First (1994). "Production of calves by transfer of nuclei from cultured inner cell mass cells." Proc Natl Acad Sci U S A **91**(13): 6143-7.

We report here the isolation and in vitro culture of bovine inner cell mass (ICM) cells and the use of ICM cells in nuclear transfer to produce totipotent blastocysts that resulted in calves born. Of 15 cell lines represented in this study, 13 were derived from immunosurgically isolated ICM of 3 in vitro produced day 9-10 bovine blastocysts, while 2 lines were derived from single blastocysts. Approximately 70% of attempted cell lines became established cell lines when started from 3 ICMs. The ability to establish cell lines was dependent on the number of ICMs starting the line. Sire differences were noted in the ability of ICMs to establish cell lines and to form blastocysts. The cell lines were cultured as a low cell density suspension in the medium CR1aa plus selenium, insulin, and transferrin (SIT) and 5% fetal calf serum (FCS) for 6-101 days before use in nuclear transfer, at which time some had multiplied to more than 2000 cells. If allowed to aggregate, cells of established cell lines formed embryoid bodies. A total of 659 nuclear transfer clones were made by fusing the ES cells into enucleated oocytes with polyethylene glycol; 460 of these fused, based on cleavage (70%). After culture of the clones for 7 days in vitro in CR1aa/SIT/5% FCS, 109 (24%) of those fused became blastocysts. Thirty-four blastocysts were transferred into uteri of 27 cows, and 13 cows (49%) became pregnant. Four of the 13 cows gave birth to 4 normal calves. DNA typing showed the calves to be derived from the respective sires of the cell lines. The calves were derived from cultures of less than 28 days.

Sinha, S., M. H. Hoofnagle, et al. (2004). "Transforming growth factor-beta1 signaling contributes to development of smooth muscle cells from embryonic stem cells." Am J Physiol Cell Physiol **287**(6): C1560-8.

Knockout of transforming growth factor (TGF)-beta1 or components of its signaling pathway leads to embryonic death in mice due to impaired yolk sac vascular development before significant smooth muscle cell (SMC) maturation occurs. Thus the role of TGF-beta1 in SMC development remains unclear. Embryonic stem cell (ESC)-derived embryoid bodies (EBs) recapitulate many of the events of early embryonic development and represent a more physiological context in which to study SMC development than most other in vitro systems. The present studies showed induction of the SMC-selective genes smooth muscle alpha-actin (SMalphaA), SM22alpha, myocardin, smoothelin-B, and smooth muscle myosin heavy chain (SMMHC) within a mouse ESC-EB model system. Significantly, SM2, the SMMHC isoform associated with fully differentiated SMCs, was expressed. Importantly, the results showed that aggregates of SMMHC-expressing cells exhibited visible contractile activity, suggesting that all regulatory pathways essential for development of contractile SMCs were functional in this in vitro model system. Inhibition of endogenous TGF-beta with an adenovirus expressing a soluble truncated TGF-beta type II receptor attenuated the increase in

SMC-selective gene expression in the ESC-EBs, as did an antibody specific for TGF-beta1. Of interest, the results of small interfering (si)RNA experiments provided evidence for differential TGF-beta-Smad signaling for an early vs. late SMC marker gene in that SMalphaA promoter activity was dependent on both Smad2 and Smad3 whereas SMMHC activity was Smad2 dependent. These results are the first to provide direct evidence that TGF-beta1 signaling through Smad2 and Smad3 plays an important role in the development of SMCs from totipotential ESCs.

Smith, C. (1992). "Retroviral vector-mediated gene transfer into hematopoietic cells: prospects and issues." J Hematother **1**(2): 155-66.

Gene therapy is a developing technology that may allow the treatment of a variety of congenital and acquired genetic disorders as well as infectious diseases through the introduction of exogenous genetic material into relevant cellular populations. Currently, the most effective method for gene transfer into cells of the hematopoietic system is with retroviral vectors. Appropriate cellular targets for gene transfer include totipotent hematopoietic stem cells as well as long-lived lineage committed cells such as T lymphocytes. Although retroviral vector-mediated gene transfer into totipotent stem cells and subsequent long-term expression of transduced genetic material in stem cell progeny has been observed in murine bone marrow transplantation experiments, similar observations have not been made in clinically relevant large-animal models. A number of recent advances in gene delivery systems, purification of stem cells, defining extramedullary sources of stem cells, characterizing the biologic processes that regulate the proliferation and developmental potential of stem cells, and construction of more effective models for assessing stem cells, may result in improvements in gene transfer into large animal and human totipotent stem cells.

Snodgrass, H. R., R. M. Schmitt, et al. (1992). "Embryonic stem cells and in vitro hematopoiesis." J Cell Biochem **49**(3): 225-30.

To study hematopoietic differentiation a variety of in vitro systems have been established using hematopoietic precursors derived from various explanted adult and fetal tissues. In this prospective we describe and discuss the potential of a novel system for studying the earliest stages of hematopoietic development. In addition, some of the applications of this system as a unique in vitro model for studying other developmental systems are discussed. Murine embryonic stem cells (ESC), which are totipotent and can be maintained undifferentiated indefinitely in vitro, have the capacity to differentiate in vitro into hematopoietic precursors of most, if not all, of the colony forming cells found in normal bone marrow. This potential can be exploited to study the control of the early stages of hematopoietic induction and differentiation. Recent results have indicated that there is a strong transcriptional activation, in a well defined temporal order, of many of the hematopoietically relevant genes. Examples of the genes expressed early during the induction of hematopoiesis include erythropoietin (Epo) and its receptor as well as the Steel (SI) factor (SLF) and its receptor (c-kit). Several other genes, including CSF-1, IL-1, and G-CSF were expressed during the later stages of hematopoietic differentiation. Contrasting with these observations, IL-3 and GM-CSF were not expressed during the first 24 days of ES cell differentiation suggesting that neither factor is necessary for the induction of hematopoietic precursors. Although these studies are just beginning, this system is easily manipulated and gives us an approach to understanding the control of the induction and differentiation of the hematopoietic system in ways not previously possible.

Solana, J., P. Lasko, et al. (2009). "Spoltud-1 is a chromatoid body component required for planarian long-term stem cell self-renewal." Dev Biol **328**(2): 410-21.

Freshwater planarians exhibit a striking power of regeneration, based on a population of undifferentiated totipotent stem cells, called neoblasts. These somatic stem cells have several characteristics resembling those of germ line stem cells in other animals, such as the presence of perinuclear RNA granules (chromatoid bodies). We have isolated a Tudor domain-containing gene in the planarian species Schmidtea polychroa, Spoltud-1, and show that it is expressed in neoblast cells, germ line cells and central nervous system, and during embryonic development. Within the neoblasts, Spoltud-1 protein is enriched in chromatoid bodies. Spoltud-1 RNAi eliminates protein expression after 3 weeks, and abolishes the power of regeneration of planarians after 7 weeks. Neoblast cells are eliminated by the RNAi treatment, disappearing at the end rather than gradually during the process. Neoblasts with no detectable Spoltud-1 protein are able to proliferate and differentiate. These results suggest that Spoltud-1 is required for long term stem cell self renewal.

Soshnikova, N. and D. Duboule (2008). "Epigenetic regulation of Hox gene activation: the waltz of methyls." Bioessays **30**(3): 199-202.

Genetic studies have revealed that the antagonistic interplay between PcG and TrxG/MLL complexes is essential for the proper maintenance of

vertebrate Hox gene expression in time and space. Hox genes must be silenced in totipotent embryonic stem cells and, in contrast, rapidly activated during embryogenesis. Here we discuss some recently published articles that propose a novel mechanism for the induction of Hox gene transcription. These studies report a new family of histone demethylases that remove H3K27me3/me2 repressive marks at Hox promoters during differentiation of stem cells. Though the overall importance of these enzymes for proper embryogenesis was demonstrated, their precise role in Hox gene epigenetic regulation during development still remains to be firmly established.

Stanworth, S. J. and A. C. Newland (2001). "Stem cells: progress in research and edging towards the clinical setting." Clin Med **1**(5): 378-82.

Mouse embryonic stem cells have been shown to differentiate into a variety of tissues in vitro and in transplantation experiments can produce many different cell types. Multipotent stem cells in adult humans have also shown a high degree of plasticity: haemopoietic stem cells, for example, have been shown to contribute to several other tissues, such as liver. From these simple observations there has been considerable extrapolation into the use of such putative totipotent stem cells in the clinical setting, with the development of 'designer' tissue engineering, whose aim is to create large tissues or even whole organs for clinical use. In practical terms, however, there are many limitations and difficulties and clinical use has been restricted to a very few settings, eg the use of fetal cells in Parkinson's disease. Nonetheless, there is enormous potential in this area, and also in the application of embryonic or adult stem cells as carriers for gene therapy; but the limitations of such treatment, in particular the stability of manipulated cells, and the problems of ageing and Ooncogenicity, not to mention a host of ethical and regulatory issues, all need to be considered.

Steeg, C. M., J. Ellis, et al. (1990). "Introduction of specific point mutations into RNA polymerase II by gene targeting in mouse embryonic stem cells: evidence for a DNA mismatch repair mechanism." Proc Natl Acad Sci U S A **87**(12): 4680-4.

We have introduced two specific point mutations, located 20 base pairs apart, into the endogenous murine gene that encodes the largest subunit of RNA polymerase II (RPII215). The first mutation conferred resistance to the mushroom toxin alpha-amanitin (amar), and the second mutation generated a restriction fragment length polymorphism without altering the protein sequence. Targeted amar clones were generated at a frequency of 1 in 30 totipotent embryonic stem cells that expressed stably integrated DNA vectors after electroporation. Thirty to 40% of these clones had acquired both mutations, whereas, surprisingly, the remaining clones had acquired the specific amar point mutation but lacked the restriction fragment length polymorphism. We suggest that the latter clones were generated by independent DNA mismatch repair rather than by double crossover or gene conversion. These results demonstrate that it is possible to introduce specific point mutations into an endogenous gene in embryonic stem cells. Thus it should be possible to introduce single base substitutions into other cellular genes, including nonselectable genes, by optimizing the efficiency of gene transfer and/or the sensitivity of screening for targeted clones.

Stevens, L. C. (1980). "Teratocarcinogenesis and spontaneous parthenogenesis in mice." Results Probl Cell Differ **11**: 265-74.

Teratomas are rare in most strains of mice. Testicular teratomas are common in some sublines of inbred strain 129. Ovarian teratomas are common in inbred strain LT. Testicular teratomas are derived from primordial germ cells and can be experimentally produced by grafting 121/2-day genital ridges to the testes of adults. They develop into testes and for some strains most have teratomas. Ovarian teratomas are derived from parthenogenetically activated ovarian oocytes that have completed the first meiotic division. Teratomas of either sex can be experimentally produced by grafting early embryos to various sites in adults. Embryo-derived teratomas originate directly from undifferentiated embryonal cells. Occasionally teratomas are malignant (teratocarcinomas) and can be maintained as transplantable tumors. Some form embryoid bodies that resemble normal early embryos. When the stem cells of some transplantable teratocarcinomas are injected into blastocysts and transferred to the uteri of pseudopregnant females, they participate in normal development and contribute to the formation of all major tissues including functional sperm and eggs. Spontaneous parthenogenesis is common in strain LT oocytes after ovulation. The eggs cleave, form blastocysts which implant in the uterus, but after the egg cylinder stage they become disorganized and are aborted. Eight-cell embryos from the pigmented LT strain were aggregated with embryos of albino strain 129 and transferred to the uteri of pseudopregnant females. They participated in development and contributed to the formation of normal chimeric tissues. Offspring from eggs derived from parthenogenetic embryonal cells were produced, demonstrating that parthenogenetic embryonic cells are totipotent. It is still a mystery why parthenogenetic embryos will not survive in utero.

Stevens, N. R., A. A. Raposo, et al. (2007). "From stem cell to embryo without centrioles." Curr Biol **17**(17): 1498-503.

Centrosome asymmetry plays a key role in ensuring the asymmetric division of Drosophila neural stem cells (neuroblasts [NBs]) and male germline stem cells (GSCs) [1-3]. In both cases, one centrosome is anchored close to a specific cortical region during interphase, thus defining the orientation of the spindle during the ensuing mitosis. To test whether asymmetric centrosome behavior is a general feature of stem cells, we have studied female GSCs, which divide asymmetrically, producing another GSC and a cystoblast. The cystoblast then divides and matures into an oocyte, a process in which centrosomes exhibit a series of complex behaviors proposed to play a crucial role in oogenesis [4-6]. We show that the interphase centrosome does not define spindle orientation in female GSCs and that DSas-4 mutant GSCs [7], lacking centrioles and centrosomes, invariably divide asymmetrically to produce cystoblasts that proceed normally through oogenesis-remarkably, oocyte specification, microtubule organization, and mRNA localization are all unperturbed. Mature oocytes can be fertilized, but embryos that cannot support centriole replication arrest very early in development. Thus, centrosomes are dispensable for oogenesis but essential for early embryogenesis. These results reveal that asymmetric centrosome behavior is not an essential feature of stem cell divisions.

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.

Stewart, C. L., I. Gadi, et al. (1994). "Stem cells from primordial germ cells can reenter the germ line." Dev Biol **161**(2): 626-8.

Embryonic stem (ES) cells are totipotent cells derived from cultured preimplantation blastocysts. When injected into embryos, they can give rise to all somatic lineages as well as functional gametes. Embryonal carcinoma (EC) cells are pluripotent cells, derived from teratocarcinomas, which contribute to somatic lineages, but only rarely to the germ line. A novel source of pluripotent cells, remarkably similar to both ES and EC cells, has been identified. These are EG cells, derived by culturing primordial germ cells isolated from postimplantation embryos, but it is not known whether they resemble ES or EC cells in their ability to contribute to the germ line. Here we show that EG cells of both sexes can form functional gametes. The derivation of such cell lines offers a new route to deriving totipotent cells and also provides insights into the timing of X-chromosome inactivation/activation and genomic imprinting in the germ line.

Stoltz, J. F., D. Bensoussan, et al. (2006). "Cell and tissue engineering and clinical applications: an overview." Biomed Mater Eng **16**(4 Suppl): S3-S18.

Most human tissues do not regenerate spontaneously; this is why cell therapies and tissue engineering are promising alternatives. The principle is simple: cells are collected in a patient and introduced in the damaged tissue or in a tridimentional porous support and harvested in a bioreactor in which the physico-chemical and mechanical parameters are controlled. Once the tissues (or the cells) are mature they may be implanted. In parallel, the development of biotherapies with stem cells is a field of research in turmoil given the hopes for clinical applications that it brings up. Embryonic stem cells are potentially more interesting since they are totipotent, but they can only be obtained at the very early stages of the embryo. The potential of adult stem cells is limited but isolating them induces no ethical problem and it has been known for more than 40 years that bone marrow does possess the regenerating functions of blood cells. Finally, the properties of foetal stem cells (blood cells from the umbilical cord) are forerunners of the haematopoietic system but the ability of these cells to

participate to the formation of other tissues is more problematic. Another field for therapeutic research is that of dendritic cells, antigen presenting cells. Their efficiency in cell therapy relies on the initiation of specific immune responses. They represent a promising tool in the development of a protective immune response against antigens which the host is usually unable to generate an efficient response (melanomas, breast against cancer, prostate cancer, ..). Finally, gene therapy, has been nourishing high hopes but few clinical applications can be envisaged in the short term, although potential applications are multiple (haemophilia, myopathies, ..). A large number of clinical areas stand as candidates for clinical applications: leukaemia and cancers, cardiac insufficiency and vascular diseases, cartilage and bone repair, ligaments and tendons, liver diseases, ophthalmology, diabetes, neurological diseases (Parkinson, Huntington disease, ..), .. Various aspects of this new regenerative therapeutic medicine are developed in this work.

Stout, C. L., D. W. Ashley, et al. (2007). "Primitive stem cells residing in the skeletal muscle of adult pigs are mobilized into the peripheral blood after trauma." Am Surg **73**(11): 1106-10.

This study was designed to determine if trauma causes the release of adult-derived blastomere-like stem cells (BLSCs) from skeletal muscle into the circulating blood of adult pigs. Experimental procedures followed the guidelines of Fort Valley State University's Institutional Animal Care and Utilization Committee. Pigs were traumatized by splenectomy followed by pancreatectomy. Blood samples and skeletal muscle biopsies were taken before and after trauma. Adult-derived BLSCs were isolated from skeletal muscle and blood samples following established procedures. Nontraumatized skeletal muscle contained approximately 277 million BLSCs per gram of muscle. After trauma, skeletal muscle contained approximately 2 million BLSCs per gram of muscle. Blood taken before trauma contained approximately 22 million BLSCs per milliliter, whereas approximately 512 million BLSCs per milliliter were present within the blood after trauma. Blood values were statistically significant with a $P < 0.05$. This report is the first demonstration that trauma causes the release of adult-derived BLSCs from skeletal muscle into blood. Further studies are required to elucidate the roles that adult-derived BLSCs play in the response to injury and in the healing process. Surgeons must take a role in this evolving field.

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.

Sun, B. W., A. C. Yang, et al. (2006). "Temporal and parental-specific expression of imprinted genes in a newly derived Chinese human embryonic stem cell line and embryoid bodies." Hum Mol Genet **15**(1): 65-75.

Although the study of imprinted genes in human development is very important, little is known about their expression and regulation in the early differentiation of human tissues due to lack of an appropriate model. In this study, a Chinese human embryonic stem (hES) cell line, SHhES1, was derived and fully characterized. Expression profiles of human imprinted genes were determined by Affymetrix Oligo micro-array in undifferentiated SHhES1 cells and SHhES1-derived embryoid bodies (EBs) at day 3, 8, 13 and 18. Thirty-two known human imprinted genes were detected in undifferentiated ES cells. Significantly, differential expression was found in nine genes at different stages of EB formation. Expression profile changes were confirmed by quantitative real-time reverse transcriptase-polymerase chain reaction in SHhES1 cells as well as in another independently derived hES cell line, HUES-7. In addition, the monoallelic expressions of four imprinted genes were examined in three different passages of undifferentiated ES cells and EBs of both hES cell lines. The monoallelic expressions of imprinted genes, H19, PEG10, NDNL1 and KCNQ1 were maintained in both undifferentiated hES cells

and derived EBs. More importantly, with the availability of maternal peripheral blood lymphocyte sample, we demonstrated that the maternal expression of KCNQ1 and the paternal expression of NDNL1 and PEG10 were maintained in SHhES1 cells. These data provide the first demonstration that the parental-specific expression of imprinted genes is stable in EBs after extensive differentiation, also indicating that in vitro fertilization protocol does not disrupt the parental monoallelic expression of the imprinted genes examined.

Sun, Y., W. Kong, et al. (2009). "CD133 (Prominin) negative human neural stem cells are clonogenic and tripotent." PLoS One **4**(5): e5498.

BACKGROUND: CD133 (Prominin) is widely used as a marker for the identification and isolation of neural precursor cells from normal brain or tumor tissue. However, the assumption that CD133 is expressed constitutively in neural precursor cells has not been examined. METHODOLOGY/PRINCIPAL FINDINGS: In this study, we demonstrate that CD133 and a second marker CD15 are expressed heterogeneously in uniformly undifferentiated human neural stem (NS) cell cultures. After fractionation by flow cytometry, clonogenic tripotent cells are found in populations negative or positive for either marker. We further show that CD133 is down-regulated at the mRNA level in cells lacking CD133 immunoreactivity. Cell cycle profiling reveals that CD133 negative cells largely reside in G1/G0, while CD133 positive cells are predominantly in S, G2, or M phase. A similar pattern is apparent in mouse NS cell lines. Compared to mouse NS cells, however, human NS cell cultures harbour an increased proportion of CD133 negative cells and display a longer doubling time. This may in part reflect a sub-population of slow- or non-cycling cells amongst human NS cells because we find that around 5% of cells do not take up BrdU over a 14-day labelling period. Non-proliferating NS cells remain undifferentiated and at least some of them are capable of re-entry into the cell cycle and subsequent continuous expansion. CONCLUSIONS: The finding that a significant fraction of clonogenic neural stem cells lack the established markers CD133 and CD15, and that some of these cells may be dormant or slow-cycling, has implications for approaches to identify and isolate neural stem cells and brain cancer stem cells. Our data also suggest the possibility that CD133 may be specifically down-regulated during G0/G1, and this should be considered when this marker is used to identify and isolate other tissue and cancer stem cells.

Suwinska, A., R. Czolowska, et al. (2008). "Blastomeres of the mouse embryo lose totipotency after the fifth cleavage division: expression of Cdx2 and Oct4 and developmental potential of inner and outer blastomeres of 16- and 32-cell embryos." Dev Biol **322**(1): 133-44.

Sixteen inner or outer blastomeres from 16-cell embryos and 32 inner or outer blastomeres from 32-cell embryos (nascent blastocysts) were reaggregated and cultured in vitro. In 24 h old blastocysts developed from blastomeres derived from 16-cell embryos the expression of Cdx2 protein was upregulated in outer cells (new trophectoderm) of the inner cells-derived aggregates and downregulated in inner cells (new inner cell mass) of the external cells-derived aggregates. After transfer to pseudopregnant recipients blastocysts originating from both inner and outer blastomeres of 16-cell embryo developed into normal, fertile mice, but the implantation rate of embryos formed from inner cell aggregates was lower. The aggregates of external blastomeres derived from 32 cell embryo usually formed trophoblastic vesicles accompanied by vacuolated cells. In contrast, the aggregates of inner blastomeres quickly compacted but cavitation was delayed. Although in the latter embryos the Cdx2 protein appeared in the new trophectoderm within 24 h of in vitro culture, these embryos formed only very small outgrowths of Troma1-positive giant trophoblastic cells and none of these embryos was able to implant in recipient females. In separate experiment we have produced normal and fertile mice from 16- and 32-cell embryos that were first disaggregated, and then the sister outer and inner blastomeres were reaggregated at random. In blastocysts developed from aggregates, within 24 h of in vitro culture, the majority of inner and outer blastomeres located themselves in their original position (internally and externally), which implies that in these embryos development was regulated mainly by cell sorting.

Szilvassy, S. J., R. K. Humphries, et al. (1990). "Quantitative assay for totipotent reconstituting hematopoietic stem cells by a competitive repopulation strategy." Proc Natl Acad Sci U S A **87**(22): 8736-40.

Although hematopoiesis is known to originate in a population of very primitive cells with both lymphopoietic and myelopoietic potential, a procedure for enumerating such cells has to date not been available. We now describe a quantitative assay for long-term repopulating stem cells with the potential for reconstituting all hematopoietic lineages. This assay has two key features. The first is the use of competitive repopulation conditions that ensure not only the detection of a very primitive class of

hematopoietic stem cells but also the survival of lethally irradiated mice transplanted with very low numbers of such cells. The second is the use of a limiting-dilution experimental design to allow stem cell quantitation. The assay involves transplanting limiting numbers of male "test" cells into lethally irradiated syngeneic female recipients together with 1-2 x 10(5) syngeneic female marrow cells whose long-term repopulating ability has been compromised by two previous cycles of marrow transplantation. The proportion of assay recipients whose regenerated hematopoietic tissues are determined to contain greater than or equal to 5% cells of test cell origin (male) greater than or equal to 5 weeks later is then used to calculate the frequency of competitive repopulating units (CRU) in the original male test cell suspension (based on Poisson statistics). Investigation of this assay system has shown that all three potential sources of stem cells (test cells, compromised cells, and the host) can under appropriate circumstances contribute to long-term hematopoietic regeneration, thus establishing both the competitive pressure of hematopoietic stem cells in the cotransplanted compromised population and in the host, and the need to use genetic markers to track the specific contribution of the injected test cells. Analysis of the frequency of CRU in test marrow suspensions that varied widely in their CRU content gave similar values when endpoints of either 5 or 10 weeks posttransplantation were used and when either recipient marrow or thymus was used to identify progeny populations. In addition, repopulation of marrow and thymus was found to be associated in most mice injected with limiting numbers of test cells. These findings are consistent with the conclusion that the assay is highly selective for a very primitive, totipotent, reconstituting hematopoietic stem cell and should therefore be particularly useful in future gene therapy-oriented research as well as for more basic studies of hematopoietic stem cell regulation and differentiation.

Talbot, N. C., T. J. Caperna, et al. (2002). "The PICM-19 cell line as an in vitro model of liver bile ductules: effects of cAMP inducers, biopeptides and pH." Cells Tissues Organs **171**(2-3): 99-116.

The PICM-19 fetal liver cell line was isolated from the primary culture and spontaneous differentiation of pig epiblast cells, i.e. embryonic stem cells. PICM-19 cells were induced to differentiate into mostly ductular formations by culturing at pH 7.6-7.8. The ductules were functionally assayed by treatment with cAMP inducing agents and bioactive peptides reported to influence the secretory activity of liver bile ductules. The secretory response of the cells was assessed by qualitative or quantitative measurement of the cross-sectional area of the ductal lumens and the appearance of biliary canaliculi in between PICM-19 cells that had formed monolayers instead of ducts. Forskolin (10 microM) and 8-bromoadenosine 3':5'-cyclic monophosphate (bcAMP; 2 mM) stimulated fluid transport and expansion of ductal structures in 15-20 min and stimulated the appearance and expansion of biliary canaliculi in 30-60 min. Cholera toxin (50 ng/ml) stimulates fluid transport in both ductules and canaliculi in 1-2 h, while 8-bromoguanosine 3':5'-cyclic monophosphate (bcGMP; 2 mM) stimulated only biliary canaliculi in 2 h. Glucagon (1.4 nM) produced a similar response in 5-10 min in ductal structures only, but the response was transitory and was almost completely reversed within 30 min. Secretin (100 pM) and vasoactive intestinal peptide (75 pM) produced a sustained response with maximal ductal lumen expansion occurring in 5-10 min and neither had an immediate effect on canaliculi. Somatostatin (0.5 microM) and gastrin (1 microM) caused marked reduction or disappearance of ductal lumens in 30-60 min, but was ineffective in reversing secretin (100 nM)-induced duct distension. Application of the adrenergic agonists, epinephrine, isoproterenol, and phenylephrine (100 microM), resulted in the complete shrinkage of ductal lumens in 20-30 min. A shift to pH 7.0-7.2 resulted in almost complete reduction of ductal lumens, while a shift to pH 7.8-8.0 resulted in expansion, although not full expansion, of the ductal lumens. PICM-19 bile duct cultures were positive for cytokeratin-7, aquaporin-1 and aquaporin-9 by Western blot analysis. The amounts of these proteins increased in the cultures as differentiation proceeded over time. Transmission electron microscopy revealed that the ductal structures were usually sandwiched between SIM mouse, thioguanine- and ouabain-resistant (STO) feeder cells that had produced a collagen matrix. Also, the ductular PICM-19 cells possessed cilia, probably occurring as a single cilium in each cell, that projected into the lumens of the ducts. The results indicated that the in vitro-produced ductal structures of the PICM-19 cell line are a functional model for biliary epithelium.

Tkemaladze, J. V. and K. N. Chichinadze (2005). "Centriolar mechanisms of differentiation and replicative aging of higher animal cells." Biochemistry (Mosc) **70**(11): 1288-303.

The centrosome (centriole) and the cytoskeleton produced by it are structures, which probably determine differentiation, morphogenesis, and switching on the mechanism of replicative aging in all somatic cells of multicellular animals. The mechanism of such programming of the events seems to include cytoskeleton influences and small RNAs

related to the centrosome. 1) If these functions are really related with centrioles, the multicellular organism's cells which: a) initially lack centrioles (e.g., higher plant cells and also zygote and early blastomeres of some animals) or cytoskeleton (e.g., embryonic stem cells); or b) generate centrioles de novo (e.g., zygote and early blastomeres of some animals), will be totipotent and lack replicative aging. Consequently, the absence (constant or temporary) of the structure determining the counting of divisions also means the absence of counting of differentiation processes. 2) Although a particular damage to centrioles or cytoskeleton (e.g., in tumor cells) fails to make the cells totipotent (because the morphogenetic status of these cells, as differentiated from that of totipotent ones, is not zero), but such a transformation can suppress the initiation of the aging mechanism induced by these structures and, thus, make such cells replicatively "immortal".

Torres-Padilla, M. E. (2008). "Cell identity in the preimplantation mammalian embryo: an epigenetic perspective from the mouse." Hum Reprod **23**(6): 1246-52.

The early preimplantation mouse embryo is a unique system where it is possible to explore the foundations of totipotency and differentiation. Following fertilization, a single cell, the zygote, will give rise to all tissues of the organism. The first signs of differentiation in the embryo are evident at the blastocyst stage with the formation of the trophectoderm, a differentiated tissue that envelopes the inner cell mass. The question of when and how the cells start to be different from each other in the embryo is central to developmental biology: as cell fate decisions are undertaken, loss of totipotency comes about. Although the blastomeres of the preimplantation embryo are totipotent, as the embryo develops some differences appear to develop between them which are, at least partially, related to the epigenetic information of each of these cells. The hypothesis of epigenetic asymmetries acting as driver for lineage allocation is presented. Although there are now some indications that epigenetic mechanisms are involved in cell fate determination, much work is needed to discover how such mechanisms are set in play upon fertilization and how they are transmitted through cell division. These considerations are further discussed in the context of preimplantation genetic diagnosis: does it matter to the embryo which cell is used for genetic diagnosis? The exquisite complexity and richness of chromatin-regulated events in the early embryo will certainly be the subject of exciting research in the future.

Towns, C. R. and D. G. Jones (2004). "Stem cells, embryos, and the environment: a context for both science and ethics." J Med Ethics **30**(4): 410-3.

Debate on the potential and uses of human stem cells tends to be conducted by two constituencies-ethicists and scientists. On many occasions there is little communication between the two, with the result that ethical debate is not informed as well as it might be by scientific insights. The aim of this paper is to highlight those scientific insights that may be of relevance for ethical debate. Environmental factors play a significant role in identifying stem cells and their various subtypes. Research related to the role of the microenvironment has led to emphasis upon "plasticity", which denotes the ability of one type of stem cell to undergo a transition to cells from other lineages. This could increase the value given to adult stem cells, in comparison with embryonic stem cell research. Any such conclusion should be treated with caution, however, since optimism of this order is not borne out by current research. The role of the environment is also important in distinguishing between the terms totipotency and pluripotency. We argue that blastocysts (early embryos) and embryonic stem cells are only totipotent if they can develop within an appropriate environment. In the absence of this, they are merely pluripotent. Hence, blastocysts in the laboratory are potentially totipotent, in contrast to their counterparts within the human body which are actually totipotent. This may have implications for ethical debate, suggesting as it does that arguments based on potential for life may be of limited relevance.

Toyooka, Y., D. Shimosato, et al. (2008). "Identification and characterization of subpopulations in undifferentiated ES cell culture." Development **135**(5): 909-18.

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass (ICM) and the epiblast, and have been suggested to be a homogeneous population with characteristics intermediate between them. These cells express Oct3/4 and Rex1 genes, which have been used as markers to indicate the undifferentiated state of ES cells. Whereas Oct3/4 is expressed in totipotent and pluripotent cells in the mouse life cycle, Rex1 expression is restricted to the ICM, and is downregulated in pluripotent cell populations in the later stages, i.e. the epiblast and primitive ectoderm (PrE). To address whether ES cells comprise a homogeneous population equivalent to a certain developmental stage of pluripotent cells or a heterogeneous population composed of cells corresponding to various stages of differentiation, we established knock-in ES cell lines in which genes for fluorescent proteins were inserted into the Rex1 and

Oct3/4 gene loci to visualize the expression of these genes. We found that undifferentiated ES cells included at least two different populations, Rex1(+)/Oct3/4(+) cells and Rex1(-)/Oct3/4(+) cells. The Rex1(-)/Oct3/4(+) and Rex1(+)/Oct3/4(+) populations could convert into each other in the presence of LIF. In accordance with our assumption that Rex1(+)/Oct3/4(+) cells and Rex1(-)/Oct3/4(+) cells have characteristics similar to those of ICM and early-PrE cells, Rex1(+)/Oct3/4(+) cells predominantly differentiated into primitive ectoderm and contributed to chimera formation, whereas Rex1(-)/Oct3/4(+) cells differentiated into cells of the somatic lineage more efficiently than non-fractionated ES cells in vitro and showed poor ability to contribute to chimera formation. These results confirmed that undifferentiated ES cell culture contains subpopulations corresponding to ICM, epiblast and PrE.

Trentin, A., C. Glavieux-Pardanaud, et al. (2004). "Self-renewal capacity is a widespread property of various types of neural crest precursor cells." Proc Natl Acad Sci U S A **101**(13): 4495-500.

In vertebrates, trunk neural crest (NC) generates glia, neurons, and melanocytes. In addition, it yields mesectodermal derivatives (connective tissues, chondrocytes, and myofibroblasts lining the blood vessels) in the head. Previous in vitro clonal analyses of avian NC cells unraveled a hierarchical succession of highly pluripotent, followed by various intermediate, progenitors, suggesting a model of progressive restrictions in the multiple potentialities of a totipotent stem cell, as prevails in the hematopoietic system. However, which progenitors are able to self-renew within the hierarchy of the NC lineages is still undetermined. Here, we explored further the stem cell properties of quail NC cells by means of in vitro serial subcloning. We identified types of multipotent and oligopotent NC progenitors that differ in their developmental repertoire, ability to self-maintain, and response to exogenous endothelin 3 according to their truncal or cephalic origin. The most striking result is that bipotent progenitors are endowed with self-renewal properties. Thus glia-melanocyte and glia-myofibroblast progenitors behave like stem cells in that they are able both to self-renew and generate a restricted progeny. In our culture conditions, glia-myofibroblast precursors display a modest capacity to self-renew, whereas glia-melanocyte precursors respond to endothelin 3 by extensive self-renewal. These findings may explain the etiology of certain multiphenotypic NC-derived tumors in humans. Moreover, the presence of multiple stem cell phenotypes along the NC-derived lineages may account for the rarity of the "totipotent NC stem cell" and may be related to the large variety and widespread dispersion of NC derivatives throughout the body.

Triffitt, J. T. (2002). "Stem cells and the philosopher's stone." J Cell Biochem Suppl **38**: 13-9.

Stem cell biology is now one of the most exciting and rapidly advancing areas of scientific endeavor. Promises of cures of a wide variety of diseases by specific replacement of damaged or malfunctional tissues by use of totipotent or multipotent stem cells is on the horizon in clinical practice. Stem cells derived from the embryo and from adult tissues have been shown to have extensive potentials for self-renewal and differentiation. In addition, the plasticities of phenotype exhibited in vivo by some of these cell populations challenge the doctrine of irreversibility of cell commitment after particular developmental stages. This brief review considers certain aspects of these recent findings of the many unexpected potentials of stem cells to differentiate into alternative processes, and their potential value for use in tissue reconstruction procedures are prominent areas that require further study. Rigorous investigation of these topics will lead to realistic approaches in the future for stem cell therapy in a variety of human diseases and other clinical problems.

Tripputi, P., B. Cassani, et al. (2001). "Chromosome 7 monosomy and deletions in myeloproliferative diseases." Leuk Res **25**(9): 735-9.

We studied deletion and monosomy of chromosome 7 in 150 patients with myeloproliferative diseases. We found 8/150 patients with monosomy 7 by cytogenetics and 4/150 with deletions of the long arm of chromosome 7 by restriction fragment length polymorphism (RFLP) analysis performed with Southern and polymerase chain reaction. To overcome limitation of RFLP analysis, we restricted loss of heterozygosity study with microsatellites to 45 patients, observing deletion 7q31.1 in 7/45 patients. In all patients with molecular alterations the deletion was observed only in myeloid cells, while the monosomy was detected in both myeloid precursor and lymphocytes. This finding suggests a CD34-totipotent stem cell origin for the monosomy and a colony forming unit - granulocyte, erythrocyte, monocyte, megakaryocytes (CFU-GEMM) stem cell origin for the deletions.

Tronik-Le Roux, D., V. Roullot, et al. (1995). "Suppression of erythro-megakaryocytopoiesis and the induction of reversible thrombocytopenia in mice transgenic for the thymidine kinase gene targeted by the platelet glycoprotein alpha IIb promoter." J Exp Med **181**(6): 2141-51.

The mechanisms that regulate the commitment of a totipotent stem cell to the megakaryocytic lineage are largely unknown. Using a molecular approach to the study of megakaryocytopoiesis and platelet production, mice in which thrombocytopoiesis could be controlled were produced by targeting the expression of the herpes simplex virus thymidine kinase toxigene to megakaryocytes using the regulatory region of the gene encoding the alpha subunit of the platelet integrin alpha IIb beta 3. The programmed eradication of the megakaryocytic lineage was induced by treating transgenic mice bearing the hybrid construct (alpha IIbtk) with the antiherpetic drug ganciclovir (GCV). After 10 d of treatment, the platelet number was reduced by > 94.6%. After discontinuing GCV, the bone marrow was repopulated with megakaryocytes and the platelet count was restored within 7 d. Prolonged GCV treatment induced erythropenia in the transgenic mice. Assays of myeloid progenitor cells in vitro demonstrated that the transgene was expressed in early erythro-megakaryocytic progenitor cells. The reversibility and facility of this system provides a powerful model to determine both the critical events in megakaryocytic and erythroid lineage development and for evaluating the precise role that platelets play in the pathogenesis of a number of vascular occlusive disorders.

Tseng, J. E., S. E. Hall, et al. (1995). "Phenotypic and functional analysis of lymphocytes in paroxysmal nocturnal hemoglobinuria." Am J Hematol **50**(4): 244-53.

The hematologic disorder paroxysmal nocturnal hemoglobinuria (PNH) arises from a somatic mutation within the Piga gene important for the biosynthesis of glycosylphosphatidylinositol (GPI) anchors. The PNH defect has been identified in all cells of the myeloerythroid lineage, but involvement of the lymphoid lineage in PNH is more controversial. We therefore analyzed lymphocytes from 22 patients with PNH to characterize phenotypically the GPI-deficient population, and to investigate the functional consequences of GPI deficiency. GPI-deficient T lymphocytes, B lymphocytes, and NK cells were identified, but at a lower percentage than granulocytes and erythrocytes. CD8+ lymphocytes were significantly more affected than CD4+ T cells, and CD45RA+ lymphocytes were significantly more affected than CD45RO+ cells. Proliferation assays demonstrated that lymphocytes from PNH patients, either unfractionated or purified GPI-deficient cells, responded normally to in vitro stimuli. When stimulated with phytohemagglutinin (PHA), naive CD45RA+ GPI-deficient T lymphocytes acquired the memory CD45RO+ phenotype. In addition, GPI-deficient T lymphocytes had a relative growth advantage as compared to normal T cells. The results demonstrate that PNH involves the lymphoid as well as the myeloerythroid lineage, and therefore arises from a totipotent bone marrow stem cell. The in vitro growth advantage of GPI-deficient lymphocytes in PNH may have important implications for the pathogenesis of some puzzling clinical aspects of PNH, including predominance of the PNH clone, defective hematopoiesis, and leukemogenesis.

Umiel, T., L. M. Nadler, et al. (1987). "Undifferentiated leukemia of infancy with t(11:17) chromosomal rearrangement. Coexpressing myeloid and B cell restricted antigens." Cancer **59**(6): 1143-9.

It has been suggested that the malignant transformation, in some of the acute leukemias, may involve totipotent stem cells resulting in a biphenotypic leukemia expressing both myeloid, and lymphoid characteristics. We describe here a hybrid cell acute leukemia, in a 16-day-old infant, in whom leukemic cells coexpressed myeloid and lymphoid B cell antigens. Blast cells in the bone marrow showed L2 morphology according to the French American British (FAB) classification, with positive periodic-acid Schiff, and nonspecific esterase staining. Sudan black, and specific esterase were negative. Terminal deoxynucleotidyl transferase, was strongly positive in 5% of blasts, and faintly reactive with the rest. Karyotypic analysis demonstrated a translocation of t(11:17);(q23;p13). Immunoglobulin gene analysis revealed rearrangement of the heavy chain genes. The blasts' phenotype was HLA/DR+ B4+ My7+ My9+ common acute lymphoblastic leukemia antigen (CALLA) B1- T11-. Dual immunofluorescence staining using anti My7, and My9 fluorescein isothiocyanate, and anti B4 pycoerythrin conjugated monoclonal antibodies, and flow cytofluorometry, revealed a labeling pattern of 25% B4+; 10% to 15% My7+; 17% My9+; and 50% of cells coexpressing B4 My7, and My9 antigens. These results provide evidence for a hybrid leukemia with lymphomyeloblasts being part of a single clone, which may indicate the origin of this leukemic clone from a pluripotent (lymphoid/myeloid) stem cell.

van de Geijn, G. J., R. Hersmus, et al. (2009). "Recent developments in testicular germ cell tumor research." Birth Defects Res C Embryo Today **87**(1): 96-113.

Testicular germ cell tumors of adolescents and adults (TGCTs; the so-called type II variant) are the most frequent malignancies found in Caucasian males between 20 and 40 years of age. The incidence has increased over the last decades. TGCTs are divided into seminomas and nonseminomas, the latter consisting of the subgroups embryonal carcinoma,

yolk-sac tumor, teratoma, and choriocarcinoma. The pathogenesis starts in utero, involving primordial germ cells/gonocytes that are blocked in their differentiation, and develops via the precursor lesion carcinoma in situ toward invasiveness. TGCTs are totipotent and can be considered as stem cell tumors. The developmental capacity of their cell of origin, the primordial germ cells/gonocyte, is demonstrated by the different tumor histologies of the invasive TGCTs. Seminoma represents the germ cell lineage, and embryonal carcinoma is the undifferentiated component, being the stem cell population of the nonseminomas. Somatic differentiation is seen in the teratomas (all lineages), whereas yolk-sac tumors and choriocarcinoma represent extra-embryonal differentiation. Seminomas are highly sensitive to irradiation and (DNA damaging) chemotherapy, whereas most nonseminomatous elements are less susceptible to radiation, although still sensitive to chemotherapy, with the exception of teratoma. To allow early diagnosis and follow up, appropriate markers are mandatory to discriminate between the different subgroups. In this review, a summary will be given related to several recent developments in TGCT research, especially selected because of their putative clinical impact.

van Inzen, W. G., M. P. Peppelenbosch, et al. (1996). "Neuronal differentiation of embryonic stem cells." Biochim Biophys Acta **1312**(1): 21-6.

Neuronal differentiation from totipotent precursors in vitro, is thought to require two signals: first a biophysical state (cellular aggregation) followed by a biochemical signal (retinoic acid treatment). In investigating the properties of retinoic acid-differentiated embryonic stem cell lines. However, we noted that retinoic acid treatment without prior aggregation, is sufficient to induce expression of the neuronal markers GAP-43 and NF-165. In agreement, immunohistochemistry revealed the presence of GAP-43 positive cells in these embryonic stem cell monolayers after three days of retinoic acid (RA) treatment. Furthermore an NF-165 positive subpopulation of cells was clearly observed after 4-5 days of RA treatment. The expression of these neuronal markers coincided with the appearance of electrically excitable cells, as assayed with whole cell patch clamp recording. We conclude that for neuronal differentiation of totipotent embryonic stem cells in vitro, one biochemical signal, i.e. retinoic acid treatment, is sufficient.

Verdeil, J. L., L. Alemanno, et al. (2007). "Pluripotent versus totipotent plant stem cells: dependence versus autonomy?" Trends Plant Sci **12**(6): 245-52.

Little is known of the mechanisms that induce the dedifferentiation of a single somatic cell into a totipotent embryogenic cell that can either be regenerated or develop into an embryo and subsequently an entire plant. In this Opinion article, we examine the cellular, physiological and molecular similarities and differences between different plant stem cell types. We propose to extend the plant stem cell concept to include single embryogenic cells as a totipotent stem cell based on their capacity to regenerate or develop into an embryo under certain conditions. Our survey suggests that differences in chromatin structure might ensure that meristem-localized stem cells have supervised freedom and are pluripotent, and that embryogenic stem cells are unsupervised, autonomous and, hence, freely totipotent.

Vittet, D., M. H. Prandini, et al. (1996). "Embryonic stem cells differentiate in vitro to endothelial cells through successive maturation steps." Blood **88**(9): 3424-31.

The mechanisms involved in the regulation of vasculogenesis still remain unclear in mammals. Totipotent embryonic stem (ES) cells may represent a suitable in vitro model to study molecular events involved in vascular development. In this study, we followed the expression kinetics of a relatively large set of endothelial-specific markers in ES-derived embryoid bodies (EBs). Results of both reverse transcription-polymerase chain reaction and/or immunofluorescence analysis show that a spontaneous endothelial differentiation occurs during EBs development. ES-derived endothelial cells express a full range of cell lineage-specific markers: platelet endothelial cell adhesion molecule (PECAM), Flk-1, tie-1, tie-2, vascular endothelial (VE) cadherin, MECA-32, and MEC-14.7. Analysis of the kinetics of endothelial marker expression allows the distinction of successive maturation steps. Flk-1 was the first to be detected; its mRNA is apparent from day 3 of differentiation. PECAM and tie-2 mRNAs were found to be expressed only from day 4, whereas VE-cadherin and tie-1 mRNAs cannot be detected before day 5. Immunofluorescence stainings of EBs with antibodies directed against Flk-1, PECAM, VE-cadherin, MECA-32, and MEC-14.7 confirmed that the expression of these antigens occurs at different steps of endothelial cell differentiation. The addition of an angiogenic growth factor mixture including erythropoietin, interleukin-6, fibroblast growth factor 2, and vascular endothelial growth factor in the EB culture medium significantly increased the development of primitive vascular-like structures within EBs. These results indicate that this in vitro system contains a large part of the endothelial cell

differentiation program and constitutes a suitable model to study the molecular mechanisms involved in vasculogenesis.

von Melchner, H., J. V. DeGregori, et al. (1992). "Selective disruption of genes expressed in totipotent embryonal stem cells." Genes Dev **6**(6): 919-27.

Two retrovirus promoter trap vectors (U3His and U3Neo) have been used to disrupt genes expressed in totipotent murine embryonal stem (ES) cells. Selection in L-histidinol or G418 produced clones in which the coding sequences for histidinol-dehydrogenase or neomycin-phosphotransferase were fused to sequences in or near the 5' exons of expressed genes, including one in the developmentally regulated REX-1 gene. Five of seven histidinol-resistant clones and three of three G418-resistant clones generated germ-line chimeras. A total of four disrupted genes have been passed to the germ line, of which two resulted in embryonic lethalities when bred to homozygosity. The ability to screen large numbers of recombinant ES cell clones for significant mutations, both in vitro and in vivo, circumvents genetic limitations imposed by the size and long generation time of mice and will facilitate a functional analysis of the mouse genome.

Vrtovec, K. T. and B. Vrtovec (2007). "Commentary: is totipotency of a human cell a sufficient reason to exclude its patentability under the European law?" Stem Cells **25**(12): 3026-8.

This article argues that totipotent character of human totipotent cells--defined as the capacity of a cell "to differentiate into all somatic lineages (ectoderm, mesoderm, endoderm), the germ line and extra-embryonic tissues such as the placenta"--is not a sufficient reason to exclude their patentability on the basis of Article 5(1) of the Directive 98/44/EC on the Legal Protection of Biotechnological Inventions (Biopatent Directive), which maintains that "the human body, at the various stages of its formation and development, [...] cannot constitute patentable inventions." Since human totipotent cells have both the potential to generate an entire new organism or to generate only different tissues or organs of an organism, they simultaneously fit the definition of the unpatentable human body at the earliest stage of its formation as well as of an element of the human body, which "may constitute a patentable invention" pursuant to Article 5(2) of the Biopatent Directive, whether that element is isolated from the human body or otherwise produced by means of a technical process. Therefore, this article suggests that, when evaluating patentability of human totipotent cells, they should be further evaluated according to their location and their method of derivation (i.e., whether human totipotent cells are located in the human body, whether they are isolated from the human body, or whether they are produced otherwise by means of a technical process). Disclosure of potential conflicts of interest is found at the end of this article.

Wada, H., T. Enomoto, et al. (1998). "Carcinosarcoma of the breast: molecular-biological study for analysis of histogenesis." Hum Pathol **29**(11): 1324-8.

The histogenesis of carcinosarcoma of the breast is controversial. In the current case, the demarcation between the carcinomatous and sarcomatous components was distinct in all microscopic fields. Immunohistochemical analysis was negative for epithelial membrane antigen (EMA) and keratin in the sarcomatous component and was negative for desmin in the carcinomatous component, suggesting that this tumor could be derived from the two different stem cells. To determine the histogenesis of this tumor, both carcinomatous and sarcomatous lesions were microdissected from formalin-fixed tissues and DNAs were prepared by proteinase K digestion. PCR amplification of the human androgen receptor (HUMARA) short tandem repeat (STR), after Hpa II digestion of the genomic DNA, indicated that the patterns of X-chromosome inactivation were identical in both components. Moreover, both components contained the identical TGT --> TTT transversion in codon 275 of the p53 gene. These observations strongly support the hypothesis that this tumor is derived from a single totipotent stem cell.

Wakao, H. (2009). "NKT cells: from totipotency to regenerative medicine." Arch Immunol Ther Exp (Warsz) **57**(2): 117-28.

The recent discovery that natural killer T (NKT) cell nuclei are totipotent opens a novel avenue for further understanding NKT cell function in normal and diseased states. The progeny of a cloned mouse harboring the in-frame rearranged Valpha14-Jalpha18 T cell receptor in one allele showed a significant increase in NKT cell number compared with wild-type or littermate control mice that possessed a different TCR. Importantly, NKT cells from such progeny produced both interferon-gamma and interleukin-4, a hallmark of NKT cells. In these progeny, NKT cell development appeared to be instructively, rather than permissively, determined. Using embryonic stem cells prepared via the somatic cell nuclear transfer of NKT nuclei, relatively mature NKT cells were induced under conditions permissible for T cell induction. Furthermore, these NKT cells matured autonomously upon injection into mice, resulting in an antigen-specific adjuvant effect.

Wakayama, T. (2003). "Cloned mice and embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Oncol Res **13**(6-10): 309-14.

Mice can now be cloned from cultured and noncultured adult-, fetus-, male-, or female-derived cells. Using the mouse as a model, research is moving towards a comprehensive description of clones generated by somatic cell nuclear transfer. In addition, embryonic stem (ES) cell lines can be generated from adult somatic cells via nuclear transfer (ntES cells). ntES cells contribute to an extensive variety of cell types including neurons in vitro and germ cells in vivo. Recent advances in mouse cloning are reported to illustrate its strengths and promise in the study of mammalian biology and biomedicine.

Walther, D. J. and M. Bader (1999). "Serotonin synthesis in murine embryonic stem cells." Brain Res Mol Brain Res **68**(1-2): 55-63.

Serotonin (5-HT) is a monoaminergic neurotransmitter involved in various processes in the mammalian nervous system with tryptophan hydroxylase (TPH) as the rate-limiting enzyme in its biosynthesis. Interestingly, there is accumulating evidence that neurotransmitters including 5-HT are directly involved in cleavage divisions and morphogenetic movements during early embryogenesis, even before neurons appear. Clonal cell models will be indispensable for investigating these pre-neuronal actions of neurotransmitter systems. Totipotent embryonic stem (ES) cells represent early embryonic stages, are amenable to genetic manipulations and can be easily induced to differentiate into cells with neuronal and glial properties enabling the recapacitation of neurulation. In this study, we used high-pressure liquid chromatography with fluorometric detection (HPLC-FD) to demonstrate the presence of 5-HT in ES cells. In addition, RNase protection assays and immunohistochemical methods detected TPH mRNA and protein, respectively, confirming the endogeneous production of 5-HT in these cells. Furthermore, TPH protein was detected in mouse zygotes after fertilization. These results indicate that ES cells may be useful for the investigation of neurotransmitters in pre-nervous embryos and their actions during ontogeny.

Wang, H., S. Wang, et al. (2009). "Oct4 is expressed in Nestin-positive cells as a marker for pancreatic endocrine progenitor." Histochem Cell Biol **131**(5): 553-63.

There are abundant progenitor cells in the developing pancreas, but molecular markers for these cells are lacking. Octamer-binding transcription factor-4 (Oct4) is an important transcription factor for keeping the features of self-renewal and pluripotency of embryonic stem cells. It's well known that Oct4, as a totipotent stem cells marker, just is expressed in totipotent stem cells. In the present study, we collected ten human fetal pancreases, and found that Oct4 mRNA and protein were expressed in human fetal pancreas samples by RT-PCR, western blot and immunohistochemistry assays. Using double-staining, we demonstrated that Oct4 was not co-expressed with Chromogranin A (a peptide expressed in endocrine cells), but partially co-expressed with Ngn3 (a transcription factor expressed in pancreatic endocrine precursor cells) and Nestin (a intermediate filament, Nestin-positive cells isolated from islets can be induced to express insulin) in human fetal pancreases. Indeed, we prepared Nestin-positive cells from human fetal pancreas by cell selection, and found that these cells expressed Oct4 and Ngn3. The Nestin-positive cells displayed a rapid duplication and could differentiate into osteoblasts, fat and endocrine cells in vitro. These results indicated that the Nestin-positive cells in the fetal age should be pancreatic progenitor cells. Overall, our study suggested that Oct4 was a marker for pancreatic endocrine progenitor.

Wang, L. and G. A. Schultz (1996). "Expression of Oct-4 during differentiation of murine F9 cells." Biochem Cell Biol **74**(4): 579-84.

Oct-4 is a transcription factor that shares a common structural motif with members of the POU family. The mRNA for Oct-4 is found in growing oocytes and in totipotent or pluripotent cells of the early mouse embryo. Oct-4 is down-regulated in embryos during differentiation events associated with blastocyst implantation and gastrulation. Oct-4 gene expression is also down-regulated when murine embryonic stem cells or embryonal carcinoma cells are induced to differentiate in the presence of retinoic acid. A polyclonal antibody that can recognize a unique peptide sequence in the C-terminus of mouse Oct-4 has been prepared. It specifically recognizes Oct-4 protein as tested by Western blots and gel mobility shift assays. This antibody has been used to measure Oct-4 protein levels during retinoic acid induced differentiation of F9 embryonal carcinoma cells, It was observed that Oct-4 protein was abundant in undifferentiated F9 cells but decreased to levels below detection as the cells differentiated, consistent with changes in levels of expression in early embryos.

Wang, Q. T., K. Piotrowska, et al. (2004). "A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo." Dev Cell **6**(1): 133-44.

The preimplantation development of the mammalian embryo encompasses a series of critical

events: the transition from oocyte to embryo, the first cell divisions, the establishment of cellular contacts, the first lineage differentiation-all the first subtle steps toward a future body plan. Here, we use microarrays to explore gene activity during preimplantation development. We reveal robust and dynamic patterns of stage-specific gene activity that fall into two major phases, one up to the 2-cell stage (oocyte-to-embryo transition) and one after the 4-cell stage (cellular differentiation). The mouse oocyte and early embryo express components of multiple signaling pathways including those downstream of Wnt, BMP, and Notch, indicating that conserved regulators of cell fate and pattern formation are likely to function at the earliest embryonic stages. Overall, these data provide a detailed temporal profile of gene expression that reveals the richness of signaling processes in early mammalian development.

Ward, C. M. and P. L. Stern (2002). "The human cytomegalovirus immediate-early promoter is transcriptionally active in undifferentiated mouse embryonic stem cells." Stem Cells **20**(5): 472-5.

It has been reported recently that the cytomegalovirus (CMV) immediate-early promoter is transcriptionally inactive in undifferentiated mouse embryonic stem (ES) cells. This result is surprising, since the CMV promoter is used to express transgenes in a variety of cell lines. We studied the expression of a human CMV-driven enhanced green fluorescent protein (EGFP) reporter gene (pEGFP-N1) in five undifferentiated mouse ES cell lines (BL/6III, D3, E14TG2a, MESC20, and 129) and found EGFP expression in all of these cell lines. Under optimal conditions, between 50%-80% transfection efficiencies could be achieved, and EGFP expression levels were maintained for at least 72 hours. Therefore, the human CMV promoter remains a useful system for transgene expression in undifferentiated ES cells.

Wartenberg, M., J. Gunther, et al. (1998). "The embryoid body as a novel in vitro assay system for antiangiogenic agents." Lab Invest **78**(10): 1301-14.

Tumor progression necessitates the induction of blood vessels that converge upon the tumor and enhance the diffusibility of oxygen and nutrients. Approaches to treat cancer by antiangiogenic therapy are therefore straightforward, and there is a great need for suitable in vitro systems to test antiangiogenic agents. In the present study, embryoid bodies (EBs) differentiated from totipotent mouse embryonic stem (ES) cells and cultivated using the spinner flask technique are introduced as an in vitro system for antiangiogenesis research. ES cells effectively differentiated endothelial cells within the three-dimensional tissue of EBs. The total area of capillary-like structures, which were positive for CD31 (platelet endothelial cell adhesion molecule, PECAM-1), was assessed by confocal laser scanning microscopy and image analysis of a series of optical sections. Endothelial differentiation occurred between Day 4-5 and Day 8 of EB development. Within 7 days, 100% of EBs contained capillary-like structures. Suramin, tamoxifen, tetrahydrocortisol, and a combination of tetrahydrocortisol and heparin were tested for their antiangiogenic capacity in the EB system and were found to efficiently inhibit endothelial differentiation. Diffusion studies of a 10-kd 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF)-dextran and the fluorescent, amphiphilic agent doxorubicin in avascular and vascularized EBs revealed that the endothelial structures formed functional vessels that facilitated diffusion. The diffusion coefficient D for doxorubicin was 296 x 10(-9) cm2 s(-1) in vascularized 8-day-old EBs, ie, about 10-fold larger than in avascular 3-day-old EBs (18 x 10(-9) cm2 s(-1)) and EBs treated with suramin (14 x 10(-9) cm2 s(-1)), tamoxifen (13.5 x 10(-9) cm2 s(-1)), and tetrahydrocortisol/heparin (18.5 x 10(-9) cm2 s(-1)). Consequently, avascular EBs treated with antiangiogenic agents developed central necrosis, which was absent in vascularized EBs. Our findings indicate that EBs are a suitable in vitro model system to study the effects of antiangiogenic agents in a three-dimensional tissue context. Furthermore, EBs provide a unique model to investigate the diffusion of anticancer agents in a tissue in both the avascular and vascularized states.

Wei, C. L., T. Miura, et al. (2005). "Transcriptome profiling of human and murine ESCs identifies divergent paths required to maintain the stem cell state." Stem Cells **23**(2): 166-85.

Human embryonic stem cells (hESCs) are an important source of stem cells in regenerative medicine, and much remains unknown about their molecular characteristics. To develop a detailed genomic profile of ESC lines in two different species, we compared transcriptomes of one murine and two different hESC lines by massively parallel signature sequencing (MPSS). Over 2 million signature tags from each line and their differentiating embryoid bodies were sequenced. Major differences and conserved similarities between species identified by MPSS were validated by reverse transcription polymerase chain reaction (RT-PCR) and microarray. The two hESC lines were similar overall, with differences that are attributable to alleles and propagation. Human-mouse comparisons, however, identified only a small (core) set of conserved genes that included genes known to be important in ESC biology, as well as additional novel genes. Identified

were major differences in leukemia inhibitory factor, transforming growth factor-beta, and Wnt and fibroblast growth factor signaling pathways, as well as the expression of genes encoding metabolic, cytoskeletal, and matrix proteins, many of which were verified by RT-PCR or by comparing them with published databases. The study reported here underscores the importance of cross-species comparisons and the versatility and sensitivity of MPSS as a powerful complement to current array technology.

Weisel, K. C., Y. Gao, et al. (2006). "Stromal cell lines from the aorta-gonado-mesonephros region are potent supporters of murine and human hematopoiesis." Exp Hematol **34**(11): 1505-16.

OBJECTIVE: The hematopoietic system is nurtured by a supportive stroma environment allowing maintenance and differentiation of hematopoietic stem cells (HSC). However, only a limited number of these stromal cell clones support hematopoiesis in the absence of cytokine supplementation. So far, only two bone marrow-derived stromal cell lines (OP9 and S17) are capable of inducing hematopoietic differentiation of totipotent murine and human embryonic stem cells (ESC). Here, the potential of more than 100 stromal cell lines developed from the aorta-gonado-mesonephros (AGM) region was investigated in supporting adult and embryonic hematopoiesis. In addition, extensive phenotypic analysis should elucidate possible mechanisms involved in maintenance of hematopoietic stem cell function. METHODS: More than 100 stromal cell clones derived from the AGM region of E10.5 mouse embryos were isolated. Hematopoietic stem cell support was tested for adult murine and human cord blood hematopoietic stem cells and hematopoietic cells derived from murine ESC. Genotypic and phenotypic characterization was performed including gene array analysis. RESULTS: It was demonstrated that multiple clones showed high efficiency in supporting maintenance and expansion of primitive murine and human hematopoietic progenitors. In addition, we demonstrated for the first time that AGM stromal cell lines are also potent inducers of hematopoietic differentiation of murine ESC. Microarray analysis of AGM lines revealed a characteristic genotype with expression of genes involved in regulating hematopoiesis as well as mesodermal and early B cell development. CONCLUSION: These AGM stromal cell lines may be of value in elucidating molecular mechanisms regulating early stem cell development and hematopoietic differentiation from ES-derived mesoderm.

Wilmut, I. and L. Paterson (2003). "Somatic cell nuclear transfer." Oncol Res **13**(6-10): 303-7.

Embryos produced by nuclear transfer from a patient's somatic cell offer one potential source of embryonic stem cells for treatment of human degenerative diseases. As with all of the approaches to such therapy, this has both strengths and weaknesses. The cells would be histocompatible with the patient's cells, be expected to have a normal life span, and in principle be a source of any other cell type. However, the time taken and the costs involved in the isolation of the appropriate cell population would probably prohibit large-scale application. Clones have been produced from the cells of adults of five species, but similar studies in at least five other species have produced early embryos, but not offspring. A variety of somatic cells have been used as successful nuclear donors. The present procedures have proved to be repeatable, but are very inefficient when typically between 1% and 4% of reconstructed embryos develop to adulthood. The inefficiency is the accumulated effect of failure at all stages of development. There may be differences between species and donor cell type in the precise pattern of loss. This outcome is assumed to reflect the inappropriate expression of a large number of genes whose lethal effect is exerted at different stages. Improvements in the efficiency may depend upon understanding those mechanisms in the early embryo that establish the precise chromatin structure that governs development.

Wulf, G. M., C. N. Adra, et al. (1993). "Inhibition of hematopoietic development from embryonic stem cells by antisense vav RNA." Embo J **12**(13): 5065-74.

The vav proto-oncogene is universally and specifically expressed in hematopoietic cells. vav contains a unique array of motifs allowing the protein to function as a signal transducer and possibly as a transcription factor. Under certain in vitro culture conditions murine embryonic stem cells develop into colonies containing multiple hematopoietic lineages. In embryonic stem cell lines, constitutively expressing high levels of antisense vav transcripts through a stably integrated transgene, differentiation into hematopoietic cells is disrupted. This observation presents the first evidence that vav has a critical role in the development of hematopoietic cells from totipotent cells.

Xu, Y., Z. He, et al. (2007). "Murine fertilized ovum, blastomere and morula cells lacking SP phenotype." Sci China C Life Sci **50**(6): 762-5.

In the field of stem cell research, SP (side population) phenotype is used to define the property that cells maintain a high efflux capability for some

fluorescent dye, such as Hoechst 33342. Recently, many researches proposed that SP phenotype is a phenotype shared by some stem cells and some progenitor cells, and that SP phenotype is regarded as a candidate purification marker for stem cells. In this research, murine fertilized ova (including conjugate and single nucleus fertilized ova), 2-cell stage and 8-cell stage blastomeres, morulas and blastocysts were isolated and directly stained by Hoechst 33342 dye. The results show that fertilized ovum, blastomere and morula cells do not demonstrate any ability to efflux the dye. However, the inner cell mass (ICM) cells of blastocyst exhibit SP phenotype, which is consistent with the result of embryonic stem cells (ESCs) in vitro. These results indicate that the SP phenotype of ICM-derived ESCs is an intrinsic property and independent of the culture condition in vitro, and that SP phenotype is one of the characteristics of at least some pluripotent stem cells, but is not shared by totipotent stem cells. In addition, the result that the SP phenotype of ICM cells disappeared when the inhibitor verapamil was added into medium implies that the SP phenotype is directly associated with ABCG2. These results suggest that not all the stem cells demonstrate SP phenotype, and that SP phenotype might act as a purification marker for partial stem cells such as some pluripotent embryonic stem cells and multipotent adult stem cells, but not for all stem cells exampled by the totipotent stem cells in the very early stage of mouse embryos.

Yeom, Y. I., G. Fuhrmann, et al. (1996). "Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells." Development **122**(3): 881-94.

The totipotent stem cells of the pregastrulation mouse embryo which give rise to all embryonic somatic tissues and germ cells express Oct-4. The expression is downregulated during gastrulation and is thereafter only maintained in the germline lineage. Oct-4/lacZ transgenes were used to determine how this pattern of expression was achieved, and resulted in the identification of two separate regulatory elements. The distal element drives Oct-4 expression in preimplantation embryos, in migratory and postmigratory primordial germ cells but is inactive in cells of the epiblast. In cell lines this element is specifically active in embryonic stem and embryonic germ cells. The proximal element directs the epiblast-specific expression pattern, including downregulation during gastrulation; in cell lines its activity is restricted to epiblast-derived cells. Thus, Oct-4 expression in the germline is regulated separately from epiblast expression. This provides the first marker for the identification of totipotent cells in the embryo, and suggests that expression of Oct-4 in the totipotent cycle is dependent on a set of factors unique to the germline.

Yin, Y., Y. K. Lim, et al. (2002). "AFP(+), ESC-derived cells engraft and differentiate into hepatocytes in vivo." Stem Cells **20**(4): 338-46.

A major problem in gene therapy and tissue replacement is accessibility of tissue-specific stem cells. One solution is to isolate tissue-specific stem cells from differentiating embryonic stem (ES) cells. Here, we show that liver progenitor cells can be purified from differentiated ES cells using alpha-fetoprotein (AFP) as a marker. By knocking the green fluorescent protein (GFP) gene into the AFP locus of ES cells and differentiating the modified ES cells in vitro, a subpopulation of GFP(+) and AFP-expressing cells was generated. When transplanted into partially hepatectomized lacZ-positive ROSA26 mice, GFP(+) cells engrafted and differentiated into lacZ-negative and albumin-positive hepatocytes. Differentiation into hepatocytes also occurred after transplantation of GFP(+) cells in apolipoprotein-E- (ApoE) or haptoglobin-deficient mice as demonstrated by the presence of ApoE-positive hepatocytes and ApoE mRNA in the liver of ApoE-deficient mice or by haptoglobin in the serum and haptoglobin mRNA in the liver of haptoglobin-deficient mice. This study describes the first isolation of ES-cell-derived liver progenitor cells that are viable mediators of liver-specific functions in vivo.

Yurugi-Kobayashi, T., H. Itoh, et al. (2003). "Effective contribution of transplanted vascular progenitor cells derived from embryonic stem cells to adult neovascularization in proper differentiation stage." Blood **101**(7): 2675-8.

We demonstrated that Flk-1(+) cells derived from mouse embryonic stem (ES) cells can differentiate into both endothelial cells (ECs) and mural cells (MCs) to suffice as vascular progenitor cells (VPCs). In the present study, we investigated the importance of the stage of ES cell differentiation on effective participation in adult neovascularization. We obtained Flk-1(+) LacZ-expressing undifferentiated VPCs. Additional culture of these VPCs with vascular endothelial growth factor (VEGF) resulted in a mixture of ECs and MCs (differentiated VPCs). We injected VPCs subcutaneously into tumor-bearing mice. Five days after the injection, whereas undifferentiated VPCs were often detected as nonvascular cells, differentiated VPCs were more specifically incorporated into developing vasculature mainly as ECs. VPC-derived MCs were also detected in vascular walls. Furthermore, transplantation of differentiated VPCs augmented tumor blood flow in nude mice. These results indicate that a specific

vascular contribution in adult neovascularization can be achieved by selective transplantation of ES cell-derived VPCs in appropriate differentiation stages, which should be the basis for vascular regeneration schemes.

Zangrossi, S., M. Marabese, et al. (2007). "Oct-4 expression in adult human differentiated cells challenges its role as a pure stem cell marker." Stem Cells **25**(7): 1675-80.

The Oct-4 transcription factor, a member of the POU family that is also known as Oct-3 and Oct3/4, is expressed in totipotent embryonic stem cells (ES) and germ cells, and it has a unique role in development and in the determination of pluripotency. ES may have their postnatal counterpart in the adult stem cells, recently described in various mammalian tissues, and Oct-4 expression in putative stem cells purified from adult tissues has been considered a real marker of stemness. In this context, normal mature adult cells would not be expected to show Oct-4 expression. On the contrary, we demonstrated, using reverse transcription-polymerase chain reaction (PCR) (total RNA, Poly A+), real-time PCR, immunoprecipitation, Western blotting, band shift, and immunofluorescence, that human peripheral blood mononuclear cells, genetically stable and mainly terminally differentiated cells with well defined functions and a limited lifespan, express Oct-4. These observations raise the question as to whether the role of Oct-4 as a marker of pluripotency should be challenged. Our findings suggest that the presence of Oct-4 is not sufficient to define a cell as pluripotent, and that additional measures should be used to avoid misleading results in the case of an embryonic-specific gene with a large number of pseudogenes that may contribute to false identification of Oct-4 in adult stem cells. These unexpected findings may provide new insights into the role of Oct-4 in fully differentiated cells. Disclosure of potential conflicts of interest is found at the end of this article.

Zeng, X. and M. S. Rao (2006). "The therapeutic potential of embryonic stem cells: A focus on stem cell stability." Curr Opin Mol Ther **8**(4): 338-44.

Most therapeutic uses of stem cells demand that large numbers of cells are maintained in a Good Manufacturing Practice (GMP) facility, and envisage the development of a master depository from which a working bank of cells can be retrieved and differentiated into an appropriate phenotype for use. Likewise for gene- and drug-discovery processes, it is assumed that stable and genetically identical cells will eventually become available in large numbers. Critical for both of these assumptions is that the stem cells are stable during periods of amplification and differentiation. This review discusses the physiological features that must be assessed to measure stem cell stability, and proposes that genomic, epigenomic and mitochondrial markers, as well as functional measures of utility, should be considered. Recent findings suggesting that the level of cell stability is not homogeneous throughout all stem cells are also discussed.

Zhang, M., B. Joseph, et al. (2005). "Embryonic mouse STO cell-derived xenografts express hepatocytic functions in the livers of nonimmunosuppressed adult rats." Stem Cells **23**(2): 186-99.

Cells derived from embryonic mouse STO cell lines differentiate into hepatocytes when transplanted into the livers of nonimmunosuppressed dipeptidylpeptidase IV (DPPIV)-negative F344 rats. Within 1 day after intrasplenic injection, donor cells moved rapidly into the liver and were found in intravascular and perivascular sites; by 1 month, they were intrasinusoidal and also integrated into hepatic plates with approximately 2% efficiency and formed conjoint bile canaliculi. Neither donor cell proliferation nor host inflammatory responses were observed during this time. Detection of intrahepatic mouse COX1 mitochondrial DNA and mouse albumin mRNA in recipient rats indicated survival and differentiation of donor cells for at least 3 months. Mouse COX1 targets were also detected intrahepatically 4-9 weeks after STO cell injection into nonimmunosuppressed wild-type rats. In contrast to STO-transplanted rats, mouse DNA or RNA was not detectable in untreated or mock-transplanted rats or in rats injected with donor cell DNA. In cultured STO donor cells, DPPIV and glucose-6-phosphatase activities were observed in small clusters; in contrast, mouse major histocompatibility complex class I H-2Kq, H-2Dq, and H-2Lq and class II I-Aq markers were undetectable in vitro before or after interferon gamma treatment. Together with H-2K allele typing, which confirmed the Swiss mouse origin of the donor cells, these observations indicate that mouse-derived STO cell lines can differentiate along hepatocytic lineage and engraft into rat liver across major histocompatibility barriers.

Zhang, X., K. T. Ebata, et al. (2006). "Aging of male germ line stem cells in mice." Biol Reprod **74**(1): 119-24.

In the present study, we investigated the effect of aging on spermatogonial stem cells (SSCs) and on the testicular somatic environment in ROSA26 mice. First, we examined testis weights at 2 mo, 6 mo, 1 yr, and 2 yr of age. At 1 and 2 yr, bilateral atrophied testes were observed in 50% and 75% of the mice,

respectively; the rest of the mice had testis weights similar to those of young mice. Next, we evaluated the number and the activity of aged SSCs using spermatogonial transplantation. Numbers of SSCs in atrophied testes decreased in an age-dependent manner to as low as 1/60 of those in testes of young mice. Numbers of SSCs in nonregressed testes were similar regardless of age. The colony length, which is indicative of the potential of SSCs to regenerate spermatogenesis, was similar with donor cells from atrophied testes of 1-yr-old mice and those from testes of young mice, suggesting that SSCs remaining in 1-yr atrophied testes were functionally intact. Colonies arising from SSCs derived from 2-yr atrophied testes were significantly shorter, however, indicating that both SSC numbers and activity declined with age. Finally, we transplanted donor cells from young animals into 1- and 2-yr atrophied testes. Although the weight of 2-yr testes did not change after transplantation, that of 1-yr testes increased significantly, indicating that 1-yr, but not 2-yr, atrophied testes are permissive for regeneration of spermatogenesis by SSCs from young mouse testes. These results demonstrate that both SSCs and somatic environment in the testis are involved in the aging process.

Zhang, Y. M., C. Hartzell, et al. (2002). "Stem cell-derived cardiomyocytes demonstrate arrhythmic potential." Circulation **106**(10): 1294-9.

BACKGROUND: Cardiomyocytes (CMs) derived from pluripotent embryonic stem cells (ESCs) and embryonal carcinoma cells (ECCs) have some but not all characteristics of adult myocytes. ESCs have shown the ability to engraft in areas of myocardial damage, which suggests their use in cell transplantation therapy for cardiomyopathy. We studied the arrhythmogenic properties of CMs differentiated from mouse ESCs and ECCs. METHODS AND RESULTS: CMs derived in vitro were studied in the whole-cell patch-clamp mode. CMs from both sources showed action potential (AP) morphology heterogeneity, with reduced maximum upstroke velocities (dV/dt) and prolonged AP durations. CMs demonstrated prolonged, spontaneous electrical activity in culture. Frequent triggered activity was observed with and without pharmacological enhancement. Phase 2 or 3 early afterdepolarizations could be induced easily by Bay K8644 plus tetraethylammonium chloride (TEA) or [TEA]o after Cs+ replacement for [K+]i, respectively. A combination of bradycardic stimulation, hypokalemia, and quinidine resulted in early afterdepolarizations. Delayed afterdepolarizations could be induced easily and reversibly by hypercalcemia or isoproterenol. CONCLUSIONS: ESCs or ECCs differentiated into at least 3 AP phenotypes. CMs showed spontaneous activity, low dV/dt, prolonged AP duration, and easily inducible triggered arrhythmias. These findings raise caution about the use of totipotent ESCs in cell transplantation therapy, because they may act as an unanticipated arrhythmogenic source from any of the 3 classic mechanisms (reentry, automaticity, or triggered activity).

Zuk, P. A. (2009). "The intracellular distribution of the ES cell totipotent markers OCT4 and Sox2 in adult stem cells differs dramatically according to commercial antibody used." J Cell Biochem **106**(5): 867-77.

To characterize ES cells, researchers have at their disposal a list of pluripotent markers, such as OCT4. In their quest to determine if adult stem cell populations, such as MSCs and ASCs, are pluripotent, several groups have begun to report the expression of these markers in these cells. Consistent with this, human ASCs (hASCs) are shown in this study to express a plethora of ES pluripotent markers at the gene and protein level, including OCT4, Sox2, and Nanog. When intracellular distribution is examined in hASCs, both OCT4 and Sox2 are expressed within the nuclei of hASCs, consistent with their expression patterns in ES cells. However, a significant amount of expression can be noted within the hASC cytoplasm and a complete absence of nuclear expression is observed for Nanog. Recent descriptions of OCT4 transcript variants may explain the cytoplasmic expression of OCT4 in hASCs and consistent with this, hASCs do express both the OCT4A and 4B transcript variants at the gene level. However, discrepancies arise when these three pluripotent markers are studied at the protein level. Specifically, distinct differences in intracellular expression patterns were noted for OCT4, Sox2, and Nanog from commercial antibody to commercial antibody. These antibody discrepancies persisted when hMSCs and rat ASCs and MSCs were examined. Therefore, confirming the expression of OCT4, Sox2, and Nanog in adult stem cells with today's commercial antibodies must be carefully considered before the designation of pluripotent can be granted.

References

1. Aiba, K., A. A. Sharov, et al. (2006). "Defining a developmental path to neural fate by global expression profiling of mouse embryonic stem cells and adult neural stem/progenitor cells." Stem Cells **24**(4): 889-95.
2. Ajjappala, B. S., M. S. Kim, et al. (2009). "Protein chip analysis of pluripotency-associated proteins in NIH3T3 fibroblast." Proteomics **9**(16): 3968-78.
3. Aladjem, M. I., L. W. Rodewald, et al. (2002). "Replication initiation patterns in the beta-globin loci of totipotent and

differentiated murine cells: evidence for multiple initiation regions." Mol Cell Biol **22**(2): 442-52.
4. Alimena, G., G. De Rossi, et al. (1980). "B cell markers in Ph1-positive acute lymphoblastic leukemia." Nouv Rev Fr Hematol **22**(3): 275-80.
5. Almeida-Porada, G., C. Porada, et al. (2001). "Adult stem cell plasticity and methods of detection." Rev Clin Exp Hematol **5**(1): 26-41.
6. Anderson, R., K. Schaible, et al. (1999). "Expression of the homophilic adhesion molecule, Ep-CAM, in the mammalian germ line." J Reprod Fertil **116**(2): 379-84.
7. Ariad, S., D. Dajee, et al. (1993). "Lack of involvement of T-lymphocytes in the leukaemic population during prolonged chronic phase of Philadelphia chromosome positive chronic myeloid leukaemia." Leuk Lymphoma **10**(3): 217-21.
8. 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.
9. Balconi, G., R. Spagnuolo, et al. (2000). "Development of endothelial cell lines from embryonic stem cells: A tool for studying genetically manipulated endothelial cells in vitro." Arterioscler Thromb Vasc Biol **20**(6): 1443-51.
10. Barker, J. E., J. Greer, et al. (1991). "Temporal replacement of donor erythrocytes and leukocytes in nonanemic W44J/W44J and severely anemic W/Wv mice." Blood **78**(6): 1432-7.
11. Barker, J. E., J. H. Wolfe, et al. (1993). "Advantages of gradient vs. 5-fluorouracil enrichment of stem cells for retroviral-mediated gene transfer." Exp Hematol **21**(1): 47-54.
12. Bernstein, A. and M. Breitman (1989). "Genetic ablation in transgenic mice." Mol Biol Med **6**(6): 523-30.
13. Best, J. B. and M. Morita (1982). "Planarians as a model system for in vitro teratogenesis studies." Teratog Carcinog Mutagen **2**(3-4): 277-91.
14. Bidaut, G. and C. J. Stoeckert, Jr. (2009). "Large scale transcriptome data integration across multiple tissues to decipher stem cell signatures." Methods Enzymol **467**: 229-45.
15. Blaese, R. M. and K. W. Culver (1992). "Gene therapy for primary immunodeficiency disease." Immunodefic Rev **3**(4): 329-49.
16. Blobel, G. A., M. C. Simon, et al. (1995). "Rescue of GATA-1-deficient embryonic stem cells by heterologous GATA-binding proteins." Mol Cell Biol **15**(2): 626-33.
17. Boiani, M. and H. R. Scholer (2005). "Regulatory networks in embryo-derived pluripotent stem cells." Nat Rev Mol Cell Biol **6**(11): 872-84.
18. Bollner, T., S. Howalt, et al. (1995). "Regeneration and post-metamorphic development of the central nervous system in the protochordate Ciona intestinalis: a study with monoclonal antibodies." Cell Tissue Res **279**(2): 421-32.
19. Bongso, A., C. Y. Fong, et al. (1994). "Isolation and culture of inner cell mass cells from human blastocysts." Hum Reprod **9**(11): 2110-7.
20. Bradley, A., R. Ramirez-Solis, et al. (1992). "Genetic manipulation of the mouse via gene targeting in embryonic stem cells." Ciba Found Symp **165**: 256-69; discussion 269-76.
21. Brehm, A., C. E. Ovitt, et al. (1998). "Oct-4: more than just a POUerful marker of the mammalian germline?" Apmis **106**(1): 114-24; discussion 124-6.
22. Brehm, A., K. Ohbo, et al. (1997). "The carboxy-terminal transactivation domain of Oct-4 acquires cell specificity through the POU domain." Mol Cell Biol **17**(1): 154-62.
23. Brinster, R. L. (1993). "Stem cells and transgenic mice in the study of development." Int J Dev Biol **37**(1): 89-99.
24. Brown, D. G., M. A. Willington, et al. (1992). "Criteria that optimize the potential of murine embryonic stem cells for in vitro and in vivo developmental studies." In Vitro Cell Dev Biol **28A**(11-12): 773-8.
25. Bryja, V., S. Bonilla, et al. (2006). "An efficient method for the derivation of mouse embryonic stem cells." Stem Cells **24**(4): 844-9.
26. Bueno, D., J. Baguna, et al. (1997). "Cell-, tissue-, and position-specific monoclonal antibodies against the planarian Dugesia (Girardia) tigrina." Histochem Cell Biol **107**(2): 139-49.
27. Bugos, O., M. Bhide, et al. (2009). "Beyond the rat models of human neurodegenerative disorders." Cell Mol Neurobiol **29**(6-7): 859-69.
28. Bukovsky, A. (2007). "Cell commitment by asymmetric division and immune system involvement." Prog Mol Subcell Biol **45**: 179-204.
29. Bukovsky, A., I. Virant-Klun, et al. (2006). "Ovarian germ cells." Methods Enzymol **419**: 208-58.
30. Bukovsky, A., M. R. Caudle, et al. (2009). "Immune physiology and oogenesis in fetal and adult humans, ovarian infertility, and totipotency of adult ovarian stem cells." Birth Defects Res C Embryo Today **87**(1): 64-89.
31. Bukovsky, A., M. Svetlikova, et al. (2005). "Oogenesis in cultures derived from adult human ovaries." Reprod Biol Endocrinol **3**: 17.
32. Bukovsky, A., P. Copas, et al. (2006). "Potential new strategies for the treatment of ovarian infertility and degenerative diseases with autologous ovarian stem cells." Expert Opin Biol Ther **6**(4): 341-65.
33. Burt, R. K., L. Verda, et al. (2004). "Embryonic stem cells as an alternate marrow donor source: engraftment without graft-versus-host disease." J Exp Med **199**(7): 895-904.
34. Cabibi, D., A. Martorana, et al. (2006). "Carcinosarcoma of monoclonal origin arising in a dermoid cyst of ovary: a case report." BMC Cancer **6**: 47.
35. Capel, B., R. G. Hawley, et al. (1990). "Long- and short-lived murine hematopoietic stem cell clones individually identified with retroviral integration markers." Blood **75**(12): 2267-70.
36. Capel, B., R. Hawley, et al. (1989). "Clonal contributions of small numbers of retrovirally marked hematopoietic stem cells engrafted in unirradiated neonatal W/Wv mice." Proc Natl Acad Sci U S A **86**(12): 4564-8.
37. Carroll, P., Y. Renoncourt, et al. (2001). "Sorting nexin-14, a gene expressed in motoneurons trapped by an in vitro preselection method." Dev Dyn **221**(4): 431-42.
38. Caruana, G. and A. Bernstein (2001). "Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene." Mol Cell Biol **21**(5): 1475-83.
39. Cauffman, G., I. Liebaers, et al. (2006). "POU5F1 isoforms show different expression patterns in human embryonic stem cells and preimplantation embryos." Stem Cells **24**(12): 2685-91.
40. Cauffman, G., M. De Rycke, et al. (2009). "Markers that define stemness in ESC are unable to identify the totipotent cells in human preimplantation embryos." Hum Reprod **24**(1): 63-70.
41. Cazillis, M., A. F. Bringuier, et al. (2004). "Disruption of MKK4 signaling reveals its tumor-suppressor role in embryonic stem cells." Oncogene **23**(27): 4735-44.
42. Chen, S. K., S. Kurdyukov, et al. (2009). "The association of homeobox gene expression with stem cell formation and morphogenesis in cultured Medicago truncatula." Planta **230**(4): 827-40.
43. Chervenick, P. A. (1985). "Methods for measuring suppression of hematopoiesis." Exp Hematol **13 Suppl 16**: 8-15.

44. Clipsham, R., K. Niakan, et al. (2004). "Nr0b1 and its network partners are expressed early in murine embryos prior to steroidogenic axis organogenesis." Gene Expr Patterns **4**(1): 3-14.
45. Cogle, C. R., S. M. Guthrie, et al. (2003). "An overview of stem cell research and regulatory issues." Mayo Clin Proc **78**(8): 993-1003.
46. Colombo, E., S. G. Giannelli, et al. (2006). "Embryonic stem-derived versus somatic neural stem cells: a comparative analysis of their developmental potential and molecular phenotype." Stem Cells **24**(4): 825-34.
47. Cooper, H. M., R. N. Tamura, et al. (1991). "The major laminin receptor of mouse embryonic stem cells is a novel isoform of the alpha 6 beta 1 integrin." J Cell Biol **115**(3): 843-50.
48. Dani, C., A. G. Smith, et al. (1997). "Differentiation of embryonic stem cells into adipocytes in vitro." J Cell Sci **110 (Pt 11)**: 1279-85.
49. Dattena, M., S. Pilichi, et al. (2009). "Sheep embryonic stem-like cells transplanted in full-thickness cartilage defects." J Tissue Eng Regen Med **3**(3): 175-87.
50. Davidson, A. J., S. A. Freeman, et al. (1997). "Expression of murine interleukin 11 and its receptor alpha-chain in adult and embryonic tissues." Stem Cells **15**(2): 119-24.
51. de Kretser, D. (2007). "Totipotent, pluripotent or unipotent stem cells: a complex regulatory enigma and fascinating biology." J Law Med **15**(2): 212-8.
52. De Mulder, K., D. Pfister, et al. (2009). "Stem cells are differentially regulated during development, regeneration and homeostasis in flatworms." Dev Biol **334**(1): 198-212.
53. De Sousa, P. A., S. J. da Silva, et al. (2004). "Neurotrophin signaling in oocyte survival and developmental competence: a paradigm for cellular toti-potency." Cloning Stem Cells **6**(4): 375-85.
54. de Vries, W. N., A. V. Evsikov, et al. (2008). "Reprogramming and differentiation in mammals: motifs and mechanisms." Cold Spring Harb Symp Quant Biol **73**: 33-8.
55. Deacon, T., J. Dinsmore, et al. (1998). "Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation." Exp Neurol **149**(1): 28-41.
56. Dean, W., F. Santos, et al. (2003). "Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer." Semin Cell Dev Biol **14**(1): 93-100.
57. Del Vecchio, F., A. Filareto, et al. (2005). "Cellular genetic therapy." Transplant Proc **37**(6): 2657-61.
58. Denker, H. W. (2006). "Potentiality of embryonic stem cells: an ethical problem even with alternative stem cell sources." J Med Ethics **32**(11): 665-71.
59. Dewey, M. J., D. W. Martin, Jr., et al. (1977). "Mosaic mice with teratocarcinoma-derived mutant cells deficient in hypoxanthine phosphoribosyltransferase." Proc Natl Acad Sci U S A **74**(12): 5564-8.
60. Do, J. T., D. W. Han, et al. (2006). "Reprogramming somatic gene activity by fusion with pluripotent cells." Stem Cell Rev **2**(4): 257-64.
61. Dominguez-Bendala, J., H. Priddle, et al. (2003). "Elevated expression of exogenous Rad51 leads to identical increases in gene-targeting frequency in murine embryonic stem (ES) cells with both functional and dysfunctional p53 genes." Exp Cell Res **286**(2): 298-307.
62. Drab, M., H. Haller, et al. (1997). "From totipotent embryonic stem cells to spontaneously contracting smooth muscle cells: a retinoic acid and db-cAMP in vitro differentiation model." Faseb J **11**(11): 905-15.
63. Dupin, E., F. Sextier-Sainte-Claire Deville, et al. (1993). "The ontogeny of the neural crest." C R Acad Sci III **316**(9): 1062-81.
64. Dutra, H. S., M. I. Rossi, et al. (1997). "Haematopoietic capacity of colony-forming cells mobilized in hepatic inflammatory reactions as compared to that of normal bone marrow cells." Res Immunol **148**(7): 437-44.
65. Eaves, C. J., H. J. Sutherland, et al. (1992). "The human hematopoietic stem cell in vitro and in vivo." Blood Cells **18**(2): 301-7.
66. Eaves, C. J., J. D. Cashman, et al. (1991). "Molecular analysis of primitive hematopoietic cell proliferation control mechanisms." Ann N Y Acad Sci **628**: 298-306.
67. Eaves, C., P. Zandstra, et al. (1998). "Changes in the cytokine regulation of stem cell self-renewal during ontogeny." Stem Cells **16 Suppl 1**: 177-84.
68. Eggan, K., K. Baldwin, et al. (2004). "Mice cloned from olfactory sensory neurons." Nature **428**(6978): 44-9.
69. Egger, B., P. Ladurner, et al. (2006). "The regeneration capacity of the flatworm Macrostomum lignano--on repeated regeneration, rejuvenation, and the minimal size needed for regeneration." Dev Genes Evol **216**(10): 565-77.
70. Enright, B. P., L. Y. Sung, et al. (2005). "Methylation and acetylation characteristics of cloned bovine embryos from donor cells treated with 5-aza-2'-deoxycytidine." Biol Reprod **72**(4): 944-8.
71. Estrov, Z. (2009). "Stem cells and somatic cells: reprogramming and plasticity." Clin Lymphoma Myeloma **9 Suppl 3**: S319-28.
72. Ferri, A. L., M. Cavallaro, et al. (2004). "Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain." Development **131**(15): 3805-19.
73. Finch, K. A., G. Fonseka, et al. (2008). "Nuclear organisation in totipotent human nuclei and its relationship to chromosomal abnormality." J Cell Sci **121**(Pt 5): 655-63.
74. First, N. L., M. M. Sims, et al. (1994). "Systems for production of calves from cultured bovine embryonic cells." Reprod Fertil Dev **6**(5): 553-62.
75. Fleischman, R. A. and B. Mintz (1984). "Development of adult bone marrow stem cells in H-2-compatible and -incompatible mouse fetuses." J Exp Med **159**(3): 731-45.
76. Fleischman, R. A., R. P. Custer, et al. (1982). "Totipotent hematopoietic stem cells: normal self-renewal and differentiation after transplantation between mouse fetuses." Cell **30**(2): 351-9.
77. Flohr, T. R., H. Bonatti, Jr., et al. (2009). "The use of stem cells in liver disease." Curr Opin Organ Transplant **14**(1): 64-71.
78. Fraser, C. C., C. J. Eaves, et al. (1990). "Expansion in vitro of retrovirally marked totipotent hematopoietic stem cells." Blood **76**(6): 1071-6.
79. Fraser, C. C., S. J. Szilvassy, et al. (1992). "Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability." Proc Natl Acad Sci U S A **89**(5): 1968-72.
80. Friedlander, M. R., C. Adamidi, et al. (2009). "High-resolution profiling and discovery of planarian small RNAs." Proc Natl Acad Sci U S A **106**(28): 11546-51.
81. Fulka, H., J. C. St John, et al. (2008). "Chromatin in early mammalian embryos: achieving the pluripotent state." Differentiation **76**(1): 3-14.
82. Furusawa, C. and K. Kaneko (2002). "Origin of multicellular organisms as an inevitable consequence of dynamical systems." Anat Rec **268**(3): 327-42.
83. Gabutti, V., F. Timeus, et al. (1993). "Expansion of cord blood progenitors and use for hemopoietic reconstitution." Stem Cells **11 Suppl 2**: 105-12.
84. Gaziova, I. and K. M. Bhat (2007). "Generating asymmetry: with and without self-renewal." Prog Mol Subcell Biol **45**: 143-78.
85. Geier, F., J. U. Lohmann, et al. (2008). "A quantitative and dynamic model for plant stem cell regulation." PLoS One **3**(10): e3553.

86. Gokhan, S. and M. F. Mehler (2001). "Basic and clinical neuroscience applications of embryonic stem cells." Anat Rec **265**(3): 142-56.
87. Gonzalez-Estevez, C., T. Momose, et al. (2003). "Transgenic planarian lines obtained by electroporation using transposon-derived vectors and an eye-specific GFP marker." Proc Natl Acad Sci U S A **100**(24): 14046-51.
88. Grabarek, J. B., F. Wianny, et al. (2003). "RNA interference by production of short hairpin dsRNA in ES cells, their differentiated derivatives, and in somatic cell lines." Biotechniques **34**(4): 734-6, 739-44.
89. Gregori, N., C. Proschel, et al. (2002). "The tripotential glial-restricted precursor (GRP) cell and glial development in the spinal cord: generation of bipotential oligodendrocyte-type-2 astrocyte progenitor cells and dorsal-ventral differences in GRP cell function." J Neurosci **22**(1): 248-56.
90. Gschwentner, R., P. Ladurner, et al. (2001). "Stem cells in a basal bilaterian. S-phase and mitotic cells in Convolutriloba longifissura (Acoela, Platyhelminthes)." Cell Tissue Res **304**(3): 401-8.
91. Guan, K., J. Rohwedel, et al. (1999). "Embryonic stem cell differentiation models: cardiogenesis, myogenesis, neurogenesis, epithelial and vascular smooth muscle cell differentiation in vitro." Cytotechnology **30**(1-3): 211-26.
92. Guo, J., A. Jauch, et al. (2005). "Multicolor karyotype analyses of mouse embryonic stem cells." In Vitro Cell Dev Biol Anim **41**(8-9): 278-83.
93. Guo, X., W. Ying, et al. (2001). "Proteomic characterization of early-stage differentiation of mouse embryonic stem cells into neural cells induced by all-trans retinoic acid in vitro." Electrophoresis **22**(14): 3067-75.
94. Guo, Y., B. Graham-Evans, et al. (2006). "Murine embryonic stem cells secrete cytokines/growth modulators that enhance cell survival/anti-apoptosis and stimulate colony formation of murine hematopoietic progenitor cells." Stem Cells **24**(4): 850-6.
95. Guo, Y., R. Costa, et al. (2002). "The embryonic stem cell transcription factors Oct-4 and FoxD3 interact to regulate endodermal-specific promoter expression." Proc Natl Acad Sci U S A **99**(6): 3663-7.
96. Haegel, H., A. Dierich, et al. (1994). "CD44 in differentiated embryonic stem cells: surface expression and transcripts encoding multiple variants." Dev Immunol **3**(4): 239-46.
97. Hall, B., A. Limaye, et al. (2009). "Overview: generation of gene knockout mice." Curr Protoc Cell Biol **Chapter 19**: Unit 19 12 19 12 1-17.
98. Handberg-Thorsager, M. and E. Salo (2007). "The planarian nanos-like gene Smednos is expressed in germline and eye precursor cells during development and regeneration." Dev Genes Evol **217**(5): 403-11.
99. Hansis, C. (2006). "Totipotency, cell differentiation and reprogramming in humans." Reprod Biomed Online **13**(4): 551-7.
100. Hawley, R. G., T. S. Hawley, et al. (1996). "Thrombopoietic potential and serial repopulating ability of murine hematopoietic stem cells constitutively expressing interleukin 11." Proc Natl Acad Sci U S A **93**(19): 10297-302.
101. Hayashi, S., T. Yamane, et al. (1998). "Commitment and differentiation of stem cells to the osteoclast lineage." Biochem Cell Biol **76**(6): 911-22.
102. Hays, E. F. and N. Margaretten (1985). "Long-term oral cadmium produces bone marrow hypoplasia in mice." Exp Hematol **13**(3): 229-34.
103. Hillmen, P., S. M. Lewis, et al. (1995). "Natural history of paroxysmal nocturnal hemoglobinuria." N Engl J Med **333**(19): 1253-8.
104. Hilton, D. J., N. A. Nicola, et al. (1991). "Distribution and comparison of receptors for leukemia inhibitory factor on murine hemopoietic and hepatic cells." J Cell Physiol **146**(2): 207-15.
105. Ho, L., J. L. Ronan, et al. (2009). "An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency." Proc Natl Acad Sci U S A **106**(13): 5181-6.
106. Hochedlinger, K. and R. Jaenisch (2002). "Nuclear transplantation: lessons from frogs and mice." Curr Opin Cell Biol **14**(6): 741-8.
107. Hochedlinger, K., W. M. Rideout, et al. (2004). "Nuclear transplantation, embryonic stem cells and the potential for cell therapy." Hematol J **5 Suppl 3**: S114-7.
108. Hoei-Hansen, C. E., A. Sehested, et al. (2006). "New evidence for the origin of intracranial germ cell tumours from primordial germ cells: expression of pluripotency and cell differentiation markers." J Pathol **209**(1): 25-33.
109. Honecker, F., H. Stoop, et al. (2006). "Germ cell lineage differentiation in non-seminomatous germ cell tumours." J Pathol **208**(3): 395-400.
110. Hong, Y. and M. Schartl (2006). "Isolation and differentiation of medaka embryonic stem cells." Methods Mol Biol **329**: 3-16.
111. Hong, Y., C. Winkler, et al. (2004). "Activation of the mouse Oct4 promoter in medaka embryonic stem cells and its use for ablation of spontaneous differentiation." Mech Dev **121**(7-8): 933-43.
112. Horii, T., Y. Nagao, et al. (2003). "Serum-free culture of murine primordial germ cells and embryonic germ cells." Theriogenology **59**(5-6): 1257-64.
113. Houldsworth, J., J. E. Korkola, et al. (2006). "Biology and genetics of adult male germ cell tumors." J Clin Oncol **24**(35): 5512-8.
114. Hubner, K., G. Fuhrmann, et al. (2003). "Derivation of oocytes from mouse embryonic stem cells." Science **300**(5623): 1251-6.
115. Hug, K. (2005). "Sources of human embryos for stem cell research: ethical problems and their possible solutions." Medicina (Kaunas) **41**(12): 1002-10.
116. Huntriss, J., M. Hinkins, et al. (2004). "Expression of mRNAs for DNA methyltransferases and methyl-CpG-binding proteins in the human female germ line, preimplantation embryos, and embryonic stem cells." Mol Reprod Dev **67**(3): 323-36.
117. Huss, R. (2000). "Perspectives on the morphology and biology of CD34-negative stem cells." J Hematother Stem Cell Res **9**(6): 783-93.
118. Jaisser, F. and A. T. Beggah (1998). "Transgenic models in renal tubular physiology." Exp Nephrol **6**(5): 438-46.
119. Jeong, D., D. J. McLean, et al. (2003). "Long-term culture and transplantation of murine testicular germ cells." J Androl **24**(5): 661-9.
120. Jordan, C. T. and I. R. Lemischka (1990). "Clonal and systemic analysis of long-term hematopoiesis in the mouse." Genes Dev **4**(2): 220-32.
121. Jordan, C. T., J. P. McKearn, et al. (1990). "Cellular and developmental properties of fetal hematopoietic stem cells." Cell **61**(6): 953-63.
122. Kaeffer, B. (2002). "Mammalian intestinal epithelial cells in primary culture: a mini-review." In Vitro Cell Dev Biol Anim **38**(3): 123-34.
123. Kanatsu, M. and S. I. Nishikawa (1996). "In vitro analysis of epiblast tissue potency for hematopoietic cell differentiation." Development **122**(3): 823-30.
124. Kang, H. and A. Sanchez Alvarado (2009). "Flow cytometry methods for the study of cell-cycle parameters of planarian stem cells." Dev Dyn **238**(5): 1111-7.
125. Karp, J. M., L. S. Ferreira, et al. (2006). "Cultivation of human embryonic stem cells without the embryoid body

step enhances osteogenesis in vitro." Stem Cells **24**(4): 835-43.
126. Kato, Y., H. Imabayashi, et al. (2004). "Nuclear transfer of adult bone marrow mesenchymal stem cells: developmental totipotency of tissue-specific stem cells from an adult mammal." Biol Reprod **70**(2): 415-8.
127. Katoh, Y. and M. Katoh (2007). "Comparative integromics on JMJD2A, JMJD2B and JMJD2C: preferential expression of JMJD2C in undifferentiated ES cells." Int J Mol Med **20**(2): 269-73.
128. Kiessling, A. A., R. Bletsa, et al. (2009). "Evidence that human blastomere cleavage is under unique cell cycle control." J Assist Reprod Genet **26**(4): 187-95.
129. Kim, J. M., M. Yamada, et al. (2003). "Functions of mammalian Cdc7 kinase in initiation/monitoring of DNA replication and development." Mutat Res **532**(1-2): 29-40.
130. Kinoshita, T., M. E. Medof, et al. (1985). "Distribution of decay-accelerating factor in the peripheral blood of normal individuals and patients with paroxysmal nocturnal hemoglobinuria." J Exp Med **162**(1): 75-92.
131. Kirby, S. L., D. N. Cook, et al. (1996). "Proliferation of multipotent hematopoietic cells controlled by a truncated erythropoietin receptor transgene." Proc Natl Acad Sci U S A **93**(18): 9402-7.
132. Kobayashi, N., J. D. Rivas-Carrillo, et al. (2005). "Gene delivery to embryonic stem cells." Birth Defects Res C Embryo Today **75**(1): 10-8.
133. Krichevsky, A. M., K. C. Sonntag, et al. (2006). "Specific microRNAs modulate embryonic stem cell-derived neurogenesis." Stem Cells **24**(4): 857-64.
134. Kucia, M., E. K. Zuba-Surma, et al. (2007). "Adult marrow-derived very small embryonic-like stem cells and tissue engineering." Expert Opin Biol Ther **7**(10): 1499-514.
135. Kucia, M., W. Wu, et al. (2007). "Bone marrow-derived very small embryonic-like stem cells: their developmental origin and biological significance." Dev Dyn **236**(12): 3309-20.
136. Ladurner, P., R. Rieger, et al. (2000). "Spatial distribution and differentiation potential of stem cells in hatchlings and adults in the marine platyhelminth macrostomum sp.: a bromodeoxyuridine analysis." Dev Biol **226**(2): 231-41.
137. Lang, D., M. M. Lu, et al. (2005). "Pax3 functions at a nodal point in melanocyte stem cell differentiation." Nature **433**(7028): 884-7.
138. Le Douarin, N. M., E. Dupin, et al. (1994). "Genetic and epigenetic control in neural crest development." Curr Opin Genet Dev **4**(5): 685-95.
139. Lee, J., B. K. Rhee, et al. (2005). "Stimulation of Oct-4 activity by Ewing's sarcoma protein." Stem Cells **23**(6): 738-51.
140. Leibfried, A., J. P. To, et al. (2005). "WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators." Nature **438**(7071): 1172-5.
141. Lelias, J. M., C. N. Adra, et al. (1993). "cDNA cloning of a human mRNA preferentially expressed in hematopoietic cells and with homology to a GDP-dissociation inhibitor for the rho GTP-binding proteins." Proc Natl Acad Sci U S A **90**(4): 1479-83.
142. Lemez, P. (1990). "Significance of lineage specific differentiation markers for complex classification of acute leukemias. I. Acute myeloid leukemias." Neoplasma **37**(3): 253-66.
143. Lemieux, M. E., V. I. Rebel, et al. (1995). "Characterization and purification of a primitive hematopoietic cell type in adult mouse marrow capable of lymphomyeloid differentiation in long-term marrow "switch" cultures." Blood **86**(4): 1339-47.
144. Lemischka, I. R. (1991). "Clonal, in vivo behavior of the totipotent hematopoietic stem cell." Semin Immunol **3**(6): 349-55.
145. Lemischka, I. R. (1992). "The haematopoietic stem cell and its clonal progeny: mechanisms regulating the hierarchy of primitive haematopoietic cells." Cancer Surv **15**: 3-18.
146. Li, L. and K. Akashi (2003). "Unraveling the molecular components and genetic blueprints of stem cells." Biotechniques **35**(6): 1233-9.
147. Li, S. and P. D. Yurchenco (2006). "Matrix assembly, cell polarization, and cell survival: analysis of peri-implantation development with cultured embryonic stem cells." Methods Mol Biol **329**: 113-25.
148. Liu, J., J. F. Schiltz, et al. (2003). "Hmga1 is required for normal sperm development." Mol Reprod Dev **66**(1): 81-9.
149. Liu, L., E. Czerwiec, et al. (2004). "Effect of ploidy and parental genome composition on expression of Oct-4 protein in mouse embryos." Gene Expr Patterns **4**(4): 433-41.
150. Loring, J. F., J. G. Porter, et al. (2001). "A gene expression profile of embryonic stem cells and embryonic stem cell-derived neurons." Restor Neurol Neurosci **18**(2-3): 81-8.
151. Louie, S. G. and B. Jung (1993). "Clinical effects of biologic response modifiers." Am J Hosp Pharm **50**(7 Suppl 3): S10-8.
152. Lu, W. G., H. Chen, et al. (2007). "Regionspecific survival and differentiation of mouse embryonic stem cell-derived implants in the adult rat brain." Sheng Li Xue Bao **59**(1): 51-7.
153. Luo, Y., C. Schwartz, et al. (2006). "A focused microarray to assess dopaminergic and glial cell differentiation from fetal tissue or embryonic stem cells." Stem Cells **24**(4): 865-75.
154. MacKinney, A. A., Jr., S. S. Clark, et al. (1993). "Simultaneous demonstration of the Philadelphia chromosome in T, B, and myeloid cells." Am J Hematol **44**(1): 48-52.
155. Magyar, J. P., M. Nemir, et al. (2001). "Mass production of embryoid bodies in microbeads." Ann N Y Acad Sci **944**: 135-43.
156. capacity to differentiate along multiple lineages and they have been suggested to be part of a 'totipotent germline cycle' that links one generation to the next.
157. Mannini, L., L. Rossi, et al. (2004). "Djeyes absent (Djeya) controls prototypic planarian eye regeneration by cooperating with the transcription factor Djsix-1." Dev Biol **269**(2): 346-59.
158. Mardanpour, P., K. Guan, et al. (2008). "Potency of germ cells and its relevance for regenerative medicine." J Anat **213**(1): 26-9.
159. Marguerie, G. and D. Tronik-Le Roux (1998). "Analysis of hematopoietic stem cell reprogramming with toxigenicity." Stem Cells **16 Suppl 2**: 85-9.
160. Marguerie, G., V. Roullot, et al. (1996). "Dissecting megakaryocytopoiesis in vivo with toxigenes." Stem Cells **14 Suppl 1**: 200-5.
161. Matin, A., G. B. Collin, et al. (1998). "Testicular teratocarcinogenesis in mice--a review." Apmis **106**(1): 174-82.
162. Metcalf, D. (2003). "The unsolved enigmas of leukemia inhibitory factor." Stem Cells **21**(1): 5-14.
163. Metzger, J. M., W. I. Lin, et al. (1995). "Myosin heavy chain expression in contracting myocytes isolated during embryonic stem cell cardiogenesis." Circ Res **76**(5): 710-9.
164. Miller, C. L., V. I. Rebel, et al. (1996). "Studies of W mutant mice provide evidence for alternate mechanisms capable of activating hematopoietic stem cells." Exp Hematol **24**(2): 185-94.
165. Mintz, B. and C. Cronmiller (1981). "METT-1: a karyotypically normal in vitro line of developmentally totipotent mouse teratocarcinoma cells." Somatic Cell Genet **7**(4): 489-505.

166. Mintz, B. and K. Illmensee (1975). "Normal genetically mosaic mice produced from malignant teratocarcinoma cells." Proc Natl Acad Sci U S A **72**(9): 3585-9.
167. Mintz, B., C. Cronmiller, et al. (1978). "Somatic cell origin of teratocarcinomas." Proc Natl Acad Sci U S A **75**(6): 2834-8.
168. Mintz, B., K. Anthony, et al. (1984). "Monoclonal derivation of mouse myeloid and lymphoid lineages from totipotent hematopoietic stem cells experimentally engrafted in fetal hosts." Proc Natl Acad Sci U S A **81**(24): 7835-9.
169. Mitalipov, S. and D. Wolf (2009). "Totipotency, pluripotency and nuclear reprogramming." Adv Biochem Eng Biotechnol **114**: 185-99.
170. Mizuno, S., Y. Sono, et al. (2006). "Expression and subcellular localization of GSE protein in germ cells and preimplantation embryos." J Reprod Dev **52**(3): 429-38.
171. Moller, E., G. Stenman, et al. (2008). "POU5F1, encoding a key regulator of stem cell pluripotency, is fused to EWSR1 in hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands." J Pathol **215**(1): 78-86.
172. Money, N. P. (2002). "Mushroom stem cells." Bioessays **24**(10): 949-52.
173. Moore, J. C., L. W. van Laake, et al. (2005). "Human embryonic stem cells: genetic manipulation on the way to cardiac cell therapies." Reprod Toxicol **20**(3): 377-91.
174. Mora, J. I., J. E. Barroeta, et al. (2004). "Paneth cell carcinoma of the ampulla of Vater." Arch Pathol Lab Med **128**(8): 908-10.
175. Morey, C., P. Navarro, et al. (2004). "The region 3' to Xist mediates X chromosome counting and H3 Lys-4 dimethylation within the Xist gene." Embo J **23**(3): 594-604.
176. Moscatelli, I., E. Pierantozzi, et al. (2009). "p75 neurotrophin receptor is involved in proliferation of undifferentiated mouse embryonic stem cells." Exp Cell Res **315**(18): 3220-32.
177. Muller, W. A., R. Teo, et al. (2004). "Totipotent migratory stem cells in a hydroid." Dev Biol **275**(1): 215-24.
178. Muller, W. E. (2006). "The stem cell concept in sponges (Porifera): Metazoan traits." Semin Cell Dev Biol **17**(4): 481-91.
179. Muller, W. E., M. Korzhev, et al. (2003). "Origin of metazoan stem cell system in sponges: first approach to establish the model (Suberites domuncula)." Biomol Eng **20**(4-6): 369-79.
180. Murphy, C. L. (2006). "Internal standards in differentiating embryonic stem cells in vitro." Methods Mol Biol **329**: 101-12.
181. Nagy, Z. P. and C. C. Chang (2005). "Current advances in artificial gametes." Reprod Biomed Online **11**(3): 332-9.
182. Nakayama, N., I. Fang, et al. (1998). "Natural killer and B-lymphoid potential in CD34+ cells derived from embryonic stem cells differentiated in the presence of vascular endothelial growth factor." Blood **91**(7): 2283-95.
183. Nakayama, N., J. Lee, et al. (2000). "Vascular endothelial growth factor synergistically enhances bone morphogenetic protein-4-dependent lymphohematopoietic cell generation from embryonic stem cells in vitro." Blood **95**(7): 2275-83.
184. Nakhei, H., A. Lingott, et al. (1998). "An alternative splice variant of the tissue specific transcription factor HNF4alpha predominates in undifferentiated murine cell types." Nucleic Acids Res **26**(2): 497-504.
185. Niemann, H., X. C. Tian, et al. (2008). "Epigenetic reprogramming in embryonic and foetal development upon somatic cell nuclear transfer cloning." Reproduction **135**(2): 151-63.
186. Nimeth, K. T., B. Egger, et al. (2007). "Regeneration in Macrostomum lignano (Platyhelminthes): cellular dynamics in the neoblast stem cell system." Cell Tissue Res **327**(3): 637-46.
187. Nimeth, K. T., M. Mahlknecht, et al. (2004). "Stem cell dynamics during growth, feeding, and starvation in the basal flatworm Macrostomum sp. (Platyhelminthes)." Dev Dyn **230**(1): 91-9.
188. Nimeth, K., P. Ladurner, et al. (2002). "Cell renewal and apoptosis in macrostomum sp. [Lignano]." Cell Biol Int **26**(9): 801-15.
189. Nishikawa, S. I., S. Nishikawa, et al. (1998). "Progressive lineage analysis by cell sorting and culture identifies FLK1+VE-cadherin+ cells at a diverging point of endothelial and hemopoietic lineages." Development **125**(9): 1747-57.
190. Ogawa, K., A. Wakayama, et al. (1998). "Identification of a receptor tyrosine kinase involved in germ cell differentiation in planarians." Biochem Biophys Res Commun **248**(1): 204-9.
191. Oh, S. K., H. S. Kim, et al. (2005). "Derivation and characterization of new human embryonic stem cell lines: SNUhES1, SNUhES2, and SNUhES3." Stem Cells **23**(2): 211-9.
192. Ohtaka, M., T. Kumasaka, et al. (2002). "Carcinosarcoma of the esophagus characterized by myoepithelial and ductal differentiations." Pathol Int **52**(10): 657-63.
193. Okoye, U. C., C. C. Malbon, et al. (2008). "Wnt and Frizzled RNA expression in human mesenchymal and embryonic (H7) stem cells." J Mol Signal **3**: 16.
194. Orii, H., T. Sakurai, et al. (2005). "Distribution of the stem cells (neoblasts) in the planarian Dugesia japonica." Dev Genes Evol **215**(3): 143-57.
195. Pagano, J. M., B. M. Farley, et al. (2009). "RNA recognition by the embryonic cell fate determinant and germline totipotency factor MEX-3." Proc Natl Acad Sci U S A **106**(48): 20252-7.
196. Parfenov, V. N., G. N. Pochukalina, et al. (2003). "Nuclear distribution of Oct-4 transcription factor in transcriptionally active and inactive mouse oocytes and its relation to RNA polymerase II and splicing factors." J Cell Biochem **89**(4): 720-32.
197. Patel, P. (2006). "A natural stem cell therapy? How novel findings and biotechnology clarify the ethics of stem cell research." J Med Ethics **32**(4): 235-9.
198. Pawliuk, R., C. Eaves, et al. (1996). "Evidence of both ontogeny and transplant dose-regulated expansion of hematopoietic stem cells in vivo." Blood **88**(8): 2852-8.
199. Pease, S. and R. L. Williams (1990). "Formation of germ-line chimeras from embryonic stem cells maintained with recombinant leukemia inhibitory factor." Exp Cell Res **190**(2): 209-11.
200. Pei, Y., J. Ma, et al. (2003). "Serum-free culture of rhesus monkey embryonic stem cells." Arch Androl **49**(5): 331-42.
201. Pelus, L. M. and S. Fukuda (2008). "Chemokine-mobilized adult stem cells; defining a better hematopoietic graft." Leukemia **22**(3): 466-73.
202. Penn, M. S. and N. Mal (2006). "Stem cells in cardiovascular disease: methods and protocols." Methods Mol Med **129**: 329-51.
203. Pesce, M. and H. R. Scholer (2001). "Oct-4: gatekeeper in the beginnings of mammalian development." Stem Cells **19**(4): 271-8.
204. Pesce, M., M. K. Gross, et al. (1998). "In line with our ancestors: Oct-4 and the mammalian germ." Bioessays **20**(9): 722-32.
205. Pessina, A. and L. Gribaldo (2006). "The key role of adult stem cells: therapeutic perspectives." Curr Med Res Opin **22**(11): 2287-300.
206. Petit, T., B. Raynal, et al. (1994). "Highly sensitive polymerase chain reaction methods show the frequent survival of residual recipient multipotent progenitors after

non-T-cell-depleted bone marrow transplantation." Blood **84**(10): 3575-83.
207. Pinho Mde, F., S. P. Hurtado, et al. (2002). "Myelopoiesis in the omentum of normal mice and during abdominal inflammatory processes." Cell Tissue Res **308**(1): 87-96.
208. Podolsky, D. K. (1993). "Regulation of intestinal epithelial proliferation: a few answers, many questions." Am J Physiol **264**(2 Pt 1): G179-86.
209. Podsakoff, G., K. K. Wong, Jr., et al. (1994). "Efficient gene transfer into nondividing cells by adeno-associated virus-based vectors." J Virol **68**(9): 5656-66.
210. Pomerantz, J. and H. M. Blau (2004). "Nuclear reprogramming: a key to stem cell function in regenerative medicine." Nat Cell Biol **6**(9): 810-6.
211. Prakash, S. K., T. A. Cormier, et al. (2002). "Loss of holocytochrome c-type synthetase causes the male lethality of X-linked dominant microphthalmia with linear skin defects (MLS) syndrome." Hum Mol Genet **11**(25): 3237-48.
212. Priddle, H., D. R. Jones, et al. (2006). "Hematopoiesis from human embryonic stem cells: overcoming the immune barrier in stem cell therapies." Stem Cells **24**(4): 815-24.
213. Psarras, S., N. Karagianni, et al. (2004). "Gene transfer and genetic modification of embryonic stem cells by Cre- and Cre-PR-expressing MESV-based retroviral vectors." J Gene Med **6**(1): 32-42.
214. Ratajczak, M. Z., E. K. Zuba-Surma, et al. (2007). "Bone-marrow-derived stem cells--our key to longevity?" J Appl Genet **48**(4): 307-19.
215. Ratajczak, M. Z., E. K. Zuba-Surma, et al. (2008). "Hunt for pluripotent stem cell -- regenerative medicine search for almighty cell." J Autoimmun **30**(3): 151-62.
216. Ray, R., K. Ray, et al. (1997). "Differential alterations in metabolic pattern of the six major UsnRNAs during development." Mol Cell Biochem **177**(1-2): 79-88.
217. Ray, W. J. and D. I. Gottlieb (1996). "Regulation of protein abundance in pluripotent cells undergoing commitment to the neural lineage." J Cell Physiol **168**(2): 264-75.
218. Raz, E. (2002). "Primordial germ cell development in zebrafish." Semin Cell Dev Biol **13**(6): 489-95.
219. Rich, I. N. (1995). "Primordial germ cells are capable of producing cells of the hematopoietic system in vitro." Blood **86**(2): 463-72.
220. Rindi, G., C. Ratineau, et al. (1999). "Targeted ablation of secretin-producing cells in transgenic mice reveals a common differentiation pathway with multiple enteroendocrine cell lineages in the small intestine." Development **126**(18): 4149-56.
221. Rinkevich, B. and I. Yankelevich (2004). "Environmental split between germ cell parasitism and somatic cell synergism in chimeras of a colonial urochordate." J Exp Biol **207**(Pt 20): 3531-6.
222. Rinkevich, B., Z. Shlemberg, et al. (1995). "Whole-body protochordate regeneration from totipotent blood cells." Proc Natl Acad Sci U S A **92**(17): 7695-9.
223. Robin, C., F. Pflumio, et al. (1999). "Identification of lymphomyeloid primitive progenitor cells in fresh human cord blood and in the marrow of nonobese diabetic-severe combined immunodeficient (NOD-SCID) mice transplanted with human CD34(+) cord blood cells." J Exp Med **189**(10): 1601-10.
224. Roche, E. and B. Soria (2004). "Generation of new islets from stem cells." Cell Biochem Biophys **40**(3 Suppl): 113-24.
225. Rodrigues, P., D. Limback, et al. (2008). "Oogenesis: Prospects and challenges for the future." J Cell Physiol **216**(2): 355-65.
226. Rodriguez, C. I., A. Galan, et al. (2006). "Derivation of clinical-grade human embryonic stem cells." Reprod Biomed Online **12**(1): 112-8.
227. Roelen, B. A. and S. M. Lopes (2008). "Of stem cells and gametes: similarities and differences." Curr Med Chem **15**(13): 1249-56.
228. 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.
229. Rossant, J. (2007). "Stem cells and lineage development in the mammalian blastocyst." Reprod Fertil Dev **19**(1): 111-8.
230. Rossi, L., A. Salvetti, et al. (2006). "DjPiwi-1, a member of the PAZ-Piwi gene family, defines a subpopulation of planarian stem cells." Dev Genes Evol **216**(6): 335-46.
231. Rossi, L., A. Salvetti, et al. (2008). "Planarians, a tale of stem cells." Cell Mol Life Sci **65**(1): 16-23.
232. Sahai, J. and S. G. Louie (1993). "Overview of the immune and hematopoietic systems." Am J Hosp Pharm **50**(7 Suppl 3): S4-9.
233. Salminen, M., B. I. Meyer, et al. (1998). "Efficient poly A trap approach allows the capture of genes specifically active in differentiated embryonic stem cells and in mouse embryos." Dev Dyn **212**(2): 326-33.
234. Salo, E. (2006). "The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes)." Bioessays **28**(5): 546-59.
235. Salvetti, A., L. Rossi, et al. (2005). "DjPum, a homologue of Drosophila Pumilio, is essential to planarian stem cell maintenance." Development **132**(8): 1863-74.
236. Santos, F. and W. Dean (2004). "Epigenetic reprogramming during early development in mammals." Reproduction **127**(6): 643-51.
237. Sato, H., K. Amagai, et al. (2009). "Stable generation of serum- and feeder-free embryonic stem cell-derived mice with full germline-competency by using a GSK3 specific inhibitor." Genesis **47**(6): 414-22.
238. Sauvageau, G., U. Thorsteinsdottir, et al. (1995). "Overexpression of HOXB4 in hematopoietic cells causes the selective expansion of more primitive populations in vitro and in vivo." Genes Dev **9**(14): 1753-65.
239. Scholtz, B., D. Kelly, et al. (1995). "Cis-regulatory elements and transcription factors involved in the regulation of the transforming growth factor-beta 2 gene." Mol Reprod Dev **41**(2): 140-8.
240. Schwartz, P. H. (2008). "Training the next generation of pluripotent stem cell researchers." J Transl Med **6**: 40.
241. Sell, S. (2004). "Stem cell origin of cancer and differentiation therapy." Crit Rev Oncol Hematol **51**(1): 1-28.
242. Senju, S., S. Hirata, et al. (2003). "Generation and genetic modification of dendritic cells derived from mouse embryonic stem cells." Blood **101**(9): 3501-8.
243. Sermon, K. D. (2006). "Preimplantation genetic diagnosis." Verh K Acad Geneeskd Belg **68**(1): 5-32.
244. Shahab, N. (2007). "Extrapulmonary small cell carcinoma of the bladder." Semin Oncol **34**(1): 15-21.
245. Shanthly, N., M. R. Aruva, et al. (2006). "Stem cells: a regenerative pharmaceutical." Q J Nucl Med Mol Imaging **50**(3): 205-16.
246. Shi, C. Z., R. N. Dhir, et al. (1995). "Mouse embryonic stem cells express receptors of the insulin family of growth factors." Mol Reprod Dev **42**(2): 173-9.
247. Shibata, N., Y. Umesono, et al. (1999). "Expression of vasa(vas)-related genes in germline cells and totipotent somatic stem cells of planarians." Dev Biol **206**(1): 73-87.
248. Shigematsu, Y., N. Yoshida, et al. (2007). "Novel embryonic stem cells expressing tdKaede protein photoconvertible from green to red fluorescence." Int J Mol Med **20**(4): 439-44.

249. Shih, C. C., D. DiGiusto, et al. (2002). "Hematopoietic potential of neural stem cells: plasticity versus heterogeneity." Leuk Lymphoma **43**(12): 2263-8.
250. Shiroi, A., M. Yoshikawa, et al. (2002). "Identification of insulin-producing cells derived from embryonic stem cells by zinc-chelating dithizone." Stem Cells **20**(4): 284-92.
251. Shostak, S. (2006). "(Re)defining stem cells." Bioessays **28**(3): 301-8.
252. Sillaber, C., S. Walchshofer, et al. (1999). "Immunophenotypic characterization of human bone marrow endosteal cells." Tissue Antigens **53**(6): 559-68.
253. Silva, J., W. Mak, et al. (2003). "Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes." Dev Cell **4**(4): 481-95.
254. Sims, M. and N. L. First (1994). "Production of calves by transfer of nuclei from cultured inner cell mass cells." Proc Natl Acad Sci U S A **91**(13): 6143-7.
255. Sinha, S., M. H. Hoofnagle, et al. (2004). "Transforming growth factor-beta1 signaling contributes to development of smooth muscle cells from embryonic stem cells." Am J Physiol Cell Physiol **287**(6): C1560-8.
256. Smith, C. (1992). "Retroviral vector-mediated gene transfer into hematopoietic cells: prospects and issues." J Hematother **1**(2): 155-66.
257. Snodgrass, H. R., R. M. Schmitt, et al. (1992). "Embryonic stem cells and in vitro hematopoiesis." J Cell Biochem **49**(3): 225-30.
258. Solana, J., P. Lasko, et al. (2009). "Spoltud-1 is a chromatoid body component required for planarian long-term stem cell self-renewal." Dev Biol **328**(2): 410-21.
259. Soshnikova, N. and D. Duboule (2008). "Epigenetic regulation of Hox gene activation: the waltz of methyls." Bioessays **30**(3): 199-202.
260. Stanworth, S. J. and A. C. Newland (2001). "Stem cells: progress in research and edging towards the clinical setting." Clin Med **1**(5): 378-82.
261. Steeg, C. M., J. Ellis, et al. (1990). "Introduction of specific point mutations into RNA polymerase II by gene targeting in mouse embryonic stem cells: evidence for a DNA mismatch repair mechanism." Proc Natl Acad Sci U S A **87**(12): 4680-4.
262. Stevens, L. C. (1980). "Teratocarcinogenesis and spontaneous parthenogenesis in mice." Results Probl Cell Differ **11**: 265-74.
263. Stevens, N. R., A. A. Raposo, et al. (2007). "From stem cell to embryo without centrioles." Curr Biol **17**(17): 1498-503.
264. 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.
265. Stewart, C. L., I. Gadi, et al. (1994). "Stem cells from primordial germ cells can reenter the germ line." Dev Biol **161**(2): 626-8.
266. Stoltz, J. F., D. Bensoussan, et al. (2006). "Cell and tissue engineering and clinical applications: an overview." Biomed Mater Eng **16**(4 Suppl): S3-S18.
267. Stout, C. L., D. W. Ashley, et al. (2007). "Primitive stem cells residing in the skeletal muscle of adult pigs are mobilized into the peripheral blood after trauma." Am Surg **73**(11): 1106-10.
268. 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.
269. Sun, B. W., A. C. Yang, et al. (2006). "Temporal and parental-specific expression of imprinted genes in a newly derived Chinese human embryonic stem cell line and embryoid bodies." Hum Mol Genet **15**(1): 65-75.
270. Sun, Y., W. Kong, et al. (2009). "CD133 (Prominin) negative human neural stem cells are clonogenic and tripotent." PLoS One **4**(5): e5498.
271. Suwinska, A., R. Czolowska, et al. (2008). "Blastomeres of the mouse embryo lose totipotency after the fifth cleavage division: expression of Cdx2 and Oct4 and developmental potential of inner and outer blastomeres of 16- and 32-cell embryos." Dev Biol **322**(1): 133-44.
272. Szilvassy, S. J., R. K. Humphries, et al. (1990). "Quantitative assay for totipotent reconstituting hematopoietic stem cells by a competitive repopulation strategy." Proc Natl Acad Sci U S A **87**(22): 8736-40.
273. Talbot, N. C., T. J. Caperna, et al. (2002). "The PICM-19 cell line as an in vitro model of liver bile ductules: effects of cAMP inducers, biopeptides and pH." Cells Tissues Organs **171**(2-3): 99-116.
274. Tkemaladze, J. V. and K. N. Chichinadze (2005). "Centriolar mechanisms of differentiation and replicative aging of higher animal cells." Biochemistry (Mosc) **70**(11): 1288-303.
275. Torres-Padilla, M. E. (2008). "Cell identity in the preimplantation mammalian embryo: an epigenetic perspective from the mouse." Hum Reprod **23**(6): 1246-52.
276. Towns, C. R. and D. G. Jones (2004). "Stem cells, embryos, and the environment: a context for both science and ethics." J Med Ethics **30**(4): 410-3.
277. Toyooka, Y., D. Shimosato, et al. (2008). "Identification and characterization of subpopulations in undifferentiated ES cell culture." Development **135**(5): 909-18.
278. Trentin, A., C. Glavieux-Pardanaud, et al. (2004). "Self-renewal capacity is a widespread property of various types of neural crest precursor cells." Proc Natl Acad Sci U S A **101**(13): 4495-500.
279. Triffitt, J. T. (2002). "Stem cells and the philosopher's stone." J Cell Biochem Suppl **38**: 13-9.
280. Tripputi, P., B. Cassani, et al. (2001). "Chromosome 7 monosomy and deletions in myeloproliferative diseases." Leuk Res **25**(9): 735-9.
281. Tronik-Le Roux, D., V. Roullot, et al. (1995). "Suppression of erythro-megakaryocytopoiesis and the induction of reversible thrombocytopenia in mice transgenic for the thymidine kinase gene targeted by the platelet glycoprotein alpha IIb promoter." J Exp Med **181**(6): 2141-51.
282. Tseng, J. E., S. E. Hall, et al. (1995). "Phenotypic and functional analysis of lymphocytes in paroxysmal nocturnal hemoglobinuria." Am J Hematol **50**(4): 244-53.
283. Umiel, T., L. M. Nadler, et al. (1987). "Undifferentiated leukemia of infancy with t(11:17) chromosomal rearrangement. Coexpressing myeloid and B cell restricted antigens." Cancer **59**(6): 1143-9.
284. van de Geijn, G. J., R. Hersmus, et al. (2009). "Recent developments in testicular germ cell tumor research." Birth Defects Res C Embryo Today **87**(1): 96-113.
285. van Inzen, W. G., M. P. Peppelenbosch, et al. (1996). "Neuronal differentiation of embryonic stem cells." Biochim Biophys Acta **1312**(1): 21-6.
286. Verdeil, J. L., L. Alemanno, et al. (2007). "Pluripotent versus totipotent plant stem cells: dependence versus autonomy?" Trends Plant Sci **12**(6): 245-52.
287. Vittet, D., M. H. Prandini, et al. (1996). "Embryonic stem cells differentiate in vitro to endothelial cells through successive maturation steps." Blood **88**(9): 3424-31.
288. von Melchner, H., J. V. DeGregori, et al. (1992). "Selective disruption of genes expressed in totipotent embryonal stem cells." Genes Dev **6**(6): 919-27.
289. Vrtovec, K. T. and B. Vrtovec (2007). "Commentary: is totipotency of a human cell a sufficient reason to exclude its patentability under the European law?" Stem Cells **25**(12): 3026-8.

290. Wada, H., T. Enomoto, et al. (1998). "Carcinosarcoma of the breast: molecular-biological study for analysis of histogenesis." Hum Pathol **29**(11): 1324-8.
291. Wakao, H. (2009). "NKT cells: from totipotency to regenerative medicine." Arch Immunol Ther Exp (Warsz) **57**(2): 117-28.
292. Wakayama, T. (2003). "Cloned mice and embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Oncol Res **13**(6-10): 309-14.
293. Walther, D. J. and M. Bader (1999). "Serotonin synthesis in murine embryonic stem cells." Brain Res Mol Brain Res **68**(1-2): 55-63.
294. Wang, H., S. Wang, et al. (2009). "Oct4 is expressed in Nestin-positive cells as a marker for pancreatic endocrine progenitor." Histochem Cell Biol **131**(5): 553-63.
295. Wang, L. and G. A. Schultz (1996). "Expression of Oct-4 during differentiation of murine F9 cells." Biochem Cell Biol **74**(4): 579-84.
296. Wang, Q. T., K. Piotrowska, et al. (2004). "A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo." Dev Cell **6**(1): 133-44.
297. Ward, C. M. and P. L. Stern (2002). "The human cytomegalovirus immediate-early promoter is transcriptionally active in undifferentiated mouse embryonic stem cells." Stem Cells **20**(5): 472-5.
298. Wartenberg, M., J. Gunther, et al. (1998). "The embryoid body as a novel in vitro assay system for antiangiogenic agents." Lab Invest **78**(10): 1301-14.
299. Wei, C. L., T. Miura, et al. (2005). "Transcriptome profiling of human and murine ESCs identifies divergent paths required to maintain the stem cell state." Stem Cells **23**(2): 166-85.
300. Weisel, K. C., Y. Gao, et al. (2006). "Stromal cell lines from the aorta-gonado-mesonephros region are potent supporters of murine and human hematopoiesis." Exp Hematol **34**(11): 1505-16.
301. Wilmut, I. and L. Paterson (2003). "Somatic cell nuclear transfer." Oncol Res **13**(6-10): 303-7.
302. Wulf, G. M., C. N. Adra, et al. (1993). "Inhibition of hematopoietic development from embryonic stem cells by antisense vav RNA." Embo J **12**(13): 5065-74.
303. Xu, Y., Z. He, et al. (2007). "Murine fertilized ovum, blastomere and morula cells lacking SP phenotype." Sci China C Life Sci **50**(6): 762-5.
304. Yeom, Y. I., G. Fuhrmann, et al. (1996). "Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells." Development **122**(3): 881-94.
305. Yin, Y., Y. K. Lim, et al. (2002). "AFP(+), ESC-derived cells engraft and differentiate into hepatocytes in vivo." Stem Cells **20**(4): 338-46.
306. Yurugi-Kobayashi, T., H. Itoh, et al. (2003). "Effective contribution of transplanted vascular progenitor cells derived from embryonic stem cells to adult neovascularization in proper differentiation stage." Blood **101**(7): 2675-8.
307. Zangrossi, S., M. Marabese, et al. (2007). "Oct-4 expression in adult human differentiated cells challenges its role as a pure stem cell marker." Stem Cells **25**(7): 1675-80.
308. Zeng, X. and M. S. Rao (2006). "The therapeutic potential of embryonic stem cells: A focus on stem cell stability." Curr Opin Mol Ther **8**(4): 338-44.
309. Zhang, M., B. Joseph, et al. (2005). "Embryonic mouse STO cell-derived xenografts express hepatocytic functions in the livers of nonimmunosuppressed adult rats." Stem Cells **23**(2): 186-99.
310. Zhang, X., K. T. Ebata, et al. (2006). "Aging of male germ line stem cells in mice." Biol Reprod **74**(1): 119-24.
311. Zhang, Y. M., C. Hartzell, et al. (2002). "Stem cell-derived cardiomyocytes demonstrate arrhythmic potential." Circulation **106**(10): 1294-9.
312. Zuk, P. A. (2009). "The intracellular distribution of the ES cell totipotent markers OCT4 and Sox2 in adult stem cells differs dramatically according to commercial antibody used." J Cell Biochem **106**(5): 867-77.
313. Christopher C. Fraser, Stephen J. Szilvassy, Connie J. Eaves, And R. Keith Humphries. Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability. Proc. Nati. Acad. Sci. USA. Vol. 89, pp. 1968-1972, 1992.
314. Fraser, C. C., Eaves, C. J., Szilvassy, S. J. & Humphries, R. K. (1990). Blood 76, 1071-1076.
315. Thomas, K. R. & Capecchi, M. R. (1987) Cell 51, 503-512.
316. Baud L, Haymann JP, Bellocq A, Fouqueray B. Contribution of stem cells to renal repair after ischemia/reperfusion. Bull Acad Natl Med. 2005;189(4):635-43.
317. Bavister BD, Wolf DP, Brenner CA. Challenges of primate embryonic stem cell research. Cloning Stem Cells 2005;7(2):82-94.
318. Bernard Lo, Patricia Zettler, Marcelle I. Cedars, Elena Gates, Arnold R. Kriegstein, Michelle Oberman, Renee Reijo Pera, Richard M. Wagner, Mary T. Wuerth, Leslie E. Wolf, Keith R. Yamamoto. A New Era in the Ethics of Human Embryonic Stem Cell Research. Stem Cells. http://www.StemCells.com. http://stemcells.alphamedpress.org/cgi/reprint/2005-0324v1.pdf. 2005.
319. Bhatt RI, Brown MD, Hart CA, Gilmore P, Ramani VAC, George NJ, Clarke NW. Novel method for the isolation and characterisation of the putative prostatic stem cell. Cytometry A. 2003;54(2):89-99.
320. Cantley LG. Adult stem cells in the repair of the injured renal tubule. Nat Clin Pract Nephrol. 2005;1(1):22-32.
321. Condorelli G, Peschle C. Stem cells for cardiac repair: state of the art. Front Biosci 2005;10:3143-50.
322. Daar AS, Sheremeta L. The science of stem cells: ethical, legal and social issues. Exp Clin Transplant. 2003;1(2):139-46.
323. Duffield JS, Bonventre JV. Kidney tubular epithelium is restored without replacement with bone marrow-derived cells during repair after ischemic injury. Kidney Int. 2005;68(5):1956-61.
324. Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. J Clin Invest. 2005;115(7):1743-55.
325. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. Int J Mol Med. 2004;14(6):1035-41.
326. Hishikawa K, Fujita T. Stem cells and kidney disease. Hypertens Res. 2006;29(10):745-9.
327. http://stemcells.nih.gov/staticresources/research/protocols/BresaGen_hESC_manual_2.1.pdf
328. Humphreys BD, Duffield JD, Bonventre JV. Renal stem cells in recovery from acute kidney injury. Minerva Urol Nefrol. 2006;58(1):13-21.
329. Kashofer K, Bonnet D. Gene Therapy Progress and Prospects: Stem cell plasticity. Gene Ther. 2005 (Epub ahead of print).
330. Kimberly Kasow. OPBMT2 Protocol: Allogeneic Hematopoietic Stem Cell Transplantation for Children Affected with Malignant Osteopetrosis - A Pilot Study. http://www.stjude.org/protocols/0,2881,450_2331_17072,00.html. 2007.

331. Kwang-Soo Kim. Stem cell research continues in Korea beyond the Hwang scandal. Stem Cells. http://www.StemCells.com. http://stemcells.alphamedpress.org/cgi/reprint/2007-0089v1.pdf. 2007.
332. Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, Igarashi P. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. J Am Soc Nephrol. 2003;14(5):1188-99.
333. Lin F. Stem cells in kidney regeneration following acute renal injury. Pediatr Res. 2006;59(4 Pt 2):74R-8R.
334. Ma H. Technique of Animal Clone. Nature and Science 2004;2(1):29-35.
335. Mitalipova et al. Stem Cells. 2003;21(5):521-6.
336. Morigi M, Benigni A, Remuzzi G, Imberti B. The regenerative potential of stem cells in acute renal failure. Cell Transplant. 2006;15 Suppl 1:S111-7.
337. Nigel Hawkes. Scientists find the secret of eternal life for stem cells. http://www.timesonline.co.uk/tol/news/uk/article1137674.ece
338. Oliver JA. Adult renal stem cells and renal repair. Curr Opin Nephrol Hypertens. 2004;13(1):17-22.
339. Paul Woodard. SCDHAP Protocol: ematopoietic Stem Cell Transplantation (HSCT) for Patients with Sickle Cell Disease and Prior Stroke or Abnormal Transcranial Doppler Ultrasound (TCD) using Reduced Conditioning and T-Cell-Depleted Hematopoietic Stem Cells from Partially Matched Family Donors - Phase I Study. http://www.stjude.org/protocols/0,2081,450_2327_18472,00.html. 2007.
340. Renee Madden. SCT521 (COG # ASCT0521) Protocol: Soluble Tumor Necrosis Factor Receptor: Enbrel (Etanercept) for the Treatment of Acute Non-Infectious Pulmonary Dysfunction (Idiopathic Pneumonia Syndrome) Following Allogeneic Stem Cell Transplantation. http://www.stjude.org/protocols/0,2881,450_2333_5873,00.html. 2007.
341. Stedman's Medical Dictionary. The American Heritage®. Houghton Mifflin Company. http://dictionary.reference.com/search?q=stem%20cell. 2002.
342. Williams D. Stem cells in medical techonology. Med Device Technol 2005;16(3):9-11.
343. Wing Leung. INFT2 Protocol: HLA - Nonidentical Stem Cell and Natural Killer Cell Transplantation for Children Less than 2 Years of Age with Hematologic Malignancies. http://www.stjude.org/protocols/0,2881,450_2330_11129,00.html. 2007.
344. Yamashita S, Maeshima A, Nojima Y. Involvement of renal progenitor tubular cells in epithelial-to-mesenchymal transition in fibrotic rat kidneys. J Am Soc Nephrol. 2005;16(7):2044-51.
345. de Almeida, M., C. V. de Almeida, E. M. Graner, G. E. Brondani and M. F. de Abreu-Tarazi (2012). "Pre-procambial cells are niches for pluripotent and totipotent stem-like cells for organogenesis and somatic embryogenesis in the peach palm: a histological study." Plant Cell Rep **31**(8): 1495-1515.
346. Hemmat, S., D. M. Lieberman and S. P. Most (2010). "An introduction to stem cell biology." Facial Plast Surg **26**(5): 343-349.
347. Sharpless, N. E. (2010). "Hot topics in stem cells and self-renewal: 2010." Aging Cell **9**(4): 457-461.
348. Shufaro, Y. and B. E. Reubinoff (2011). "Cell cycle synchronization for the purpose of somatic cell nuclear transfer (SCNT)." Methods Mol Biol **761**: 239-247.
349. Surani, M. A. and P. Hajkova (2010). "Epigenetic reprogramming of mouse germ cells toward totipotency." Cold Spring Harb Symp Quant Biol **75**: 211-218.
350. Wang, X. D., K. E. Nolan, R. R. Irwanto, M. B. Sheahan and R. J. Rose (2011). "Ontogeny of embryogenic callus in Medicago truncatula: the fate of the pluripotent and totipotent stem cells." Ann Bot **107**(4): 599-609.
351. Pesce M, Schöler HR. Oct-4: control of totipotency and germline determination. Mol Reprod Dev. 2000 Apr;55(4):452-7.
352. Ma H, Chen G (2005). Stem Cell. J Am Sci. 1(2):90-92. http://www.sciencepub.net/american/0102/14-mahongbao.pdf.
353. Ma H, Chenrg S (2007). Eternal Life and Stem Cell. Nat Sci. 5(1):81-96. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
354. Ma H, Chenrg S (2007). Review of Stem Cell Studies. Nat Sci. 5(2):45-65. http://www.sciencepub.net/nature/0502/09-0247-mahongbao-stem-ns.pdf.
355. Yang Y, Ma H (2010**).** Germ Stem Cell. Stem Cell. 1(2):38-60]. http://www.sciencepub.net/stem/stem0102/07_1348stem0102_38_60.pdf.
356. Pubmed. Stem Cell. http://www.ncbi.nlm.nih.gov/pubmed/?term=stem+cell.
357. Wikipedia. Stem Cell. http://en.wikipedia.org/wiki/Stem_cell.

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