

Totipotent Stem Cell Research Literatures

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

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

Introduction

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

Totipotent stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.

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

Almeida-Porada, G., 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.

Annas, G. J., et al. (1999). "Stem cell politics, ethics and medical progress." *Nat Med* **5**(12): 1339-1341.

Tremendous controversy has surrounded efforts to undertake research on totipotent human stem cells. To date public policy in the United States has attempted to skirt the ethical and social questions raised by this research. Annas et al. argue that research using human embryos as a source of totipotent stem cells can secure broad public support if there is an open and public discussion about the ethical justification for undertaking such research and the assurance of adequate federal regulation and oversight.

Baker, R. K., 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): 201-214.

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

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

Biava, P. M., et al. (2017). "Stem Cell Differentiation Stage Factors and Their Role in Triggering Symmetry Breaking Processes during Cancer Development: A Quantum Field Theory Model

for Reprogramming Cancer Cells to Healthy Phenotypes." *Curr Med Chem*.

A long history of research has pursued the use of embryonic factors isolated during cell differentiation processes for the express purpose of transforming cancer cells back to healthy phenotypes. Recent results have clarified that the substances present at different stages of cell differentiation-which we call stem cell differentiation stage factors (SCDSFs)-are proteins with low molecular weight and nucleic acids that regulate genomic expression. The present review summarizes how these substances, taken at different stages of cellular maturation, are able to retard proliferation of many human tumor cell lines and thereby reprogram cancer cells to healthy phenotypes. The model presented here is a quantum field theory (QFT) model in which SCDSFs are able to trigger symmetry breaking processes during cancer development. These symmetry breaking processes, which lie at the root of many phenomena in elementary particle physics and condensed matter physics, govern the phase transitions of totipotent cells to higher degrees of diversity and order, resulting in cell differentiation. In cancers, which share many genomic and metabolic similarities with embryonic stem cells, stimulated re-differentiation often signifies the phenotypic reversion back to health and non-proliferation. In addition to acting on key components of the cellular cycle, SCDSFs are able to reprogram cancer cells by delicately influencing the cancer microenvironment, modulating the electrochemistry and thus the collective electrodynamic behaviors between dipole networks in biomacromolecules and the interstitial water field. Coherent effects in biological water, which are derived from a dissipative QFT framework, may offer new diagnostic and therapeutic targets at a systemic level, before tumor instantiation occurs in specific tissues or organs. Thus, by including the environment as an essential component of our model, we may push the prevailing paradigm of mutation-driven oncogenesis toward a closer description of reality.

Bidaut, G. and C. J. Stoeckert, Jr. (2009). "Large scale transcriptome data integration across multiple tissues to decipher stem cell signatures." *Methods Enzymol* **467**: 229-245.

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 studying 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>.

Blazquez Fernandez, E. (2004). "[Therapeutic implications of the differentiation of adult stem cells into other cell types]." *An R Acad Nac Med (Madr)* **121**(4): 533-546; discussion 546-539.

During last years stem cells have generated a great interest because its potential therapeutic use. These are unspecialised cells, with ability to self-renewal and to differentiate into other cell types, being embryo cells totipotent and of restrictive use for ethical reasons, while adult stem cells are multipotent and of potential clinical use. Between the latter the best studied are the cells of the bone marrow: hematopoietic producing blood cell lines and mesenchymal that may be transformed into hepatocytes, chondrocytes, osteocytes, adipocytes, cardiocytes and neural cells. Although these facts are promising many studies must be done until to get stem cells therapies, as well as verify these treatments in animal models. It is believed that in this century besides traditional therapies we will have to take into account cell therapies with stem cells, in whose development will be very important the scientific, clinical and ethical parameters.

Brehm, K. (2010). "Echinococcus multilocularis as an experimental model in stem cell research and molecular host-parasite interaction." *Parasitology* **137**(3): 537-555.

Totipotent somatic stem cells (neoblasts) are key players in the biology of flatworms and account for their amazing regenerative capability and developmental plasticity. During recent years, considerable progress has been made in elucidating molecular features of neoblasts from free-living flatworms, whereas their role in parasitic species has so far merely been addressed by descriptive studies. Very recently, however, significant advances have been made in the in vitro culture of neoblasts from the cestode *Echinococcus multilocularis*. The isolated

cells proved capable of generating mature metacystode vesicles under laboratory conditions in a manner that closely resembles the oncosphere-metacystode transition during natural infections. Using the established neoblast cultivation protocols, combined with targeted manipulation of *Echinococcus* genes by RNA-interference, several fundamental questions of host-dependent parasite development can now be addressed. Here, I give an overview of current cultivation techniques for *E. multilocularis* neoblasts and present experimental approaches to study their function. Furthermore, I introduce the *E. multilocularis* genome sequencing project that is presently in an advanced stage. The combined input of data from the *E. multilocularis* sequencing project, stem cell cultivation, and recently initiated attempts to genetically manipulate *Echinococcus* will provide an ideal platform for hypothesis-driven research into cestode development in the next years.

Brustle, O., et al. (1999). "Embryonic stem cell-derived glial precursors: a source of myelinating transplants." *Science* **285**(5428): 754-756.

Self-renewing, totipotent embryonic stem (ES) cells may provide a virtually unlimited donor source for transplantation. A protocol that permits the in vitro generation of precursors for oligodendrocytes and astrocytes from ES cells was devised. Transplantation in a rat model of a human myelin disease shows that these ES cell-derived precursors interact with host neurons and efficiently myelinate axons in brain and spinal cord. Thus, ES cells can serve as a valuable source of cell type-specific somatic precursors for neural transplantation.

Capel, B., et al. (1990). "Long- and short-lived murine hematopoietic stem cell clones individually identified with retroviral integration markers." *Blood* **75**(12): 2267-2270.

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/W^v-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.

Chen, S. K., et al. (2009). "The association of homeobox gene expression with stem cell formation and morphogenesis in cultured *Medicago truncatula*." *Planta* **230**(4): 827-840.

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.

Choi, Y. J., et al. (2017). "Deficiency of microRNA miR-34a expands cell fate potential in pluripotent stem cells." *Science* **355**(6325).

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) efficiently generate all embryonic cell lineages but rarely generate

extraembryonic cell types. We found that microRNA miR-34a deficiency expands the developmental potential of mouse pluripotent stem cells, yielding both embryonic and extraembryonic lineages and strongly inducing MuERV-L (MERVL) endogenous retroviruses, similar to what is seen with features of totipotent two-cell blastomeres. miR-34a restricts the acquisition of expanded cell fate potential in pluripotent stem cells, and it represses MERVL expression through transcriptional regulation, at least in part by targeting the transcription factor Gata2. Our studies reveal a complex molecular network that defines and restricts pluripotent developmental potential in cultured ESCs and iPSCs.

Cogle, C. R., 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.

Corley, L. S. and M. D. Lavine (2006). "A review of insect stem cell types." *Semin Cell Dev Biol* **17**(4): 510-517.

Insect stem cells have been described from both embryonic and adult tissues from a diversity of insect species, although much of the focus in insect stem cell research has been on *Drosophila*. Insects are a vast and

diverse group and it is surprising that a critical aspect of their development like stem cells has not received more attention. In this review we discuss the current state of knowledge of insect stem cell types. We examine what stem cell types have been identified from insects, and briefly discuss what is known about their regulation.

Coulombel, L. (2009). "[Stem cell therapy: an update]." *Bull Acad Natl Med* **193**(3): 535-536.

Medicine will be faced with a major challenge in coming years, namely how to treat for tissue dysfunction due to disease and aging. There are two basic options: drug therapy and cell therapy. Stem cells have been the subject of intense speculation and controversy for several years, as they open up radically new therapeutic possibilities. Classical drugs can only smoothen consequences of tissue dysfunction, whereas cell therapy has the potential to restore tissue function by providing fresh cells. Cell therapy is totally different from organ transplantation, which can only benefit a limited number of patients. The use of the generic term "stem cells" to designate a whole variety of cell types that are present throughout life, is a source of confusion and ambiguity. It will take years of cognitive research to unravel the molecular mechanisms that govern a stem cell's multi- or totipotent status before we can fully exploit this therapeutic tool to the full. The younger a stem cell the greater its potential and, probably, the more durable its benefits, but the use of embryonic stem cells raises ethical issues. The redundancy or equivalence of different categories of cells is another source of controversy, yet researchers must be able to study stem cells in all their diversity, as complementary rather than competitive alternatives, in an acceptable ethical and regulatory environment. We briefly describe the three types of stem cells: pluripotent embryonic stem cells, fetal and adult stem cells, and pluripotent reprogrammed adult somatic cells. Only the former two categories have physiological functions: the first gives rise to tissues and organs while the second maintains tissue function during adulthood.

D'Anselmi, F., et al. (2011). "Zebrafish stem cell differentiation stage factors suppress Bcl-xL release and enhance 5-Fu-mediated apoptosis in colon cancer cells." *Curr Pharm Biotechnol* **12**(2): 261-267.

Stem cell differentiation stage factors (SCDSF), taken from Zebrafish embryos during the stage in which totipotent stem cells are differentiating into pluripotent stem cells, have been shown to inhibit proliferation and induce apoptosis in colon tumors. In order to ascertain if these embryonic factors could synergistically/additively interact with 5-Fluorouracil (5-Fu), whole cell-count, flow-cytometry analysis and

apoptotic parameters were recorded in human colon cancer cells (Caco2) treated with Zebrafish stem cell differentiation stage factors (SCDSF 3 microg/ml) in association or not with 5-Fu in the sub-pharmacological therapeutic range (0.01 mg/ml). Cell proliferation was significantly reduced by SCDSF, meanwhile SCDSF+5-Fu leads to an almost complete growth-inhibition. SCDSF produces a significant apoptotic effect, meanwhile the association with 5-FU leads to an enhanced additive apoptotic rate at both 24 and 72 hrs. SCDSF alone and in association with 5-Fu trigger both the extrinsic and the intrinsic apoptotic pathways, activating caspase-8, -3 and -7. SCDSF and 5-Fu alone exerted opposite effects on Bax and Bcl-xL proteins, meanwhile SCDSF+5-Fu induced an almost complete suppression of Bcl-xL release and a dramatic increase in the Bax/Bcl-xL ratio. These data suggest that zebrafish embryo factors could improve chemotherapy efficacy by reducing anti-apoptotic proteins involved in drug-resistance processes.

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

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

Eaves, C., et al. (1998). "Changes in the cytokine regulation of stem cell self-renewal during ontogeny." *Stem Cells* **16 Suppl 1**: 177-184.

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., et al. (1992). "The human hematopoietic stem cell in vitro and in vivo." *Blood Cells* **18**(2): 301-307.

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.

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

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

Franchi, S., et al. (2014). "Adult stem cell as new advanced therapy for experimental neuropathic pain treatment." *Biomed Res Int* **2014**: 470983.

Neuropathic pain (NP) is a highly invalidating disease resulting as consequence of a lesion or disease affecting the somatosensory system. All the pharmacological treatments today in use give a long lasting pain relief only in a limited percentage of patients before pain reappears making NP an incurable disease. New approaches are therefore needed and research is testing stem cell usage. Several papers have been written on experimental neuropathic pain treatment using stem cells of different origin and species to treat experimental NP. The original idea was based on the capacity of stem cell to offer a totipotent cellular source for replacing injured neural cells and for delivering trophic factors to lesion site; soon the researchers agreed that the capacity of stem cells to contrast NP was not dependent upon their regenerative effect but was mostly linked to a bidirectional interaction between the stem cell and damaged microenvironment resident cells. In this paper we review the preclinical studies produced in the last years assessing the effects induced by several stem cells in different models of neuropathic pain. The

overall positive results obtained on pain remission by using stem cells that are safe, of easy isolation, and which may allow an autologous transplant in patients may be encouraging for moving from bench to bedside, although there are several issues that still need to be solved.

Fulka, J., Jr., et al. (2008). "Cybrid human embryos--warranting opportunities to augment embryonic stem cell research." *Trends Biotechnol* **26**(9): 469-474.

The recent vote in the British Parliament allows scientists in principle to create hybrid embryos by transferring human somatic cell nuclei into animal oocytes. This vote opens a fascinating new area of research with the central aim of generating interspecific lines of embryonic stem cells (ESCs) that could potentially be used to understand development, differentiation, gene expression and genomic compatibility. It will also promote human cell therapies, as well as the pharmaceutical industry's search for new drug targets. If this approach is to be successful, many biological questions need to be answered and, in addition, some moral and ethical aspects must be taken into account.

Funayama, N. (2013). "The stem cell system in demosponges: suggested involvement of two types of cells: archeocytes (active stem cells) and choanocytes (food-entrapping flagellated cells)." *Dev Genes Evol* **223**(1-2): 23-38.

Major questions about stem cell systems include what type (s) of stem cells are involved (unipotent/totipotent/pluripotent/multipotent stem cells) and how the self-renewal and differentiation of stem cells are regulated. Sponges, the sister group of all other animals and probably the earliest branching multicellular lineage of extant animals, are thought to possess totipotent stem cells. This review introduces what is known about the stem cells in sponges based on histological studies and also on recent molecular biological studies that have started to reveal the molecular and cellular mechanisms of the stem cell system in sponges (mainly in demosponges). The currently proposed model of the stem cell system in demosponges is described, and the possible applicability of this model to other classes of sponges is discussed. Finally, a possible scenario of the evolution of stem cells, including how migrating stem cells arose in the urmetazoan (the last common ancestor of metazoans) and the evolutionary origin of germ line cells in the urbilaterian (the last common ancestor of bilaterians), are discussed.

Funayama, N. (2018). "The cellular and molecular bases of the sponge stem cell systems

underlying reproduction, homeostasis and regeneration." *Int J Dev Biol* **62**(6-7-8): 513-525.

The evolution of multicellular organisms is generally thought (and seems likely) to have been accompanied by the evolution of a stem cell system. Sponges, some of the early-evolved metazoans, have totipotent/pluripotent stem cells. Thus, uncovering the cellular and molecular bases of the sponge stem cells will not only be crucial for understanding the ancestral gene repertoire of animal stem cells, but will also give us clues to understanding the evolution of molecular mechanisms for maintaining multipotency (pluripotency) and differentiation ability during animal evolution. Sponges (Porifera) are a large phylum that includes an enormous number of species, whose cellular compositions and life cycles show striking variations. In the last decade, methodologies for molecular studies and sequencing resources have dramatically advanced and made it possible to clearly define stem cells in sponges in cellular and molecular terms. In this review, together with recent studies of sponges in various classes, the following issues will be discussed: i) recent findings that revealed that the previously proposed model that "archeocytes and choanocytes are the two types of stem cells" originally based on work in demosponges can be applied as a unified view of the stem cell system in sponges that have various cellular organizations, ii) the fact that sponge cells are more plastic than previously thought, as shown by recent studies of sponge regeneration both from dissociated cells and upon injury, and iii) the importance of transdifferentiation in sponge stem cell systems and regeneration.

Galloni, M. (2012). "Global irradiation effects, stem cell genes and rare transcripts in the planarian transcriptome." *Int J Dev Biol* **56**(1-3): 103-116.

Stem cells are the closest relatives of the totipotent primordial cell, which is able to spawn millions of daughter cells and hundreds of cell types in multicellular organisms. Stem cells are involved in tissue homeostasis and regeneration, and may play a major role in cancer development. Among animals, planarians host a model stem cell type, called the neoblast, which essentially confers immortality. Gaining insights into the global transcriptional landscape of these exceptional cells takes an unprecedented turn with the advent of Next Generation Sequencing methods. Two Digital Gene Expression transcriptomes of *Schmidtea mediterranea* planarians, with or without neoblasts lost through irradiation, were produced and analyzed. Twenty one bp NlaIII tags were mapped to transcripts in the *Schmidtea* and *Dugesia taxidis*. Differential representation of tags in normal versus irradiated animals reflects differential gene expression. Canonical and non-canonical tags

were included in the analysis, and comparative studies with human orthologs were conducted. Transcripts fell into 3 categories: invariant (including housekeeping genes), absent in irradiated animals (potential neoblast-specific genes, IRDOWN) and induced in irradiated animals (potential cellular stress response, IRUP). Different mRNA variants and gene family members were recovered. In the IR-DOWN class, almost all of the neoblast-specific genes previously described were found. In irradiated animals, a larger number of genes were induced rather than lost. A significant fraction of IRUP genes behaved as if transcript versions of different lengths were produced. Several novel potential neoblast-specific genes have been identified that varied in relative abundance, including highly conserved as well as novel proteins without predicted orthologs. Evidence for a large body of antisense transcripts, for example regulated antisense for the *Smed-piwi1* gene, and evidence for RNA shortening in irradiated animals is presented. Novel neoblast-specific candidates include a peroxiredoxin protein that appears to be preferentially expressed in human embryonic stem cells.

Geier, F., 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.

Giri, T. K., et al. (2018). "Current status of stem cell therapies in tissue repair and regeneration." Curr Stem Cell Res Ther.

Tissue engineering is a multi-disciplinary field such as material science, life science, and bioengineering that are necessary to make artificial tissue or rejuvenate damaged tissue. Numerous tissue repair techniques and substitute now exist even though it has several shortcomings; these shortcomings give a good reason for the continuous research for more acceptable tissue-engineered substitutes. The search for and use of a suitable stem cell in tissue engineering is a promising concept. Stem cells have a distinctive capability to differentiate and self-renew that make more suitable for cell-based therapies in tissue repair and regeneration. This review article focuses on stem cell for tissue engineering and their methods of manufacture with their application in nerve, bone, skin, cartilage, bladder, cardiac, liver tissue repair and regeneration.

Guan, K., et al. (1999). "Embryonic stem cell differentiation models: cardiogenesis, myogenesis, neurogenesis, epithelial and vascular smooth muscle cell differentiation in vitro." Cytotechnology **30**(1-3): 211-226.

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, Y., 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): 3663-3667.

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., 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): 850-856.

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.

Hemmat, S., et al. (2010). "An introduction to stem cell biology." *Facial Plast Surg* **26**(5): 343-349.

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. This article will serve as an overview of the current state of stem cell research as it applies to scientific and medical applications. Included in the discussion is a review of the many different types of stem cells, including but not limited to adult, embryonic, and perinatal stem cells. Also, this article describes somatic cell nuclear transfer, an exciting technology that allows the production of totipotent stem cells from fully differentiated cells, thereby eliminating the use of embryonic sources. This discussion should serve as a review of the field of stem cell biology and provide a foundation for the reader to better understand the interface of stem cell technology and facial plastic and reconstructive surgery.

Ho, L., 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): 5181-5186.

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., et al. (2004). "Nuclear transplantation, embryonic stem cells and the potential for cell therapy." *Hematol J* **5 Suppl 3**: S114-117.

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.

Hug, K. (2005). "Sources of human embryos for stem cell research: ethical problems and their possible solutions." *Medicina (Kaunas)* **41**(12): 1002-1010.

UNLABELLED: 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. However, there already theoretical possibilities of embryonic stem cell research exist, the application of which could decrease the ethical objections to such research. This should be taken into consideration when making decisions on the regulation of embryonic stem cell research. But so far there is no consensus on these questions, and the article presents both favorable and unfavorable opinions regarding this research.

Ikeda, M. and M. Ohme-Takagi (2014). "TCPs, WUSs, and WINDs: families of transcription factors that regulate shoot meristem formation, stem cell maintenance, and somatic cell differentiation." *Front Plant Sci* **5**: 427.

In contrast to somatic mammalian cells, which cannot alter their fate, plant cells can dedifferentiate to form totipotent callus cells and regenerate a whole plant, following treatment with specific phytohormones. However, the regulatory mechanisms and key factors that control differentiation-dedifferentiation and cell totipotency have not been completely clarified in plants. Recently, several plant transcription factors that regulate meristem formation and dedifferentiation have been identified and include members of the TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR (TCP), WUSCHEL (WUS), and WOUND INDUCED DEDIFFERENTIATION (WIND1) families. WUS and WIND positively control plant cell totipotency, while TCP negatively controls it. Interestingly, TCP is a transcriptional activator that acts as a negative regulator of shoot meristem formation, and WUS is a transcriptional repressor that positively maintains totipotency of the stem cells of the shoot meristem. We describe here the functions of TCP, WUS, and WIND transcription factors in the regulation of differentiation-dedifferentiation by positive and negative transcriptional regulators.

Ilic, D., et al. (2009). "Derivation of human embryonic stem cell lines from biopsied blastomeres on human feeders with minimal exposure to xenomaterials." *Stem Cells Dev* **18**(9): 1343-1350.

In a continuous effort to improve the generation of therapeutic grade human embryonic stem cell (hESC) lines, we focused on preserving developmental

capacity of the embryos, minimizing the exposure to xenomaterials, increasing derivation efficacy, and reducing the complexity of the derivation procedure. In this study, we describe an improved method for efficient derivation of hESC lines from blastomeres of biopsied embryos. Our protocol substituted feeder cells of mouse origin with human foreskin fibroblasts (HFFs), limited serum exposure of cells to formation of the initial outgrowth, and increased derivation efficacy from 12.5% (one hESC line out of 13 biopsies) to 50% (3 out of 6 biopsies) by using early population doubling (PD) HFFs. In addition, it eliminated a need for embryo-blastomere coculture, thus reducing the complexity of the culture and enabling continued development of the biopsied embryo under optimal conditions. All derived lines maintained normal karyotype and expressed totipotent phenotype including the ability to differentiate into trophectoderm and all three germ layers.

Irie, N., et al. (2018). "What Can Stem Cell Models Tell Us About Human Germ Cell Biology?" *Curr Top Dev Biol* **129**: 25-65.

Fusion of sperm and egg generates a totipotent zygote that develops into a whole organism. Accordingly, the "immortal" germline transmits genetic and epigenetic information to subsequent generations with consequences for human health and disease. In mammals, primordial germ cells (PGCs) originate from peri-gastrulation embryos. While early human embryos are inaccessible for research, in vitro model systems using pluripotent stem cells have provided critical insights into human PGC specification, which differs from that in mice. This might stem from significant differences in early embryogenesis at the morphological and molecular levels, including pluripotency networks. Here, we discuss recent advances and experimental systems used to study mammalian germ cell development. We also highlight key aspects of germ cell disorders, as well as mitochondrial and potentially epigenetic inheritance in humans.

Ivanova, N. B., et al. (2002). "A stem cell molecular signature." *Science* **298**(5593): 601-604.

Mechanisms regulating self-renewal and cell fate decisions in mammalian stem cells are poorly understood. We determined global gene expression profiles for mouse and human hematopoietic stem cells and other stages of the hematopoietic hierarchy. Murine and human hematopoietic stem cells share a number of expressed gene products, which define key conserved regulatory pathways in this developmental system. Moreover, in the mouse, a portion of the genetic program of hematopoietic stem cells is shared with embryonic and neural stem cells. This

overlapping set of gene products represents a molecular signature of stem cells.

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): 1111-1117.

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.

Khanlarkhani, N., et al. (2016). "Multipotent Stem Cell and Reproduction." *J Stem Cells* **11**(4): 219-229.

Stem cells are self-renewing and undifferentiated cell types that can be differentiated into functional cells. Stem cells can be classified into two main types based on their source of origin: Embryonic and Adult stem cells. Stem cells also classified based on the range of differentiation potentials into Totipotent, Pluripotent, Multipotent, and Unipotent. Multipotent stem cells have the ability to differentiate into all cell types within one particular lineage. There are plentiful advantages and usages for multipotent stem cells. Multipotent Stem cells act as a significant key in procedure of development, tissue repair, and protection. The accessibility and adaptability of these amazing cells create them a great therapeutic choice for different part of medical approaches, and it becomes interesting topic in the scientific researches to found obvious method for the most advantageous use of MSC-based therapies. Recent studies in the field of stem cell biology have provided new perspectives and opportunities for the treatment of infertility disorders.

Koziol, U., et al. (2014). "The unique stem cell system of the immortal larva of the human parasite *Echinococcus multilocularis*." *Evodevo* **5**(1): 10.

BACKGROUND: It is believed that in tapeworms a separate population of undifferentiated

cells, the germinative cells, is the only source of cell proliferation throughout the life cycle (similar to the neoblasts of free living flatworms). In *Echinococcus multilocularis*, the metacestode larval stage has a unique development, growing continuously like a mass of vesicles that infiltrate the tissues of the intermediate host, generating multiple protoscoleces by asexual budding. This unique proliferation potential indicates the existence of stem cells that are totipotent and have the ability for extensive self-renewal. **RESULTS:** We show that only the germinative cells proliferate in the larval vesicles and in primary cell cultures that undergo complete vesicle regeneration, by using a combination of morphological criteria and by developing molecular markers of differentiated cell types. The germinative cells are homogeneous in morphology but heterogeneous at the molecular level, since only sub-populations express homologs of the post-transcriptional regulators *nanos* and *argonaute*. Important differences are observed between the expression patterns of selected neoblast marker genes of other flatworms and the *E. multilocularis* germinative cells, including widespread expression in *E. multilocularis* of some genes that are neoblast-specific in planarians. Hydroxyurea treatment results in the depletion of germinative cells in larval vesicles, and after recovery following hydroxyurea treatment, surviving proliferating cells grow as patches that suggest extensive self-renewal potential for individual germinative cells. **CONCLUSIONS:** In *E. multilocularis* metacestodes, the germinative cells are the only proliferating cells, presumably driving the continuous growth of the larval vesicles. However, the existence of sub-populations of the germinative cells is strongly supported by our data. Although the germinative cells are very similar to the neoblasts of other flatworms in function and in undifferentiated morphology, their unique gene expression pattern and the evolutionary loss of conserved stem cell regulators suggest that important differences in their physiology exist, which could be related to the unique biology of *E. multilocularis* larvae.

Krichevsky, A. M., et al. (2006). "Specific microRNAs modulate embryonic stem cell-derived neurogenesis." *Stem Cells* **24**(4): 857-864.

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.

Lang, D., et al. (2005). "Pax3 functions at a nodal point in melanocyte stem cell differentiation." *Nature* **433**(7028): 884-887.

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.

Larijani, B., et al. (2012). "Stem cell therapy in treatment of different diseases." *Acta Med Iran* **50**(2): 79-96.

Stem cells are undifferentiated cells with the ability of proliferation, regeneration, conversion to differentiated cells and producing various tissues. Stem cells are divided into two categories of embryonic and adult. In another categorization stem

cells are divided to Totipotent, Multipotent and Unipotent cells. So far usage of stem cells in treatment of various blood diseases has been studied (such as lymphoblastic leukemia, myeloid leukemia, thalassemia, multiple myeloma and sickle cell anemia). In this paper the goal is evaluation of cell therapy in treatment of Parkinson's disease, Amyotrophic lateral sclerosis, Alzheimer, Stroke, Spinal Cord Injury, Multiple Sclerosis, Radiation Induced Intestinal Injury, Inflammatory Bowel Disease, Liver Disease, Duchenne Muscular Dystrophy, Diabetes, Heart Disease, Bone Disease, Renal Disease, Chronic Wounds, Graft-Versus-Host Disease, Sepsis and Respiratory diseases. It should be mentioned that some disease that are the target of cell therapy are discussed in this article.

Lemischka, I. R. (1991). "Clonal, in vivo behavior of the totipotent hematopoietic stem cell." *Semin Immunol* **3**(6): 349-355.

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, 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**: 113-125.

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.

Liang, Z., et al. (2015). "Calpain-Mediated Positional Information Directs Cell Wall Orientation to Sustain Plant Stem Cell Activity, Growth and Development." *Plant Cell Physiol* **56**(9): 1855-1866.

Eukaryotic development and stem cell control depend on the integration of cell positional sensing with cell cycle control and cell wall positioning, yet few factors that directly link these events are known. The DEFECTIVE KERNEL1 (DEK1) gene encoding the unique plant calpain protein is fundamental for development and growth, being essential to confer and maintain epidermal cell identity that allows development beyond the globular embryo stage. We show that DEK1 expression is highest in the actively dividing cells of seeds, meristems and vasculature. We further show that eliminating Arabidopsis DEK1 function leads to changes in developmental cues from the first zygotic division onward, altered microtubule patterns and misshapen cells, resulting in early embryo abortion. Expression of the embryonic marker genes *WOX2*, *ATML1*, *PIN4*, *WUS* and *STM*, related to axis organization, cell identity and meristem functions, is also altered in *dek1* embryos. By monitoring cell layer-specific DEK1 down-regulation, we show that L1- and 35S-induced down-regulation mainly affects stem cell functions, causing severe shoot apical meristem phenotypes. These results are consistent with

a requirement for DEK1 to direct layer-specific cellular activities and set downstream developmental cues. Our data suggest that DEK1 may anchor cell wall positions and control cell division and differentiation, thereby balancing the plant's requirement to maintain totipotent stem cell reservoirs while simultaneously directing growth and organ formation. A role for DEK1 in regulating microtubule-orchestrated cell wall orientation during cell division can explain its effects on embryonic development, and suggests a more general function for calpains in microtubule organization in eukaryotic cells.

Livraghi, T., et al. (2011). "Complete response in 5 out of 38 patients with advanced hepatocellular carcinoma treated with stem cell differentiation stage factors: case reports from a single centre." Curr Pharm Biotechnol **12**(2): 254-260.

Hepatocellular carcinoma (HCC) represents the third cause of cancer-related death. Because HCC is multi-centric with time, excluding the few transplanted patients, sooner or later it becomes untreatable with loco-regional therapies and, until some years ago, it was not responsive to systemic therapies. In 2005 a randomized trial indicated the efficacy of a product containing stem cell differentiation stage factors (SCDSF) taken from zebra fish embryos during the stage in which the totipotent stem cells are differentiating into the pluripotent adult stem cells. In such a trial the patients, with "intermediate" and "advanced" HCC according to BCLC/AASLD guidelines, presented benefit in terms of performance status (PS) and objective tumoral response, with some cases (2.4%) of complete response (CR). The aim of this cohort study is to report the experience of a tertiary referral center on the evidence of cases of CR in patients with "advanced" stage HCC treated with SCDSF as supportive care. CR was regarded as sustained disappearance of the neoplastic areas or blood supply therein, accompanied by normalization of AFP levels. Out of 49 patients consecutively recruited and retrospectively evaluated, 38 had "advanced" stage and 11 "terminal" stage. In 5 patients with "advanced" stage a sustained CR was reported (13.1%). Improvement on PS was obtained in 17 patients (34.6%). No side effects occurred. SCDSF treatment confirmed its efficacy in patients with "advanced" HCC, in terms of PS and tumoral response.

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

Embryonic stem (ES) cells have the ability to differentiate into a variety of cell lineages. We are examining ES cell differentiation in vitro by using

cDNA microarrays to generate a molecular phenotype for each cell type. E14 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 Mpl) 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 well 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.

Lu, W. G., 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): 51-57.

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., et al. (2006). "A focused microarray to assess dopaminergic and glial cell differentiation from fetal tissue or embryonic stem cells." *Stem Cells* **24**(4): 865-875.

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.

Lysdahl, H., et al. (2006). "Derivation and characterization of four new human embryonic stem cell lines: the Danish experience." *Reprod Biomed Online* **12**(1): 119-126.

In September 2003, legislation approved in Denmark legalized work on surplus human embryos from IVF for clinical purposes to establish human

embryonic stem (ES) cell cultures. The aim of this study was to establish such stem cell lines. Fresh surplus embryos were donated after informed consent from the donors. Embryos were cultured into blastocysts and using the immunosurgery procedure, inner cell masses were isolated and cultured on irradiated human foreskin fibroblasts in KnockOut D-MEM supplemented with KnockOut Serum Replacement, bFGF, and LIF. Within a period of 12 months, 198 embryos were donated. Four isolated inner cell masses developed into putative ES cell lines, CLS1, CLS2, CLS3, CLS4, which have now been continuously cultured for eight months, corresponding to 30 passages. These cells expressed markers for undifferentiated human ES cells: stage-specific embryonic antigen-4, tumour-related antigen (TRA)-1-60, TRA-1-81, OCT4, NANOG, SOX2, and FGF4. The cells expressed high levels of telomerase activity, had a normal karyotype, and have been successfully cryopreserved and thawed. Finally, the cells displayed the potential to differentiate in vitro into cell types originating from all three germ layers. It is thought that the cell lines described in this study are the first human ES cells established in Denmark.

Macfarlan, T. S., et al. (2012). "Embryonic stem cell potency fluctuates with endogenous retrovirus activity." *Nature* **487**(7405): 57-63.

Embryonic stem (ES) cells are derived from blastocyst-stage embryos and are thought to be functionally equivalent to the inner cell mass, which lacks the ability to produce all extraembryonic tissues. Here we identify a rare transient cell population within mouse ES and induced pluripotent stem (iPS) cell cultures that expresses high levels of transcripts found in two-cell (2C) embryos in which the blastomeres are totipotent. We genetically tagged these 2C-like ES cells and show that they lack the inner cell mass pluripotency proteins Oct4 (also known as Pou5f1), Sox2 and Nanog, and have acquired the ability to contribute to both embryonic and extraembryonic tissues. We show that nearly all ES cells cycle in and out of this privileged state, which is partially controlled by histone-modifying enzymes. Transcriptome sequencing and bioinformatic analyses showed that many 2C transcripts are initiated from long terminal repeats derived from endogenous retroviruses, suggesting this foreign sequence has helped to drive cell-fate regulation in placental mammals.

Maeda, T., et al. (2011). "Telomerase inhibition promotes an initial step of cell differentiation of primate embryonic stem cell." *Biochem Biophys Res Commun* **407**(3): 491-494.

Embryonic stem (ES) cell is well known as a totipotent cell, which is derived from a blastocyst and has potential to differentiate into every kind of somatic cell. ES cell bears self-renewal characteristic as well as differentiation potential. ES cell bears telomerase activity to avoid telomere shortening, which is a characteristic of differentiated somatic cells. As the differentiation of ES cells proceeds, their telomerase activity is losing. However, it has not been convinced whether suppression of the telomerase activity promotes progression of ES cell differentiation. The effect of telomerase inhibitor on the differentiation potential of marmoset ES cell was assessed, counting cells expressing embryonic markers (alkaline phosphatase and TPA-1-60) under existence of a telomerase inhibitor. Telomerase inhibitor showed a promotional effect for the marmoset ES cell differentiation. This result suggests that exogenous inhibition of telomerase activity leads to induction of an early differentiation of primate ES cell.

Marguerie, G. and D. Tronik-Le Roux (1998). "Analysis of hematopoietic stem cell reprogramming with toxigenicity." *Stem Cells* **16 Suppl 2**: 85-89.

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 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.

Matthews, W., et al. (1991). "A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations." *Cell* **65**(7): 1143-1152.

To elucidate the molecular biology of the hematopoietic stem cell, we have begun to isolate genes from murine cell populations enriched in stem cell activity. One such cDNA encodes a novel receptor tyrosine kinase, designated fetal liver kinase-2 or flk-2, which is related to the W locus gene product c-kit. Expression analyses suggest an extremely restricted distribution of flk-2. It is expressed in populations enriched for stem cells and primitive uncommitted progenitors, and is absent in populations containing more mature cells. Therefore, this receptor may be a key signal transducing component in the totipotent hematopoietic stem cell and its immediate self-renewing progeny.

Meng, G. L., et al. (2002). "[Discussion of the methods for establishing embryonic stem cell lines from 129/ter. C57BL/6J mice with high efficiency]." *Sheng Wu Gong Cheng Xue Bao* **18**(6): 740-743.

A new method for establishing ES cell lines from 129/ter. C57BL/6J mice was set up which was characterized by the murine embryonic fibroblast cell (MEF) feeder, the medium of rat heart cell-conditioned medium (RH-CM) for ES cells, and the consecutive digestion by the digestion liquid containing 1% serum. Every group of improved experiments was done with a control of routine method. The results showed that, compared with routine method, the improved way increased the ratio of ES cell lines of 129/ter mice from 11.8% to 33.3%, and of C57BL/6J from 3.7% to 13.3%. The difference is distinct. The passage culture of ES cells showed that, compared with medium added LIF, RH-CM not only inhibited the differentiation of murine ES cells, maintained its diploid karyotype, but also promote its adherence growth. This kind of culture condition not only maintained the ES cells in an undifferentiated state and their normal diploid karyotype, but also a series of other characteristics of totipotent embryonic stem cells during extended culture period.

Metzger, J. M., et al. (1995). "Myosin heavy chain expression in contracting myocytes isolated during embryonic stem cell cardiogenesis." *Circ Res* **76**(5): 710-719.

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.

Mishra, P. K., et al. (2013). "Cardiac stem cell niche, MMP9, and culture and differentiation of embryonic stem cells." *Methods Mol Biol* **1035**: 153-163.

Embryonic stem cells (ESC) are totipotent, self-renewing, and clonogenic, having potential to differentiate into a wide variety of cell types. Due to regenerative capability, it has tremendous potential for treating myocardial infarction (death of myocardial tissue) and type 1 diabetes (death of pancreatic beta cells). Understanding the components regulating ESC differentiation is the key to unlock the regenerative potential of ESC-based therapies. Both the stiffness of extracellular matrix (ECM) and surrounding niche/microenvironment play pivotal roles in ESC differentiation. Matrix metalloproteinase-9 (MMP9) induces fibrosis that causes stiffness of the ECM and impairs differentiation of cardiac stem cells into cardiomyocytes. Here, we describe the method of ESC culture and differentiation, and the expression of

MMP9 and its inhibitor, tissue inhibitor of metalloproteinase-4 (TIMP4) in differentiating ESC.

Moller, E., 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.

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

Almost 7 years after their first derivation from human embryos, a pressing urgency to deliver the promises of therapies based on human embryonic stem cells (hESC) has arisen. Protocols have been developed to support long-term growth of undifferentiated cells and partially direct differentiation to specific cell lineages. The stage has almost been set for the next step: transplantation in animal models of human disease. Here, we review the state-of-the-art with respect to the transplantation of embryonic stem cell-derived heart cells in animals. One problem affecting progress in this area and functional analysis in vivo in general, is the availability of genetically marked hESC. There are only a few cell lines that express reporter genes ubiquitously, and none is associated with particular

lineages; a major hurdle has been the resistance of hESC to established infection and chemical transfection methodologies to introduce ectopic genes. The methods that have been successful are reviewed. We also describe the processes for generating a new, genetically-modified hESC line that constitutively expresses GFP as well as some of its characteristics, including its ability to form cardiomyocytes with electrophysiological properties of ventricular-like cells.

Muller, W. E. (2006). "The stem cell concept in sponges (Porifera): Metazoan traits." *Semin Cell Dev Biol* **17**(4): 481-491.

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., et al. (2003). "Origin of metazoan stem cell system in sponges: first approach to establish the model (*Suberites domuncula*)." *Biomol Eng* **20**(4-6): 369-379.

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.

Nakayama, N., et al. (2003). "Macroscopic cartilage formation with embryonic stem-cell-derived mesodermal progenitor cells." *J Cell Sci* **116**(Pt 10): 2015-2028.

The totipotent embryonic stem cell generates various mesodermal cells when stimulated with BMP4. Among the resulting cells, those expressing *flk-1* and/or PDGFRalpha displayed chondrogenic activity in the presence of TGFbeta3 and expressed cartilage-specific genes in 7 to 16 day pellet cultures. Depositions of cartilage matrix and type II collagen were detected by day 14. TGFbeta-stimulated chondrogenesis was synergistically enhanced by PDGF-BB, resulting in a larger cartilage particle filled with a cartilaginous area containing type II collagen, with a surface cell layer expressing type I collagen. In contrast, *noggin* inhibited both the TGFbeta- and TGFbeta+PDGF-stimulated cartilage formation, suggesting that a BMP-dependent pathway is involved. In fact, replacement of TGFbeta3 with BMP4 on days 10 to 12 markedly elevated the cartilage matrix deposition during the following 7 to 8 days. Moreover, culture with TGFbeta3 and PDGF-BB, followed by the incubation with BMP4 alone, resulted in a cartilage particle lacking type I collagen in the matrix and the surface layer, which suggests hyaline cartilage formation. Furthermore, such hyaline cartilage particles were mineralized. These studies indicate that the PDGFRalpha+ and/or *flk-1*+ cells derived from embryonic stem cells possess the full developmental potential toward chondrocytes, in common with embryonic mesenchymal cells.

Nakayama, N., 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): 2275-2283.

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.

Nimeth, K. T., et al. (2007). "Regeneration in *Macrostomum lignano* (Platyhelminthes): cellular dynamics in the neoblast stem cell system." *Cell Tissue Res* **327**(3): 637-646.

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., et al. (2004). "Stem cell dynamics during growth, feeding, and starvation in the basal flatworm *Macrostomum* sp. (Platyhelminthes)." *Dev Dyn* **230**(1): 91-99.

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.

Oh, S. K., et al. (2005). "Derivation and characterization of new human embryonic stem cell lines: SNUhES1, SNUhES2, and SNUhES3." *Stem Cells* **23**(2): 211-219.

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.

Pan, G. J., et al. (2002). "Stem cell pluripotency and transcription factor Oct4." *Cell Res* **12**(5-6): 321-329.

Mammalian cell totipotency is a subject that has fascinated scientists for generations. A long lasting question whether some of the somatic cells retains

totipotency was answered by the cloning of Dolly at the end of the 20th century. The dawn of the 21st has brought forward great expectations in harnessing the power of totipotency in medicine. Through stem cell biology, it is possible to generate any parts of the human body by stem cell engineering. Considerable resources will be devoted to harness the untapped potentials of stem cells in the foreseeable future which may transform medicine as we know today. At the molecular level, totipotency has been linked to a singular transcription factor and its expression appears to define whether a cell should be totipotent. Named Oct4, it can activate or repress the expression of various genes. Curiously, very little is known about Oct4 beyond its ability to regulate gene expression. The mechanism by which Oct4 specifies totipotency remains entirely unresolved. In this review, we summarize the structure and function of Oct4 and address issues related to Oct4 function in maintaining totipotency or pluripotency of embryonic stem cells.

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): 235-239.

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.

Pomerantz, J. and H. M. Blau (2004). "Nuclear reprogramming: a key to stem cell function in regenerative medicine." *Nat Cell Biol* **6**(9): 810-816.

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.

Priddle, H., et al. (2006). "Hematopoiesis from human embryonic stem cells: overcoming the immune barrier in stem cell therapies." *Stem Cells* **24**(4): 815-824.

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. This review proposes that more efficient differentiation of hESCs to the tolerogenic cell types required is most likely to occur through applying knowledge gained of the ontogeny of complex regulatory signals used by the embryo for definitive hematopoietic development in vivo. Stepwise formation of mesoderm, induction of definitive hematopoietic stem cells, and the application of factors key to their self-renewal may improve in vitro production both quantitatively and qualitatively.

Ratajczak, M. Z., et al. (2016). "Stem cells and clinical practice: new advances and challenges at the time of emerging problems with induced pluripotent stem cell therapies." *Pol Arch Med Wewn* **126**(11): 879-890.

Humans, like other species that reproduce sexually, originate from a fertilized oocyte (zygote), which is a totipotent stem cell giving rise to an adult organism. During the process of embryogenesis, stem cells at different levels of the developmental hierarchy establish all 3 germ layers and give rise to tissue-committed stem cells, which are responsible for rejuvenation of a given tissue or organ. The robustness of the stem cell compartment is one of the major factors that directly impact life quality as well as lifespan. Stem cells continuously replace cells and tissues that are used up during life; however, this replacement occurs at a different pace in various organs. The rapidly developing field of regenerative medicine is taking advantage of these physiological properties of stem cells and is attempting to employ them in clinical settings to regenerate damaged organs (eg, the heart, liver or bone). For this purpose, the stem cells most successfully employed so far are adult tissue-derived stem cells isolated mainly from bone marrow, mobilized peripheral blood, umbilical cord blood, fat tissue, and even myocardial biopsies. At the same time, attempts to employ embryonic stem cells and induced pluripotent stem cells in the clinic have failed due to their genomic instability and the risk of tumor formation. In this review, we will discuss the various potential sources of stem cells that are currently employed in regenerative medicine and the mechanisms that explain their beneficial effects. We will also highlight the preliminary results of clinical trials as well as the emerging problems relating to stem cell therapies in cardiology.

Ratajczak, M. Z., et al. (2008). "Hunt for pluripotent stem cell -- regenerative medicine search for almighty cell." *J Autoimmun* **30**(3): 151-162.

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.

Regitnig, P., et al. (2001). "Insulinoma of the pancreas with insular-ductular differentiation in its liver metastasis--indication of a common stem-cell origin of the exocrine and endocrine components." *Virchows Arch* **438**(6): 624-628.

We describe an insulinoma of the pancreas in a 56-year-old patient, which showed insular-ductular differentiation in its liver metastasis. Although the primary tumor was uniformly endocrine in nature with insulin production, the metastasis contained two distinct cell types in organoid arrangement. One cell type was insulin-positive and was arranged in islet-like structures; the other was insulin-negative but distinctly pan-cytokeratin and cytokeratin 7 positive and arranged in ducts. In the primary tumor and the metastasis, the tumor cells were surrounded by a desmoplastic stroma. As to the histogenesis of the tumor and its metastasis, we discuss the following possibilities: (1) the tumor cells might derive from a common stem cell that matures into two phenotypically different cell lines, resembling the situation in embryogenesis and (2) one tumor cell type originates from the other by transdifferentiation (metaplasia). We conclude that the parallel occurrence of endocrine and ductal differentiation supports the concept that, under certain conditions, islet cells and ductular cells may also originate from islets and that mixed endocrine/exocrine pancreatic tumors do not necessarily arise from totipotent duct cells but might also have a primary endocrine cell origin.

Resnik, D. B. (2007). "Embryonic stem cell patents and human dignity." *Health Care Anal* **15**(3): 211-222.

This article examines the assertion that human embryonic stem cells patents are immoral because they violate human dignity. After analyzing the concept of human dignity and its role in bioethics debates, this article argues that patents on human embryos or totipotent embryonic stem cells violate human dignity, but that patents on pluripotent or multipotent stem cells do not. Since patents on pluripotent or multipotent stem cells may still threaten human dignity by encouraging people to treat embryos as property, patent agencies should carefully monitor and control these patents to ensure that patents are not inadvertently awarded on embryos or totipotent stem cells.

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

Embryonic germ (EG) cells of line EG-1 derived from mouse primordial germ cells were investigated for their *in vitro* differentiation capacity. By cultivation as embryo-like aggregates EG-1 cells differentiated into cardiac, skeletal muscle and neuronal cells accompanied by the expression of tissue-specific genes and proteins as shown by RT-PCR analysis and indirect immunofluorescence. In comparison to embryonic stem (ES) cells of line D3 the efficiency of differentiation into cardiac and muscle cells was comparatively low, whereas spontaneous neuronal differentiation was more efficient than in D3 cells. Furthermore, the distribution of cell cycle phases as a parameter for the differentiation state was analysed in undifferentiated EG cells and ES cells and compared to data obtained for embryonic carcinoma (EC) cells of line P19 and differentiated, epithelioid EPI-7 cells. Flow cytometric analysis revealed similar cell cycle phase distributions in EG, EC and ES cells. In contrast, the somatic differentiated EPI-7 cells showed a longer G1-phase and shorter S- and G2/M-phases. Together, our results demonstrate that the differentiation state and capacity of EG cells *in vitro* resemble that of totipotent ES cells.

Salo, E. (2006). "The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes)." *Bioessays* **28**(5): 546-559.

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.

Salveti, A., et al. (2005). "DjPum, a homologue of *Drosophila* Pumilio, is essential to planarian stem cell maintenance." *Development* **132**(8): 1863-1874.

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.

Sato, H., 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): 414-422.

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.

Schlatt, S. (2002). "Spermatogonial stem cell preservation and transplantation." *Mol Cell Endocrinol* **187**(1-2): 107-111.

Spermatogonia are the male germ line stem cells. Their life long expansion is needed for permanent production of male germ cells. Spermatogonia are the only cells of the germ line, which proliferate in

adulthood and offer interesting applications as they are potentially totipotent and immortal cells. This review presents some of the recent breakthroughs, which have led to a better understanding of spermatogonial physiology and opened new fields of basic research and of clinical applications in veterinary and medical science.

Schoorlemmer, J., et al. (2014). "Regulation of Mouse Retroelement MuERV-L/MERVL Expression by REX1 and Epigenetic Control of Stem Cell Potency." *Front Oncol* 4: 14.

About half of the mammalian genome is occupied by DNA sequences that originate from transposable elements. Retrotransposons can modulate gene expression in different ways and, particularly retrotransposon-derived long terminal repeats, profoundly shape expression of both surrounding and distant genomic loci. This is especially important in pre-implantation development, during which extensive reprogramming of the genome takes place and cells pass through totipotent and pluripotent states. At this stage, the main mechanism responsible for retrotransposon silencing, i.e., DNA methylation, is inoperative. A particular retrotransposon called muERV-L/MERVL is expressed during pre-implantation stages and contributes to the plasticity of mouse embryonic stem cells. This review will focus on the role of MERVL-derived sequences as controlling elements of gene expression specific for pre-implantation development, two-cell stage-specific gene expression, and stem cell pluripotency, the epigenetic mechanisms that control their expression, and the contributions of the pluripotency marker REX1 and the related Yin Yang 1 family of transcription factors to this regulation process.

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 sur le Traitement du Cancer, etc. Paris. (1829); Editorial 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 Chirurgia 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.

Sharif, T., et al. (2011). "Selective proapoptotic activity of polyphenols from red wine on teratocarcinoma cell, a model of cancer stem-like cell." *Invest New Drugs* **29**(2): 239-247.

Cancer stem cells are expected to be responsible for tumor initiation and metastasis. These cells are therefore potential targets for innovative anticancer therapies. However, the absence of bona fide cancer stem cell lines is a real problem for the development of such approaches. Since teratocarcinoma cells are totipotent stem cells with a high degree of malignancy, we used them as a model of cancer stem cells in order to evaluate the anticancer chemopreventive activity of red wine polyphenols (RWPs) and to determine the underlying cellular and molecular mechanisms. We therefore investigated the effects of RWPs on the embryonal carcinoma (EC) cell line P19 which was grown in the same culture conditions as the most appropriate normal cell line counterpart, the pluripotent embryonic fibroblast cell line NIH/3T3. The present study indicates that RWPs selectively inhibited the proliferation of P19 EC cells and induced G1 cell cycle arrest in a dose-dependent manner. Moreover, RWPs treatment specifically triggered apoptosis of P19 EC cells in association with a dramatic upregulation of the tumor suppressor gene p53 and caspase-3 activation. Our findings suggest that the chemopreventive activity of RWPs on tumor initiation and development is related to a growth inhibition and a p53-dependent induction of apoptosis in teratocarcinoma cells. In addition, this study also shows that the EC cell line is a convenient source for studying the responses of cancer stem cells to new potential anticancer agents.

Sobhani, A., et al. (2017). "Multipotent Stem Cell and Current Application." *Acta Med Iran* **55**(1): 6-23.

Stem cells are self-renewing and undifferentiated cell types that can be differentiate into functional cells. Stem cells can be classified into two main types based on their source of origin: Embryonic and Adult stem cells. Stem cells also classified based on the range of differentiation potentials into Totipotent, Pluripotent, Multipotent, and Unipotent. Multipotent stem cells have the ability to differentiate into all cell types within one particular lineage. There are plentiful advantages and usages for multipotent stem cells. Multipotent Stem cells act as a significant key in procedure of development, tissue repair, and protection. Multipotent Stem cells have been applying in treatment of different disorders such as spinal cord injury, bone fracture, autoimmune diseases,

rheumatoid arthritis, hematopoietic defects, and fertility preservation.

Solana, J., et al. (2009). "Spoltud-1 is a chromatoid body component required for planarian long-term stem cell self-renewal." *Dev Biol* **328**(2): 410-421.

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.

Stevens, N. R., et al. (2007). "From stem cell to embryo without centrioles." *Curr Biol* **17**(17): 1498-1503.

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., et al. (2000). "Herpesvirus saimiri-based gene delivery vectors maintain heterologous expression throughout mouse embryonic stem cell differentiation in vitro." *Gene Ther* 7(6): 464-471.

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.

Sukoyan, M. A., 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): 535-542.

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., 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.

Tabansky, I. and J. N. H. Stern (2016). Basics of Stem Cell Biology as Applied to the Brain. *Stem Cells in Neuroendocrinology*. D. Pfaff and Y. Christen. Cham (CH): 11-24.

Stem cell technology can allow us to produce human neuronal cell types outside the body, but what exactly are stem cells, and what challenges are

associated with their use? Stem cells are a kind of cell that has the capacity to self-renew to produce additional stem cells by mitosis, and also to differentiate into other-more mature-cell types. Stem cells are usually categorized as multipotent (able to give rise to multiple cells within a lineage), pluripotent (able to give rise to all cell types in an adult) and totipotent (able to give rise to all embryonic and adult lineages). Multipotent adult stem cells are found throughout the body, and they include neural stem cells. The challenge in utilizing adult stem cells for disease research is obtaining cells that are genetically matched to people with disease phenotypes, and being able to differentiate them into the appropriate cell types of interest. As adult neural stem cells reside in the brain, their isolation would require considerably invasive and dangerous procedures. In contrast, pluripotent stem cells are easy to obtain, due to the paradigm-shifting work on direct reprogramming of human skin fibroblasts into induced pluripotent stem cells.

Talbot, N. C., et al. (2013). "Growth and Development Symposium: Development, characterization, and use of a porcine epiblast-derived liver stem cell line: ARS-PICM-19." *J Anim Sci* **91**(1): 66-77.

Totipotent embryonic stem cell lines have not been established from ungulates; however, we have developed a somatic stem cell line from the in vitro culture of pig epiblast cells. The cell line, ARS-PICM-19, was isolated via colony cloning and was found to spontaneously differentiate into hepatic parenchymal epithelial cell types, namely hepatocytes and bile duct cells. Hepatocytes form as monolayers and bile duct cells as 3-dimensional bile ductules. Transmission electron microscopy revealed that the ductules were composed of radially arranged, monociliated cells with their cilia projecting into the lumen of the ductule whereas hepatocytes were arranged in monolayers with lateral canalicular structures containing numerous microvilli and connected by tight junctions and desmosomes. Extensive Golgi and rough endoplasmic reticulum networks were also present, indicative of active protein synthesis. Analysis of conditioned medium by 2-dimensional electrophoresis and mass spectrometry indicated a spectrum of serum-protein secretion by the hepatocytes. The PICM-19 cell line maintains a range of inducible cytochrome P450 activities and, most notably, is the only nontransformed cell line that synthesizes urea in response to ammonia challenge. The PICM-19 cell line has been used for several biomedical- and agricultural-related purposes, such as the in vitro replication of hepatitis E virus, a zoonotic virus of pigs, and a spaceflight experiment to evaluate somatic stem

cell differentiation and liver cell function in microgravity. The cell line was also evaluated as a platform for toxicity testing and has been used in a commercial artificial liver rescue device bioreactor. A PICM-19 subclone, PICM-19H, which only differentiates into hepatocytes, was isolated and methods are currently under development to grow PICM-19 cells without feeder cells. Feeder-cell-independent growth will facilitate the study of mesenchymal-parenchymal interactions that influence the divergent differentiation of the PICM-19 cells, enhance our ability to genetically modify the cells, and provide a better model system to investigate porcine hepatic metabolism.

Talbot, N. C., et al. (2017). "Derivation and Characterization of a Pig Embryonic-Stem-Cell-Derived Exocrine Pancreatic Cell Line." *Pancreas* **46**(6): 789-800.

OBJECTIVES: The aim of this study was to identify an epithelial cell line isolated from the spontaneous differentiation of totipotent pig epiblast cells. **METHODS:** PICM-31 and its colony-cloned derivative cell line, PICM-31A, were established from the culture and differentiation of an epiblast mass isolated from an 8-day-old pig blastocyst. The cell lines were analyzed by transmission electron microscopy, marker gene expression, and mass spectroscopy-based proteomics. **RESULTS:** The PICM-31 cell lines were continuously cultured and could be successively colony cloned. They spontaneously self-organized into acinarlike structures. Transmission electron microscopy indicated that the cell lines' cells were epithelial and filled with secretory granules. Candidate gene expression analysis of the cells showed an exocrine pancreatic profile that included digestive enzyme expression, for example, carboxypeptidase A1, and expression of the fetal marker, alpha-fetoprotein. Pancreatic progenitor marker expression included pancreatic and duodenal homeobox 1, NK6 homeobox 1, and pancreas-specific transcription factor 1a, but not neurogenin 3. Proteomic analysis of cellular proteins confirmed the cells' production of digestive enzymes and showed that the cells expressed cytokeratins 8 and 18. **CONCLUSIONS:** The PICM-31 cell lines provide in vitro models of fetal pig pancreatic exocrine cells. They are the first demonstration of continuous cultures, that is, cell lines, of nontransformed pig pancreas cells.

Tandon, P. N. (2009). "Transplantation and stem cell research in neurosciences: where does India stand?" *Neurol India* **57**(6): 706-714.

The nearly absent ability of the neurons to regenerate or multiply has prompted neuroscientists to search for the mean to replace damaged or dead cells.

The failed attempts using adult tissue, initiated nearly a century ago, ultimately brought rays of hope when developing fetal neurons were used for transplantation in 1970s. The initial excitement was tempered by limited success and ethical issues. But these efforts unequivocally established the feasibility of successful neural transplantation provided appropriate tissue was available. The ability to derive embryonic stem cells with their totipotent potential by Thomson in 1998 rekindled the interest in their use for replacement therapy for damaged brain tissue. The present review surveys the current status of this promising field of stem cell research especially in respect to their therapeutic potentials for purposes of neural transplantation. A brief account is provided of the ongoing Indian efforts in this direction.

Wahlestedt, M., et al. (2013). "An epigenetic component of hematopoietic stem cell aging amenable to reprogramming into a young state." *Blood* **121**(21): 4257-4264.

Aging of hematopoietic stem cells (HSCs) leads to several functional changes, including alterations affecting self-renewal and differentiation. Although it is well established that many of the age-induced changes are intrinsic to HSCs, less is known regarding the stability of this state. Here, we entertained the hypothesis that HSC aging is driven by the acquisition of permanent genetic mutations. To examine this issue at a functional level in vivo, we applied induced pluripotent stem (iPS) cell reprogramming of aged hematopoietic progenitors and allowed the resulting aged-derived iPS cells to reform hematopoiesis via blastocyst complementation. Next, we functionally characterized iPS-derived HSCs in primary chimeras and after the transplantation of re-differentiated HSCs into new hosts, the gold standard to assess HSC function. Our data demonstrate remarkably similar functional properties of iPS-derived and endogenous blastocyst-derived HSCs, despite the extensive chronological and proliferative age of the former. Our results, therefore, favor a model in which an underlying, but reversible, epigenetic component is a hallmark of HSC aging.

Wakayama, T. (2003). "Cloned mice and embryonic stem cell lines generated from adult somatic cells by nuclear transfer." *Oncol Res* **13**(6-10): 309-314.

Mice can now be cloned from cultured and noncultured adult-, fetus-, male-, or female-derived cells. Using the mouse as a model, research is moving towards a comprehensive description of clones generated by somatic cell nuclear transfer. In addition, embryonic stem (ES) cell lines can be generated from adult somatic cells via nuclear transfer (ntES cells).

ntES cells contribute to an extensive variety of cell types including neurons in vitro and germ cells in vivo. Recent advances in mouse cloning are reported to illustrate its strengths and promise in the study of mammalian biology and biomedicine.

Wei, C. L., et al. (2005). "Transcriptome profiling of human and murine ESCs identifies divergent paths required to maintain the stem cell state." *Stem Cells* **23**(2): 166-185.

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.

Williams, K., et al. (2013). "CD44 integrates signaling in normal stem cell, cancer stem cell and (pre)metastatic niches." *Exp Biol Med (Maywood)* **238**(3): 324-338.

The stem cell niche provides a regulatory microenvironment for cells as diverse as totipotent embryonic stem cells to cancer stem cells (CSCs) which exhibit stem cell-like characteristics and have the capability of regenerating the bulk of tumor cells while maintaining self-renewal potential. The transmembrane glycoprotein CD44 is a common component of the stem cell niche and exists as a standard isoform (CD44s) and a range of variant isoforms (CD44v) generated through alternative splicing. CD44 modulates signal transduction through post-translational modifications as well as interactions with hyaluronan, extracellular matrix molecules and growth factors and their cognate receptor tyrosine kinases. While the function of CD44 in hematopoietic

stem cells has been studied in considerable detail, our knowledge of CD44 function in tissue-derived stem cell niches remains limited. Here we review CD44s and CD44v in both hematopoietic and tissue-derived stem cell niches, focusing on their roles in regulating stem cell behavior including self-renewal and differentiation in addition to cell-matrix interactions and signal transduction during cell migration and tumor progression. Determining the role of CD44 and CD44v in normal stem cell, CSC and (pre)metastatic niches and elucidating their unique functions could provide tools and therapeutic strategies for treating diseases as diverse as fibrosis during injury repair to cancer progression.

Yang, Y., et al. (2015). "Heightened potency of human pluripotent stem cell lines created by transient BMP4 exposure." *Proc Natl Acad Sci U S A* **112**(18): E2337-2346.

Human pluripotent stem cells (PSCs) show epiblast-type pluripotency that is maintained with ACTIVIN/FGF2 signaling. Here, we report the acquisition of a unique stem cell phenotype by both human ES cells (hESCs) and induced pluripotent stem cells (iPSCs) in response to transient (24-36 h) exposure to bone morphogenetic protein 4 (BMP4) plus inhibitors of ACTIVIN signaling (A83-01) and FGF2 (PD173074), followed by trypsin dissociation and recovery of colonies capable of growing on a gelatin substratum in standard medium for human PSCs at low but not high FGF2 concentrations. The self-renewing cell lines stain weakly for CDX2 and strongly for NANOG, can be propagated clonally on either Matrigel or gelatin, and are morphologically distinct from human PSC progenitors on either substratum but still meet standard in vitro criteria for pluripotency. They form well-differentiated teratomas in immune-compromised mice that secrete human chorionic gonadotropin (hCG) into the host mouse and include small areas of trophoblast-like cells. The cells have a distinct transcriptome profile from the human PSCs from which they were derived (including higher expression of NANOG, LEFTY1, and LEFTY2). In nonconditioned medium lacking FGF2, the colonies spontaneously differentiated along multiple lineages, including trophoblast. They responded to PD173074 in the absence of both FGF2 and BMP4 by conversion to trophoblast, and especially syncytiotrophoblast, whereas an A83-01/PD173074 combination favored increased expression of HLA-G, a marker of extravillous trophoblast. Together, these data suggest that the cell lines exhibit totipotent potential and that BMP4 can prime human PSCs to a self-renewing alternative state permissive for trophoblast development. The results may have implications for regulation of lineage decisions in the early embryo.

Zangrossi, S., et al. (2007). "Oct-4 expression in adult human differentiated cells challenges its role as a pure stem cell marker." *Stem Cells* **25**(7): 1675-1680.

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.

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): 867-877.

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.

Zwaka, T. P. and J. A. Thomson (2005). "Differentiation of human embryonic stem cells occurs through symmetric cell division." *Stem Cells* **23**(2): 146-149.

Embryonic (ES) stem cells can be expanded indefinitely, yet retain the ability to form all cell types of the body. Here we report that human ES cells differentiate exclusively by symmetric cell division in each of four distinct differentiation conditions examined. This suggests that, in some respects, ES cells more closely resemble precursor or transit amplifying cells rather than adult stem cells.

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