Adult Stem Cell Literatures

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

Life is a physical and chemical process. From ontology aspect, the world is timeless and the life exists forever as any other body in the nature. The nature of life is that life is a process of negative entropy, evolution, autopoiesis (auto-organizing), adaptation, emergence and living hierarchy (Hongbao Ma 2005). To the life, the most important are two points: live and die. Conventionally, everybody of us thinks that all the life has a beginning as the birth and the end as the die. All plants and animals, including all the people must die (Ma Hongbao 2009). Stem Cell is the original of life. All cells come from stem cells (Hongbao Ma 2005). It is stem cell offers the hope for the life body eternal.

The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Somatic stem cells are generally believed to differentiate only into cells characteristic of the tissue wherein they reside. In the stem cell field, the terms somatic stem cell has the same meaning as adult stem cell. In this article, the terms somatic stem cell and adult stem cell point same concept.

It normally says that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their original tissue. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem cells or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells. Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (stem cell niche) (Bavister, 2005; Bernard, 2005).

An adult stem cell is thought to be an undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cells. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue. Somatic stem cells are defined by their origin (Cantley, 2005).

In the 1950s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the types of blood cells in the body. A second population, called bone marrow stromal stem cells, were discovered to make up a small proportion of the stromal cell population in the bone marrow, and can generate bone, cartilage, fat, cells that support the formation of blood, and fibrous connective tissue. In the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells that ultimately become nerve cells (Littlefield, Travis et al. 1997).

Typically, there is a very small number of stem cells in each tissue, and once removed from the body, their capacity to divide is limited, making generation of large quantities of stem cells difficult. The following methods normally to be used to identify adult stem cells: (1) label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate; (2) remove the cells from a living animal, label them in cell culture, and transplant them back into another animal to

determine whether the cells replace their tissue of origin. It is possible to produce large amount stem cells by cell culture technique.

Generally to say, a single adult stem cell can generate a line of genetically identical cells that gives rise to all the appropriate differentiated cell types of the tissue. To confirm experimentally that a putative adult stem cell is indeed a stem cell, scientists tend to show either that the cell can give rise to these genetically identical cells in culture, and/or that a purified population of these candidate stem cells can repopulate or reform the tissue after transplant into an animal. In a living animal, adult stem cells are available to divide and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue. Adult stem cells occur in many tissues and that they enter normal differentiation pathways to form the specialized cell types of the tissue in which they reside. Animal and preliminary human studies of adult cell therapy following acute myocardial infarction have shown an overall improvement of cardiac function (Gnecchi, Zhang et al. 2008).

Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, and macrophages (Condorelli, 2005).

Mesenchymal stem cells give rise to a variety of cell types: bone cells (osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and other kinds of connective tissue cells such as those in tendons.

Neural stem cells in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of non-neuronal cells—astrocytes and oligodendrocytes.

Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, paneth cells, and enteroendocrine cells.

Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and to the epidermis.

Certain adult stem cell types can differentiate into cell types seen in organs or tissues other than those expected from the cells' predicted lineage (i.e., brain stem cells that differentiate into blood cells or blood-forming cells that differentiate into cardiac muscle cells, and so forth). This is called transdifferentiation. Although isolated instances of transdifferentiation have been observed in some vertebrate species, whether this phenomenon actually occurs in humans is under debate by the scientific community. Instead of transdifferentiation, the observed instances may involve fusion of a donor cell with a recipient cell. Another possibility is that transplanted stem cells are secreting factors that encourage the recipient's own stem cells to begin the repair process. Even when transdifferentiation has been detected, only a very small percentage of cells undergo the process. In a variation of transdifferentiation experiments, certain adult cell types can be reprogrammed into other cell types in vivo using a well-controlled process of genetic modification. This strategy may offer a way to reprogram available cells into other cell types that have been lost or damaged due to disease. In addition to reprogramming cells to become a specific cell type, it is now possible to reprogram adult somatic cells to become like embryonic stem cells (induced pluripotent stem cells) through the introduction of embryonic genes. Thus, a source of cells can be generated that are specific to the donor, thereby avoiding issues of histocompatibility (van der Bogt, Sheikh et al. 2008).

Many important questions about adult stem cells remain to be answered. They include:

1 How many kinds of adult stem cells exist, and in which tissues do they exist?
2 How do adult stem cells evolve during development and how are they maintained in the adult? Are they "leftover" embryonic stem cells, or do they arise in some other way?
3 Why do stem cells remain in an undifferentiated state when all the cells around them have differentiated? What are the characteristics of their “niche” that controls their behavior?
4 Do adult stem cells have the capacity to transdifferentiate, and is it possible to control this process to improve its reliability and efficiency?
5 If the beneficial effect of adult stem cell transplantation is a trophic effect, what are the mechanisms? Is donor cell-recipient cell contact required, secretion of factors by the donor cell, or both?
6 What are the factors that control adult stem cell proliferation and differentiation?
7 What are the factors that stimulate stem cells to relocate to sites of injury or damage, and how can this process be enhanced for better healing?

One major difference between embryonic and adult stem cells is their different abilities in the number and type of differentiated cell types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are limited to differentiating into different cell types of their tissue of origin. Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is comparative difficult. Somatic nuclei can be reprogrammed to pluripotency through fusion with embryonic stem cells (Ma, Chiang et al. 2008).

Reprogramming of differentiated somatic cells into induced pluripotent stem cells has potential for derivation of patient-specific cells for therapy as well as for development of models with which to study disease progression. Derivation of pluripotent stem cells from human somatic cells has been achieved by viral transduction of human fibroblasts with early developmental genes. Because forced expression of these genes by viral transduction results in transgene integration with unknown and unpredictable potential mutagenic effects, identification of cell culture conditions that can induce endogenous expression of these genes is desirable (Page, Ambady et al. 2009).

Adult stem cells and tissues derived from them are less rejection after transplantation. This is because a patient's own cells could be expanded in culture, coaxed into assuming a specific cell type (differentiation), and then reintroduced into the patient. The adult stem cells and tissues derived from the patient's own adult stem cells are less rejected by the immune system. This represents a significant advantage.

Literatures

Adams, J. G., C. L. Hardy, et al. (1991). "Disappearance of the protein of a somatic mutation: a possible example of stem cell inactivation." Am J Physiol **261**(3 Pt 1): C448-54.

The low concentration of the hemoglobin variant, Hb Vicksburg (leucine-beta-75 deleted), and a profound deficit of its mRNA led us to postulate that a beta(+)-thalassemia mutation existed in cis to the coding region mutation, suppressing its synthesis. We examined blood from this patient 6, 8, and 10 yr after our initial studies, using methods of analysis unavailable initially. We found 1) mutations causing beta(+)-(-88 C----T) and beta 0-(849 A----G) thalassemia; 2) that the proportion of Hb Vicksburg in erythrocytes fell over time, from 8 to 4%, and ultimately disappeared; and 3) that the mutation causing Hb Vicksburg was not detectable in genomic DNA isolated from blood leukocytes when this variant was present in hemolysate. We postulate that Hb Vicksburg arose from a somatic mutation of a beta(+)-thalassemia gene in an erythroid-committed stem cell. Its gradual disappearance suggests the cycling of stem cells, with inactivation of different clones over time.

Anisimov, S. V. (2009). "[Cell therapy for Parkinson's disease: II. Somatic stem cell-based applications]." Adv Gerontol **22**(1): 150-66.

Motor dysfunctions in Parkinson's disease are believed to be primarily due to the degeneration of dopaminergic neurons located in the substantia nigra pars compacta. Numerous cell replacement therapy approaches have been developed and tested, including these based on donor cell transplantation (embryonic and adult tissue-derived), adult mesenchymal stem cells (hMSCs)-, neural stem cells (hNSCs)- and finally human embryonic stem cells (hESCs)-based. Despite the progress achieved, numerous difficulties prevent wider practical application of stem cell-based therapy approaches for the treatment of Parkinson's disease. Among the latter, ethical, safety and technical issues stand out. Current series of reviews (Cell therapy for Parkinson's disease: I. Embryonic and adult donor tissue-based applications; II. Adult stem cell-based applications; III. Neonatal, fetal and embryonic stem cell-based applications; IV. Risks and future trends) aims providing a balanced and updated view on various issues associated with cell types (including stem cells) in regards to their potential in the treatment of Parkinson's disease. Essential features of the individual cell subtypes, principles of available cell handling protocols, transplantation, and safety issues are discussed extensively.

Anne Cook, H., D. Williams, et al. (2000). "Crypt-restricted metallothionein immunopositivity in murine colon: validation of a model for studies of somatic stem cell mutation." J Pathol **191**(3): 306-12.

The ability to visualize the cellular effects of a somatic mutation is relevant to studies of cell kinetics and carcinogenesis. In the colon, mutagen administration leads to scattered crypt-restricted loss of activity of the X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD); it has been shown that this is due to somatic mutation in the G6PD gene. Mutagen-induced crypt-restricted immunopositivity for metallothionein (MT) has been reported in one study in the mouse colon; if this is also due to somatic mutation, it provides a simple method for studying the phenomenon which could be carried out on paraffin sections. This study shows that, as in the G6PD model, the frequency of crypt-restricted immunopositivity for MT is very low in untreated animals, but increases proportionately with the dose of mutagen administered. There is a good overall

correlation of a range of MT-positive crypt frequencies with those derived from studies using G6PD. As with the G6PD model, the MT-positive crypt phenotype evolves over time after mutagen administration; initially individual crypts include both positive and negative phenotype cells, but later almost all involved crypts are composed entirely of MT-positive cells. The frequency of MT-positive crypts stabilizes after a few weeks and remains at the same level 6 months later. All these observations are qualitatively identical to those found using the G6PD model and provide strong evidence that stable, crypt-restricted immunopositivity for MT results from a mutation affecting expression of the metallothionein gene in a colonic stem cell. This model will provide a useful tool to study factors influencing stem cell mutation frequency and cell kinetics in the colon.

Arnaud, D., M. G. Mattei, et al. (1993). "A panel of deleted mouse X chromosome somatic cell hybrids derived from the embryonic stem cell line HD3 shows preferential breakage in the Hprt-DXHX254E region." Genomics **18**(3): 520-6.

A panel of 91 somatic cell hybrids containing deleted mouse X chromosomes and falling into seven nested intervals has been isolated and characterized from fusions involving the murine embryonic stem cell HD3. Many of the X chromosome breakpoints present in these hybrids fall within regions in which few or no other hybrids were previously available. The apparent enrichment for breakpoints lying within the Hprt-DXHX254E region is discussed in relation to both the nature of the embryonic stem cell fusions and the presence of the Fmr1 gene associated with FRAXA in man within this span.

Bosse, R., M. Singhofer-Wowra, et al. (1997). "Good manufacturing practice production of human stem cells for somatic cell and gene therapy." Stem Cells **15 Suppl 1**: 275-80.

Peripheral blood stem cells (PBSC) are used for transplantation to reconstitute the hematopoietic system after high-dose chemotherapy. PBSC are harvested from peripheral blood upon successful mobilization by cytokines and/or chemotherapy. Further in vitro manipulation steps like enrichment of CD34+ PBSC or gene transfer can be performed. To ensure the quality and safety of the final cell preparations intended for transplantation, national and international guidelines and regulations have been issued. Herein the implementation of a quality assurance program including the principles of good manufacturing practice (GMP) and a quality control (QC) system is one major concern. GMP regulations apply to all phases of cell collection, processing and storage as well as documentation, training of personnel, and the laboratory facility. QC measures have to be taken to ensure consistent quality and safety with an emphasis on preventing any deficiencies.

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

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

Byrne, J. A., S. Simonsson, et al. (2003). "Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes." Curr Biol **13**(14): 1206-13.

Nuclear reprogramming by the transplantation of somatic cell nuclei to eggs (in second meiotic metaphase) is always followed by a phase of chromosome replication and cell division before new gene expression is seen. To help understand the mechanism of nuclear reprogramming, we have asked whether the nuclei of normal, nontransformed, nondividing, and terminally differentiated mammalian cells can be directly reprogrammed, without DNA replication, by Xenopus oocytes. We find that nuclei of adult mouse thymocytes and of adult human blood lymphocytes, injected into Xenopus oocytes, are induced to extinguish a differentiation marker and to strongly express oct-4, the most diagnostic mammalian stem cell/pluripotency marker. In the course of 2 days at 18 degrees C, the mammalian oct-4 transcripts are spliced to mature mRNA. We conclude that normal mammalian nuclei can be directly reprogrammed by the nucleus (germinal vesicle) of amphibian oocytes

to express oct-4 at a rate comparable to that of oct-4 in mouse ES cells. To our knowledge, this is the first demonstration of a stem cell marker being induced in a differentiated adult human cell nucleus. This is an early step toward the long-term aim of developing a procedure for reprogramming readily accessible human adult cells for cell replacement therapy.

Campbell, F., M. A. Appleton, et al. (1998). "No difference in stem cell somatic mutation between the background mucosa of right- and left-sided sporadic colorectal carcinomas." J Pathol **186**(1): 31-5.

Epidemiological, morphological, and molecular differences exist between carcinomas of the right and left sides of the large bowel. To investigate whether this is reflected in differences in somatic mutation frequency in the background mucosa, mutation of the neutral O-acetyltransferase gene (oat) was quantified in histologically normal resection margins from 20 informative (heterozygous) patients with caecal or ascending colon cancer (11 males, median age 75 years) and 20 with sigmoid colon or rectal cancer (10 males, median age 70 years). Mutant discordant crypts lacking O-acetyltransferase activity were visualized by mPAS staining and classified as wholly or partially involved by the mutant phenotype; median frequencies (x10(-4) were compared (Mann-Whitney U-test) after assessing a sample of more than 10,000 crypts per case. No significant difference was found between the frequencies of wholly involved mPAS-positive crypts in background mucosa of left- and right-sided cancers ($p = 0.4569$), indicating that tumours on both sides of the colon are associated with similar levels of lifetime-accumulated stem cell mutational load. However, partially involved mPAS-positive crypts were significantly more frequent in mucosa from left-sided cancers ($p < 0.04$), indicating increased mutational activity during the previous 12 months. Analysis of mucosa proximal and distal to left-sided cancers showed that this increase was due to a statistically higher frequency of partially involved crypts in proximal mucosa, which probably resulted from the obstructive effects of the tumour causing increased exposure of the proximal mucosa to luminal carcinogens and/or epithelial regeneration in response to low-grade inflammation or ischaemia. The findings indicate that although left-sided colonic cancer is commoner than right-sided cancer in the British population, carcinomas on both sides of the large bowel arise in a background of similar levels of stem cell mutational activity.

Campbell, F., J. M. Geraghty, et al. (1998). "Increased stem cell somatic mutation in the non-neoplastic colorectal mucosa of patients with familial adenomatous polyposis." Hum Pathol **29**(12): 1531-5.

Colorectal tumorigenesis in familial adenomatous polyposis (FAP) results from somatic mutation of either the normal APC allele or another growth control gene in epithelial cells bearing a germline APC defect. The rate at which tumors develop is therefore dependent on the somatic mutation frequency; it is not known whether this is normal or elevated in FAP. We aimed to quantify stem cell somatic mutation in FAP, comparing it with hereditary nonpolyposis colorectal cancer (HNPCC) and Crohn's disease (CD). Stem cell somatic mutation frequency was studied in 47 FAP patients, 5 HNPCC patients, and 13 CD patients, all younger than 49 years, by quantifying crypt-restricted loss of O-acetyltransferase activity in sections of morphologically normal colonic mucosa from individuals heterozygous for this monogenically inherited polymorphism. Median stem cell somatic mutation frequency was significantly higher in FAP than HNPCC (4.2 x 10(-4) v 1.4 x 10(-4), Mann-Whitney U, $P < .02$). The level in CD (4.0 x 10(-4)) was similar to FAP. Mutated crypts occurred in groups more frequently in FAP (22%) than HNPCC (12%) or CD (10%), suggesting an increase in stem cell division associated with crypt fission in FAP. We conclude that stem cell somatic mutation frequency is raised in non-neoplastic colorectal mucosa in FAP. This is probably related to increased stem cell proliferation and contributes to the high rate of tumor formation in this condition.

Cibelli, J. B., S. L. Stice, et al. (1998). "Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells." Nat Biotechnol **16**(7): 642-6.

We have developed a method, using nuclear transplantation, to produce transgenic embryonic stem (ES)-like cells from fetal bovine fibroblasts. These cells, when reintroduced into preimplantation embryos, differentiated into derivatives from the three embryonic germ layers, ectoderm, mesoderm, and endoderm, in 5-month-old animals. Six out of seven (86%) calves born were found to be chimeric for at least one tissue. These experiments demonstrate that somatic cells can be genetically modified and then de-differentiated by nuclear transfer into ES-like cells, opening the possibility of using them in differentiation studies and human cell therapy.

Corti, S., F. Locatelli, et al. (2004). "Somatic stem cell research for neural repair: current evidence and emerging perspectives." J Cell Mol Med **8**(3): 329-37.

Recent evidence supports the existence of adult mammalian stem cell subpopulations, particularly within the bone marrow, that may be able to "transdifferentiate" across tissue lineage

boundaries, thus offering an accessible source for therapeutic applications even for neural tissue repair. However, the difficulties in reproducing some experimental data, the rarity of the transdifferentiation events and observations that cell fusion may be an alternative explanation argue against the idea of stem cell plasticity. Investigations going beyond descriptive experiments and more mechanicistic approaches may provide a more solid foundation to adult stem cell therapeutic potential.

Dabelsteen, S., P. Hercule, et al. (2009). "Epithelial cells derived from human embryonic stem cells display p16INK4A senescence, hypermotility, and differentiation properties shared by many P63+ somatic cell types." Stem Cells **27**(6): 1388-99.

Human embryonic stem (hES) cells can generate cells expressing p63, K14, and involucrin, which have been proposed to be keratinocytes. Although these hES-derived, keratinocyte-like (hESderK) cells form epithelioid colonies when cultured in a fibroblast feeder system optimal for normal tissue-derived keratinocytes, they have a very short replicative lifespan unless engineered to express HPV16 E6E7. We report here that hESderK cells undergo senescence associated with p16(INK4A) expression, unrelated to telomere status. Transduction to express bmi1, a repressor of the p16(INK4A)/p14(ARF) locus, conferred upon hESderK cells and keratinocytes a substantially extended lifespan. When exposed to transforming growth factor beta or to an incompletely processed form of Laminin-332, three lifespan-extended or immortalized hESderK lines that we studied became directionally hypermotile, a wound healing and invasion response previously characterized in keratinocytes. In organotypic culture, hESderK cells stratified and expressed involucrin and K10, as do epidermal keratinocytes in vivo. However, their growth requirements were less stringent than keratinocytes. We then extended the comparison to endoderm-derived, p63(+)/K14(+) urothelial and tracheobronchial epithelial cells. Primary and immortalized lines of these cell types had growth requirements and hypermotility responses similar to keratinocytes and bmi1 expression facilitated their immortalization by engineering to express the catalytic subunit of telomerase (TERT). In organotypic culture, they stratified and exhibited squamous metaplasia, expressing involucrin and K10. Thus, hESderK cells proved to be distinct from all three normal p63(+) cell types tested. These results indicate that hESderK cells cannot be identified conclusively as keratinocytes or even as ectodermal cells, but may represent an incomplete form of, or deviation from, normal p63(+) lineage development.

Faast, R., S. J. Harrison, et al. (2006). "Use of adult mesenchymal stem cells isolated from bone marrow and blood for somatic cell nuclear transfer in pigs." Cloning Stem Cells **8**(3): 166-73.

Mesenchymal stem cells (MSCs) isolated from bone marrow were used to examine the hypothesis that a less differentiated cell type could increase adult somatic cell nuclear transfer (SCNT) efficiencies in the pig. SCNT embryos were produced using a fusion before activation protocol described previously and the rate at which these developed to the blastocyst stage compared with that using fibroblasts obtained from ear tissue from the same animal. The use of bone marrow MSCs did not increase cleavage rates compared with adult fibroblasts. However, the percentage of embryos that developed to the blastocyst stage was almost doubled, providing support for the hypothesis that a less differentiated cell can increase cloning efficiencies. As MSCs are relatively difficult to isolate from the bone marrow of live animals, a second experiment was undertaken to determine whether MSCs could be isolated from the peripheral circulation and used for SCNT. Blood MSCs were successfully isolated from four of the five pigs sampled. These cells had a similar differentiation capacity and marker profile to those isolated from bone marrow but did not result in increased rates of development. This is the first study to our knowledge, to report that MSCs can be derived from peripheral blood and used for SCNT for any species. These cells can be readily obtained under relatively sterile conditions compared with adult fibroblasts and as such, may provide an alternative cell type for cloning live animals.

Fang, Z. F., H. Gai, et al. (2006). "Rabbit embryonic stem cell lines derived from fertilized, parthenogenetic or somatic cell nuclear transfer embryos." Exp Cell Res **312**(18): 3669-82.

Embryonic stem cells were isolated from rabbit blastocysts derived from fertilization (conventional rbES cells), parthenogenesis (pES cells) and nuclear transfer (ntES cells), and propagated in a serum-free culture system. Rabbit ES (rbES) cells proliferated for a prolonged time in an undifferentiated state and maintained a normal karyotype. These cells grew in a monolayer with a high nuclear/cytoplasm ratio and contained a high level of alkaline phosphate activity. In addition, rbES cells expressed the pluripotent marker Oct-4, as well as EBAF2, FGF4, TDGF1, but not antigens recognized by antibodies against SSEA-1, SSEA-3, SSEA-4, TRA-1-10 and TRA-1-81. All 3 types of ES cells formed embryoid bodies and generated teratoma that contained tissue types of all three germ layers.

rbES cells exhibited a high cloning efficiency, were genetically modified readily and were used as nuclear donors to generate a viable rabbit through somatic cell nuclear transfer. In combination with genetic engineering, the ES cell technology should facilitate the creation of new rabbit lines.

Frydman, H. M., J. M. Li, et al. (2006). "Somatic stem cell niche tropism in Wolbachia." Nature **441**(7092): 509-12.

Wolbachia are intracellular bacteria found in the reproductive tissue of all major groups of arthropods. They are transmitted vertically from the female hosts to their offspring, in a pattern analogous to mitochondria inheritance. But Wolbachia phylogeny does not parallel that of the host, indicating that horizontal infectious transmission must also occur. Insect parasitoids are considered the most likely vectors, but the mechanism for horizontal transfer is largely unknown. Here we show that newly introduced Wolbachia cross several tissues and infect the germline of the adult Drosophila melanogaster female. Through investigation of bacterial migration patterns during the course of infection, we found that Wolbachia reach the germline through the somatic stem cell niche in the D. melanogaster germarium. In addition, our data suggest that Wolbachia are highly abundant in the somatic stem cell niche of long-term infected hosts, implying that this location may also contribute to efficient vertical transmission. This is, to our knowledge, the first report of an intracellular parasite displaying tropism for a stem cell niche.

Ghule, P. N., Z. Dominski, et al. (2009). "The subnuclear organization of histone gene regulatory proteins and 3' end processing factors of normal somatic and embryonic stem cells is compromised in selected human cancer cell types." J Cell Physiol **220**(1): 129-35.

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

Kania, G., D. Corbeil, et al. (2005). "Somatic stem cell marker prominin-1/CD133 is expressed in embryonic stem cell-derived progenitors." Stem Cells **23**(6): 791-804.

Prominin-1/CD133 is a plasma membrane marker found in several types of somatic stem cells, including hematopoietic and neural stem cells. To study its role during development and with differentiation, we analyzed its temporal and spatial expression (mRNA and protein) in preimplantation embryos, undifferentiated mouse embryonic stem (ES) cells, and differentiated ES cell progeny. In early embryos, prominin-1 was expressed in trophoblast but not in cells of the inner cell mass; however, prominin-1 transcripts were detected in undifferentiated ES cells. Both ES-derived cells committed to differentiation and early progenitor cells coexpressed prominin-1 with early lineage markers, including the cytoskeletal markers (nestin, cytokeratin 18, desmin), fibulin-1, and valosin-containing protein. After spontaneous differentiation at terminal stages, prominin-1 expression was downregulated and no coexpression with markers characteristic for neuroectodermal, mesodermal, and endodermal cells was found. Upon induction of neuronal differentiation, some prominin-1-positive cells, which coexpressed nestin and showed the typical morphology of neural progenitor cells, persisted until terminal stages of differentiation. However, no coexpression of prominin-1 with markers of differentiated neural cells was detected. In conclusion, we present the somatic stem cell marker prominin-1 as a new parameter to define ES-derived committed and early progenitor cells.

Katiyar, S., X. Jiao, et al. (2007). "Somatic excision demonstrates that c-Jun induces cellular migration and invasion through induction of stem cell factor." Mol Cell Biol **27**(4): 1356-69.

Cancer cells arise through sequential acquisition of mutations in tumor suppressors and

oncogenes. c-Jun, a critical component of the AP-1 complex, is frequently overexpressed in diverse tumor types and has been implicated in promoting cellular proliferation, migration, and angiogenesis. Functional analysis of candidate genetic targets using germ line deletion in murine models can be compromised through compensatory mechanisms. As germ line deletion of c-jun induces embryonic lethality, somatic deletion of the c-jun gene was conducted using floxed c-jun (c-jun(f/f)) conditional knockout mice. c-jun-deleted cells showed increased cellular adhesion, stress fiber formation, and reduced cellular migration. The reduced migratory velocity and migratory directionality was rescued by either c-Jun reintroduction or addition of secreted factors from wild-type cells. An unbiased analysis of cytokines and growth factors, differentially expressed and showing loss of secretion upon c-jun deletion, identified stem cell factor (SCF) as a c-Jun target gene. Immunoneutralizing antibody to SCF reduced migration of wild-type cells. SCF addition rescued the defect in cellular adhesion, cellular velocity, directional migration, transwell migration, and cellular invasion of c-jun(-/-) cells. c-Jun induced SCF protein, mRNA, and promoter activity. Induction of the SCF promoter required the c-Jun DNA-binding domain. c-Jun bound to the SCF promoter in chromatin immunoprecipitation assays. Mutation of the c-Jun binding site abolished c-Jun-mediated induction of the SCF promoter. These studies demonstrate an essential role of c-Jun in cellular migration through induction of SCF.

Kiger, A. A., H. White-Cooper, et al. (2000). "Somatic support cells restrict germline stem cell self-renewal and promote differentiation." Nature **407**(6805): 750-4.

Stem cells maintain populations of highly differentiated, short-lived cell-types, including blood, skin and sperm, throughout adult life. Understanding the mechanisms that regulate stem cell behaviour is crucial for realizing their potential in regenerative medicine. A fundamental characteristic of stem cells is their capacity for asymmetric division: daughter cells either retain stem cell identity or initiate differentiation. However, stem cells are also capable of symmetric division where both daughters remain stem cells, indicating that mechanisms must exist to balance self-renewal capacity with differentiation. Here we present evidence that support cells surrounding the stem cells restrict self-renewal and control stem cell number by ensuring asymmetric division. Loss of function of the Drosophila Epidermal growth factor receptor in somatic cells disrupted the balance of self-renewal versus differentiation in the male germline, increasing the number of germline stem cells. We propose that activation of this receptor specifies normal behaviour of somatic support cells; in turn, the somatic cells play a guardian role, providing information that prevents self-renewal of stem cell identity by the germ cell they enclose.

Kim, B. O., H. Tian, et al. (2005). "Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model." Circulation **112**(9 Suppl): I96-104.

BACKGROUND: Cell transplantation offers the promise in the restoration of ventricular function after an extensive myocardial infarction, but the optimal cell type remains controversial. Human unrestricted somatic stem cells (USSCs) isolated from umbilical cord blood have great potential to differentiate into myogenic cells and induce angiogenesis. The present study evaluated the effect of USSCs on myocardial regeneration and improvement of heart function after myocardial infarction in a porcine model. METHOD AND RESULTS: The distal left anterior descending artery of Yorkshire pigs (30 to 35 kg) was occluded by endovascular implantation of a coil. Four weeks after infarction, single-photon emission computed tomography technetium 99m sestamibi scans (MIBI) and echocardiography were performed. USSCs (100 x 10(6)) or culture media were then directly injected into the infarcted region (n=8 per group). Pigs were immunosuppressed by daily administration of cyclosporin A. At 4 weeks after transplantation, MIBI and echocardiography were repeated and heart function was also assessed with a pressure-volume catheter. The infarcted myocardium and implanted cells were studied histologically. MIBI showed improved regional perfusion ($P<0.05$) and wall motion ($P<0.05$) of the infarct region in the transplant group compared with the control. Ejection fraction evaluated by both MIBI and echocardiography decreased in the control group but increased in the transplant group ($P<0.01$). Scar thickness of the transplant group was higher than the control. The grafted cells were detected 4 weeks after transplantation by both immunohistochemistry and in situ hybridization. CONCLUSIONS: Engrafted USSCs were detected in the infarct region 4 weeks after cell transplantation, and the implanted cells improved regional and global function of the porcine heart after a myocardial infarction. This study suggests that the USSC implantation will be efficacious for cellular cardiomyoplasty.

Kim, J. Y., S. Tavare, et al. (2005). "Counting human somatic cell replications: methylation mirrors

endometrial stem cell divisions." Proc Natl Acad Sci U S A **102**(49): 17739-44.

Cell proliferation may be altered in many diseases, but it is uncertain exactly how to measure total numbers of divisions. Although it is impossible to count every division directly, potentially total numbers of stem cell divisions since birth may be inferred from numbers of somatic errors. The idea is that divisions are surreptitiously recorded by random errors that occur during replication. To test this "molecular clock" hypothesis, epigenetic errors encoded in certain methylation patterns were counted in glands from 30 uteri. Endometrial divisions can differ among women because of differences in estrogen exposures or numbers of menstrual cycles. Consistent with an association between mitotic age and methylation, there was an age-related increase in methylation with stable levels after menopause, and significantly less methylation was observed in lean or older multiparous women. Methylation patterns were diverse and more consistent with niche rather than immortal stem cell lineages. There was no evidence for decreased stem cell survival with aging. An ability to count lifetime numbers of stem cell divisions covertly recorded by random replication errors provides new opportunities to link cell proliferation with aging and cancer.

Kim, K., K. Ng, et al. (2007). "Recombination signatures distinguish embryonic stem cells derived by parthenogenesis and somatic cell nuclear transfer." Cell Stem Cell **1**(3): 346-52.

Parthenogenesis and somatic cell nuclear transfer (SCNT) are two methods for deriving embryonic stem (ES) cells that are genetically matched to the oocyte donor or somatic cell donor, respectively. Using genome-wide single nucleotide polymorphism (SNP) analysis, we demonstrate distinct signatures of genetic recombination that distinguish parthenogenetic ES cells from those generated by SCNT. We applied SNP analysis to the human ES cell line SCNT-hES-1, previously claimed to have been derived by SCNT, and present evidence that it represents a human parthenogenetic ES cell line. Genome-wide SNP analysis represents a means to validate the genetic provenance of an ES cell line.

Kim, M. K. (2009). "Oversight framework over oocyte procurement for somatic cell nuclear transfer: comparative analysis of the Hwang Woo Suk case under South Korean bioethics law and U.S. guidelines for human embryonic stem cell research." Theor Med Bioeth **30**(5): 367-84.

We examine whether the current regulatory regime instituted in South Korea and the United States would have prevented Hwang's potential transgressions in oocyte procurement for somatic cell nuclear transfer, we compare the general aspects and oversight framework of the Bioethics and Biosafety Act in South Korea and the US National Academies' Guidelines for Human Embryonic Stem Cell Research, and apply the relevant provisions and recommendations to each transgression. We conclude that the Act would institute centralized oversight under governmental auspices while the Guidelines recommend politically-independent, decentralized oversight bodies including a special review body for human embryonic stem cell research at an institutional level and that the Guidelines would have provided more vigorous protection for the women who had undergone oocyte procurement for Hwang's research than the Act. We also suggest additional regulations to protect those who provide oocytes for research in South Korea.

King, F. J. and H. Lin (1999). "Somatic signaling mediated by fs(1)Yb is essential for germline stem cell maintenance during Drosophila oogenesis." Development **126**(9): 1833-44.

Drosophila oogenesis starts when a germline stem cell divides asymmetrically to generate a daughter germline stem cell and a cystoblast that will develop into a mature egg. We show that the fs(1)Yb gene is essential for the maintenance of germline stem cells during oogenesis. We delineate fs(1)Yb within a 6.4 kb genomic region by transgenic rescue experiments. fs(1)Yb encodes a 4.1 kb RNA that is present in the third instar larval, pupal and adult stages, consistent with its role in regulating germline stem cells during oogenesis. Germline clonal analysis shows that all fs(1)Yb mutations are soma-dependent. In the adult ovary, fs(1)Yb is specifically expressed in the terminal filament cells, suggesting that fs(1)Yb acts in these signaling cells to maintain germline stem cells. fs(1)Yb encodes a novel hydrophilic protein with no potential signal peptide or transmembrane domains, suggesting that this protein is not itself a signal but a key component of the signaling machinery for germline stem cell maintenance.

Kirilly, D., E. P. Spana, et al. (2005). "BMP signaling is required for controlling somatic stem cell self-renewal in the Drosophila ovary." Dev Cell **9**(5): 651-62.

BMP signaling is essential for promoting self-renewal of mouse embryonic stem cells and Drosophila germline stem cells and for repressing stem cell proliferation in the mouse intestine and skin. However, it remains unknown whether BMP signaling can promote self-renewal of adult somatic stem cells. In this study, we show that BMP signaling is necessary and sufficient for promoting self-renewal

and proliferation of somatic stem cells (SSCs) in the Drosophila ovary. BMP signaling is required in SSCs to directly control their maintenance and division, but is dispensable for proliferation of their differentiated progeny. Furthermore, BMP signaling is required to control SSC self-renewal, but not survival. Moreover, constitutive BMP signaling prolongs the SSC lifespan. Therefore, our study clearly demonstrates that BMP signaling directly promotes SSC self-renewal and proliferation in the Drosophila ovary. Our work further suggests that BMP signaling could promote self-renewal of adult stem cells in other systems.

Kishigami, S., S. Wakayama, et al. (2006). "Cloned mice and embryonic stem cell establishment from adult somatic cells." Hum Cell **19**(1): 2-10.

Cloning methods are now well described and becoming routine. Yet the frequency at which cloned offspring are produced remains below 2% irrespective of nucleus donor species or cell type. Especially in the mouse, few laboratories can make clones from adult somatic cells, and most mouse strains never succeed to produce cloned mice. On the other hand, nuclear transfer can be used to generate embryonic stem (ntES) cell lines from a patient's own somatic cells. We have shown that ntES cells can be generated relatively easily from a variety of mouse genotypes and cell types of both sexes, even though it may be more difficult to generate clones directly. Several reports have already demonstrated that ntES cells can be used in regenerative medicine in order to rescue immune deficient or infertile phenotypes. However, it is unclear whether ntES cells are identical to fertilized embryonic stem (ES) cells. In general, ntES cell techniques are expected to be applicable to regenerative medicine, however, these techniques can also be used for the preservation of the genetic resources of mouse strains instead of preserving such resources in embryos, oocytes or spermatozoa. This review seeks to describe the phenotype, application, and possible abnormalities of cloned mice and ntES cell lines.

Kogler, G., S. Sensken, et al. (2004). "A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential." J Exp Med **200**(2): 123-35.

Here a new, intrinsically pluripotent, CD45-negative population from human cord blood, termed unrestricted somatic stem cells (USSCs) is described. This rare population grows adherently and can be expanded to 10(15) cells without losing pluripotency. In vitro USSCs showed homogeneous differentiation into osteoblasts, chondroblasts, adipocytes, and hematopoietic and neural cells including astrocytes and neurons that express neurofilament, sodium channel protein, and various neurotransmitter phenotypes. Stereotactic implantation of USSCs into intact adult rat brain revealed that human Tau-positive cells persisted for up to 3 mo and showed migratory activity and a typical neuron-like morphology. In vivo differentiation of USSCs along mesodermal and endodermal pathways was demonstrated in animal models. Bony reconstitution was observed after transplantation of USSC-loaded calcium phosphate cylinders in nude rat femurs. Chondrogenesis occurred after transplanting cell-loaded gelfoam sponges into nude mice. Transplantation of USSCs in a noninjury model, the preimmune fetal sheep, resulted in up to 5% human hematopoietic engraftment. More than 20% albumin-producing human parenchymal hepatic cells with absence of cell fusion and substantial numbers of human cardiomyocytes in both atria and ventricles of the sheep heart were detected many months after USSC transplantation. No tumor formation was observed in any of these animals.

Kues, W. A., J. W. Carnwath, et al. (2005). "From fibroblasts and stem cells: implications for cell therapies and somatic cloning." Reprod Fertil Dev **17**(1-2): 125-34.

Pluripotent embryonic stem cells (ESCs) from the inner cell mass of early murine and human embryos exhibit extensive self-renewal in culture and maintain their ability to differentiate into all cell lineages. These features make ESCs a suitable candidate for cell-replacement therapy. However, the use of early embryos has provoked considerable public debate based on ethical considerations. From this standpoint, stem cells derived from adult tissues are a more easily accepted alternative. Recent results suggest that adult stem cells have a broader range of potency than imagined initially. Although some claims have been called into question by the discovery that fusion between the stem cells and differentiated cells can occur spontaneously, in other cases somatic stem cells have been induced to commit to various lineages by the extra- or intracellular environment. Recent data from our laboratory suggest that changes in culture conditions can expand a subpopulation of cells with a pluripotent phenotype from primary fibroblast cultures. The present paper critically reviews recent data on the potency of somatic stem cells, methods to modify the potency of somatic cells and implications for cell-based therapies.

Kuraguchi, M., H. Cook, et al. (2001). "Differences in susceptibility to colonic stem cell somatic mutation in three strains of mice." J Pathol **193**(4): 517-21.

Different species and different strains of animals commonly show very different sensitivities to

carcinogenic regimes, which are often unexplained. A major possible contributory factor is variation in susceptibility to mutation, but this has not been directly demonstrated. This study therefore quantified the colonic stem cell mutation frequency in three strains of mice using two carcinogens. Stem cell mutations were identified using loss of function of glucose 6-phosphate dehydrogenase (G6PD) in individual crypts, a technique validated by several previous studies. The carcinogens dimethylhydrazine (DMH) and ethyl nitrosurea (ENU) were given to Balb/C, C57BL/6J, and C3H mice. In response to DMH, Balb/C mice were most susceptible, with approximately double the stem cell mutation frequency found in C3H and more than ten-fold that found in C57BL/6J (3.3+/-0.71 vs. 1.5+/-0.52 vs. 0.28+/-0.8x10(-4)). In response to ENU, Balb/C mice and C3H mice were equally susceptible, showing a stem cell mutation frequency approximately twice that of C57BL/6J (3.1+/-0.4 vs. 3.1+/-0.65 vs. 1.63+/-0.28x10(-4)). The observed differences among the strains with respect to somatic mutation following DMH treatment are likely to be due to the previously documented differences in metabolic conversion to the active metabolite. However, as ENU is a directly acting, rapidly inactivated mutagen, strain differences in response to ENU are unlikely to be due to strain-dependent metabolism of the mutagen and are likely to reflect differences in DNA repair efficiency, or possibly in stem cell kinetics among the strains studied. Susceptibility to the induction of colonic stem cell mutation is an important factor in susceptibility to carcinogens, whether due to differences in DNA repair or to other factors. Direct quantification of stem cell mutation frequency allows the separate identification of this component of the carcinogenic cascade and shows that it can make a major contribution to the differing susceptibility of different mouse strains.

Leatherman, J. L. and S. Dinardo (2008). "Zfh-1 controls somatic stem cell self-renewal in the Drosophila testis and nonautonomously influences germline stem cell self-renewal." Cell Stem Cell **3**(1): 44-54.

The ability of adult stem cells to maintain their undifferentiated state depends upon residence in their niche. While simple models of a single self-renewal signal are attractive, niche-stem cell interactions are likely to be more complex. Many niches have multiple cell types, and the Drosophila testis is one such complex niche with two stem cell types, germline stem cells (GSCs) and somatic cyst progenitor cells (CPCs). These stem cells require chemokine activation of Jak/STAT signaling for self-renewal. We identified the transcriptional repressor Zfh-1 as a presumptive somatic target of Jak/STAT signaling, demonstrating that it is necessary and sufficient to maintain CPCs. Surprisingly, sustained zfh-1 expression or intrinsic STAT activation in somatic cells caused neighboring germ cells to self-renew outside their niche. In contrast, germline-intrinsic STAT activation was insufficient for GSC renewal. These data reveal unexpected complexity in cell interactions in the niche, implicating CPCs in GSC self-renewal.

Liang, L. and J. R. Bickenbach (2002). "Somatic epidermal stem cells can produce multiple cell lineages during development." Stem Cells **20**(1): 21-31.

It has been demonstrated that several types of somatic stem cells have the remarkable capacity to differentiate into other types of tissues. We demonstrate here that stem cells from the skin, the largest organ of the body, have the capacity to form multiple cell lineages during development. Using our recently developed sorting technique, we isolated viable homogeneous populations of somatic epidermal stem and transient amplifying cells from the skin of 3-day old transgenic mice, who carried the enhanced green fluorescent protein transgene, and injected stem, TA, or unsorted basal epidermal cells into 3.5-day C57BL/6 blastocysts. Only the stem-injected blastocysts produced mice with GFP(+) cells in their tissues. We found GFP(+) cells in ectodermal, mesenchymal, and neural-crest-derived tissues in E13.5 embryos, 13-day-old neonates, and 60-day-old adult mice, proving that epidermal stem cells survived the blastocyst injection and multiplied during development. Furthermore, the injected stem cells altered their epidermal phenotype and expressed the appropriate proteins for the tissues into which they developed, demonstrating that somatic epidermal stem cells have the ability to produce cells of different lineages during development. These data suggest that somatic epidermal stem cells may show a generalized plasticity expected only of embryonic stem cells and that environmental (extrinsic) factors may influence the lineage pathway for somatic stem cells. Thus, the skin could be a source of easily accessible stem cells that are able to be reprogrammed to form multiple cell lineages.

Loeffler, M., A. Birke, et al. (1993). "Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt." J Theor Biol **160**(4): 471-91.

Among highly proliferating tissues the intestinal tissue is of particular interest. Techniques are available that permit an insight into how intestinal crypts as the basic macroscopic tissue unit are

regenerated from a small population of self-maintaining stem cells. However, neither the precise number of these stem cells nor their properties are known. We have recently suggested a model of stem cell organization which explains the life cycle of murine intestinal crypts, their birth (by crypt fission) and extinction rates, as well as their size distribution on a quantitative basis (Loeffler & Grossman, 1991). The model assumptions involve two stochastic branching processes, one for the growth of several independent indistinguishable stem cells and a second for a threshold dependent crypt fission process. New data have now become available challenging the above concept. They relate to the conversion of crypts to monoclonal phenotypic expression after mutagenic events, presumably taking place in single stem cells. A detailed analysis of these data is shown here utilizing a more elaborate version of the above model. The new data are consistent with this model within the range of parameters predicted previously. We conclude that the cellular regeneration of intestinal crypts can be explained on the basis of several indistinguishable stem cells which can replace each other.

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

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

Markoulaki, S., A. Meissner, et al. (2008). "Somatic cell nuclear transfer and derivation of embryonic stem cells in the mouse." Methods **45**(2): 101-14.

Addressing the fundamental questions of nuclear equivalence in somatic cells has fascinated scientists for decades and has resulted in the development of somatic cell nuclear transfer (SCNT) or animal cloning. SCNT involves the transfer of the nucleus of a somatic cell into the cytoplasm of an egg whose own chromosomes have been removed. In the mouse, SCNT has not only been successfully used to address the issue of nuclear equivalence, but has been used as a model system to test the hypothesis that embryonic stem cells (ESCs) derived from NT blastocysts have the potential to correct--through genetic manipulations--degenerative diseases. This paper aims to provide a comprehensive description of SCNT in the mouse and the derivation of ESCs from blastocysts generated by this technique. SCNT is a very challenging and inefficient procedure because it is technically complex, it bypasses the normal events of gamete interactions and egg activation, and it depends on adequate reprogramming of the somatic cell nucleus in vivo. Improvements in any or all those aspects may enhance the efficiency and applicability of SCNT. ESC derivation from SCNT blastocysts, on the other hand, requires the survival of only a few successfully reprogrammed cells, which have the capacity to proliferate indefinitely in vitro, maintain correct genetic and epigenetic status, and differentiate into any cell type in the body--characteristics that are essential for transplantation therapy or any other in vivo application.

Mayani, H. (2003). "A glance into somatic stem cell biology: basic principles, new concepts, and clinical relevance." Arch Med Res **34**(1): 3-15.

Somatic stem cells are undifferentiated cells with a high capacity for self-renewal that can give rise to one or more specialized cell types with specific functions in the body. Profound characterization of these cells has been difficult due to the fact that their frequency in different tissues of the body is extremely low; furthermore, their identification is not based on their morphology but on immunophenotypic and functional assays. Nevertheless, significant advances in the study of these cells at both cellular and

molecular levels have been achieved during the last decade. The majority of what we know concerning somatic stem cell biology has come from work on hematopoietic stem cells. More recently, however, there has been a great amount of information on neural and epithelial stem cells. The importance of stem cell research has gone beyond basic biology and is currently contributing to the development of new medical approaches for treatment of hematologic, neurologic, autoimmune, and metabolic disorders (cellular therapy).

Neganova, I. and M. Lako (2008). "G1 to S phase cell cycle transition in somatic and embryonic stem cells." J Anat **213**(1): 30-44.

It is well known that G1 to S phase transition is tightly regulated by the expression and phosphorylation of a number of well-characterized cyclins, cyclin-dependent kinases and members of the retinoblastoma gene family. In this review we discuss the role of these components in regulation of G1 to S phase transition in somatic cells and human embryonic stem cells. Most importantly, we discuss some new tenable links between maintenance of pluripotency and cell cycle regulation in embryonic stem cells by describing the role that master transcription factors play in this process. Finally, the differences in cell cycle regulation between murine and human embryonic stem cells are highlighted, raising interesting questions regarding their biology and stages of embryonic development from which they have been derived.

Oda, M., S. Tanaka, et al. (2009). "Establishment of trophoblast stem cell lines from somatic cell nuclear-transferred embryos." Proc Natl Acad Sci U S A **106**(38): 16293-7.

Placental abnormalities occur frequently in cloned animals. Here, we attempted to isolate trophoblast stem (TS) cells from mouse blastocysts produced by somatic cell nuclear transfer (NT) at the blastocyst stage (NT blastocysts). Despite the predicted deficiency of the trophoblast cell lineage, we succeeded in isolating cell colonies with typical morphology of TS cells and cell lines from the NT blastocysts (ntTS cell lines) with efficiency as high as that from native blastocysts. The established 10 ntTS cell lines could be maintained in the undifferentiated state and induced to differentiate into several trophoblast subtypes in vitro. A comprehensive analysis of the transcriptional and epigenetic traits demonstrated that ntTS cells were indistinguishable from control TS cells. In addition, ntTS cells contributed exclusively to the placenta and survived until term in chimeras, indicating that ntTS cells have developmental potential as stem cells. Taken together, our data show that NT blastocysts contain cells that can produce TS cells in culture, suggesting that proper commitment to the trophoblast cell lineage in NT embryos occurs by the blastocyst stage.

Pralong, D., K. Mrozik, et al. (2005). "A novel method for somatic cell nuclear transfer to mouse embryonic stem cells." Cloning Stem Cells **7**(4): 265-71.

Nuclear reprogramming by somatic cell nuclear transfer (SCNT) provides a practical approach for generating autologous pluripotent cells from adult somatic cells. It has been shown that murine somatic cells can also be reprogrammed to a pluripotent-like state by fusion with embryonic stem (ES) cells. Typically, the first step in SCNT involves enucleation of the recipient cell. However, recent evidence suggests that enucleated diploid ES cells may lack reprogramming capabilities. Here we have developed methods whereby larger tetraploid ES cells are first generated by fusion of two mouse ES cell lines transfected with plasmids carrying different antibiotic-resistance cassettes, followed by double antibiotic selection. Tetraploid ES cells grown on tissue culture disks or wells can be efficiently enucleated (up to 99%) using a combination of cytochalasin B treatment and centrifugation, with cytoplasts generated from these cells larger than those obtained from normal diploid ES cells. Also, we show that the enucleation rate is dependent on centrifugation time and cell ploidy. Further, we demonstrate that normal diploid ES cells can be fused to tetraploid ES cells to form heterokaryons, and that selective differential centrifugation conditions can be applied where the tetraploid nucleus is removed while the diploid donor nucleus is retained. This technology opens new avenues for generating autologous, diploid pluripotent cells, and provides a dynamic model for studying nuclear reprogramming in ES cells.

Reubinoff, B. E., M. F. Pera, et al. (2000). "Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro." Nat Biotechnol **18**(4): 399-404.

We describe the derivation of pluripotent embryonic stem (ES) cells from human blastocysts. Two diploid ES cell lines have been cultivated in vitro for extended periods while maintaining expression of markers characteristic of pluripotent primate cells. Human ES cells express the transcription factor Oct-4, essential for development of pluripotential cells in the mouse. When grafted into SCID mice, both lines give rise to teratomas containing derivatives of all three embryonic germ layers. Both cell lines differentiate in vitro into extraembryonic and somatic cell lineages. Neural progenitor cells may be isolated from

differentiating ES cell cultures and induced to form mature neurons. Embryonic stem cells provide a model to study early human embryology, an investigational tool for discovery of novel growth factors and medicines, and a potential source of cells for use in transplantation therapy.

Seipel, K., N. Yanze, et al. (2004). "The germ line and somatic stem cell gene Cniwi in the jellyfish Podocoryne carnea." Int J Dev Biol **48**(1): 1-7.

In most animal phyla from insects to mammals, there is a clear division of somatic and germ line cells. This is however not the case in plants and some animal phyla including tunicates, flatworms and the basal phylum Cnidaria, where germ stem cells arise de novo from somatic cells. Piwi-like genes represent essential stem cell genes in diverse multicellular organisms. The cnidarian Piwihomolog Cniwiwas cloned from Podocoryne carnea, a hydrozoan with a full life cycle. CniwiRNA is present in all developmental stages with highest levels in the egg and the medusa. In the adult medusa, Cniwi expression is prominent in the gonads where it likely functions as a germ stem cell gene. The gene is also expressed, albeit at low levels, in differentiated somatic cells like the striated muscle of the medusa. Isolated striated muscle cells can be induced to transdifferentiate into smooth muscle cells which proliferate and differentiate into nerve cells. Cniwi expression is upregulated transiently after induction of transdifferentiation and again when the emerging smooth muscle cells proliferate and differentiate. The continuous low-level expression of an inducible stem cell gene in differentiated somatic cells may underlie the ability to form medusa buds from polyp cells and explain the extraordinary transdifferentation and regeneration potential of Podocoryne carnea.

Sidorov, I., M. Kimura, et al. (2009). "Leukocyte telomere dynamics and human hematopoietic stem cell kinetics during somatic growth." Exp Hematol **37**(4): 514-24.

OBJECTIVE: A central question in stem cell research is knowing the frequency of human hematopoietic stem cells (HSC) replication in vivo. MATERIALS AND METHODS: We have constructed a model that characterizes HSC kinetics and the relative sizes of the hematopoietic progenitor cell (HPC) and HSC pools from birth onward. The model capitalizes on leukocyte telomere length (LTL) data and body weight-gain charts from birth to the age of 20 years. The core premise of the model is that during human growth, LTL dynamics (birth LTL and age-dependent LTL shortening afterward) chronicle the expansions of the HSC and HPC pools. RESULTS: The model estimates that by the end of the first year of life, HSC have replicated approximately 17 times and they replicate approximately 2.5 times/year between the ages of 3 and 13 years. Subsequently, HSC replication slows considerably. In adults HSC replicate at a rate of approximately 0.6 times/year. In addition, the model predicts that newborns with small birth weight would have shorter LTL as adults and that women would have longer LTL than men. CONCLUSION: Our findings will be useful in bone marrow transplantations and might explain a body of clinical observations related to LTL distribution in the general population.

Song, X. and T. Xie (2002). "DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the Drosophila ovary." Proc Natl Acad Sci U S A **99**(23): 14813-8.

Evidence from many systems has shown that stem cells are maintained in "niches" or specific regulatory microenvironments formed by stromal cells. The question of how stem cells are maintained in their niches is important, and further studies will lead to a better understanding of stem cell regulation and enhance the future use of stem cells in regenerative medicine. Here we show that cadherin-mediated cell adhesion is required for anchoring somatic stem cells (SSCs) to their niches in the Drosophila ovary. DE-cadherin and Armadillo/beta-catenin accumulate in the junctions between SSCs and their neighboring cells, inner germarial sheath cells. Removal of DE-cadherin from SSCs results in stem cell loss in the adult ovary. Furthermore, the cadherin-mediated adhesion is also important for maintaining SSCs in their niches before adulthood. This study provides further support that SSCs are located in a niche formed by their neighboring cells. We have previously shown that DE-cadherin-mediated cell adhesion is essential for anchoring germ-line stem cells to their niches in the Drosophila ovary. This study further implicates cadherin-mediated cell adhesion as a general mechanism for anchoring stem cells to their niches in a variety of systems.

Strelchenko, N., V. Kukharenko, et al. (2006). "Reprogramming of human somatic cells by embryonic stem cell cytoplast." Reprod Biomed Online **12**(1): 107-11.

Somatic cell nuclear transfer (SCNT) provides the basis for the development of patient-specific stem cell lines. Recent progress in SCNT suggested the presence of reprogramming factors in human embryonic stem (hES) cells, although no method is currently available for replacement of nuclei of hES cells by somatic cell nuclei. An original technique has been developed, involving the fusion of different types of somatic cells with hES cells, which

allowed a complete replacement of the nuclei of hES cells by nuclei of somatic cells. The resulting 'cybrids' were demonstrated to have the genotype of the donor somatic cells and 'stemness' of the recipient hES cells. However, the colonies isolated from the resulting fusion contained a mixture of these cybrid cells with the cells with the recipient nuclei, as well as hybrid cells containing both donor and recipient nuclei, so future purification will be necessary before the technique can be considered for future practical application.

Subramanian, V. (1989). "A malignant, stem cell-like somatic hybrid between a mouse teratocarcinoma and a rat ascitic hepatoma is differentiation competent." Cell Differ Dev **27**(3): 197-214.

A hybrid clone was developed by the fusion of a pluripotent mouse teratocarcinoma cell line PCC-4 AzaR to the Zajdela ascitic hepatoma (ZAH) of rat origin. This hybrid cell line, F2231A, possessed a predominantly teratocarcinoma morphology with a large nucleus and prominent nucleoli, and grew in nests. F2231A cells formed undifferentiated tumours in irradiated Sv/129 mice. It formed aggregates when subcultured at high densities in bacteriological Petri dishes. The hybrid cell line differentiated in response to retinoic acid and also underwent spontaneous differentiation upon overgrowth. Karyological analysis showed the presence of several rat chromosomes in the hybrid and upon isozyme analysis it was found that only the rat variant of the X-linked enzyme HGPRT was expressed. Analysis of the genomic DNA with a cloned probe, specific for rat repetitive sequences, gave strong positive signals in the hepatoma parent and F2231A cells while the parental embryonal carcinoma (EC) cells were negative. The hybrid cell line, like the PCC-4 cells, expressed the SSEA-1 surface marker but not SSEA-3, intercellular fibronectin and EGF receptors. Upon differentiation of F2231A cells there was a loss of expression of SSEA-1. The mRNA for alpha-fetoprotein was expressed by the hybrid cell line and in this respect it resembled the hepatoma parent. Albumin mRNA was not detectable in the hybrid cell line. The mRNA for the transformation-related protein, p53, was expressed at a high level in F2231A cells. The hybrid cell line F2231A retained several of the biochemical and immunological properties of the teratocarcinoma cells.

Sullivan, S., S. Pells, et al. (2006). "Nuclear reprogramming of somatic cells by embryonic stem cells is affected by cell cycle stage." Cloning Stem Cells **8**(3): 174-88.

Hybrid embryonic stem (ES)-like clones were generated by fusion of murine ES cells with somatic cells that carried a neo resistance gene under the transcriptional control of the Oct-4 promoter. The Oct-4 promoter was reactivated in hybrid ES cells formed by fusion with fetal fibroblasts, and all hybrid colonies were of ES rather than fibroblast phenotype, suggesting efficient reprogramming of fibroblast chromosomes. Like normal diploid murine ES cells, hybrid lines expressed alkaline phosphatase activity and formed differentiated cells derived from the three embryonic germ layers both in vitro and in vivo. Treatments thought to affect nuclear transfer efficiency (ES cell confluence and serum starvation of primary embryonic fibroblasts) were investigated to determine whether they had an effect on reprogramming in cell hybrids. Serum starvation of primary embryonic fibroblasts increased hybrid colony number 50-fold. ES cells were most effective at reprogramming when they contained a high proportion of cells in the S and G2/M phases of the cell cycle. These data suggest that nuclear reprogramming requires an initial round of somatic DNA replication of quiescent chromatin in the presence of ES-derived factors produced during S and G2/M phases.

Sung, L. Y., S. Gao, et al. (2006). "Differentiated cells are more efficient than adult stem cells for cloning by somatic cell nuclear transfer." Nat Genet **38**(11): 1323-8.

Since the creation of Dolly via somatic cell nuclear transfer (SCNT), more than a dozen species of mammals have been cloned using this technology. One hypothesis for the limited success of cloning via SCNT (1%-5%) is that the clones are likely to be derived from adult stem cells. Support for this hypothesis comes from the findings that the reproductive cloning efficiency for embryonic stem cells is five to ten times higher than that for somatic cells as donors and that cloned pups cannot be produced directly from cloned embryos derived from differentiated B and T cells or neuronal cells. The question remains as to whether SCNT-derived animal clones can be derived from truly differentiated somatic cells. We tested this hypothesis with mouse hematopoietic cells at different differentiation stages: hematopoietic stem cells, progenitor cells and granulocytes. We found that cloning efficiency increases over the differentiation hierarchy, and terminally differentiated postmitotic granulocytes yield cloned pups with the greatest cloning efficiency.

Tsao, J. L., J. Zhang, et al. (1998). "Tracing cell fates in human colorectal tumors from somatic microsatellite mutations: evidence of adenomas with stem cell architecture." Am J Pathol **153**(4): 1189-200.

Occult aspects of tumor proliferation are likely recorded genetically as their microsatellite (MS) loci become polymorphic. However, MS mutations generated by division may also be eliminated with death as noncoding MS loci lack selective value. Therefore, highly polymorphic MS loci cannot exist unless mutation rates are high, or unless mutation losses are inherently minimized. Mutations accumulate differently when cell fates are determined intrinsically before or extrinsically after division. Stem cell (asymmetrical division as in intestinal crypts) and random (asymmetrical and symmetrical division) proliferation, respectively, represent simulated cell fates determined before or after division. Whereas mutations regardless of selection systematically persist once inherited with stem cell proliferation, mutations are eliminated by the symmetrical losses of both daughter cells with random proliferation. Therefore, greater genetic diversity or MS variance accumulate with stem cell compared with random proliferation. MS loci in normal murine intestinal mucosa and xenografts of cancer cell lines accumulated mutations, respectively, consistent with stem cell and random proliferation. Tumors from patients with hereditary nonpolyposis colorectal cancer (HNPCC) demonstrated polymorphic MS loci. Overall, three of five adenomas and one of six cancers exhibited high MS variances. Assuming mutation rates are not significantly greater in adenomas than in cancers, these studies suggest the stem cell proliferation and hierarchy of normal intestines persists in many HNPCC adenomas and some cancers. An adenoma stem cell architecture can explain the complex polymorphic MS loci observed in HNPCC adenomas and account for many adenoma features. In contrast, cancers may lose intrinsic control of cell fate. These studies illustrate a feasible phylogenetic approach to unravel and describe occult aspects of human tumor proliferation. The switch from predominantly stem cell to random proliferation may be a critical and defining characteristic of malignancy.

Vasilkova, A. A., H. A. Kizilova, et al. (2007). "Dominant manifestation of pluripotency in embryonic stem cell hybrids with various numbers of somatic chromosomes." Mol Reprod Dev **74**(8): 941-51.

Developmental potential was assessed in 8 intra-specific and 20 inter-specific hybrid clones obtained by fusion of embryonic stem (ES) cells with either splenocytes or fetal fibroblasts. Number of chromosomes derived from ES cells in these hybrid clones was stable while contribution of somatic partner varied from single chromosomes to complete complement. This allowed us to compare pluripotency of the hybrid cells with various numbers of somatic chromosomes. Three criteria were used for the assessment: (i) expression of Oct-4 and Nanog genes; (ii) analyses of teratomas generated by subcutaneous injections of the tested cells into immunodeficient mice; (iii) contribution of the hybrid cells in chimeras generated by injection of the tested cells into C57BL blastocysts. All tested hybrid clones showed expression of Oct-4 and Nanog at level comparable to ES cells. Histological and immunofluorescent analyses demonstrated that most teratomas formed from the hybrid cells with different number of somatic chromosomes contained derivatives of three embryonic layers. Tested hybrid clones make similar contribution in various tissues of chimeras in spite of significant differences in the number of somatic chromosomes they contained. The data indicate that pluripotency is manifested as a dominant trait in the ES hybrid cells and does not depend substantially on the number of somatic chromosomes. The latter suggests that the developmental potential derived from ES cells is maintained in ES-somatic cell hybrids by cis-manner and is rather resistant to trans-acting factors emitted from the somatic one.

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

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

Wakayama, T. (2006). "Establishment of nuclear transfer embryonic stem cell lines from adult somatic cells by nuclear transfer and its application." Ernst Schering Res Found Workshop(60): 111-23.

Nuclear transfer can be used to generate embryonic stem cell (ntESC) lines from a patient's own somatic cells. We have shown that ntESCs can be generated relatively easily from a variety of mouse genotypes and cell types of both sexes, even though it may be more difficult to generate clones directly. Several reports have already demonstrated that ntESCs can be used in regenerative medicine in order to rescue immunodeficient or infertile phenotypes. However, it is unclear whether ntES cells are identical to fertilized embryonic stem cells (ESCs). This review

seeks to describe the phenotype and possible abnormalities of ntESC lines.

Wakayama, T., V. Tabar, et al. (2001). "Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Science **292**(5517): 740-3.

Embryonic stem (ES) cells are fully pluripotent in that they can differentiate into all cell types, including gametes. We have derived 35 ES cell lines via nuclear transfer (ntES cell lines) from adult mouse somatic cells of inbred, hybrid, and mutant strains. ntES cells contributed to an extensive variety of cell types, including dopaminergic and serotonergic neurons in vitro and germ cells in vivo. Cloning by transfer of ntES cell nuclei could result in normal development of fertile adults. These studies demonstrate the full pluripotency of ntES cells.

Williams, E. D., A. P. Lowes, et al. (1992). "A stem cell niche theory of intestinal crypt maintenance based on a study of somatic mutation in colonic mucosa." Am J Pathol **141**(4): 773-6.

In both large and small intestine, mutagen administration leads to the occurrence of isolated crypts that are completely populated by a mutated phenotype; therefore, it has been proposed that crypts are maintained by a single stem cell. We show in mice that a single dose of mutagen leads to an early transient increase in frequency of colonic crypts that show a partial mutated phenotype and a later increase in frequency of crypts that show a complete mutated phenotype. This increase reaches a plateau at about the same time as the disappearance of partially mutated crypts. The same is true in the small intestine, but the time course is much slower. We propose an explanation based on multiple crypt stem cells that occupy a "stem cell niche," with random cell loss after stem cell division. A small difference in the number of crypt stem cells that occupy the niche provides a simple explanation for the surprisingly large difference in the time course of phenotypic changes in the large and small intestines after administration of a single dose of mutagen.

Zhang, Y. and D. Kalderon (2001). "Hedgehog acts as a somatic stem cell factor in the Drosophila ovary." Nature **410**(6828): 599-604.

Secreted signalling molecules of the Hedgehog (Hh) family have many essential patterning roles during development of diverse organisms including Drosophila and humans. Although Hedgehog proteins most commonly affect cell fate, they can also stimulate cell proliferation. In humans several distinctive cancers, including basal-cell carcinoma, result from mutations that aberrantly activate Hh signal transduction. In Drosophila, Hh directly stimulates proliferation of ovarian somatic cells. Here we show that Hh acts specifically on stem cells in the Drosophila ovary. These cells cannot proliferate as stem cells in the absence of Hh signalling, whereas excessive Hh signalling produces supernumerary stem cells. We deduce that Hh is a stem-cell factor and suggest that human cancers due to excessive Hh signalling might result from aberrant expansion of stem cell pools.

Glossary of Stem Cell

1 Adult stem cell—Also called somatic stem cell, a relatively rare undifferentiated cell found in many organs and differentiated tissues with a limited capacity for both self renewal and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. This is an active area of investigation.
2 Astrocyte—A type of glial cell existed in the nervous system.
3 Blastocoel—The fluid-filled cavity inside the blastocyst, an early, preimplantation stage of the developing embryo.
4 Blastocyst—A preimplantation embryo of about 150 cells produced by cell division following fertilization. The blastocyst is a sphere made up of an outer layer of cells (trophoblast), a fluid-filled cavity (blastocoel), and a cluster of cells on the interior (inner cell mass).
5 Bone marrow stromal cells—A population of cells found in bone marrow that are different from blood cells.
6 Bone marrow stromal stem cells (skeletal stem cells)—A multipotent subset of bone marrow stromal cells able to form bone, cartilage, stromal cells that support blood formation, fat, and fibrous tissue.
7 Cell-based therapies—Treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cells or tissues.
8 Cell culture—Growth of cells in vitro in an artificial medium for research or medical treatment.
9 Cell division—Method by which a single cell divides to create two cells. There are two main types of cell division depending on what happens to the chromosomes: mitosis and meiosis.
10 Chromosome—A structure consisting of DNA and regulatory proteins found in the nucleus of the cell. The DNA in the nucleus is usually divided up among several chromosomes. The number of chromosomes in the nucleus varies depending on the species of the organism. Humans have 46 chromosomes, 23 pairs.
11 Clone— (v) To generate identical copies of a region of a DNA molecule or to generate genetically identical copies of a cell, or organism; (n) The identical molecule, cell, or organism that results from the cloning process.

(1) In reference to DNA: To clone a gene, one finds the region where the gene resides on the DNA and copies that section of the DNA using laboratory techniques.
(2) In reference to cells grown in a tissue culture dish: a clone is a line of cells that is genetically identical to the originating cell. This cloned line is produced by cell division (mitosis) of the original cell.
(3) In reference to organisms: Many natural clones are produced by plants and (mostly invertebrate) animals. The term clone may also be used to refer to an animal produced by somatic cell nuclear transfer or parthenogenesis.

12 Cloning—Clone. (v) To generate identical copies of a region of a DNA molecule or to generate genetically identical copies of a cell, or organism; (n) The identical molecule, cell, or organism that results from the cloning process.
13 Cord blood stem cells—See Umbilical cord blood stem cells.
14 Culture medium—The liquid that covers cells in a culture dish and contains nutrients to nourish and support the cells. Culture medium may also include growth factors added to produce desired changes in the cells.
15 Differentiation—The process whereby an unspecialized embryonic cell acquires the features of a specialized cell such as a heart, liver, or muscle cell. Differentiation is controlled by the interaction of a cell's genes with the physical and chemical conditions outside the cell, usually through signaling pathways involving proteins embedded in the cell surface.
16 Directed differentiation—The manipulation of stem cell culture conditions to induce differentiation into a particular cell type.
17 DNA—Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions or blueprint for making all the structures and materials the body needs to function. DNA consists of both genes and non-gene DNA in between the genes.
18 Ectoderm—The outermost germ layer of cells derived from the inner cell mass of the blastocyst; gives rise to the nervous system, sensory organs, skin, and related structures.
19 Embryo—In humans, the developing organism from the time of fertilization until the end of the eighth week of gestation, when it is called a fetus.
20 Embryoid bodies—Rounded collections of cells that arise when embryonic stem cells are cultured in suspension. Embryoid bodies contain cell types derived from all 3 germ layers.
21 Embryonic germ cells—Pluripotent stem cells that are derived from early germ cells (those that would become sperm and eggs). Embryonic germ cells are thought to have properties similar to embryonic stem cells.
22 Embryonic stem cells—Primitive (undifferentiated) cells that are derived from preimplantation-stage embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. Embryonic stem cells are isolated from cells in a blastocyst, a very early stage embryo. Once isolated from the blastocyst, these cells form colonies in culture (closely packed groups of cells) and can become cells of the three germ layers, which later make up the adult body.
23 Embryonic stem cell line—Embryonic stem cells, which have been cultured under in vitro conditions that allow proliferation without differentiation for months to years.
24 Endoderm—The innermost layer of the cells derived from the inner cell mass of the blastocyst; it gives rise to lungs, other respiratory structures, and digestive organs, or generally "the gut."
25 Enucleated—Having had its nucleus removed.
26 Epigenetic—Having to do with the process by which regulatory proteins can turn genes on or off in a way that can be passed on during cell division.
27 Feeder layer—Cells used in co-culture to maintain pluripotent stem cells. For human embryonic stem cell culture, typical feeder layers include mouse embryonic fibroblasts or human embryonic fibroblasts that have been treated to prevent them from dividing.
28 Fertilization—The joining of the male gamete (sperm) and the female gamete (egg).
29 Fetus—In humans, the developing human from approximately eight weeks after conception until the time of its birth.
30 Gamete—An egg (in the female) or sperm (in the male) cell. See also Somatic cell.
31 Gastrulation—The process in which cells proliferate and migrate within the embryo to transform the inner cell mass of the blastocyst stage into an embryo containing all three primary germ layers.
32 Gene—A functional unit of heredity that is a segment of DNA found on chromosomes in the nucleus of a cell. Genes direct the formation of an enzyme or other protein.
33 Germ layers—After the blastocyst stage of embryonic development, the inner cell mass of the blastocyst goes through gastrulation, a period when the inner cell mass becomes organized into three distinct cell layers, called germ layers. The three layers are the ectoderm, the mesoderm, and the endoderm.
34 Hematopoietic stem cell—A stem cell that gives rise to all red and white blood cells and platelets.
35 Human embryonic stem cell (hESC)—A type of pluripotent stem cells derived from early stage human embryos, up to and including the blastocyst stage, that are capable of dividing without differentiating for a prolonged period in

culture, and are known to develop into cells and tissues of the three primary germ layers.

36 Induced pluripotent stem cell (iPSC)—A type of pluripotent stem cell, similar to an embryonic stem cell, formed by the introduction of certain embryonic genes into a somatic cell.

37 In vitro—Latin for "in glass"; in a laboratory dish or test tube; an artificial environment.

38 In vitro fertilization—A technique that unites the egg and sperm in a laboratory instead of inside the female body.

39 Inner cell mass (ICM)—The cluster of cells inside the blastocyst. These cells give rise to the embryo and ultimately the fetus. The ICM may be used to generate embryonic stem cells.

40 Long-term self-renewal—The ability of stem cells to replicate themselves by dividing into the same non-specialized cell type over long periods (many months to years) depending on the specific type of stem cell.

41 Mesenchymal stem cells—A term that is currently used to define non-blood adult stem cells from a variety of tissues, although it is not clear that mesenchymal stem cells from different tissues are the same.

42 Meiosis—The type of cell division a diploid germ cell undergoes to produce gametes (sperm or eggs) that will carry half the normal chromosome number. This is to ensure that when fertilization occurs, the fertilized egg will carry the normal number of chromosomes rather than causing aneuploidy (an abnormal number of chromosomes).

43 Mesoderm—Middle layer of a group of cells derived from the inner cell mass of the blastocyst; it gives rise to bone, muscle, connective tissue, kidneys, and related structures.

44 Microenvironment—The molecules and compounds such as nutrients and growth factors in the fluid surrounding a cell in an organism or in the laboratory, which play an important role in determining the characteristics of the cell.

45 Mitosis—The type of cell division that allows a population of cells to increase its numbers or to maintain its numbers. The number of chromosomes remains the same in this type of cell division.

46 Multipotent—Having the ability to develop into more than one cell type of the body. See also pluripotent and totipotent.

47 Neural stem cell—A stem cell found in adult neural tissue that can give rise to neurons and glial (supporting) cells. Examples of glial cells include astrocytes and oligodendrocytes.

48 Neurons—Nerve cells, the principal functional units of the nervous system. A neuron consists of a cell body and its processes—an axon and one or more dendrites. Neurons transmit information to other neurons or cells by releasing neurotransmitters at synapses.

49 Oligodendrocyte—A supporting cell that provides insulation to nerve cells by forming a myelin sheath (a fatty layer) around axons.

50 Parthenogenesis—The artificial activation of an egg in the absence of a sperm; the egg begins to divide as if it has been fertilized.

51 Passage—In cell culture, the process in which cells are disassociated, washed, and seeded into new culture vessels after a round of cell growth and proliferation. The number of passages a line of cultured cells has gone through is an indication of its age and expected stability.

52 Pluripotent—Having the ability to give rise to all of the various cell types of the body. Pluripotent cells cannot make extra-embryonic tissues such as the amnion, chorion, and other components of the placenta. Scientists demonstrate pluripotency by providing evidence of stable developmental potential, even after prolonged culture, to form derivatives of all three embryonic germ layers from the progeny of a single cell and to generate a teratoma after injection into an immunosuppressed mouse.

53 Polar Body—A polar body is a structure produced when an early egg cell, or oogonium, undergoes meiosis. In the first meiosis, the oogonium divides its chromosomes evenly between the two cells but divides its cytoplasm unequally. One cell retains most of the cytoplasm, while the other gets almost none, leaving it very small. This smaller cell is called the first polar body. The first polar body usually degenerates. The ovum, or larger cell, then divides again, producing a second polar body with half the amount of chromosomes but almost no cytoplasm. The second polar body splits off and remains adjacent to the large cell, or oocyte, until it (the second polar body) degenerates. Only one large functional oocyte, or egg, is produced at the end of meiosis.

54 Preimplantation—With regard to an embryo, preimplantation means that the embryo has not yet implanted in the wall of the uterus. Human embryonic stem cells are derived from preimplantation-stage embryos fertilized outside a woman's body (in vitro).

55 Proliferation—Expansion of the number of cells by the continuous division of single cells into two identical daughter cells.

56 Regenerative medicine—A field of medicine devoted to treatments in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cell populations or tissues. (See also cell-based therapies).

57 Reproductive cloning—The process of using somatic cell nuclear transfer (SCNT) to produce a normal, full grown organism (e.g., animal) genetically identical to the organism (animal) that donated the somatic cell nucleus. In mammals, this would require implanting the resulting embryo in a uterus where it would undergo normal development to become a live independent being.

The first mammal to be created by reproductive cloning was Dolly the sheep, born at the Roslin Institute in Scotland in 1996. See also Somatic cell nuclear transfer (SCNT).

58 Signals—Internal and external factors that control changes in cell structure and function. They can be chemical or physical in nature.

59 Somatic cell—Any body cell other than gametes (egg or sperm); sometimes referred to as "adult" cells. See also Gamete.

60 Somatic cell nuclear transfer (SCNT)—A technique that combines an enucleated egg and the nucleus of a somatic cell to make an embryo. SCNT can be used for therapeutic or reproductive purposes, but the initial stage that combines an enucleated egg and a somatic cell nucleus is the same. See also therapeutic cloning and reproductive cloning.

61 Somatic (adult) stem cells—A relatively rare undifferentiated cell found in many organs and differentiated tissues with a limited capacity for both self renewal and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. This is an active area of investigation.

62 Stem cells—Cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells.

63 Stromal cells—Connective tissue cells found in virtually every organ. In bone marrow, stromal cells support blood formation.

64 Subculturing—Transferring cultured cells, with or without dilution, from one culture vessel to another.

65 Surface markers—Proteins on the outside surface of a cell that are unique to certain cell types and that can be visualized using antibodies or other detection methods.

66 Telomere—The end of a chromosome, associated with a characteristic DNA sequence that is replicated in a special way. A telomere counteracts the tendency of the chromosome to shorten with each round of replication.

67 Teratoma—A multi-layered benign tumor that grows from pluripotent cells injected into mice with a dysfunctional immune system. Scientists test whether they have established a human embryonic stem cell (hESC) line by injecting putative stem cells into such mice and verifying that the resulting teratomas contain cells derived from all three embryonic germ layers.

68 Tetraploid complementation assay—An assay that can be used to test a stem cell's potency. Scientists studying mouse chimeras (mixing cells of two different animals) noted that fusing two 8-cell embryos produces cells with 4 sets of chromosomes (tetraploid cells) that are biased toward developing into extra-embryonic tissues such as the placenta. The tetraploid cells do not generate the embryo itself; the embryo proper develops from injected diploid stem cells. This tendency has been exploited to test the potency of a stem cell. Scientists begin with a tetraploid embryo. Next, they inject the stem cells to be tested. If the injected cells are pluripotent, then an embryo develops. If no embryo develops, or if the resultant embryo cannot survive until birth, the scientists conclude that the cells were not truly pluripotent.

69 Therapeutic cloning—The process of using somatic cell nuclear transfer (SCNT) to produce cells that exactly match a patient. By combining a patient's somatic cell nucleus and an enucleated egg, a scientist may harvest embryonic stem cells from the resulting embryo that can be used to generate tissues that match a patient's body. This means the tissues created are unlikely to be rejected by the patient's immune system. See also Somatic cell nuclear transfer (SCNT).

70 Totipotent—Having the ability to give rise to all the cell types of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta. (See also Pluripotent and Multipotent).

71 Transdifferentiation—The process by which stem cells from one tissue differentiate into cells of another tissue.

72 Trophectoderm—The outer layer of the preimplantation embryo in mice. It contains trophoblast cells.

73 Trophoblast—The outer cell layer of the blastocyst. It is responsible for implantation and develops into the extraembryonic tissues, including the placenta, and controls the exchange of oxygen and metabolites between mother and embryo.

74 Umbilical cord blood stem cells—Stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body (hematopoietic). Cord blood is currently used to treat patients who have undergone chemotherapy to destroy their bone marrow due to cancer or other blood-related disorders.

75 Undifferentiated—A cell that has not yet developed into a specialized cell type.

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