Reverse of Life, Immortality and Stem Cell

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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 the reverse of life, immortality and stem cell.
[Smith MH. **Reverse of Life, Immortality and Stem Cell Literatures.** *Stem Cell* 2013;4(1):119-140] (ISSN 1545-4570). http://www.sciencepub.net/stem. 6

Key words: stem cell; life; gene; DNA; protein; reverse of life, immortality

As the whole, the universe is unlimited, no origin, no termination. However, each body and each event in the universe has a beginning and a terminating. The life in the earth, no matter as a species or a living body, has a birth and death naturally. Life looks at one direction in the timeline, from younger to older, from birth to death. However, is life line reversible? Can life become younger from older? For this question, my answer is yes.

In the universe, all the results come from the reasons, and all the reasons create results. In the timeline of the universe, all the current events (or universe condition) come from the past and become the future. Under a certain determined condition, all the factors exist. As the reason, it creates new condition. The younger condition of a life body changes to old under certain condition. Under other condition, the life body could change to the younger.

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

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

Totipotent stem cell can differentiate into all the human body's cells (about 200 types). In most animals, the only true totipotent stem cell is the fertilized egg and its immediate descendants. A totipotent stem cell can potentially generate a complete organism. Differentiation results from differential gene expression.

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

Cellular determination results from the asymmetric segregation of cellular determinants. However, in most cases, determination is the result of inductive signaling between cells. Asymmetric segregation of cellular determinants is caused from the asymmetric localization of cytoplasmic molecules within the cell before dividing. During cell division,

one daughter cell receives more localized molecules and the other daughter cell may receive less of these molecules, which results in two different daughter cells taking on different cell fates based on differences in gene expression. The localized cytoplasmic determinants are often transcription factors or mRNAs encoding by the transcription factors.

The field of stem cell biology has undergone tremendous expansion over the past two decades. Scientific investigation has continued to expand our understanding of these complex cells at a rapidly increasing rate. This understanding has produced a vast array of potential clinical applications (Hemmat, Lieberman et al. 2010).

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

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

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

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

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

Literatures:

Ambrosi, D. J., B. Tanasijevic, et al. (2007). "Genome-wide reprogramming in hybrids of somatic cells and embryonic stem cells." Stem Cells **25**(5): 1104-13.

Recent experiments demonstrate that somatic nuclei can be reprogrammed to a pluripotent state when fused to ESCs. The resulting hybrids are pluripotent as judged by developmental assays, but detailed analyses of the underlying molecular-genetic control of reprogrammed transcription in such hybrids are required to better understand fusion-mediated reprogramming. We produced hybrids of mouse ESCs and fibroblasts that, although nearly tetraploid, exhibit characteristics of normal ESCs, including apparent immortality in culture, ESC-like colony morphology, and pluripotency. Comprehensive analysis of the mouse embryonic fibroblast/ESC hybrid transcriptome revealed global patterns of gene expression reminiscent of ESCs. However, combined analysis of variance and hierarchical clustering analyses revealed at least seven distinct classes of differentially regulated genes in comparisons of hybrids, ESCs, and somatic cells. The largest class includes somatic genes that are silenced in hybrids and ESCs, but a smaller class includes genes that are expressed at nearly equivalent levels in hybrids and ESCs that contain many genes implicated in pluripotency and chromatin function. Reprogrammed genes are distributed throughout the genome. Reprogramming events include both transcriptional silencing and activation of genes residing on chromosomes of somatic origin. Somatic/ESC hybrid cell lines resemble their pre-fusion ESC partners in terms of behavior in culture and pluripotency. However, they contain unique expression profiles that are similar but not identical to normal ESCs. ESC fusion-mediated reprogramming provides a tractable system for the investigation of mechanisms of reprogramming. Disclosure of potential conflicts of interest is found at the end of this article.

Amit, M., V. Margulets, et al. (2003). "Human feeder layers for human embryonic stem cells." Biol Reprod **68**(6): 2150-6.

Human embryonic stem (hES) cells hold great promise for future use in various research areas, such as human developmental biology and cell-based therapies. Traditionally, these cells have been cultured on mouse embryonic fibroblast (MEF) feeder layers, which permit continuous growth in an undifferentiated stage. To use these unique cells in human therapy, an animal-free culture system must be used, which will prevent exposure to mouse retroviruses. Animal-free culture systems for hES cells enjoy three major advantages in the basic culture conditions: 1). the ability to grow these cells under serum-free conditions, 2). maintenance of the cells in an undifferentiated state on Matrigel matrix with 100% MEF-conditioned medium, and 3). the use of either human embryonic fibroblasts or adult fallopian tube epithelial cells as feeder layers. In the present study, we describe an additional animal-free culture system for hES cells, based on a feeder layer derived from foreskin and a serum-free medium. In this culture condition, hES cells maintain all embryonic stem cell features (i.e., pluripotency, immortality, unlimited undifferentiated

proliferation capability, and maintenance of normal karyotypes) after prolonged culture of 70 passages (>250 doublings). The major advantage of foreskin feeders is their ability to be continuously cultured for more than 42 passages, thus enabling proper analysis for foreign agents, genetic modification such as antibiotic resistance, and reduction of the enormous workload involved in the continuous preparation of new feeder lines.

Ashkenazi, R., S. N. Gentry, et al. (2008). "Pathways to tumorigenesis--modeling mutation acquisition in stem cells and their progeny." Neoplasia **10**(11): 1170-82.

Most adult tissues consist of stem cells, progenitors, and mature cells, and this hierarchical architecture may play an important role in the multistep process of carcinogenesis. Here, we develop and discuss the important predictions of a simple mathematical model of cancer initiation and early progression within a hierarchically structured tissue. This work presents a model that incorporates both the sequential acquisition of phenotype altering mutations and tissue hierarchy. The model simulates the progressive effect of accumulating mutations that lead to an increase in fitness or the induction of genetic instability. A novel aspect of the model is that symmetric self-renewal, asymmetric division, and differentiation are all incorporated, and this enables the quantitative study of the effect of mutations that deregulate the normal, homeostatic stem cell division pattern. The model is also capable of predicting changes in both tissue composition and in the progression of cells along their lineage at any given time and for various sequences of mutations. Simulations predict that the specific order in which mutations are acquired is crucial for determining the pace of cancer development. Interestingly, we find that the importance of genetic stability differs significantly depending on the physiological expression of mutations related to symmetric self-renewal and differentiation of stem and progenitor cells. In particular, mutations that lead to the alteration of the stem cell division pattern or the acquisition of some degree of immortality in committed progenitors lead to an early onset of cancer and diminish the impact of genetic instability.

Blagosklonny, M. V. (2006). "Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition." Cell Cycle **5**(18): 2087-102.

While ruling out programmed aging, evolutionary theory predicts a quasi-program for aging, a continuation of the developmental program that is not turned off, is constantly on, becoming hyper-functional and damaging, causing diseases of aging. Could it be switched off pharmacologically? This would require identification of a molecular target involved in cell senescence, organism aging and diseases of aging. Notably, cell senescence is associated with activation of the TOR (target of rapamycin) nutrient- and mitogen-sensing pathway, which promotes cell growth, even though cell cycle is blocked. Is TOR involved in organism aging? In fact, in yeast (where the cell is the organism), caloric restriction, rapamycin and mutations that inhibit TOR all slow down aging. In animals from worms to mammals caloric restrictions, life-extending agents, and numerous mutations that increase longevity all converge on the TOR pathway. And, in humans, cell hypertrophy, hyper-function and hyperplasia, typically associated with activation of TOR, contribute to diseases of aging. Theoretical and clinical considerations suggest that rapamycin may be effective against atherosclerosis, hypertension and hyper-coagulation (thus, preventing myocardial infarction and stroke), osteoporosis, cancer, autoimmune diseases and arthritis, obesity, diabetes, macula-degeneration, Alzheimer's and Parkinson's diseases. Finally, I discuss that extended life span will reveal new causes for aging (e.g., ROS, 'wear and tear', Hayflick limit, stem cell exhaustion) that play a limited role now, when quasi-programmed senescence kills us first.

Blagosklonny, M. V. (2007). "Cancer stem cell and cancer stemloids: from biology to therapy." Cancer Biol Ther **6**(11): 1684-90.

It has become a cliche that cancer therapy fails because it does not target rare cancer stem cells (CSCs). Here we are discuss that this is not how therapy fails and not any cancer cell with stem-like properties is CSC. Paradoxically, CSCs must be resting to explain their resistance to therapy yet must be cycling to explain their persistence in cell culture. To solve contradictions, this article introduces the term cancer stemloids (or stem cell-like cells) to describe proliferating self-renewing cells. The stem cell hierarchy (stem--proliferating--terminal cells) exists exactly to separate self-renewal (immortality) from proliferation. Cancer stemloids break the stem cell hierarchy and eventually may replace other cells. While CSC is shielded from any selective pressure and therefore unable to drive tumor progression, cancer stemloids undergo clonal selection, accumulate mutations, thus determining tumor progression and therapeutic failures. Unlike CSC, cancer stemloids are a crucial target for cancer therapy, exactly because they proliferate. Furthermore, two normally mutually-exclusive properties (proliferation and stemness) provide a means to design therapy to kill cancer stemloids selectively without killing normal stem and

non-stem cells. In contrast, true CSCs are not only a difficult, but also an insufficient and perhaps even an unnecessary therapeutic target, especially in advanced malignancies.

Borges, R. M. (2009). "Phenotypic plasticity and longevity in plants and animals: cause and effect?" J Biosci **34**(4): 605-11.

Immobile plants and immobile modular animals outlive unitary animals. This paper discusses competing but not necessarily mutually exclusive theories to explain this extreme longevity, especially from the perspective of phenotypic plasticity. Stem cell immortality, vascular autonomy, and epicormic branching are some important features of the phenotypic plasticity of plants that contribute to their longevity. Monocarpy versus polycarpy can also influence the kind of senescent processes experienced by plants. How density-dependent phenomena affecting the establishment of juveniles in these immobile organisms can influence the evolution of senescence, and consequently longevity, is reviewed and discussed. Whether climate change scenarios will favour long-lived or short-lived organisms, with their attendant levels of plasticity, is also presented.

Bosch, T. C. (2009). "Hydra and the evolution of stem cells." Bioessays **31**(4): 478-86.

Hydra are remarkable because they are immortal. Much of immortality can be ascribed to the asexual mode of reproduction by budding, which requires a tissue consisting of stem cells with continuous self-renewal capacity. Emerging novel technologies and the availability of genomic resources enable for the first time to analyse these cells in vivo. Stem cell differentiation in Hydra is governed through the coordinated actions of conserved signaling pathways. Studies of stem cells in Hydra, therefore, promise critical insights of general relevance into stem cell biology including cellular senescence, lineage programming and reprogramming, the role of extrinsic signals in fate determination and tissue homeostasis, and the evolutionary origin of these cells. With these new facts as a backdrop, this review traces the history of studying stem cells in Hydra and offers a view of what the future may hold.

Brown, D. (2009). "The energy body and its functions: immunosurveillance, longevity, and regeneration." Ann N Y Acad Sci **1172**: 312-37.

There are three interrelated levels of a macromolecular energy-information relay system in the human body, each generated by a specific type of semiconductant tissue and each with a specific function. The surface layer of the energy body, generated by fluid connective tissue and known as the ordinary channel system or meridian system in traditional Chinese medicine (TCM), functions in the service of immunosurveillance through detection of distress signals and transmitting energy-information regarding immunoresponse. The middle layer of the energy body, generated by semiconductant hard and spongy bone tissue, known as the extraordinary channel system in TCM, functions in the service of longevity and regeneration, as described in Bodhidharma's classic, Bone Marrow Washing. The bone marrow energy-information system has direct relevance to modern stem-cell research on the role of stem cells in regeneration of injured tissue. The deepest layer of the energy body generated by semiconductant nervous system tissue notably the vagus nerve and spinal column, functions in the service of awakening consciousness and in immortality. This system is described in the Tibetan Inner Fire meditations as well as in the Taoist shen breathing practices. There is very little scientific understanding of the central channel system.

Cowell, J. K. (1999). "Telomeres and telomerase in ageing and cancer." Age (Omaha) **22**(2): 59-64.

Telomeres lie at the ends of human chromosomes and contain long tandem repeats of a simple nucleotide sequence. Because DNA replication cannot proceed to the very end of chromosomes, copies of these repeats are lost at each cell division. If the telomeres shorten below a critical length, the cells will eventually die as a result of genomic instability. Aging cells usually avoid death by entering senescence before the critical telomere length is reached. Malignantly transformed, immortal cells overcome senescence but they must still avoid the final, critical shortening of telomeres to survive. In the vast majority of cases, tumor cells achieve this by activating the telomerase enzyme, a ribonucleoprotein complex which repairs the end of chromosomes and prevents telomere shortening. Normal mortal cells do not normally express telomerase, although some stem cell populations which must regenerate thought the life span of the organism, retain enzyme activity. Cellular senescence can be overcome by inducing telomerase expression in mortal cells, firmly establishing the role of telomere length in the senescence signaling pathway. In tumor cells, the evidence of a role for telomerase in immortality is still largely correlative, with 80-90% of tumors expressing telomerase activity. To establish whether telomerase activity is important in maintaining the malignant phenotype, attempts have been made to inactivate it in tumor cells, using a variety of approaches, where there is evidence that disrupting telomerase function can result in the induction of apoptosis. The background and implications of these observations is discussed.

de Caralt, S., M. J. Uriz, et al. (2007). "Cell culture from sponges: pluripotency and immortality." Trends Biotechnol **25**(10): 467-71.

Sponges are a source of compounds with potential pharmaceutical applications. In this article, methods of sponge cell culture for production of these bioactive compounds are reviewed, and new approaches for overcoming the problem of metabolite supply are examined. The use of embryos is proposed as a new source of sponge material for cell culture. Stem cells are present in high amounts in embryos and are more versatile and resistant to infections than adult cells. Additionally, genetic engineering and cellular research on apoptotic mechanisms are promising new fields that might help to improve cell survival in sponge-cell lines. We propose that one topic for future research should be how to reduce apoptosis, which appears to be very high in sponge cell cultures.

Dennis, J. E., A. Merriam, et al. (1999). "A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse." J Bone Miner Res **14**(5): 700-9.

Adult marrow contains mesenchymal progenitor cells (MPCs) that have multiple differentiation potentials. A conditionally immortalized MPC clone, BMC9, has been identified that exhibits four mesenchymal cell phenotypes: chondrocyte, adipocyte, stromal (support osteoclast formation), and osteoblast. The BMC9 clone, control brain fibroblasts and another marrow-derived clone, BMC10, were isolated from a transgenic mouse (H-2Kb-tsA58) containing a gene for conditional immortality. To test for chondrogenic potential, cells were cultured in defined medium containing 10 ng/ml transforming growth factor beta and 10-7 M dexamethasone in 15-ml polypropylene tubes ("aggregate cultures"). Adipogenic potential was quantitated by flow cytometry of Nile Red-stained cells cultured for 1 and 2 weeks in medium containing isobutyl methylxanthine, indomethacin, insulin, and dexamethasone. Support of osteoclast formation was measured by quantitating multinucleated tartrate-resistant acid phosphatase-positive cells in spleen cell cocultures of test clones (immortomouse clones and positive control ST2 cells) cultured in the presence of 10-7 M vitamin D3 and 150 mM ascorbate-2-phosphate. In vivo osteogenic potential was assayed by histologic examination of bone formation in subcutaneous implants, into athymic mouse hosts, of a composite of cells combined with porous calcium phosphate ceramics. The bone marrow-derived clone BMC9 has the potential to express each of the four mesenchymal characteristics tested, while brain fibroblasts, tested under identical conditions, did not exhibit any of these four mesenchymal characteristics. BMC10 cells exhibited osteogenic and chondrogenic phenotypes, but showed only minimal expression of adipocytic or osteoclast-supportive phenotypes. Clone BMC9 is, minimally, a quadripotential MPC isolated from the marrow of an adult mouse that can differentiate into cartilage and adipose, support osteoclast formation, and form bone. The BMC9 clone is an example of an adult-derived multipotential progenitor cell that is situated early in the mesenchymal lineage.

Drexler, H. G., Y. Matsuo, et al. (1998). "Proposals for the characterization and description of new human leukemia-lymphoma cell lines." Hum Cell **11**(1): 51-60.

Continuous human leukemia-lymphoma cell lines have become invaluable tools for hematological research as they provide an unlimited amount of cellular material. The first human lymphoma cell line Raji was established in 1963; since then several hundred leukemia-lymphoma cell lines spanning almost the whole spectrum of hematopoietic cell lineages (except for dendritic cells) have been described. The cardinal features of leukemia-lymphoma cell lines are their monoclonal origin, arrest of differentiation, and (growth factor-independent or -dependent) unlimited proliferation. Categorization of cell lines usually follows the physiological stages of hematopoietic differentiation in the various cell lineages. For an adequate classification, a detailed characterization of both primary and cultured cells in absolutely necessary. New cell lines, in particular, must be adequately, characterized; while cell culture data and immunological and cytogenetic features are essential, cell lines should be described in as much detail as possible. In addition to this recommended multiparameter characterization and the obligatory immortality of the culture, authentication of the true origin of the cells, novelty, scientific significance and availability of the cell line for other investigators are of utmost importance. It is still extremely difficult to establish new leukemia-lymphoma cell lines (except for some subtypes), and most attempts fail. Paramount to the lack of our understanding as to why certain cells start to proliferate in culture and others do not (thus implying a random process), is probably the difficulty of mimicking in vitro the physiological in vivo microenvironment. Attempts to improve the efficiency of cell line establishment should focus on examining the appropriateness of the in vitro culture conditions; these conditions should emulate as closely as possible the in vivo situation. In summary, leukemia-lymphoma cell lines have the potential to greatly facilitate diverse studies of normal and malignant

hematopoiesis; to that end, these cell lines must be extensively characterized and adequately described.

Franzese, O., A. Comandini, et al. (1998). "Effect of prostaglandin A1 on proliferation and telomerase activity of human melanoma cells in vitro." Melanoma Res **8**(4): 323-8.

Previous studies have shown that cyclopentenone prostaglandins are endowed with antitumour activity in various murine and human tumour models. In the present investigation four human melanoma cell lines were treated with graded concentrations (4-16microg/ml) of prostaglandin A1 (PGA1) for 24 or 48 h in vitro. At the end of the treatment, cell proliferation (measured in terms of DNA synthesis) and telomerase activity were determined. The results showed that PGA1 induced concentration-dependent inhibition of DNA synthesis at 48 h but not at 24 h in SK-MEL-28 cells. In contrast, marked inhibition of telomerase activity was detected after only 24 h of PGA1 treatment. Moreover, after 48h of treatment with the agent, inhibition of telomerase was more pronounced than inhibition of cell proliferation. Additional studies performed with three freshly generated melanoma cell lines confirmed that PGA1 produced early inhibition of cell growth accompanied by marked impairment of telomerase activity. These results suggest that PGA1 could be of potential value as antitumour agent, on the basis of two distinct mechanisms: direct cytostatic/cytotoxic effects on melanoma cells, and inhibitory activity on a tumour-associated enzymatic function (i.e. telomerase) that is responsible for cancer cell immortality.

Fuller, M. T. and A. C. Spradling (2007). "Male and female Drosophila germline stem cells: two versions of immortality." Science **316**(5823): 402-4.

Drosophila male and female germline stem cells (GSCs) are sustained by niches and regulatory pathways whose common principles serve as models for understanding mammalian stem cells. Despite striking cellular and genetic similarities that suggest a common evolutionary origin, however, male and female GSCs also display important differences. Comparing these two stem cells and their niches in detail is likely to reveal how a common heritage has been adapted to the differing requirements of male and female gamete production.

Hiyama, E. and K. Hiyama (2007). "Telomere and telomerase in stem cells." Br J Cancer **96**(7): 1020-4.

Telomeres, guanine-rich tandem DNA repeats of the chromosomal end, provide chromosomal stability, and cellular replication causes their loss. In somatic cells, the activity of telomerase, a reverse transcriptase that can elongate telomeric repeats, is usually diminished after birth so that the telomere length is gradually shortened with cell divisions, and triggers cellular senescence. In embryonic stem cells, telomerase is activated and maintains telomere length and cellular immortality; however, the level of telomerase activity is low or absent in the majority of stem cells regardless of their proliferative capacity. Thus, even in stem cells, except for embryonal stem cells and cancer stem cells, telomere shortening occurs during replicative ageing, possibly at a slower rate than that in normal somatic cells. Recently, the importance of telomere maintenance in human stem cells has been highlighted by studies on dyskeratosis congenital, which is a genetic disorder in the human telomerase component. The regulation of telomere length and telomerase activity is a complex and dynamic process that is tightly linked to cell cycle regulation in human stem cells. Here we review the role of telomeres and telomerase in the function and capacity of the human stem cells.

Hoffman, L. M. and M. K. Carpenter (2005). "Human embryonic stem cell stability." Stem Cell Rev **1**(2): 139-44.

Human embryonic stem cells (hESCs) are derived from human preimplantation embryos, and exhibit the defining characteristics of immortality and pluripotency. Indeed, these cell populations can be maintained for several years in continuous culture, and undergo hundreds of population doublings. hESCs are thus likely candidates for source of cells for cell replacement therapies. Although hESC lines appear stable in their expression of cytokine markers, expression of telomerase, ability to differentiate, and maintenance of a stable karyotype, several other aspects of stability have not yet been addressed, including mitochondrial sequencing, methylation patterns, and fine resolution cytogenetic analysis. Because of the potential utility of hESCs, it will be of utmost importance to evaluate the stability of these aspects of ESC biology.

Hohaus, S., M. T. Voso, et al. (1997). "Telomerase activity in human hematopoietic progenitor cells." Haematologica **82**(3): 262-8.

BACKGROUND AND OBJECTIVE: Telomerase is the enzyme that stabilizes and elongates the telomeric ends of chromosomes. It is expressed in germline and malignant cells and absent in most human somatic cells. The selective expression of telomerase has thus been proposed to be a basis for the immortality of germline and malignant cells. Recently, telomerase activity has been observed in human bone marrow (BM) and peripheral blood (PB) samples. The objective of our study was to further characterize the telomerase-expressing population in BM and PB.

METHODS: CD34+ cells were isolated from BM and PB, cultured in vitro, and telomerase activity was assessed by the PCR-based TRAP assay. RESULTS: Telomerase activity in human BM and PB could be almost exclusively assigned to the hematopoietic progenitor cell fraction expressing the CD34 antigen. We observed telomerase activity in CD34+ cells from BM and cytokine-mobilized PB. CD34+ cells lacking co-expression of CD33 demonstrated higher levels of telomerase than myeloid committed CD34+/CD33+ cells. In vitro culture of CD34+ cells in the presence of a cocktail of growth factors inducing differentiation resulted in a decrease of telomerase activity. Telomerase activity increased in peripheral blood during cytokine-induced mobilization of hematopoietic progenitor cells. INTERPRETATION AND CONCLUSIONS: Our data demonstrate that at least a portion of the hematopoietic stem/progenitor cell fraction expresses telomerase and downregulates its expression through differentiation.

Holm, T. M., L. Jackson-Grusby, et al. (2005). "Global loss of imprinting leads to widespread tumorigenesis in adult mice." Cancer Cell **8**(4): 275-85.

Loss of imprinting (LOI), commonly observed in human tumors, refers to loss of monoallelic gene regulation normally conferred by parent-of-origin-specific DNA methylation. To test the function of LOI in tumorigenesis, we developed a model by using transient demethylation to generate imprint-free mouse embryonic stem cells (IF-ES cells). Embryonic fibroblasts derived from IF-ES cells (IF-MEFs) display TGFbeta resistance and reduced p19 and p53 expression and form tumors in SCID mice. IF-MEFs exhibit spontaneous immortalization and cooperate with H-Ras in cellular transformation. Chimeric animals derived from IF-ES cells develop multiple tumors arising from the injected IF-ES cells within 12 months. These data demonstrate that LOI alone can predispose cells to tumorigenesis and identify a pathway through which immortality conferred by LOI lowers the threshold for transformation.

Isfort, R. J. and R. A. LeBoeuf (1995). "The Syrian hamster embryo (SHE) cell transformation system: a biologically relevant in vitro model--with carcinogen predicting capabilities--of in vivo multistage neoplastic transformation." Crit Rev Oncog **6**(3-6): 251-60.

Neoplastic transformation is a multistep process that can be modeled in vitro using Syrian hamster embryo (SHE) cells. SHE cells multistage transformation involves several intermediate stages, including morphological transformation, immortality, acquisition of tumorigenicity, and malignant progression. Analysis of the molecular alterations that occur at each stage indicated that morphological transformation results from both carcinogen-induced irreversible chromosomal/genetic mutations and reversible genetic events, including altered DNA methylation. Morphological transformation results from a block in the cellular differentiation of progenitor and determined stem-like cells in the SHE cell population via alternation in the expression of the H19 tumor suppressor gene and other genes. Immortality results from genetic mutations in growth factor responsiveness, including loss of growth suppression by TGF beta and autocrine growth factor production, and genomic stability, resulting in genomic instability and an increased mutation rate. Acquisition of tumorigenicity involves loss of tumor suppressor gene function, altered mitogenic signal transduction, mutation of oncogenes, acquisition of anchorage independent growth, and chromosomal aberrations. Malignant progression is associated with alterations in extracellular matrix growth characteristics, alterations in cytoskeleton structure, elevated fibrinolytic activity, secretion of proteases, and changes in extracellular matrix protein secretion. Together, these changes model the alterations observed during in vivo neoplastic transformation and possibly explain why the SHE assay, as a carcinogen screening tool, is able to identify carcinogens with a 80 to 85% accuracy.

Jaishankar, A., M. Barthelery, et al. (2009). "Human embryonic and mesenchymal stem cells express different nuclear proteomes." Stem Cells Dev **18**(5): 793-802.

Human embryonic stem cells (hESCs) are characterized by their immortality and pluripotency. Human mesenchymal stem cells (hMSC), on the other hand, have limited self-renewal and differentiation capabilities. The underlying molecular differences that account for this characteristic self-renewal and plasticity are, however, poorly understood. This study reports a nuclear proteomic analysis of human embryonic and bone marrow-derived mesenchymal stem cells. Our proteomic screen highlighted a 5-fold difference in the expression of Reptin52. We show, using two-dimensional difference gel electrophoresis (2-DIGE), western analysis, and quantitative reverse transcriptase polymerase chain reaction, that Reptin52 is more abundantly expressed in hESC than hMSC. Moreover, we observed differential expression of Pontin52 and beta-catenin-proteins known to interact with Reptin52. This difference in the expression of Reptin52 and Pontin52 (known regulators of beta-catenin) further supports a role for Wnt signaling in stem cell self-renewal and proliferation.

Jones, D. L. (2007). "Aging and the germ line: where mortality and immortality meet." Stem Cell Rev **3**(3): 192-200.

Germ cells are highly specialized cells that form gametes, and they are the only cells within an organism that contribute genes to offspring. Germline stem cells (GSCs) sustain gamete production, both oogenesis (egg production) and spermatogenesis (sperm production), in many organisms. Since the genetic information contained within germ cells is passed from generation to generation, the germ line is often referred to as immortal. Therefore, it is possible that germ cells possess unique strategies to protect and transmit the genetic information contained within them indefinitely. However, aging often leads to a dramatic decrease in gamete production and fecundity. In addition, single gene mutations affecting longevity often have a converse effect on reproduction. Recent studies examining age-related changes in GSC number and activity, as well as changes to the stem cell microenvironment, provide insights into the mechanisms underlying the observed reduction in gametogenesis over the lifetime of an organism.

Keith, W. N. (2004). "From stem cells to cancer: balancing immortality and neoplasia." Oncogene **23**(29): 5092-4.

In this issue of Oncogene, Serakinci et al show that adult stem cells can be targets for neoplastic transformation. After transducing human adult mesenchymal stem cells (hMSC) with the telomerase hTERT gene, and growing them for many population doublings in culture, Serakinci et al observed that the transduced cells developed characteristics consistent with transformation including loss of contact inhibition, anchorage independence and tumour formation in mice. Underlying these changes were alterations to genes involved in cell cycle regulation and senescence as well as oncogene activation. The importance of these observations is twofold. Firstly, showing that stem cells can become tumours raises a note of caution for stem cell therapeutics. Secondly, the findings lend support to the stem cell hypothesis of cancer development, and provide an experimental system in which the tantalizing hint of new diagnostic, prognostic, and therapeutic opportunities offered by this concept can be explored further.

Klingler, K., G. R. Johnson, et al. (1988). "Transformation of single myeloid precursor cells by the malignant histiocytosis sarcoma virus (MHSV): generation of growth-factor-independent myeloid colonies and permanent cell lines." J Cell Physiol **135**(1): 32-8.

Direct single-cell assays for oncogenic transformation are available for fibroblasts but not for other cell types. Using malignant histiocytosis sarcoma virus (MHSV), a member of the ras family of retroviruses, in vivo-infected granulocyte/macrophage and macrophage precursor cells lost the requirement for externally added hematopoietic growth factors. Factor-independent growth was demonstrated by colony-transfer experiments. More than 25% of the independent colonies were established as permanent macrophage cell lines following a phase of adaptation to tissue culture conditions. Factor-independent colony growth was also obtained by in vitro infection of single cells. As many as 50% of all myeloid precursor cells were target cells for MHSV as measured by this assay. About 2 x 10(-3) of these colony-forming cells acquired growth factor independence and immortality after in vitro infection. Cell lines derived from these colonies did not require adaptation to tissue culture conditions.

Kopper, L. and M. Hajdu (2004). "Tumor stem cells." Pathol Oncol Res **10**(2): 69-73.

Stem cells possess two basic characteristics: they are able to renew themselves and to develop into different cell types. The link between normal stem cells and tumor cells could be examined in three aspects: what are the differences and similarities in the control of self-renewal capacity between stem cells and tumor cells; whether tumor cells arise from stem cells; do tumorous stem cells exist? Since tumor cells also exhibit self-renewal capacity, it seems plausible that their regulation is similar to that of the stem cells. The infinite self-renewal ability (immortalization) is assured by several, so far only partly known, mechanisms. One of these is telomerase activity, another important regulatory step for survival is the inhibition of apoptosis. Other signal transduction pathways in stem cell regulation may also play certain roles in carcinogenesis: e.g. Notch, Sonic hedgehog (SHH), and Wnt signals. Existence of tumor stem cells was suggested since it is simpler to retain the self-renewal capacity than to reactivate the immortality program in an already differentiated cell. Moreover, stem cells live much longer than the differentiated ones, and so they are exposed for a long period of time to impairments, collecting gene errors leading to the breakdown of the regulation. However, it is still an open question whether all cells in the tumor possess the capacity that produces this tissue or not, that is: are there tumor stem cells or there are not. If tumor stem cells exist, they would be the main target for therapy: only these must be killed since the other tumor cells possess limited proliferative capacity, therefore limited life span. The only problem is that during tumor progression stem-like cells can develop continuously and the identification but mainly the prevention of their formation is still a great challenge.

Kraemer, P. M., F. A. Ray, et al. (1986). "Spontaneous immortalization rate of cultured Chinese hamster cells." J Natl Cancer Inst **76**(4): 703-9.

Chinese hamster cell cultures derived from either fetal cell suspensions or adult ear clippings invariably became permanent cell lines during conventional subcultivation. The immortal cell cultures arose from rare spontaneous cellular events during the in vitro cultivation of cells with limited proliferative capacity. Immortality was not related to rare, precommitted cells from the animals. The expansion of clones of cells with limited life-span to form permanent cell lines was routinely successful only when the initial, unsubdivided culture achieved a total number in excess of 10(6) cells. On the basis of this observation, a serial clonogenicity assay was developed for determining the life-span of the cells with limited proliferative capacity and for determining whether a cell population is immortal. In addition, the technique of clonal expansion was used for a fluctuation analysis to determine the rate of immortalization. This analysis yielded a rate of 1.9 X 10(6) per cell per generation.

Kroll, J. (2005). "Chaperones and longevity." Biogerontology **6**(5): 357-61.

That evolution of longevity may depend on alterations in the expression of relatively few regulatory genes has been inferred from the rapid increase in lifespan during evolution of the hominid species (Cutler RG (1979) Mech Ageing Dev 9: 337-354). Also the inherent immortality of the embryonic stem cells implies that replicative senescence (Hayflick L (1997) Biochem Mosc 62: 1180-1190) as possibly aging of species are epigenetic phenomena. Evidence is presented to suggest that the epigenetic changes of the longevity determinants to a significant extend concerns the molecular chaperones. Specific involvement of RNA chaperones in cell immortalization and defective RecQ-DNA chaperones in syndromes of premature aging suggest that DNA/RNA - chaperones probably rank high among the determinants of cellular and species longevity.

Kroll, J. (2007). "Molecular chaperones and the epigenetics of longevity and cancer resistance." Ann N Y Acad Sci **1100**: 75-83.

The inherent immortality of embryonic stem cells demonstrates that replicative senescence as possibly the aging of species are epigenetic phenomena. The cellular level of expression of the housekeeping molecular chaperones correlates with longevity and cancer resistance of species. The chaperones are cancer antagonists by acting as genetic buffers, stabilizing the normal phenotype. Probably the progressive age-related silencing of the housekeeping genes contributes to the phenotype of aging, with the associated increase in cancer incidence. The present review concerns epigenetic chemical, immunological, and hormonal mechanisms, activating chaperone- and immune-response genes, which have proved effective in increasing longevity and cancer resistance. The relation of steroid hormone levels to species longevity, the anticarcinogenic activity of pregnancy hormones, and the influence of hormones on the longevity of social insects, illustrates the importance of hormonal mechanisms for the activation of longevity genes.

Lawrenz, B., H. Schiller, et al. (2004). "Highly sensitive biosafety model for stem-cell-derived grafts." Cytotherapy **6**(3): 212-22.

BACKGROUND: The recent success in the derivation of differentiated cell types from stem cells has raised prospects for the application of regenerative cell therapy. In particular, embryonic stem cells are attractive sources for cell transplantation, due to their immortality and rapid growth. These cells, however, also possess tumorigenic properties, which raises serious safety concerns and makes biosafety testing mandatory. Our goal was to establish a highly sensitive animal model for testing the proliferative potential of stem-cell grafts. METHODS: BALB/c nude mice received cell grafts of non-neoplastic MRC-5 cells containing defined numbers of mouse embryonic stem cells. We either injected 1 million viable cells into the kidney capsule, or mixed 2 million cells with Matrigel for s.c. transplantation. To analyze the possible impact of an intact immune response on tumor development, we also transplanted the cells into immunocompetent mice. Animals were sacrificed when the tumors became >1 cm and were analyzed in detail. RESULTS: The nude mouse model reproducibly allowed detection of 20 tumorigenic cells, and even as few as 2 ES cells were found to form teratoma. Interestingly, the administration of cell grafts at two different application sites resulted in different growth kinetics and tumor phenotypes. The highest level of sensitivity (100% detection of 20 tumorigenic ES cells) was achieved by s.c. injection of cells mixed with Matrigel. The influence of the immune system on tumor-cell development was demonstrated by a higher tumor rate of transplants in immunodeficient nude mice compared with immunocompetent mice. DISCUSSION: We have established a reliable animal model for routine assessment of the biosafety profile of stem-cell-derived cell transplants. This model will facilitate the generation of homogenous non-tumorigenic cell populations, and will help to integrate standardized

safety systems into the application of stem-cell-derived grafts for clinical purposes.

Lin, K. W. and J. Yan (2005). "The telomere length dynamic and methods of its assessment." J Cell Mol Med **9**(4): 977-89.

Human telomeres are composed of long repeating sequences of TTAGGG, associated with a variety of telomere-binding proteins. Its function as an end-protector of chromosomes prevents the chromosome from end-to-end fusion, recombination and degradation. Telomerase acts as reverse transcriptase in the elongation of telomeres, which prevent the loss of telomeres due to the end replication problems. However, telomerase activity is detected at low level in somatic cells and high level in embryonic stem cells and tumor cells. It confers immortality to embryonic stem cells and tumor cells. In most tumor cells, telomeres are extremely short and stable. Telomere length is an important indicator of the telomerase activity in tumor cells and it may be used in the prognosis of malignancy. Thus, the assessment of telomeres length is of great experimental and clinical significance. This review describes the role of telomere and telomerase in cancer pathogenesis and the dynamics of the telomeres length in different cell types. The various methods of measurement of telomeres length, i.e. southern blot, hybridization protection assay, fluorescence in situ hybridization, primed in situ, quantitative PCR and single telomere length analysis are discussed. The principle and comparative evaluation of these methods are reviewed. The detection of G-strand overhang by telomeric-oligonucleotide ligation assay, primer extension/nick translation assay and electron microscopy are briefly discussed.

Lo, K. C., S. Whirledge, et al. (2005). "Stem cells: implications for urology." Curr Urol Rep **6**(1): 49-54.

Stem cells are characterized by their potential immortality and are capable of self-renewal and differentiation. Stem cells are proposed to provide the potential to cure degenerative diseases and to give important clues regarding human development and aging. However, stem cell research has evoked enthusiasm and passionate debate regarding the ethics of their use in medicine and reproduction. In this article, the current understanding of the biology of stem cells, their application in urology, and some of the controversies regarding their use are discussed. Although the clinical application of stem cell technologies to urologic practice is likely to be well in the future, advances in this field hold great promise for the correction of a number of illnesses. Nevertheless, scientists and ethicists will continue to struggle with their ethical responsibilities to the patient and society.

Marczynska, B., N. Khoobyarian, et al. (1991). "Phorbol ester promotes growth and transformation of carcinogen-exposed nonhuman primate cells in vitro." Anticancer Res **11**(5): 1711-7.

Kidney cells established in vitro from a white-lipped marmoset (106) were exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) alone or in combination with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Low (0.1 micrograms/ml, 4 times), intermediate (1 microgram/ml) and high (1 microgram/ml, 4 times) doses of MNNG resulted in 100%, 50% and 2.8% of cell survival, respectively. High and low doses of MNNG had no effect on cell transformation. Upon exposure of cells to an intermediate dose of MNNG, 106 cells ecquired immortality and evolved into permanent cell line, 106-1M. However, the cells retained normal morphology and anchorage dependence. Chronic applications of TPA (0.1 micrograms/ml, 13 times) promoted 106-1M cells to morphological transformation and anchorage-independent growth but not to tumorigenicity in nude mice (106-1MT cell line). Chromosome analysis revealed only numerical changes in 106 cells and both numerical and structural aberrations in transformed 106-1MT cells. These changes in marmoset cells usually reflected cell culture instability leading to either senescence or to longer survival of cells in vitro. Chronic treatment with TPA did not result in downregulation of protein kinase C (PKC) in transformed 106-1MT cells. Instead, an additional species of PKC appeared in these cells.

McLaren, A. (2001). "Mammalian germ cells: birth, sex, and immortality." Cell Struct Funct **26**(3): 119-22.

The germ cell lineage in the mouse is not predetermined but is established during gastrulation, in response to signalling molecules acting on a subset of epiblast cells that move through the primitive streak together with extra-embryonic mesoderm precursors. After migration to the site of the future gonads, germ cell sex determination is achieved, with germ cell phenotype in male and female embryos diverging. Evidence suggests that all germ cells spontaneously take the female pathway, entering prophase of the first meiotic division five or six days after the birth of the germ cell lineage, with the exception of those located in the embryonic testis, which exit the cell cycle in response to some inhibitory signal and remain in Go until after birth, when spermatogenesis begins. In culture, germ cells respond to certain growth factors by proliferating indefinitely. These immortalized embryonic germ (EG) cell lines are chromosomally stable and pluripotent, closely resembling the embryonic stem (ES) cell lines derived from blastocyst-stage embryos. Human EG and ES cell

lines have recently been made, raising the hope that their differentiation could be directed to specific cell types, of value in the clinical treatment of degenerative diseases.

Merle, P. and C. Trepo (2009). "Molecular mechanisms underlying hepatocellular carcinoma." Viruses **1**(3): 852-72.

Hepatocarcinogenesis is a complex process that remains still partly understood. That might be explained by the multiplicity of etiologic factors, the genetic/epigenetic heterogeneity of tumors bulks and the ignorance of the liver cell types that give rise to tumorigenic cells that have stem cell-like properties. The DNA stress induced by hepatocyte turnover, inflammation and maybe early oncogenic pathway activation and sometimes viral factors, leads to DNA damage response which activates the key tumor suppressive checkpoints p53/p21(Cip1) and p16(INK4a)/pRb responsible of cell cycle arrest and cellular senescence as reflected by the cirrhosis stage. Still obscure mechanisms, but maybe involving the Wnt signaling and Twist proteins, would allow pre-senescent hepatocytes to bypass senescence, acquire immortality by telomerase reactivation and get the last genetic/epigenetic hits necessary for cancerous transformation. Among some of the oncogenic pathways that might play key driving roles in hepatocarcinogenesis, c-myc and the Wnt/beta-catenin signaling seem of particular interest. Finally, antiproliferative and apoptosis deficiencies involving TGF-beta, Akt/PTEN, IGF2 pathways for instance are prerequisite for cancerous transformation. Of evidence, not only the transformed liver cell per se but the facilitating microenvironment is of fundamental importance for tumor bulk growth and metastasis.

Monk, M., C. Holding, et al. (2001). "Isolation of novel developmental genes from human germ cell, oocyte and embryo cDNA by differential display." Reprod Fertil Dev **13**(1): 51-7.

Due to the difficulties inherent in research on human embryos, almost nothing is known about genes active in human early development. Although the human genome project will provide resources that theoretically provide access to every human gene, those genes specific to human early development may be difficult to define. Also, by definition, genes specific to early development will not be represented in cDNA databases derived from human somatic cells. Yet these unknown human developmental genes are likely to be of key importance for several areas of human health, including assisted reproduction and contraception, embryo stem cell research and tissue transplantation, ageing and cancer. In order to identify and isolate these human developmental genes, we have prepared amplified cDNA from human primordial germ cells, oocytes and embryos, and used differential display to compare patterns of gene expression in these embryonic cells and in the cells of somatic tissues of a 10-week human fetus. This paper reviews the highly sensitive procedures used to create amplified cDNA representing expressed genes in a single cell and the use of differential display to identify developmental genes. Several such genes have been isolated, but their full-length sequences and function are yet to be elucidated. Genes active in human early development are expected to play key roles in the maintenance of the archetypal stem cell state, potential immortality and the invasiveness of trophectoderm and primordial germ cells. They represent candidate genes regulating these functions for targeting in clinical research in human reproduction, stem cell differentiation and cancer.

Mummery, C. (2004). "Stem cell research: immortality or a healthy old age?" Eur J Endocrinol **151 Suppl 3**: U7-12.

Stem cell research holds the promise of treatments for many disorders resulting from disease or trauma where one or at most a few cell types have been lost or do not function. In combination with tissue engineering, stem cells may represent the greatest contribution to contemporary medicine of the present century. Progress is however being hampered by the debate on the origin of stem cells, which can be derived from human embryos and some adult tissues. Politics, religious beliefs and the media have determined society's current perception of their relative value while the ethical antipathy towards embryonic stem cells, which require destruction of a human embryo for their derivation, has in many countries biased research towards adult stem cells. Many scientists believe this bias may be premature and basic research on both cell types is still required. The media has created confusion about the purpose of stem cell research: treating chronic ailments or striving for immortality. Here, the scientific state of the art on adult and embryonic stem cells is reviewed as a basis for a debate on whether research on embryonic stem cells is ethically acceptable.

Negishi, Y., A. Kudo, et al. (2000). "Multipotency of a bone marrow stromal cell line, TBR31-2, established from ts-SV40 T antigen gene transgenic mice." Biochem Biophys Res Commun **268**(2): 450-5.

Bone marrow is believed to contain multipotential stromal stem cells which can differentiate into osteoblasts, chondrocytes, adipocytes, and myoblasts (Prockop, D. J. Science 276, 71-74, 1997). Therefore, characterization and identification of the stem-like cell within the stromal cells are

important to understand bone marrow function in relation to the hematopoietic microenvironment, and repair/regeneration of tissue defects. TBR31-2 cell, a bone marrow stromal cell line established from bone marrow of transgenic mice harboring temperature-sensitive (ts) simian virus (SV) 40T-antigen gene for immortality, is induced toward both adipocytic and osteogenic cells under conditions of the inactivation of T-antigen (Okuyama, R., Yanai, N., Obinata, M. Exp. Cell Res. 218, 424-429, 1995). In this work, using a semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, mRNA expressions of tissue-specific differentiation markers for adipocyte (lipoprotein lipase), osteoblast (type I collagen and osteocalcin), chondrocyte (type II and X collagen), and muscle cell (desmin) were examined during a long-term culture of the cell. In addition, histochemical studies showed the appearance of adipocytic, osteoblastic, chondrocytic, and muscle cells during this long-term culture. Thus, TBR31-2, which has characteristics of an undifferentiated cell, has the potential to express the multipotential cell lineages. These results indicated that a multipotential progenitor cell including potential to differentiate into a muscle cell and which is situated in the mesenchymal cell lineage was first obtained.

Oshimura, M., D. J. Fitzgerald, et al. (1988). "Cytogenetic changes in rat tracheal epithelial cells during early stages of carcinogen-induced neoplastic progression." Cancer Res **48**(3): 702-8.

The cytogenetic changes in enhanced growth (EG) variants of rat tracheal epithelial cells in culture were examined. These variants which are detectable at 35 days after carcinogen exposure are the first phenotypic alteration in the multistep neoplastic process studied in this model system. Karyotypic analysis of N-methyl-N'-nitro-N-nitrosoguanidine-induced EG variants at Day 35 was made possible by the development of an in situ method of cytogenetic analysis on intact colonies containing too few cells for conventional chromosome preparation methods. Of the transformed EG variant colonies in both control and N-methyl-N'-nitro-N-nitrosoguanidine-treated groups, 62-78% had abnormal karyotypes which included numerical and structural changes. There were no specific chromosome changes, although aberrations of chromosomes 3 and 4 were recurrently observed. However, some colonies of even the most morphologically transformed EG variants were composed of only diploid cells. To confirm this finding 10 EG variant colonies were bisected and half of the clone was prepared for chromosome analysis and the other half was subcultured to measure the clonogenicity and karyotypes of the cells. Cells from 3 colonies plated very poorly on 3T3 feeders and therefore no karyotypic analysis of the colony-forming cells was possible; the cells of the 3 parental colonies were diploid. Three other parental colonies were predominantly diploid (80-90%) but upon replating the resultant daughter colonies had progressively smaller fractions of diploid cells indicating a selection for cells with abnormal karyotypes. When more selective conditions were used (i.e., growth after removal of the feeder cells), the percentage of abnormal cells increased even further. In one case the parental cells had a karyotypic alteration in the long arm of chromosome 4 and this karyotypic alteration was accentuated in the daughter colonies. Thus, selection of cells with increased growth ability upon subculturing or growth in the absence of feeder cells (properties associated with the acquisition of immortality) resulted in concomitant selection for cells with abnormal karyotypes. Since some of the carcinogen-induced rat tracheal epithelial cells expressing the EG variant phenotype were diploid, it is possible that the first step in this transformation process is an epigenetic change. However, most of the diploid cells became terminal. The aneuploid subpopulations present in these colonies have a selective growth advantage and comprise the cell compartment that expresses continued growth, immortality, and ultimately tumorigenicity.

Park, W. C., H. Liu, et al. (2005). "Deregulation of estrogen induced telomerase activity in tamoxifen-resistant breast cancer cells." Int J Oncol **27**(5): 1459-66.

Telomerase, a ribonucleoprotein enzyme that functions as a reverse transcriptase, is detected exclusively in immortal cells such as germ cells, stem cells and cancer cells. Telomerase activity is present in almost all human cancers. Telomerase activation is considered to be essential to maintain the integrity of the replicating tumor cell and to establish immortality. Based on this concept antiestrogen should initially regulate estrogen-stimulated telomerase but the enzyme would be expected to be constitutive in tamoxifen-resistant tumor cells. We have studied the estrogen regulation of telomerase in T47D:A18 breast cancer cells with a TRAPEZE Telomerase detection kit. Estradiol significantly increased telomerase activity after a 2-day treatment. Telomerase activity induced by estradiol was up to 10-fold higher within 4 days. Antiestrogens 4-hydroxytamoxifen (4-OHT) and ICI 182,780 were inactive alone and significantly blocked estradiol-stimulated increase in telomerase. These effects were correlated with changes in cell replications and changes in the cell cycle. In contrast, 4-OHT resistant T47D:A18 cells (T47D:A18/4-OHT, cultured in 1 microM 4-OHT for 6 months) grew spontaneously and had no changes in the cell cycle

with estrogen treatment. The estrogen receptor (ERalpha) was present and still regulated at an estrogen responsive luciferase reporter gene with estrogen despite the fact that progesterone receptor was not increased in response to estradiol in T47D:A18/4-OHT cells. However, telomerase activity was increased about 40-fold in T47D:A18/4-OHT cells and this was not regulated by ICI 182,780. We conclude that the differential regulation of telomerase gene might be an important transition for tamoxifen resistance in T47D:A18 breast cancer cells.

Peng, W. M., L. L. Yu, et al. (2002). "Transplanted neuronal precursors migrate and differentiate in the developing mouse brain." Cell Res **12**(3-4): 223-8.

The subventricular zone (SVZ), lining the lateral ventricle in forebrain, retains a population of neuronal precursors with the ability of proliferation in adult mammals. To test the potential of neuronal precursors in adult mice, we transplanted adult SVZ cells labeled with fluorescent dye PKH26 into the lateral ventricle of the mouse brain in different development stages. The preliminary results indicated that the grafted cells were able to survive and migrate into multiple regions of the recipient brain, including SVZ, the third ventricle, thalamus, superior colliculus, inferior colliculus, cerebellum and olfactory bulb etc; and the amount of survival cells in different brain regions was correlated with the development stage of the recipient brain. Immunohistochemical studies showed that most of the grafted cells migrating into the specific target could express neuronal or astrocytic marker. Our results revealed that the neuronal precursors in adult SVZ still retained immortality and ability of proliferation, which is likely to be induced by some environmental factors.

Pfeffer, N. (2008). "What British women say matters to them about donating an aborted fetus to stem cell research: a focus group study." Soc Sci Med **66**(12): 2544-54.

This is the first investigation into what matters to British women when they think about donating an aborted fetus to research, and how stem cell research and therapies might influence their views. Tissue derived from the aborted fetus is considered "the right tool for the job" in some stem cell laboratories. Research using tissue derived from aborted fetuses is permitted in Britain, while deliberate abortion to provide fetal tissue for research is illegal. Investigators are advised to seek women's agreement to donate the fetus after they have signed the consent form for the abortion, and stem cell researchers seek fetuses aborted under the 'social' grounds of the Abortion Act 1967. This research was based on focus groups with women who had both had a termination and had not had a termination. It found that initial enthusiasm for the donation of the aborted fetus for medical research, which was understood as a good thing, diminished as participants gained information and thought more carefully about the implications of such a decision. Lack of knowledge about how aborted fetuses are treated as scientific objects in the stem cell laboratory provoked concerns about mishandling, and invoked in some participants what we have called the duty of care which women feel towards babies and children. The duty of care might apply to other research using aborted fetuses. But what makes stem cell research more troubling is its association with renewal, regeneration, and immortality which participants understood as somehow reinstating and even developing the fetus' physical existence and social biography, the very thing abortion is meant to eliminate. By the end of the focus groups, participants had co-produced a tendency to refuse to donate aborted fetuses.

Postovit, L. M., F. F. Costa, et al. (2007). "The commonality of plasticity underlying multipotent tumor cells and embryonic stem cells." J Cell Biochem **101**(4): 908-17.

Aggressive cancer cells and pluripotent stem cells converge in their capacity for self-renewal, proliferation and plasticity. Recent studies have capitalized on these similarities by demonstrating that tumors arise from specific cancer stem cell populations that, in a manner reminiscent of normal stem cells, are able to both self-renew and give rise to a heterogeneous tumor population. This stem cell like function of aggressive cancer cells is likely attributable to the ectopic expression of embryonic factors such as Nodal and Cancer Testis Specific Antigens (CTAs), which maintain a functional plasticity by promoting pluripotency and immortality. During development, the expression of these embryonic factors is tightly regulated by a dynamic array of mediators, including the spatial and temporal expression of inhibitors such as Lefty, and the epigenetic modulation of the genome. In aggressive cancer cells, particularly melanoma, this balance of regulatory mediators is disrupted, leading to the aberrant expression of pluripotency-associated genes. By exposing aggressive cancer cells to embryonic microenvironments, this balance of regulatory mediators is restored, thereby reprogramming tumor cells to a more benign phenotype. These stem cell-derived mediators, as well as the genes they regulate, provide therapeutic targets designed to specifically differentiate and eradicate aggressive cancers.

Rahman, R., R. Heath, et al. (2009). "Cellular immortality in brain tumours: an integration of the

cancer stem cell paradigm." Biochim Biophys Acta **1792**(4): 280-8.

Brain tumours are a diverse group of neoplasms that continue to present a formidable challenge in our attempt to achieve curable intervention. Our conceptual framework of human brain cancer has been redrawn in the current decade. There is a gathering acceptance that brain tumour formation is a phenotypic outcome of dysregulated neurogenesis, with tumours viewed as abnormally differentiated neural tissue. In relation, there is accumulating evidence that brain tumours, similar to leukaemia and many solid tumours, are organized as a developmental hierarchy which is maintained by a small fraction of cells endowed with many shared properties of tissue stem cells. Proof that neurogenesis persists throughout adult life, compliments this concept. Although the cancer cell of origin is unclear, the proliferative zones that harbour stem cells in the embryonic, post-natal and adult brain are attractive candidates within which tumour-initiation may ensue. Dysregulated, unlimited proliferation and an ability to bypass senescence are acquired capabilities of cancerous cells. These abilities in part require the establishment of a telomere maintenance mechanism for counteracting the shortening of chromosomal termini. A strategy based upon the synthesis of telomeric repeat sequences by the ribonucleoprotein telomerase, is prevalent in approximately 90% of human tumours studied, including the majority of brain tumours. This review will provide a developmental perspective with respect to normal (neurogenesis) and aberrant (tumourigenesis) cellular turnover, differentiation and function. Within this context our current knowledge of brain tumour telomere/telomerase biology will be discussed with respect to both its developmental and therapeutic relevance to the hierarchical model of brain tumourigenesis presented by the cancer stem cell paradigm.

Rando, T. A. (2006). "Stem cells, ageing and the quest for immortality." Nature **441**(7097): 1080-6.

Adult stem cells reside in most mammalian tissues, but the extent to which they contribute to normal homeostasis and repair varies widely. There is an overall decline in tissue regenerative potential with age, and the question arises as to whether this is due to the intrinsic ageing of stem cells or, rather, to the impairment of stem-cell function in the aged tissue environment. Unravelling these distinct contributions to the aged phenotype will be critical to the success of any therapeutic application of stem cells in the emerging field of regenerative medicine with respect to tissue injury, degenerative diseases or normal functional declines that accompany ageing.

Rashid-Doubell, F., T. R. Kershaw, et al. (1994). "Effects of basic fibroblast growth factor and gamma interferon on hippocampal progenitor cells derived from the H-2Kb-tsA58 transgenic mouse." Gene Ther **1 Suppl 1**: S63.

Many workers have immortalised neural precursor cells by applying a variety of techniques including transfection and retroviral-mediated gene insertion using a variety of oncogenes including c-myc, neu, and the SV40 T antigen. This study made use of a conditionally immortalised hippocampal cell population derived from the H-2Kb-tsA58 transgenic mouse. In this mouse the tsA58 gene is under the control of the H-2Kb major histocompatibility complex class I promoter. Enabling the mouse to possess an established integrated copy of the early region of the large tumour antigen (TAg) gene from the temperature sensitive simian virus 40 (SV40) mutant strain tsA58. The H-2Kb promoter used in this insert allows expression in many tissues with an up-regulation of its effect produced on the addition of interferon, which assists cellular proliferation. The temperature sensitive gene allows immortality to be controlled at the permissive temperature of 33 degrees C, the non-permissive temperature being 39.5 degrees C4.

Rha, S. Y., E. Izbicka, et al. (2000). "Effect of telomere and telomerase interactive agents on human tumor and normal cell lines." Clin Cancer Res **6**(3): 987-93.

Shortening of telomeres along with an up-regulation of telomerase is implicated in the immortality of tumor cells. Targeting either telomeres or telomerase with specific compounds has been proposed as an anticancer strategy. Because telomerase activity and telomeres are found in normal cells, telomere or telomerase targeting agents could induce side effects in normal tissues. We evaluated the effects of telomere and telomerase interactive agents in human tumor and normal cell lines to try to determine the potential side effects those agents might induce in patients. Toxicity of the G-quadruplex interactive porphyrins (TMPyP4, TMPyP2) and azidothymidine (AZT) were tested using a cell-counting technique against normal human cell lines (CRL-2115 and CRL-2120, fibroblasts; NHEK-Ad, adult keratinocytes; CCL-241, small intestinal cells; NCM 460, colonic mucosal epithelial cells) and human tumor cell lines (MDA-MB 231 and Hs 578T, breast cancer; SK-N-FI, neuroblastoma; HeLa, cervix cancer; MIA PaCa-2, pancreatic cancer; HT-29 and HCT-116, colon cancer; DU 145, prostatic cancer cell line). Telomerase activity of these cell lines was measured by a non-PCR-based conventional assay.

The effects of TMPgammaP2, TMPyP4, and AZT were also evaluated against normal human bone marrow specimens, using a granulocyte-macrophage colony-forming assay (CFU-GM). AZT showed very low cytotoxic effects against normal and tumor cell lines, with the IC50 values above 200 microM. The IC50 values for TMPyP2 and TMPyP4 in normal human cell lines were in the range of 2.9-48.3 microM and 1.7-15.5 microM, respectively, whereas in tumor cell lines the IC50 values were 11.4-53 microM and 9.0-28.2 microM, respectively. Within the tissue types, keratinocytes were more sensitive to TMPyP4 than fibroblasts, and small intestinal cells were more sensitive than colonic mucosal epithelial cells. The IC50 for TMPyP2 and TMPyP4 in the normal marrow colony-forming assays were 19.3 +/- 5.1 microM and 47.9 +/-1.0 microM, respectively. In conclusion, the in vitro cytotoxicity of the telomere interactive agent TMPyP4 is comparable in human tumor and normal cell lines, which indicates that TMPyP4 could have effects on normal tissues.

Rosenberger, R. F. (1995). "The initiation of senescence and its relationship to embryonic cell differentiation." Bioessays **17**(3): 257-60.

Mouse embryonic stem cells have an unlimited lifespan in cultures if they are prevented from differentiating. After differentiating, they produce cells which divide only a limited number of times. These changes seen in cultures parallel events that occur in the developing embryo, where immortal embryonic cells differentiate and produce mortal somatic ones. The data strongly suggest that differentiation initiates senescence, but this view entails additional assumptions in order to explain how the highly differentiated sexual gametes manage to remain potentially immortal. Cells differentiate by blocking expression from large parts of their genome and it is suggested that losses or gains of genetic totipotency determine cellular lifespans. Cells destined to be somatic do not regain totipotency and senesce, while germ-line cells regain complete genome expression and immortality after meiosis and gamete fusions. Losses of genetic totipotency could induce senescence by lowering the levels of repair and maintenance enzymes.

Rushing, E. J., K. Yashima, et al. (1997). "Expression of telomerase RNA component correlates with the MIB-1 proliferation index in ependymomas." J Neuropathol Exp Neurol **56**(10): 1142-6.

Although there is general agreement that certain morphologic subtypes of ependymoma are benign, the biologic behavior of other ependymal neoplasms is poorly understood and not clearly related to conventional histopathologic criteria. The absence of universally accepted standards has prompted the search for more objective biologic markers. Telomerase is an RNA-containing enzyme associated with immortality in proliferating stem cells and many tumors. We investigated the proliferative activity of 26 ependymomas as determined by MIB-1 immunolabeling and compared the results with the in situ expression of human telomerase RNA (hTR) and WHO tumor grade. The study included 9 WHO grade I ependymomas (6 subependymomas and 3 myxopapillary ependymomas), 13 WHO grade II ependymomas, and 4 anaplastic (WHO grade III) ependymomas. The proliferation index (PI) and telomerase RNA expression were significantly increased in grade III ependymomas ($p < 0.0001$ for PI and $p = 0.0015$ for hTR). In these tumors, the PI and hTR expression were highly correlated ($p = 0.0001$). Of note, a single case designated grade II showed both increased proliferative activity and the highest hTR expression detected in this series of ependymal neoplasms. Our results suggest that the PI and hTR expression may be important biologic markers, independent of other histopathologic criteria of tumor grade. Future studies examining the correlation of MIB-1 cell kinetics and hTR expression with clinical parameters in selected ependymoma subtypes are needed to determine the prognostic relevance of these markers.

Saretzki, G. (2009). "Telomerase, mitochondria and oxidative stress." Exp Gerontol **44**(8): 485-92.

Telomerase plays an important role in cellular proliferation capacity and survival under conditions of stress. A large part of this protective function is due to telomere capping and maintenance. Thus it contributes to cellular immortality in stem cells and cancer. Recently, evidence has accumulated that telomerase can contribute to cell survival and stress resistance in a largely telomere-independent manner. Telomerase has been shown to shuttle dynamically between different cellular locations. Under increased oxidative stress telomerase is excluded from the nucleus and can be found within the mitochondria. This phenotype correlates with decreased oxidative stress within telomerase expressing cells and improved mitochondrial function by currently largely unknown mechanisms. Our data suggest that mitochondrial protection could be an important non-canonical function for telomerase in cell survival and ageing. This review summarises briefly our knowledge about extra-telomeric functions of telomerase and discusses the potential significance of its mitochondrial localisation.

Schofield, R. (1978). "The relationship between the spleen colony-forming cell and the haemopoietic stem cell." Blood Cells **4**(1-2): 7-25.

Several experimental findings that are inconsistent with the view that the spleen colony-forming cell (CFU-S) is the primary haemopoietic stem cell are reviewed. Recovery of CFU-S, both quantitatively and qualitatively, can proceed differently depending upon the cytotoxic agent or regime used to bring about the depletion. The virtual immortality of the stem cell population is at variance with evidence that the CFU-S population has an 'age-structure' which has been invoked by several workers to explain experimental and clinical observations. To account for these inconsistencies, a hypothesis is proposed in which the stem cell is seen in association with other cells which determine its behaviour. It becomes essentially a fixed tissue cell. Its maturation is prevented and, as a result, its continued proliferation as a stem cell is assured. Its progeny, unless they can occupy a similar stem cell 'niche', are first generation colony-forming cells, which proliferate and mature to acquire a high probability of differentiation, i.e., they have an age-structure. Some of the experimental situations reviewed are discussed in relation to the proposed hypothesis.

Sell, S. and H. L. Leffert (2008). "Liver cancer stem cells." J Clin Oncol **26**(17): 2800-5.

In an effort to review the evidence that liver cancer stem cells exist, two fundamental questions must be addressed. First, do hepatocellular carcinomas (HCC) arise from liver stem cells? Second, do HCCs contain cells that possess properties of cancer stem cells? For many years the finding of preneoplastic nodules in the liver during experimental induction of HCCs by chemicals was interpreted to support the hypothesis that HCC arose by dedifferentiation of mature liver cells. More recently, recognition of the role of small oval cells in the carcinogenic process led to a new hypothesis that HCC arises by maturation arrest of liver stem cells. Analysis of the cells in HCC supports the presence of cells with stem-cell properties (ie, immortality, transplantability, and resistance to therapy). However, definitive markers for these putative cancer stem cells have not yet been found and a liver cancer stem cell has not been isolated.

Stolzing, A., J. Hescheler, et al. (2007). "Fusion and regenerative therapies: is immortality really recessive?" Rejuvenation Res **10**(4): 571-86.

Harnessing cellular fusion as a potential tool for regenerative therapy has been under tentative investigation for decades. A look back the history of fusion experiments in gerontology reveals that whereas some studies indicate that aging-related changes are conserved in fused cells, others have demonstrated that fusion can be used as a tool to revoke cellular senescence and induce tissue regeneration. Recent findings about the role of fusion processes in tissue homeostasis, replenishment, and repair link insights from fusion studies of previous decades with modern developments in stem cell biology and regenerative medicine. We suggest that age-associated loss of regenerative capacity is associated with a decline of effectiveness in stem cell fusion. We project how studies into the fusion of stem cells with tissue cells, or the fusion between activator stem cells and patient cells might help in the development of applications that "rejuvenate" certain target cells, thereby strategically reinstating a regeneration cascade. The outlook is concluded with a discussion of the next research milestones and the potential hazards of fusion therapies.

Sugihara, M., K. Ohshima, et al. (1999). "Decreased expression of telomerase-associated RNAs in the proliferation of stem cells in comparison with continuous expression in malignant tumors." Int J Oncol **15**(6): 1075-80.

Telomerase, an enzyme associated with cellular immortality, is expressed by malignant tumor and stem cells, especially germ cells. Normal somatic cells, however, usually do not express telomerase. In the malignant tumor, deregulation of telomerase is thought to facilitate tumorigenesis and cellular immortality by providing cancer cells unlimited proliferation capacity. We investigated the relationship between proliferation activity and in situ expression of the telomerase RNA component (human telomerase RNA component, hTERC). In addition, in situ hybridization of the telomerase-associated proteins (telomerase-associated protein 1, TEP1; human telomerase reverse transcriptase, TERT), and MIB-1 immunohistochemistry for proliferation activity were performed, using the malignant tumors of adenocarcinoma, squamous cell carcinoma, and malignant lymphoma, and somatic tissues of testis, endometrium, stomach, skin, and lymph nodes. In the somatic tissues, the stem cells expressed telomerase-associated RNA, but no proliferation activity. When the proliferation activity of the stem cells increased, however, the telomerase-associated expressions decreased. In the malignant tumors, both proliferation activity and expression of the telomerase-associated RNA significantly increased. Deregulation of telomerase, in addition to proliferation activity, is associated with tumorigenesis.

Sugiki, T., T. Uyama, et al. (2007). "Hyaline cartilage formation and enchondral ossification modeled with

KUM5 and OP9 chondroblasts." J Cell Biochem **100**(5): 1240-54.

What is it that defines a bone marrow-derived chondrocyte? We attempted to identify marrow-derived cells with chondrogenic nature and immortality without transformation, defining "immortality" simply as indefinite cell division. KUM5 mesenchymal cells, a marrow stromal cell line, generated hyaline cartilage in vivo and exhibited enchondral ossification at a later stage after implantation. Selection of KUM5 chondroblasts based on the activity of the chondrocyte-specific cis-regulatory element of the collagen alpha2(XI) gene resulted in enhancement of their chondrogenic nature. Gene chip analysis revealed that OP9 cells, another marrow stromal cell line, derived from macrophage colony-stimulating factor-deficient osteopetrotic mice and also known to be niche-constituting cells for hematopoietic stem cells expressed chondrocyte-specific or -associated genes such as type II collagen alpha1, Sox9, and cartilage oligomeric matrix protein at an extremely high level, as did KUM5 cells. After cultured OP9 micromasses exposed to TGF-beta3 and BMP2 were implanted in mice, they produced abundant metachromatic matrix with the toluidine blue stain and formed type II collagen-positive hyaline cartilage within 2 weeks in vivo. Hierarchical clustering and principal component analysis based on microarray data of the expression of cell surface markers and cell-type-specific genes resulted in grouping of KUM5 and OP9 cells into the same subcategory of "chondroblast," that is, a distinct cell type group. We here show that these two cell lines exhibit the unique characteristics of hyaline cartilage formation and enchondral ossification in vitro and in vivo.

Takahashi, K., K. Mitsui, et al. (2003). "Role of ERas in promoting tumour-like properties in mouse embryonic stem cells." Nature **423**(6939): 541-5.

Embryonic stem (ES) cells are pluripotent cells derived from early mammalian embryos. Their immortality and rapid growth make them attractive sources for stem cell therapies; however, they produce tumours (teratomas) when transplanted, which could preclude their therapeutic usage. Why ES cells, which lack chromosomal abnormalities, possess tumour-like properties is largely unknown. Here we show that mouse ES cells specifically express a Ras-like gene, which we have named ERas. We show that human HRasp, which is a recognized pseudogene, does not contain reported base substitutions and instead encodes the human orthologue of ERas. This protein contains amino-acid residues identical to those present in active mutants of Ras and causes oncogenic transformation in NIH 3T3 cells. ERas interacts with phosphatidylinositol-3-OH kinase but not with Raf. ERas-null ES cells maintain pluripotency but show significantly reduced growth and tumorigenicity, which are rescued by expression of ERas complementary DNA or by activated phosphatidylinositol-3-OH kinase. We conclude that the transforming oncogene ERas is important in the tumour-like growth properties of ES cells.

Toshima, S., T. Arai, et al. (1999). "Cytological diagnosis and telomerase activity of cells in effusions of body cavities." Oncol Rep **6**(1): 199-203.

Telomerase is a ribonucleoprotein that synthesizes telometric DNA on chromosome ends, and may be related to the aging and immortality of cells. Recently, a telometric repeat amplification protocol (TRAP) assay for telomerase activity, using the polymerase chain reaction, was developed. We examined the limitations of TRAP assay by applying it to a cultured colon cancer cell line (COLO320) and 58 human cytological materials from body cavity effusions, and obtained the following results; i) The limits of the TRAP assay were 20-50 cells for the COLO320 cell line; ii) One COLO320 cell per 100 normal blood white cells was detectable; iii) Seventeen of 58 samples were positive for telomerase activity in this study. The sensitivity was 69% (9/13) and the specificity was 87.5% (28/32) between cytological diagnosis and telomerase activity; iv) Among 29 malignant cases, 15 were positive for telomerase activity, while there were 11 cytologically positive cases. The positive cases detected by the combination of cytology and telomerase activity accounted for 21 of the total 29 cases (72.4%). These results suggest that the measurement of telomerase activity in body cavity effusions may be useful as an adjunctive tool for cytological and clinicopathological diagnosis and that this technique is potentially applicable to remnant cytological materials.

Utikal, J., J. M. Polo, et al. (2009). "Immortalization eliminates a roadblock during cellular reprogramming into iPS cells." Nature **460**(7259): 1145-8.

The overexpression of defined transcription factors in somatic cells results in their reprogramming into induced pluripotent stem (iPS) cells. The extremely low efficiency and slow kinetics of in vitro reprogramming suggest that further rare events are required to generate iPS cells. The nature and identity of these events, however, remain elusive. We noticed that the reprogramming potential of primary murine fibroblasts into iPS cells decreases after serial passaging and the concomitant onset of senescence. Consistent with the notion that loss of replicative potential provides a barrier for reprogramming, here we show that cells with low endogenous p19(Arf)

(encoded by the Ink4a/Arf locus, also known as Cdkn2a locus) protein levels and immortal fibroblasts deficient in components of the Arf-Trp53 pathway yield iPS cell colonies with up to threefold faster kinetics and at a significantly higher efficiency than wild-type cells, endowing almost every somatic cell with the potential to form iPS cells. Notably, the acute genetic ablation of Trp53 (also known as p53) in cellular subpopulations that normally fail to reprogram rescues their ability to produce iPS cells. Our results show that the acquisition of immortality is a crucial and rate-limiting step towards the establishment of a pluripotent state in somatic cells and underscore the similarities between induced pluripotency and tumorigenesis.

Varga, A. C. and J. L. Wrana (2005). "The disparate role of BMP in stem cell biology." Oncogene **24**(37): 5713-21.

Stem cells share several characteristics of cancer cells including loss of contact inhibition and immortality. Therefore, stem cells represent an excellent model system in which to define the molecular mechanisms underlying cancer development and progression. Several signal transduction pathways including leukemia inhibitory factor, Wnt and FGF have been demonstrated to function in stem cell self-renewal and differentiation. However, more recently bone morphogenetic proteins (BMPs) have emerged as key regulators of stem cell fate commitment. Intriguingly, BMPs have disparate roles in regulating the biology of embryonic stem (ES) cells compared with neural crest stem cells (NCSCs). Furthermore, although BMPs block neural differentiation of ES cells from both mouse and human, they contribute to self-renewal specifically in mouse ES cells. These observations strongly suggest that combinations of extracellular factors regulate stem cells, and that crosstalk between intracellular signaling pathways precisely defines stem cell fate commitment. In this review, we focus on the role of BMP signaling in mouse and human ES cells compared with NCSCs. We then discuss how the molecular effectors of BMP signaling may contribute to cancer, and thus represent potential targets for therapeutic intervention.

Vinnitsky, V. B. (1993). "Oncogerminative hypothesis of tumor formation." Med Hypotheses **40**(1): 19-27.

The oncogerminative hypothesis of tumor formation states that during malignant transformation of somatic cells part of the germinative cell genome is activated. This part determines the phenotype property of the germinative cell: its potential immortality realized during its life cycle. In malignant cells this activated part of the genome also determines immortality in its life cycle. The life cycle of the cell may be divided into five stages: 1) the reproduction stage under the influence of promotors; 2) the stage of multicellular oncospheroid formation (the parody of blastocyst) characterized by heterogeneous composition of cellular population consisting of three major phenotypically different cells: oncogerminative ones (stem), oncotrophoblast (fulfilling trophic function) and oncosomatic ones (differentiated) imitating germinative, trophoblast and somatic cells of the embryo respectively; 3) the stage of malignant tumor formation which consist of the vascularization of the oncospheroid and its growth under the conditions of anatomic contacts with the organism; 4) the stage of disaggregation of the oncogerminative cells which manifested in the organism by process of metastatic spreading; 5) the stage of formation of metastatic tumors. The change of the ratio of oncogerminative, oncotrophoblast and oncosomatic cells in metastatic tumors is a basis of tumor progression.

Wai, L. K. (2004). "Telomeres, telomerase, and tumorigenesis--a review." MedGenMed **6**(3): 19.

Human telomeres function as a protective structure capping both ends of the chromosome. They are composed of long, repetitive sequences of TTAGGG, associated with a variety of telomere-binding proteins. Telomeres protect the chromosomes from end-to-end fusion, recombination, and degradation, all events that can lead to cell death. At cell replication, telomeres cannot be completely replicated. They are gradually shortened, and when the telomeres reach a critical threshold, cell replication is arrested in what is called "replicative senescence." Thus, telomeres act as an intrinsic "counting" mechanism of the cell's aging process. Telomerase is an enzymatic ribonucleoprotein complex that acts as a reverse transcriptase in the elongation of telomeres. Telomerase activity is almost absent in somatic cells, but it is detected in embryonic stem cells and in the vast majority of tumor cells. Tumor cells, in fact, may contain short and stable telomeres that confer immortality to the cancer cells, which are thus able to replicate indefinitely. The deregulation of telomeres thus plays an important role in the relationship between premature aging syndrome and cancer. This review describes the recent advances in the molecular characterization of telomeres, the regulation of telomerase

Walford, R. L. (1979). "Multigene families, histocompatibility systems, transformation, meiosis, stem cells, and DNA repair." Mech Ageing Dev **9**(1-2): 19-26.

Aging is probably not directly traceable to changes along the whole genome, but to a small portion thereof. The main histocompatibility complex appears to be one among the postulated sets of multigene families responsible. The immortality of transformed cells, the germ line, and possibly certain pluripotential stem cells may suggest common qualitative and/or quantitative differences in DNA repair mechanisms between these cell populations and committed, normal cell populations. A relationship between HLA and at least two diseases showing defective DNA-repair suggests that the same chromosome carrying the main histocompatibility complex may control some repair processes. The correspondence of variation in lifespans in different mouse strains with the DNA repair capabilities and degrees of autoimmune susceptibility of the same strains lends further support to the idea that DNA repair, immune dysfunction and aging in higher animals may be intimately related.

Watanabe, H., V. T. Hoang, et al. (2009). "Immortality and the base of multicellular life: Lessons from cnidarian stem cells." Semin Cell Dev Biol **20**(9): 1114-25.

Cnidarians are phylogenetically basal members of the animal kingdom (>600 million years old). Together with plants they share some remarkable features that cannot be found in higher animals. Cnidarians and plants exhibit an almost unlimited regeneration capacity and immortality. Immortality can be ascribed to the asexual mode of reproduction that requires cells with an unlimited self-renewal capacity. We propose that the basic properties of animal stem cells are tightly linked to this archaic mode of reproduction. The cnidarian stem cells can give rise to a number of differentiated cell types including neuronal and germ cells. The genomes of Hydra and Nematostella, representatives of two major cnidarian classes indicate a surprising complexity of both genomes, which is in the range of vertebrates. Recent work indicates that highly conserved signalling pathways control Hydra stem cell differentiation. Furthermore, the availability of genomic resources and novel technologies provide approaches to analyse these cells in vivo. Studies of stem cells in cnidarians will therefore open important insights into the basic mechanisms of stem cell biology. Their critical phylogenetic position at the base of the metazoan branch in the tree of life makes them an important link in unravelling the common mechanisms of stem cell biology between animals and plants.

Yui, J., C. P. Chiu, et al. (1998). "Telomerase activity in candidate stem cells from fetal liver and adult bone marrow." Blood **91**(9): 3255-62.

Telomerase is a ribonucleoprotein polymerase that synthesizes telomeric repeats onto the 3' ends of eukaryotic chromosomes. Activation of telomerase may prevent telomeric shortening and correlates with cell immortality in the germline and certain tumor cells. Candidate hematopoietic stem cells (HSC) from adult bone marrow express low levels of telomerase, which is upregulated with proliferation and/or differentiation. To address this issue, we stimulated purified candidate HSC from human adult bone marrow with stem cell factor (SCF), interleukin-3 (IL-3), and Flt3-ligand (FL). After 5 days in culture, activity was detected in total cell extracts from IL-3-, SCF + FL-, SCF + IL-3-, FL + IL-3-, and SCF + IL-3 + FL-stimulated cultures, but not from cells cultured in SCF or FL alone. Within the CD34(+) fraction of the cultured cells, significant activity was found in the CD34(+)CD71(+) fraction. In addition, PKH26 staining confirmed that detectable telomerase activity was present in dividing PKH26(lo) cells, whereas nondividing PKH26(hi) cells were telomerase negative. Because in these experiments no distinction could be made between cycling "candidate" stem cells that had retained or had lost self-renewal properties, fetal liver cells with a CD34(+)CD38(-) phenotype, highly enriched for cycling stem cells, were also examined and found to express readily detectable levels of telomerase activity. Given the replication-dependent loss of telomeric DNA in hematopoietic cells, these observations suggest that the observed telomerase activity in candidate stem cells is either expressed in a minor subset of stem cells or, more likely, is not sufficient to prevent telomere shortening.

Zeng, X. (2007). "Human embryonic stem cells: mechanisms to escape replicative senescence?" Stem Cell Rev **3**(4): 270-9.

Human embryonic stem cells (hESCs) are unique in that they can proliferate indefinitely in culture in an undifferentiated state as well as differentiate into any somatic cells. Undifferentiated hESCs do not appear to undergo senescence and remain nontransformed over multiple passages. Culture hESCs maintain telomere length and exhibit high telomerase activity after prolonged in vitro culture. The ability of hESCs to bypass senescence is lost as hESCs differentiate into fully differentiated somatic cells. This loss of immortality upon differentiation may be due to a variety aging related factors such as reduction in telomere length, alteration of telomerase activity, changes in cell cycle regulation and decrease in DNA repair ability. Absence of such aging factors as well as the lack of genomic, mitochondrial and epigenetic changes, may contribute to the lack of senescence in hESCs. In this review, we will summarize recent advances in determining

changes in these aspects in prolonged hESC cultures. We will in particular discuss the potential roles of several cellular pathways including the telomerase, p53, and Rb pathways in escaping senescence in hESCs. We will also discuss the genomic and epigenetic changes in long-term hESC culture and their potential roles in bypassing senescence, as well as alternative sources of pluripotent stem cells.

Zimmermann, S., M. Voss, et al. (2003). "Lack of telomerase activity in human mesenchymal stem cells." Leukemia **17**(6): 1146-9.

Telomerase activity and telomere maintenance have been associated with immortality in tumor and embryonic stem cells. Whereas most normal somatic cells are telomerase negative, low levels of this enzyme have been found in adult stem cells from the skin, gut and the hematopoietic system. Here, we show that telomerase activity is not detectable in human mesenchymal stem cells (hMSCs), which have the phenotype SH2+, SH3+, SH4+, CD29+, CD44+, CD14-, CD34- and CD45-, and have the capacity to differentiate into adipocytes, chondrocytes and osteoblasts. These data suggest that hMSCs have a different telomere biology compared to other adult stem cells. Alternatively, true mesenchymal stem cells might be a very rare subpopulation that have a detection level that is below the sensitivity of the TRAP assay.

References

1. Ambrosi, D. J., B. Tanasijevic, et al. (2007). "Genome-wide reprogramming in hybrids of somatic cells and embryonic stem cells." Stem Cells **25**(5): 1104-13.
2. Amit, M., V. Margulets, et al. (2003). "Human feeder layers for human embryonic stem cells." Biol Reprod **68**(6): 2150-6.
3. Ashkenazi, R., S. N. Gentry, et al. (2008). "Pathways to tumorigenesis--modeling mutation acquisition in stem cells and their progeny." Neoplasia **10**(11): 1170-82.
4. Bernardi, R. and P. P. Pandolfi (2003). "The nucleolus: at the stem of immortality." Nat Med **9**(1): 24-5.
5. Blagosklonny, M. V. (2006). "Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition." Cell Cycle **5**(18): 2087-102.
6. Blagosklonny, M. V. (2007). "Cancer stem cell and cancer stemloids: from biology to therapy." Cancer Biol Ther **6**(11): 1684-90.
7. Borges, R. M. (2009). "Phenotypic plasticity and longevity in plants and animals: cause and effect?" J Biosci **34**(4): 605-11.
8. Bosch, T. C. (2009). "Hydra and the evolution of stem cells." Bioessays **31**(4): 478-86.
9. Brown, D. (2009). "The energy body and its functions: immunosurveillance, longevity, and regeneration." Ann N Y Acad Sci **1172**: 312-37.
10. Cowell, J. K. (1999). "Telomeres and telomerase in ageing and cancer." Age (Omaha) **22**(2): 59-64.
11. de Caralt, S., M. J. Uriz, et al. (2007). "Cell culture from sponges: pluripotency and immortality." Trends Biotechnol **25**(10): 467-71.
12. Dennis, J. E., A. Merriam, et al. (1999). "A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse." J Bone Miner Res **14**(5): 700-9.
13. Drexler, H. G., Y. Matsuo, et al. (1998). "Proposals for the characterization and description of new human leukemia-lymphoma cell lines." Hum Cell **11**(1): 51-60.
14. Franzese, O., A. Comandini, et al. (1998). "Effect of prostaglandin A1 on proliferation and telomerase activity of human melanoma cells in vitro." Melanoma Res **8**(4): 323-8.
15. Fuller, M. T. and A. C. Spradling (2007). "Male and female Drosophila germline stem cells: two versions of immortality." Science **316**(5823): 402-4.
16. Hiyama, E. and K. Hiyama (2007). "Telomere and telomerase in stem cells." Br J Cancer **96**(7): 1020-4.
17. Hoffman, L. M. and M. K. Carpenter (2005). "Human embryonic stem cell stability." Stem Cell Rev **1**(2): 139-44.
18. Hohaus, S., M. T. Voso, et al. (1997). "Telomerase activity in human hematopoietic progenitor cells." Haematologica **82**(3): 262-8.
19. Holland, A. M. and E. G. Stanley (2009). "Stems cells and the price of immortality." Stem Cell Res **2**(1): 26-8.
20. Holm, T. M., L. Jackson-Grusby, et al. (2005). "Global loss of imprinting leads to widespread tumorigenesis in adult mice." Cancer Cell **8**(4): 275-85.
21. Isfort, R. J. and R. A. LeBoeuf (1995). "The Syrian hamster embryo (SHE) cell transformation system: a biologically relevant in vitro model--with carcinogen predicting capabilities--of in vivo multistage neoplastic transformation." Crit Rev Oncog **6**(3-6): 251-60.
22. Jaishankar, A., M. Barthelery, et al. (2009). "Human embryonic and mesenchymal stem cells express different nuclear proteomes." Stem Cells Dev **18**(5): 793-802.
23. Jones, D. L. (2007). "Aging and the germ line: where mortality and immortality meet." Stem Cell Rev **3**(3): 192-200.
24. Keith, W. N. (2004). "From stem cells to cancer: balancing immortality and neoplasia." Oncogene **23**(29): 5092-4.
25. Klingler, K., G. R. Johnson, et al. (1988). "Transformation of single myeloid precursor cells by the malignant histiocytosis sarcoma virus (MHSV): generation of growth-factor-independent myeloid colonies and permanent cell lines." J Cell Physiol **135**(1): 32-8.
26. Kopper, L. and M. Hajdu (2004). "Tumor stem cells." Pathol Oncol Res **10**(2): 69-73.
27. Kraemer, P. M., F. A. Ray, et al. (1986). "Spontaneous immortalization rate of cultured Chinese hamster cells." J Natl Cancer Inst **76**(4): 703-9.
28. Kroll, J. (2005). "Chaperones and longevity." Biogerontology **6**(5): 357-61.
29. Kroll, J. (2007). "Molecular chaperones and the epigenetics of longevity and cancer resistance." Ann N Y Acad Sci **1100**: 75-83.
30. Lawrenz, B., H. Schiller, et al. (2004). "Highly sensitive biosafety model for stem-cell-derived grafts." Cytotherapy **6**(3): 212-22.
31. Lepperdinger, G. (2009). "Open-ended question: is immortality exclusively inherent to the germline?--A mini-review." Gerontology **55**(1): 114-7.
32. Lin, K. W. and J. Yan (2005). "The telomere length dynamic and methods of its assessment." J Cell Mol Med **9**(4): 977-89.
33. Lo, K. C., S. Whirledge, et al. (2005). "Stem cells: implications for urology." Curr Urol Rep **6**(1): 49-54.
34. Matzuk, M. M. (2004). "Germ-line immortality." Proc Natl Acad Sci U S A **101**(47): 16395-6.
35. McCulloch, E. A., T. Motoji, et al. (1984). "Hemopoietic stem cells: their roles in human leukemia and certain continuous cell lines." J Cell Physiol Suppl **3**: 13-20.
36. McLaren, A. (1992). "Embryology. The quest for immortality." Nature **359**(6395): 482-3.
37. McLaren, A. (2001). "Mammalian germ cells: birth, sex, and immortality." Cell Struct Funct **26**(3): 119-22.
38. Merle, P. and C. Trepo (2009). "Molecular mechanisms underlying hepatocellular carcinoma." Viruses **1**(3): 852-72.

39. Monk, M., C. Holding, et al. (2001). "Isolation of novel developmental genes from human germ cell, oocyte and embryo cDNA by differential display." Reprod Fertil Dev **13**(1): 51-7.
40. Mummery, C. (2004). "Stem cell research: immortality or a healthy old age?" Eur J Endocrinol **151 Suppl 3**: U7-12.
41. Negishi, Y., A. Kudo, et al. (2000). "Multipotency of a bone marrow stromal cell line, TBR31-2, established from ts-SV40 T antigen gene transgenic mice." Biochem Biophys Res Commun **268**(2): 450-5.
42. Oshimura, M., D. J. Fitzgerald, et al. (1988). "Cytogenetic changes in rat tracheal epithelial cells during early stages of carcinogen-induced neoplastic progression." Cancer Res **48**(3): 702-8.
43. Park, W. C., H. Liu, et al. (2005). "Deregulation of estrogen induced telomerase activity in tamoxifen-resistant breast cancer cells." Int J Oncol **27**(5): 1459-66.
44. Peng, W. M., L. L. Yu, et al. (2002). "Transplanted neuronal precursors migrate and differentiate in the developing mouse brain." Cell Res **12**(3-4): 223-8.
45. Pfeffer, N. (2008). "What British women say matters to them about donating an aborted fetus to stem cell research: a focus group study." Soc Sci Med **66**(12): 2544-54.
46. Postovit, L. M., F. F. Costa, et al. (2007). "The commonality of plasticity underlying multipotent tumor cells and embryonic stem cells." J Cell Biochem **101**(4): 908-17.
47. Price, J. E., A. J. Syms, et al. (1986). "Cellular immortality, clonogenicity, tumorigenicity and the metastatic phenotype." Eur J Cancer Clin Oncol **22**(3): 349-55.
48. Rahman, R., R. Heath, et al. (2009). "Cellular immortality in brain tumours: an integration of the cancer stem cell paradigm." Biochim Biophys Acta **1792**(4): 280-8.
49. Rando, T. A. (2006). "Stem cells, ageing and the quest for immortality." Nature **441**(7097): 1080-6.
50. Rashid-Doubell, F., T. R. Kershaw, et al. (1994). "Effects of basic fibroblast growth factor and gamma interferon on hippocampal progenitor cells derived from the H-2Kb-tsA58 transgenic mouse." Gene Ther **1 Suppl 1**: S63.
51. Rha, S. Y., E. Izbicka, et al. (2000). "Effect of telomere and telomerase interactive agents on human tumor and normal cell lines." Clin Cancer Res **6**(3): 987-93.
52. Rosenberger, R. F. (1995). "The initiation of senescence and its relationship to embryonic cell differentiation." Bioessays **17**(3): 257-60.
53. Rothstein, J. D. and E. Y. Snyder (2004). "Reality and immortality--neural stem cells for therapies." Nat Biotechnol **22**(3): 283-5.
54. Rushing, E. J., K. Yashima, et al. (1997). "Expression of telomerase RNA component correlates with the MIB-1 proliferation index in ependymomas." J Neuropathol Exp Neurol **56**(10): 1142-6.
55. Saretzki, G. (2009). "Telomerase, mitochondria and oxidative stress." Exp Gerontol **44**(8): 485-92.
56. Schofield, R. (1978). "The relationship between the spleen colony-forming cell and the haemopoietic stem cell." Blood Cells **4**(1-2): 7-25.
57. Sell, S. and H. L. Leffert (2008). "Liver cancer stem cells." J Clin Oncol **26**(17): 2800-5.
58. Stolzing, A., J. Hescheler, et al. (2007). "Fusion and regenerative therapies: is immortality really recessive?" Rejuvenation Res **10**(4): 571-86.
59. Sugihara, M., K. Ohshima, et al. (1999). "Decreased expression of telomerase-associated RNAs in the proliferation of stem cells in comparison with continuous expression in malignant tumors." Int J Oncol **15**(6): 1075-80.
60. Sugiki, T., T. Uyama, et al. (2007). "Hyaline cartilage formation and enchondral ossification modeled with KUM5 and OP9 chondroblasts." J Cell Biochem **100**(5): 1240-54.
61. Takahashi, K., K. Mitsui, et al. (2003). "Role of ERas in promoting tumour-like properties in mouse embryonic stem cells." Nature **423**(6939): 541-5.
62. Toshima, S., T. Arai, et al. (1999). "Cytological diagnosis and telomerase activity of cells in effusions of body cavities." Oncol Rep **6**(1): 199-203.
63. Utikal, J., J. M. Polo, et al. (2009). "Immortalization eliminates a roadblock during cellular reprogramming into iPS cells." Nature **460**(7259): 1145-8.
64. Varga, A. C. and J. L. Wrana (2005). "The disparate role of BMP in stem cell biology." Oncogene **24**(37): 5713-21.
65. Vinnitsky, V. B. (1993). "Oncogerminative hypothesis of tumor formation." Med Hypotheses **40**(1): 19-27.
66. Vogel, G. (2005). "Stem cells. Scientists chase after immortality in a petri dish." Science **309**(5743): 1982-3.
67. Wai, L. K. (2004). "Telomeres, telomerase, and tumorigenesis--a review." MedGenMed **6**(3): 19.
68. Walford, R. L. (1979). "Multigene families, histocompatibility systems, transformation, meiosis, stem cells, and DNA repair." Mech Ageing Dev **9**(1-2): 19-26.
69. Watanabe, H., V. T. Hoang, et al. (2009). "Immortality and the base of multicellular life: Lessons from cnidarian stem cells." Semin Cell Dev Biol **20**(9): 1114-25.
70. Yui, J., C. P. Chiu, et al. (1998). "Telomerase activity in candidate stem cells from fetal liver and adult bone marrow." Blood **91**(9): 3255-62.
71. Zeng, X. (2007). "Human embryonic stem cells: mechanisms to escape replicative senescence?" Stem Cell Rev **3**(4): 270-9.
72. Zimmermann, S., M. Voss, et al. (2003). "Lack of telomerase activity in human mesenchymal stem cells." Leukemia **17**(6): 1146-9.
73. Christopher C. Fraser, Stephen J. Szilvassy, Connie J. Eaves, And R. Keith Humphries. Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability. Proc. Nati. Acad. Sci. USA. Vol. 89, pp. 1968-1972, 1992.
74. Fraser, C. C., Eaves, C. J., Szilvassy, S. J. & Humphries, R. K. (1990). Blood 76, 1071-1076.
75. Thomas, K. R. & Capecchi, M. R. (1987) Cell 51, 503-512.
76. Baud L, Haymann JP, Bellocq A, Fouqueray B. Contribution of stem cells to renal repair after ischemia/reperfusion. Bull Acad Natl Med. 2005;189(4):635-43.
77. Bavister BD, Wolf DP, Brenner CA. Challenges of primate embryonic stem cell research. Cloning Stem Cells 2005;7(2):82-94.
78. Bernard Lo, Patricia Zettler, Marcelle I. Cedars, Elena Gates, Arnold R. Kriegstein, Michelle Oberman, Renee Reijo Pera, Richard M. Wagner, Mary T. Wuerth, Leslie E. Wolf, Keith R. Yamamoto. A New Era in the Ethics of Human Embryonic Stem Cell Research. Stem Cells. http://www.StemCells.com. http://stemcells.alphamedpress.org/cgi/reprint/2005-0324v1.pdf. 2005.
79. Bhatt RI, Brown MD, Hart CA, Gilmore P, Ramani VAC, George NJ, Clarke NW. Novel method for the isolation and characterisation of the putative prostatic stem cell. Cytometry A. 2003;54(2):89-99.
80. Cantley LG. Adult stem cells in the repair of the injured renal tubule. Nat Clin Pract Nephrol. 2005;1(1):22-32.
81. Condorelli G, Peschle C. Stem cells for cardiac repair: state of the art. Front Biosci 2005;10:3143-50.
82. Daar AS, Sheremeta L. The science of stem cells: ethical, legal and social issues. Exp Clin Transplant. 2003;1(2):139-46.
83. Duffield JS, Bonventre JV. Kidney tubular epithelium is restored without replacement with bone marrow-derived cells during repair after ischemic injury. Kidney Int. 2005;68(5):1956-61.
84. Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs

independently of bone marrow-derived stem cells. J Clin Invest. 2005;115(7):1743-55.
85. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. Int J Mol Med. 2004;14(6):1035-41.
86. Hishikawa K, Fujita T. Stem cells and kidney disease. Hypertens Res. 2006;29(10):745-9.
87. http://stemcells.nih.gov/staticresources/research/protocols/BresaGen_hESC_manual_2.1.pdf
88. Humphreys BD, Duffield JD, Bonventre JV. Renal stem cells in recovery from acute kidney injury. Minerva Urol Nefrol. 2006;58(1):13-21.
89. Kashofer K, Bonnet D. Gene Therapy Progress and Prospects: Stem cell plasticity. Gene Ther. 2005 (Epub ahead of print).
90. Kimberly Kasow. OPBMT2 Protocol: Allogeneic Hematopoietic Stem Cell Transplantation for Children Affected with Malignant Osteopetrosis - A Pilot Study. http://www.stjude.org/protocols/0,2881,450_2331_17072,00.html. 2007.
91. Kwang-Soo Kim. Stem cell research continues in Korea beyond the Hwang scandal. Stem Cells. http://www.StemCells.com. http://stemcells.alphamedpress.org/cgi/reprint/2007-0089v1.pdf. 2007.
92. Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, Igarashi P. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. J Am Soc Nephrol. 2003;14(5):1188-99.
93. Lin F. Stem cells in kidney regeneration following acute renal injury. Pediatr Res. 2006;59(4 Pt 2):74R-8R.
94. Ma H. Technique of Animal Clone. Nature and Science 2004;2(1):29-35.
95. Mitalipova et al. Stem Cells. 2003;21(5):521-6.
96. Morigi M, Benigni A, Remuzzi G, Imberti B. The regenerative potential of stem cells in acute renal failure. Cell Transplant. 2006;15 Suppl 1:S111-7.
97. Nigel Hawkes. Scientists find the secret of eternal life for stem cells. http://www.timesonline.co.uk/tol/news/uk/article1137674.ece
98. Oliver JA. Adult renal stem cells and renal repair. Curr Opin Nephrol Hypertens. 2004;13(1):17-22.
99. Paul Woodard. SCDHAP Protocol: ematopoietic Stem Cell Transplantation (HSCT) for Patients with Sickle Cell Disease and Prior Stroke or Abnormal Transcranial Doppler Ultrasound (TCD) using Reduced Conditioning and T-Cell-Depleted Hematopoietic Stem Cells from Partially Matched Family Donors - Phase I Study. http://www.stjude.org/protocols/0,2081,450_2327_18472,00.html. 2007.
100. Renee Madden. SCT521 (COG # ASCT0521) Protocol: Soluble Tumor Necrosis Factor Receptor: Enbrel (Etanercept) for the Treatment of Acute Non-Infectious Pulmonary Dysfunction (Idiopathic Pneumonia Syndrome) Following Allogeneic Stem Cell Transplantation. http://www.stjude.org/protocols/0,2881,450_2333_5873,00.html. 2007.
101. Stedman's Medical Dictionary. The American Heritage®. Houghton Mifflin Company. http://dictionary.reference.com/search?q=stem%20cell. 2002.
102. Williams D. Stem cells in medical techonology. Med Device Technol 2005;16(3):9-11.
103. Wing Leung. INFT2 Protocol: HLA - Nonidentical Stem Cell and Natural Killer Cell Transplantation for Children Less than 2 Years of Age with Hematologic Malignancies. http://www.stjude.org/protocols/0,2881,450_2330_11129,00.html. 2007.
104. Yamashita S, Maeshima A, Nojima Y. Involvement of renal progenitor tubular cells in epithelial-to-mesenchymal transition in fibrotic rat kidneys. J Am Soc Nephrol. 2005;16(7):2044-51.
105. de Almeida, M., C. V. de Almeida, E. M. Graner, G. E. Brondani and M. F. de Abreu-Tarazi (2012). "Pre-procambial cells are niches for pluripotent and totipotent stem-like cells for organogenesis and somatic embryogenesis in the peach palm: a histological study." Plant Cell Rep **31**(8): 1495-1515.
106. Hemmat, S., D. M. Lieberman and S. P. Most (2010). "An introduction to stem cell biology." Facial Plast Surg **26**(5): 343-349.
107. Sharpless, N. E. (2010). "Hot topics in stem cells and self-renewal: 2010." Aging Cell **9**(4): 457-461.
108. Shufaro, Y. and B. E. Reubinoff (2011). "Cell cycle synchronization for the purpose of somatic cell nuclear transfer (SCNT)." Methods Mol Biol **761**: 239-247.
109. Surani, M. A. and P. Hajkova (2010). "Epigenetic reprogramming of mouse germ cells toward totipotency." Cold Spring Harb Symp Quant Biol **75**: 211-218.
110. Wang, X. D., K. E. Nolan, R. R. Irwanto, M. B. Sheahan and R. J. Rose (2011). "Ontogeny of embryogenic callus in Medicago truncatula: the fate of the pluripotent and totipotent stem cells." Ann Bot **107**(4): 599-609.
111. Ma H, Chen G (2005). Stem Cell. J Am Sci. 1(2):90-92. http://www.sciencepub.net/american/0102/14-mahongbao.pdf.
112. Ma H, Chenrg S (2007). Eternal Life and Stem Cell. Nat Sci. 5(1):81-96. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
113. Ma H, Chenrg S (2007). Review of Stem Cell Studies. Nat Sci. 5(2):45-65. http://www.sciencepub.net/nature/0502/09-0247-mahongbao-stem-ns.pdf.
114. Yang Y, Ma H (2010). Germ Stem Cell. Stem Cell. 1(2):38-60]. http://www.sciencepub.net/stem/stem0102/07_1348stem0102_38_60.pdf.
115. Pubmed. Stem Cell. http://www.ncbi.nlm.nih.gov/pubmed/?term=stem+cell.
116. Wikipedia. Stem Cell. http://en.wikipedia.org/wiki/Stem_cell.

7/7/2012