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Stem Cell

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Stem Cell and Immortality Research Literatures

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

Introduction

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

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

Ackermann, M. (2008). "Bacteria as a new model system for aging studies: investigations using light microscopy." Biotechniques **44**(4): 564-567.

Aging-the decline in an individual's condition over time-is at the center of an active research field in medicine and biology. Some very basic questions have, however, remained unresolved, the most fundamental being: do all organisms age? Or are there organisms that would continue to live forever if not killed by external forces? For a long time it was believed that aging only affected organisms such as animals, plants, and fungi. Bacteria, in contrast, were assumed to be potentially immortal and until recently this assertion remained untested. We used phase-contrast microscopy (on an Olympus BX61) to follow individual bacterial cells over many divisions to prove that some bacteria show a distinction between an aging mother cell and a rejuvenated daughter, and that these bacteria thus age. This indicates that aging is a more fundamental property of organisms than was previously assumed. Bacteria can now be used as very simple model system for investigating why and how organisms age.

Aghajani, M., et al. (2019). "New emerging roles of CD133 in cancer stem cell: Signaling pathway and miRNA regulation." J Cell Physiol **234**(12): 21642-21661.

Cancer stem cells (CSC) are rare immortal cells within a tumor that are able to initiate tumor progression, development, and resistance. Advances studies show that, like normal stem cells, CSCs can be both self-renewed and given rise to many cell types, therefore form tumors. A number of cell surface markers, such as CD44, CD24, and CD133 are frequently used to identify CSCs. CD133, a transmembrane glycoprotein, either alone or in collaboration with other markers, has been mainly considered to identify CSCs from different solid tumors. However, the exactness of CD133 as a cancer stem cell biomarker has not been approved yet. The clinical importance of CD133 is as a CSC marker in many cancers. Also, it contributes to shorter survival, tumor progression, and tumor recurrence. The expression of CD133 is controlled by many extracellular or intracellular factors, such as tumor microenvironment, epigenetic factors, signaling pathways, and miRNAs. In this study, it was attempted to determine: 1) CD133 function; 2) the role of CD133

in cancer; 3) CD133 regulation; 4) the therapeutic role of CD133 in cancers.

Ahmadbeigi, N., et al. (2011). "Early spontaneous immortalization and loss of plasticity of rabbit bone marrow mesenchymal stem cells." Cell Prolif **44**(1): 67-74.

OBJECTIVES: Bone marrow-derived mesenchymal stem cells (BM-MSC) have been widely used for cell therapy and tissue engineering purposes. However, there are still controversies concerning safety of application of these cells after in vitro expansion. Therefore, we aimed to investigate the characteristics of rabbit BM-MSC during long-term culture. MATERIALS AND METHODS: In this study, we have examined growth kinetics, morphological changes, differentiation potential and chromosomal abnormalities, as well as tumour formation potential of rabbit BM-MSC in long-term culture. RESULTS AND CONCLUSION: We found that shortly after isolation, proliferation rate of rabbit BM-MSC decreases until they enter a dormant phase. During this period of quiescence, the cells are large and multinucleate. After some weeks of dormancy we found that several small mononuclear cells originated from each large multinucleate cell. These newly formed cells proliferated rapidly but had inferior differentiation potential. Although they were immortal, they did not have the capability for tumour formation in soft agar assay or in nude mice. This is the first report of spontaneous, non-tumorigenic immortalization of BM-MSC in rabbits. The phenomenon raises more concern for meticulous monitoring and quality control for using rabbit BM-MSC in cell-based therapies and tissue engineering experiments.

Ahmadbeigi, N., et al. (2011). "Dormant phase and multinuclear cells: two key phenomena in early culture of murine bone marrow mesenchymal stem cells." Stem Cells Dev **20**(8): 1337-1347.

Special features of mesenchymal stem cells (MSCs) have made them a popular tool in cell therapy and tissue engineering. Although mouse animal models and murine MSCs are common tools in this field, our understanding of the effect of in vitro expansion on the behavior of these cells is poor and controversial. In addition, in comparison to human, isolation of MSCs from mouse has been reported to be more difficult and some unexplained features such as heterogeneity and slow growth rate in the culture of these cells have been observed. Here we followed mouse bone marrow MSCs for >1 year after isolation and examined the effect of expansion on changes in morphology, growth kinetics, plasticity, and chromosomal structure during in vitro culture. Shortly after isolation, the growth rate of the cells decreased until they stopped dividing and entered a dormant state. In this state the size of the cells increased and they became multinuclear. These large multinuclear cells then gave origin to small mononuclear cells, which after a while resumed proliferation and could be expanded immortally. The immortal cells had diminished plasticity and were aneuploid but could not form tumors in nude mice. These results suggest that mouse bone marrow MSCs bear several modifications when expanded in vitro, and therefore, the interpretation of the data obtained with these cells should be done more cautiously.

Ahn, E. H., et al. (2009). "Loss of anti-proliferative effect of all-trans retinoic acid in advanced stage of breast carcinogenesis." Anticancer Res **29**(8): 2899-2904.

BACKGROUND: Mechanisms by which the inhibitory effect of retinoic acid on tumor growth is attenuated as tumors progress to more advanced stages are unclear. MATERIALS AND METHODS: This study utilizes a novel cell culture system of human breast epithelial cells (HBEC). Immortal (M13SV1), weakly tumorigenic (M13SV1-R2), and highly tumorigenic (M13SV1-R2N1) transformed Type I HBEC were derived sequentially from the same parental Type I HBEC (stem cells) developed from reduction mammoplasty of healthy women. Effects of all-trans retinoic acid (AT-RA) on the growth, protein expression of RAR-alpha, beta and gamma, and RARE transcriptional activation were determined. RESULTS AND CONCLUSION: AT-RA reduces proliferation rates of immortal and weakly tumorigenic cells, but not highly tumorigenic cells. This loss of response of highly tumorigenic cells to AT-RA is associated with overexpression of p185(c-erbB2/neu). It is not associated with decreased RAR-alpha, beta or gamma expression, or activation by AT-RA; RAR-alpha, beta and gamma are expressed and AT-RA increases RARE transcriptional activity in all cell lines tested in this study.

Ahn, E. H., et al. (2016). "Decreased Mitochondrial Mutagenesis during Transformation of Human Breast Stem Cells into Tumorigenic Cells." Cancer Res **76**(15): 4569-4578.

Rare stochastic mutations may accumulate during dormancy of stem-like cells, but technical limitations in DNA sequencing have limited exploring this possibility. In this study, we employed a recently established deep-sequencing method termed Duplex Sequencing to conduct a genome-wide analysis of mitochondrial (mt) DNA mutations in a human breast stem cell model that recapitulates the sequential stages of breast carcinogenesis. Using this method, we found significant differences in mtDNA among normal stem cells, immortal/preneoplastic cells, and tumorigenic cells. Putative cancer stem-like cell (CSC) populations and mtDNA copy numbers increased as normal stem cells become tumorigenic cells. Transformed cells exhibited lower rare mutation frequencies of whole

mtDNA than did normal stem cells. The predicted mtDNA rare mutation pathogenicity was significantly lower in tumorigenic cells than normal stem cells. Major rare mutation types in normal stem cells are C>T/G>A and T>C/A>G transitions, while only C>T/G>A are major types in transformed cells. We detected a total of 1,220 rare point mutations, 678 of which were unreported previously. With only one possible exception (m10342T>C), we did not find specific mutations characterizing mtDNA in human breast CSCs; rather, the mitochondrial genome of CSCs displayed an overall decrease in rare mutations. On the basis of our work, we suggest that this decrease (in particular T>C/A>G transitions), rather than the presence of specific mitochondrial mutations, may constitute an early biomarker for breast cancer detection. Our findings support the hypothesis that the mitochondrial genome is altered greatly as a result of the transformation of normal stem cells to CSCs, and that mtDNA mutation signatures may aid in delineating normal stem cells from CSCs. Cancer Res; 76(15); 4569-78. (c)2016 AACR.

Ahn, E. H., et al. (2019). "Evaluation of chemotherapeutic and cancer-protective properties of sphingosine and C2-ceramide in a human breast stem cell derived carcinogenesis model." Int J Oncol **54**(2): 655-664.

The overall goal of the present study was to evaluate the chemotherapeutic and cancerprotective properties of Derythrosphingosine (sphingosine) and C2ceramide using a human breast epithelial cell (HBEC) culture system, which represents multiplestages of breast carcinogenesis. The HBEC model includes Type I HBECs (normal stem), Type II HBECs (normal differentiated) and transformed cells (immortal/nontumorigenic cells and tumorigenic cells, which are transformed from the same parental normal stem cells). The results of the present study indicate that sphingosine preferentially inhibits proliferation and causes death of normal stem cells (Type I), tumorigenic cells, and MCF7 breast cancer cells, but not normal differentiated cells (Type II). In contrast to the selective antiproliferative effects of sphingosine, C2ceramide inhibits proliferation of normal differentiated cells as well as normal stem cells, tumorigenic cells, and MCF7 cancer cells with similar potency. Both sphingosine and C2ceramide induce apoptosis in tumorigenic cells. Among the sphingosine stereoisomers (Derythro, Dthreo, Lerythro, and Lthreo) and sphinganine that were tested, Lerythrosphingosine most potently inhibits proliferation of tumorigenic cells. The inhibition of breast tumorigenic/cancer cell proliferation by sphingosine was accompanied by inhibition of telomerase activity. Sphingosine at noncytotoxic concentrations, but not C2ceramide, induces differentiation of normal stem cells (Type I), thereby reducing the number of stem cells that are more susceptible to neoplastic transformation. To the best of our knowledge, the present study demonstrates one of the first results that sphingosine can be a potential chemotherapeutic and cancerprotective agent, whereas C2ceramide is not an ideal chemotherapeutic and cancerprotective agent due to its antiproliferative effects on Type II HBECs and its inability to induce the differentiation of Type I to Type II HBECs.

Akiyama, K., et al. (2006). "Rad54 is dispensable for the ALT pathway." Genes Cells **11**(11): 1305-1315.

Some immortal cells use the alternative lengthening of telomeres (ALT) pathway to maintain their telomeres instead of telomerase. Previous studies revealed that homologous recombination (HR) contributes to the ALT pathway. To further elucidate molecular mechanisms, we inactivated Rad54 involved in HR, in mouse ALT embryonic stem (ES) cells. Although Rad54-deficient ALT ES cells showed radiosensitivity in line with expectation, cell growth and telomeres were maintained for more than 200 cell divisions. Furthermore, although MMC-stimulated sister chromatid exchange (SCE) was suppressed in the Rad54-deficient ALT ES cells, ALT-associated telomere SCE was not affected. This is the first genetic evidence that mouse Rad54 is dispensable for the ALT pathway.

Alcolea, M. P., et al. (2014). "Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change." Nat Cell Biol **16**(6): 615-622.

Multiple cancers may arise from within a clonal region of preneoplastic epithelium, a phenomenon termed 'field change'. However, it is not known how field change develops. Here we investigate this question using lineage tracing to track the behaviour of scattered single oesophageal epithelial progenitor cells expressing a mutation that inhibits the Notch signalling pathway. Notch is frequently subject to inactivating mutation in squamous cancers. Quantitative analysis reveals that cell divisions that produce two differentiated daughters are absent from mutant progenitors. As a result, mutant clones are no longer lost by differentiation and become functionally immortal. Furthermore, mutant cells promote the differentiation of neighbouring wild-type cells, which are then lost from the tissue. These effects lead to clonal expansion, with mutant cells eventually replacing the entire epithelium. Notch inhibition in progenitors carrying p53 stabilizing mutations creates large confluent regions of doubly mutant epithelium. Field change is thus a consequence of imbalanced differentiation in individual progenitor cells.

Al-Sadik, H., et al. (2020). "Effects of Diesel Exhaust Particles on Mouse Gastric Stem Cells." Life (Basel) **10**(8).

Stem cells have attracted many scientists because of their unique properties and therapeutic applications. However, very little is known on the environmental toxins that could affect their biological features. This study focuses on the consequences of the exposure of a cell line representative of the mouse gastric stem/progenitor (mGS) cells to diesel exhaust particles (DEPs). These immortal cells were cultured using routine protocols. The DEPs were added to the culture media at 1, 10, and 100 microg/mL for 1 to 72 h. The cells were assayed for their viability, migration, oxidative stress, and the expression of genes specific for cell proliferation, pluripotency, and death. DEPs induced a reduction in the metabolic activity of mGS cells, only at a high concentration of 100 microg/mL. However, no significant effects were detected on cell migration, oxidative stress markers (glutathione and thiobarbituric acid reactive substances), and cell death related proteins/genes. Interestingly, these findings were associated with down-regulation of Notch 2 and 3 and Bmi-1 proteins and activation of STAT3 involved in the regulation of the fate of stem cells. In conclusion, this study demonstrates that mGS cells have some resistance to oxidative stress and apoptosis when exposed to DEPs at the expense of their stemness.

Amit, M. and J. Itskovitz-Eldor (2002). "Derivation and spontaneous differentiation of human embryonic stem cells." J Anat **200**(Pt 3): 225-232.

Embryonic stem (ES) cells are unique cells derived from the inner cell mass of the mammalian blastocyst. These cells are immortal and pluripotent, retain their developmental potential after prolonged culture, and can be continuously cultured in an undifferentiated state. Many in vitro differentiation systems have been developed for mouse ES cells, including reproducible methods for mouse ES cell differentiation into haematopoietic and neural precursors, cardiomyocytes, insulin-secreting cells, endothelial cells and various other cell types. The derivation of new human ES cell lines provides the opportunity to develop unique models for developmental research and for cell therapies. In this review we consider the derivation and spontaneous differentiation of human ES cells.

Amit, M. and J. Itskovitz-Eldor (2006). "Sources, derivation, and culture of human embryonic stem cells." Semin Reprod Med **24**(5): 298-303.

Human embryonic stem cells (hESCs) are immortal cells capable of perpetual self-renewal in culture while maintaining their undifferentiated state, high telomerase activity, normal karyotype, and specific pattern expression of embryonic surface markers and pluripotent transcription factors such as Oct-4 and Nanog. Since their first derivation in 1998, hundreds of hESC lines have been derived and characterized. Normal surplus embryos from IVF programs are the main source for the derivation of hESC lines but cell lines from poor-quality discarded embryos or embryos carrying genetic defects following preimplantation genetic diagnosis were also isolated. Such isolation is usually accomplished by either mechanical or immunosurgical removal of the trophectoderm and culture of the inner cell mass on inactivated feeder cells. In light of the future need for clinical-grade cells, the subject of defining specific culture conditions has been addressed widely. Indeed, derivation and maintenance of hESCs without feeder cells and in media free of animal products have been attained recently. This well-defined culture system may facilitate research and clinical applications, and use the remarkable potential of these exceptional cells to its fullest in both the laboratory and the clinic.

Ando, Y., et al. (2017). "Can Human Embryonic Stem Cell-Derived Stromal Cells Serve a Starting Material for Myoblasts?" Stem Cells Int **2017**: 7541734.

A large number of myocytes are necessary to treat intractable muscular disorders such as Duchenne muscular dystrophy with cell-based therapies. However, starting materials for cellular therapy products such as myoblasts, marrow stromal cells, menstrual blood-derived cells, and placenta-derived cells have a limited lifespan and cease to proliferate in vitro. From the viewpoints of manufacturing and quality control, cells with a long lifespan are more suitable as a starting material. In this study, we generated stromal cells for future myoblast therapy from a working cell bank of human embryonic stem cells (ESCs). The ESC-derived CD105(+) cells with extensive in vitro proliferation capability exhibited myogenesis and genetic stability in vitro. These results imply that ESC-derived CD105(+) cells are another cell source for myoblasts in cell-based therapy for patients with genetic muscular disorders. Since ESCs are immortal, mesenchymal stromal cells generated from ESCs can be manufactured at a large scale in one lot for pharmaceutical purposes.

Antoniou, A., et al. (2013). "Cancer stem cells, a fuzzy evolving concept: a cell population or a cell property?" Cell Cycle **12**(24): 3743-3748.

The cancer stem cells (CSC) hypothesis represents a pathological extrapolation of the physiological concept of embryonic and somatic stem cells. In its initial definition, it encompassed the hypothesis of a qualitatively distinct population of immortal cancer cells originating from somatic stem cells, which generate in xenotransplants by a deterministic irreversible process, the hierarchy of more differentiated finite lifespan derived cells, which constitute, themselves, the bulk of the cancer. These

CSC would express specific biomarkers and gene expressions related to chemo- and radioresistance, stemness, epithelial-mesenchymal transition, etc. No convincing congruence of several of these properties in one cell population has been demonstrated. The concept has greatly evolved with time and with different authors ("the plasticity of cancer stem cells"), leading to a minimal definition of cells generating a hierarchy of derived cells. In this article these concepts are analyzed. It is proposed that stemness is a property, more or less reversible, a hallmark of some cells at some time in a cancer cell population, as immortality, dormancy, chemo- or radioresistance, epithelial-mesenchymal transition etc. These phenotypic properties represent the result of independent, linked, or more or less congruent, genetic, epigenetic, or signaling programs.

Aravalli, R. N., et al. (2015). "Gene expression profiling of MYC-driven tumor signatures in porcine liver stem cells by transcriptome sequencing." World J Gastroenterol **21**(7): 2011-2029.

AIM: To identify the genes induced and regulated by the MYC protein in generating tumors from liver stem cells. METHODS: In this study, we have used an immortal porcine liver stem cell line, PICM-19, to study the role of c-MYC in hepatocarcinogenesis. PICM-19 cells were converted into cancer cells (PICM-19-CSCs) by overexpressing human MYC. To identify MYC-driven differential gene expression, transcriptome sequencing was carried out by RNA sequencing, and genes identified by this method were validated using real-time PCR. In vivo tumorigenicity studies were then conducted by injecting PICM-19-CSCs into the flanks of immunodeficient mice. RESULTS: Our results showed that MYC-overexpressing PICM-19 stem cells formed tumors in immunodeficient mice demonstrating that a single oncogene was sufficient to convert them into cancer cells (PICM-19-CSCs). By using comparative bioinformatics analyses, we have determined that > 1000 genes were differentially expressed between PICM-19 and PICM-19-CSCs. Gene ontology analysis further showed that the MYC-induced, altered gene expression was primarily associated with various cellular processes, such as metabolism, cell adhesion, growth and proliferation, cell cycle, inflammation and tumorigenesis. Interestingly, six genes expressed by PICM-19 cells (CDO1, C22orf39, DKK2, ENPEP, GPX6, SRPX2) were completely silenced after MYC-induction in PICM-19-CSCs, suggesting that the absence of these genes may be critical for inducing tumorigenesis. CONCLUSION: MYC-driven genes may serve as promising candidates for the development of hepatocellular carcinoma therapeutics that would not have deleterious effects on other cell types in the liver.

Arifin, M., et al. (2010). "Carcinogenesis and cellular immortalization without persistent inactivation of p16/Rb pathway in lung cancer." Int J Oncol **36**(5): 1217-1227.

Existence of cancer stem cells (CSCs) is still hypothetical and their practical marker is not available yet in lung cancer. To verify the possible existence of CSCs and to find their markers in lung cancer, we compared the p16/Rb and telomerase status in 83 lung cancer tissues and 15 lung cancer cell lines, since inactivation of p16/Rb pathway is considered to be a prerequisite for normal somatic cells to become immortal cancer cells. We found that 7 of 14 adenocarcinoma, but not squamous cell carcinoma, tissues with high telomerase activity and 3 adenocarcinoma cell lines likely had intact p16/Rb. Such cell lines showed higher colony formation capacity in soft agar compared with inactivated ones with similar growth rate. Moreover, cisplatin-resistant cell line PC9/CDDP with intact p16/Rb, but not PC14/CDDP with its inactivation, increased the colony formation capacity compared with the parent cells. Since CSCs are considered to be resistant to conventional anticancer drugs, they could have been concentrated as long as CSCs existed. We propose that half of immortal lung adenocarcinomas are derived from innately telomerase-positive stem cells, which might be the origin of CSCs, and that high telomerase activity with intact p16/Rb could be a marker of stem cell origin.

Baghbaderani, B. A., et al. (2016). "Detailed Characterization of Human Induced Pluripotent Stem Cells Manufactured for Therapeutic Applications." Stem Cell Rev Rep **12**(4): 394-420.

We have recently described manufacturing of human induced pluripotent stem cells (iPSC) master cell banks (MCB) generated by a clinically compliant process using cord blood as a starting material (Baghbaderani et al. in Stem Cell Reports, 5(4), 647-659, 2015). In this manuscript, we describe the detailed characterization of the two iPSC clones generated using this process, including whole genome sequencing (WGS), microarray, and comparative genomic hybridization (aCGH) single nucleotide polymorphism (SNP) analysis. We compare their profiles with a proposed calibration material and with a reporter subclone and lines made by a similar process from different donors. We believe that iPSCs are likely to be used to make multiple clinical products. We further believe that the lines used as input material will be used at different sites and, given their immortal status, will be used for many years or even decades. Therefore, it will be important to develop assays to monitor the state of the cells and their drift in culture. We suggest that a detailed characterization of the initial status of the cells, a comparison with some calibration material and

the development of reporter sublcones will help determine which set of tests will be most useful in monitoring the cells and establishing criteria for discarding a line.

Bahmani, B., et al. (2015). "The Lcn2-engineered HEK-293 cells show senescence under stressful condition." Iran J Basic Med Sci **18**(5): 459-464.

OBJECTIVES: Lipocalin2 (Lcn2) gene is highly expressed in response to various types of cellular stresses. The precise role of Lcn2 has not been fully understood yet. However, it plays a key role in controlling vital cellular processes such as proliferation, apoptosis and metabolism. Recently it was shown that Lcn2 decreases senescence and increases proliferation of mesenchymal stem cells (MSC) with finite life span under either normal or oxidative stress conditions. However, Lcn2 effects on immortal cell line with infinite proliferation are not defined completely. Materials and. MATERIALS AND METHODS: HEK-293 cells were transfected with recombinant pcDNA3.1 containing Lcn2 fragment (pcDNA3.1-Lcn2). Expression of lipocalin2 in transfected cells was evaluated by RT-PCR, real time RT-PCR, and ELISA. Different cell groups were treated with H2O2 and WST-1 assay was performed to determine their proliferation rate. Senescence was studied by beta-galactosidase and gimsa staining methods as well as evaluation of the expression of senescence-related genes by real time RT-PCR. RESULTS: Lcn2 increased cell proliferation under normal culture condition, while the proliferation slightly decreased under oxidative stress. This decrease was further found to be attributed to senescence. CONCLUSION: Our findings indicated that under harmful conditions, Lcn2 gene is responsible for the regulation of cell survival through senescence.

Barnett, S. C., et al. (1995). "Differential regulation of AP-1 and novel TRE-specific DNA-binding complexes during differentiation of oligodendrocyte-type-2-astrocyte (O-2A) progenitor cells." Development **121**(12): 3969-3977.

AP-1 is an ubiquitous transcription factor which is composed of the Jun and Fos proto-oncogene proteins and is thought to play a role in both cell proliferation and differentiation. We have used an immortal, bipotential oligodendrocyte-type-2 astrocyte progenitor cell line (O-2A/c-myc) which can differentiate into oligodendrocytes or type-2 astrocytes in vitro, to investigate whether AP-1 DNA-binding activity fluctuates during glial cell differentiation. Unexpectedly, DNA-mobility shift assays using a TRE-containing oligonucleotide derived from the promoter of the glial-specific gene, glial fibrillary acidic protein (GFAP/AP-1), revealed that O-2A/c-myc progenitor cells were devoid of conventional AP-1 DNA-binding complexes. O-2A/c-myc cells did however contain several novel GFAP/AP-1-specific DNA-binding complexes, which we have termed APprog. APprog complexes recognise the TRE consensus motif present in the GFAP/AP-1 oligonucleotide together with adjacent 3' sequences but do not contain c-Jun or any other known Jun-related proteins. When O-2A/c-myc cells underwent terminal differentiation APprog complexes were lost and conventional AP-1 DNA-binding activity became evident, particularly in astrocytes. These changes appear to be closely linked to the differentiation process since they did not occur in a derivative of the O-2A/c-myc cell line that contains an activated v-ras oncogene and which fails to differentiate under appropriate culture conditions. The inverse regulation of conventional AP-1 and APprog complexes within the O-2A lineage suggests that these factors may play a role in the regulation of glial cell differentiation or glial cell-specific gene expression.

Barsov, E. V. (2011). "Telomerase and primary T cells: biology and immortalization for adoptive immunotherapy." Immunotherapy **3**(3): 407-421.

Telomeres are specialized repeats, present at the end of chromosomes, whose loss during cell division is followed by growth arrest, a central mechanism of replicative senescence in human cells. Telomere length in stem cells is maintained by telomerase, a specialized reverse transcriptase, whose function is to restore shortening telomeres. Unlike most somatic cell types, human T lymphocytes are capable of briefly reactivating telomerase expression at the time of stimulation. Telomerase expression in T lymphocytes is modulated by a variety of external stimuli and by viral infections. However, telomerase reactivation in stimulated, proliferating human T lymphocytes is limited and cannot prevent the ultimate onset of senescence. Ectopic telomerase expression can rescue human and macaque antigen-specific T cells from senescence. Primary T cells have been engineered with telomerase to have substantially extended replicative lifespans without the loss of primary cell functions or malignant transformation. 'Immortal' antigen-specific T-cell lines and clones overexpressing telomerase are an invaluable source of well-characterized quasi-primary T cells for research of T-cell biology and are potentially useful for immunotherapy of cancer and AIDS.

Bell, J. C., et al. (1986). "Lineage-specific transformation after differentiation of multipotential murine stem cells containing a human oncogene." Mol Cell Biol **6**(2): 617-625.

We transfected the human EJ bladder carcinoma oncogene (Ha-rasEJ-1) into multipotential embryonal carcinoma cell line P19. The transgenic P19(ras+) cells expressed high levels of both the mRNA and the p21EJ protein derived from the

oncogene. When cultured in the presence of retinoic acid, P19(ras+) cells differentiated and developed into the same spectrum of differentiated cell types as the parental P19 cells (namely, neurons, astrocytes, and fibroblast-like cells). Thus, it seems unlikely that the Ha-ras-1 proto-oncogene product plays a role in initiation of differentiation or in the choice of differentiated cell lineage. Most of the P19(ras+)-derived differentiated cells contained relatively low levels of p21EJ and were nontransformed, whereas certain cells with fibroblast-like morphology continued to express the Ha-rasEJ-1 gene at high levels and were transformed (i.e., immortal and anchorage independent). Fibroblasts derived from P19 cells did not become transformed following transfection of the Ha-rasEJ-1 oncogene, suggesting that transformation of the fibroblast cells only occurred if the oncogene was present and expressed during the early stages of the developmental lineage.

Bennett, D. C., et al. (1998). "recessive spotting: a linked locus that interacts with W/Kit but is not allelic." Genes Cells **3**(4): 235-244.

BACKGROUND: The murine coat-colour mutation recessive spotting (rs) maps very closely to the W/Kit locus, encoding the proto-oncoprotein Kit, the protein tyrosine kinase receptor for stem cell factor. Kit is important in the development of melanocytes, germ cells, interstitial cells of Cajal (ICC) and haemopoietic lineages, including mast cells. rs has never been genetically separated from Kit, and interacts with Kit mutations, suggesting that it is a recessive allele of Kit. Here we have tested this possibility. We have shown previously that diploid rs/rs melanocytes proliferated more slowly than did +/+ melanocytes, as did an immortal line of rs/rs melanocytes, melan-rs. RESULTS: The Kit mRNA level in rs/rs melanocytes was indistinguishable from that of other melanocyte lines. The Kit cDNA sequence from rs/rs melanocytes and the kinase activity of Kit in rs/rs mast cells appeared to be normal. No deficiency of mast cells or ICC was observed in rs/rs mice. Moreover, following the overexpression of a normal Kit cDNA, proliferation of rs/rs melanocytes was retarded further, but that of +/+ melanocytes was increased, indicating an intracellular interaction between rs and Kit. Of other closely linked tyrosine kinase genes, melanocytes and melanoblasts did not express mRNA for Pdgfra, Flk-1 or Txk, but both expressed Tec, encoding a nonreceptor kinase that interacts with Kit. CONCLUSIONS: rs is not a mutation in Kit, although we have confirmed that rs interacts with Kit. It seems unlikely that rs affects Pdgfra, Flk-1 or Txk, but Tec remains a candidate for rs.

Ben-Yehudah, A., et al. (2010). "Systems biology discoveries using non-human primate pluripotent stem and germ cells: novel gene and genomic imprinting interactions as well as unique expression patterns." Stem Cell Res Ther **1**(3): 24.

The study of pluripotent stem cells has generated much interest in both biology and medicine. Understanding the fundamentals of biological decisions, including what permits a cell to maintain pluripotency, that is, its ability to self-renew and thereby remain immortal, or to differentiate into multiple types of cells, is of profound importance. For clinical applications, pluripotent cells, including both embryonic stem cells and adult stem cells, have been proposed for cell replacement therapy for a number of human diseases and disorders, including Alzheimer's, Parkinson's, spinal cord injury and diabetes. One challenge in their usage for such therapies is understanding the mechanisms that allow the maintenance of pluripotency and controlling the specific differentiation into required functional target cells. Because of regulatory restrictions and biological feasibilities, there are many crucial investigations that are just impossible to perform using pluripotent stem cells (PSCs) from humans (for example, direct comparisons among panels of inbred embryonic stem cells from prime embryos obtained from pedigreed and fertile donors; genomic analysis of parent versus progeny PSCs and their identical differentiated tissues; intraspecific chimera analyses for pluripotency testing; and so on). However, PSCs from nonhuman primates are being investigated to bridge these knowledge gaps between discoveries in mice and vital information necessary for appropriate clinical evaluations. In this review, we consider the mRNAs and novel genes with unique expression and imprinting patterns that were discovered using systems biology approaches with primate pluripotent stem and germ cells.

Bernasconi, D. P., et al. (2016). "Survival probabilities with time-dependent treatment indicator: quantities and non-parametric estimators." Stat Med **35**(7): 1032-1048.

The 'landmark' and 'Simon and Makuch' non-parametric estimators of the survival function are commonly used to contrast the survival experience of time-dependent treatment groups in applications such as stem cell transplant versus chemotherapy in leukemia. However, the theoretical survival functions corresponding to the second approach were not clearly defined in the literature, and the use of the 'Simon and Makuch' estimator was criticized in the biostatistical community. Here, we review the 'landmark' approach, showing that it focuses on the average survival of patients conditional on being failure free and on the treatment status assessed at the landmark time. We argue that the 'Simon and Makuch' approach represents counterfactual survival probabilities where treatment status is forced to be fixed: the patient is thought as under chemotherapy without possibility to switch

treatment or as under transplant since the beginning of the follow-up. We argue that the 'Simon and Makuch' estimator leads to valid estimates only under the Markov assumption, which is however less likely to occur in practical applications. This motivates the development of a novel approach based on time rescaling, which leads to suitable estimates of the counterfactual probabilities in a semi-Markov process. The method is also extended to deal with a fixed landmark time of interest.

Bickenbach, J. R., et al. (1998). "Telomerase is not an epidermal stem cell marker and is downregulated by calcium." J Invest Dermatol **111**(6): 1045-1052.

The ribonucleoprotein complex telomerase, which was found to be active in germ line, immortal, and tumor cells, and in cells from continuously renewing normal tissues such as epidermis or bone marrow, is thought to be correlated with an indefinite life span. Therefore, it has been postulated that in the normal tissues, telomerase activity may be restricted to stem cells, the possible precursors of tumor cells. Here, we demonstrate that a 56% enriched population of epidermal stem cells exhibited less telomerase activity than the more actively proliferating transit amplifying cells, which are destined to differentiate after a finite number of cell divisions. Thus telomerase is not a stem cell marker. In human epidermis we found a heterogeneous expression of the telomerase RNA component (hTR) within the basal layer, with clusters of hTR-positive cells showing variable activities. Histone-3 expressing S-phase basal cells were distributed evenly, illustrating that hTR upregulation may not strictly be correlated with proliferation. We further show for human epidermal cells that differentiation-dependent downregulation of telomerase correlates with Ca++-induced cell differentiation and that increasing the amount of Ca++ but not Mg++ or Zn++ reduced telomerase activity in a dose-dependent manner in a cell-free system (differentiation-independent). Furthermore, addition of ethyleneglycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid completely reversed this Ca++-induced inhibition. These data indicate that Ca++ is not only an important regulator of epidermal differentiation but also a key regulator of telomerase.

Bignold, L. P. (2003). "Initiation of genetic instability and tumour formation: a review and hypothesis of a nongenotoxic mechanism." Cell Mol Life Sci **60**(6): 1107-1117.

Genetic instability in tumours results in cell-to-cell variability of genome which parallels the cell-to-cell variability of microscopic morphology and of behaviour (tumour cell heterogeneity) of these lesions. Genetic instability is therefore strongly supported as the fundamental process by which normal tissue cells become neoplastic. The commonest current suggestion for the mechanism of initiation of carcinogenesis is a 'direct hit' mutation of a 'cancer critical' gene in a somatic cell by carcinogenic agents. However, this mechanism does not account for the activity of carcinogens which are not mutagens, and does not explain why many mutagens are not carcinogens. This paper proposes a nonmutational (nongenotoxic) mechanism of initiation of genetic instability in previously normal cells as follows: 1) During S phase of local tissue stem cells, carcinogen binds to and disables the proofreading enzyme for a new DNA strand. 2) While it is disabled, the proofreading enzyme fails to correct illicit changes in the nucleotide sequence(s) for one or more genes for proofreading fidelity or repair of DNA in the new strand of DNA, which passes to one daughter cell. 3) When this daughter cell is a continuing stem cell, the resulting cell line remains immortal, and retains its prior differentiation commitment to produce daughter cells of a particular type. However, the acquired genetic instability in this cell line causes secondary mutations which lead to uncontrolled growth, and the heterogeneous morphologic and behavioural features of a tumour resembling the parent cell type.

Boehm, A. M., et al. (2012). "FoxO is a critical regulator of stem cell maintenance in immortal Hydra." Proc Natl Acad Sci U S A **109**(48): 19697-19702.

Hydra's unlimited life span has long attracted attention from natural scientists. The reason for that phenomenon is the indefinite self-renewal capacity of its stem cells. The underlying molecular mechanisms have yet to be explored. Here, by comparing the transcriptomes of Hydra's stem cells followed by functional analysis using transgenic polyps, we identified the transcription factor forkhead box O (FoxO) as one of the critical drivers of this continuous self-renewal. foxO overexpression increased interstitial stem cell and progenitor cell proliferation and activated stem cell genes in terminally differentiated somatic cells. foxO down-regulation led to an increase in the number of terminally differentiated cells, resulting in a drastically reduced population growth rate. In addition, it caused down-regulation of stem cell genes and antimicrobial peptide (AMP) expression. These findings contribute to a molecular understanding of Hydra's immortality, indicate an evolutionarily conserved role of FoxO in controlling longevity from Hydra to humans, and have implications for understanding cellular aging.

Brambrink, T., et al. (2006). "ES cells derived from cloned and fertilized blastocysts are transcriptionally and functionally indistinguishable." Proc Natl Acad Sci U S A **103**(4): 933-938.

Reproductive cloning is uniformly rejected as a valid technology in humans because of the severely abnormal phenotypes seen in cloned animals. Gene

expression aberrations observed in tissues of cloned animals have also raised concerns regarding the therapeutic application of "customized" embryonic stem (ES) cells derived by nuclear transplantation (NT) from a patient's somatic cells. Although previous experiments in mice have demonstrated that the developmental potential of ES cells derived from cloned blastocysts (NT-ES cells) is identical to that of ES cells derived from fertilized blastocysts, a systematic molecular characterization of NT-ES cell lines is lacking. To investigate whether transcriptional aberrations, similar to those observed in tissues of cloned mice, also occur in NT-ES cells, we have compared transcriptional profiles of 10 mouse NT- and fertilization-derived-ES cell lines. We report here that the ES cell lines derived from cloned and fertilized mouse blastocysts are indistinguishable based on their transcriptional profiles, consistent with their normal developmental potential. Our results indicate that, in contrast to embryonic and fetal development of clones, the process of NT-ES cell derivation rigorously selects for those immortal cells that have erased the "epigenetic memory" of the donor nucleus and, thus, become functionally equivalent. Our findings support the notion that ES cell lines derived from cloned or fertilized blastocysts have an identical therapeutic potential.

Brinster, R. L. and M. Nagano (1998). "Spermatogonial stem cell transplantation, cryopreservation and culture." Semin Cell Dev Biol **9**(4): 401-409.

Testis cells of a fertile male mouse can be transplanted to the seminiferous tubules of an infertile male, where the donor spermatogonial stem cells will establish spermatogenesis and produce spermatozoa that transmit the donor haplotype to progeny. In addition, stem cells can be cryopreserved for long periods, thereby making male germ lines immortal. Recently, mouse testis cells have been cultured for longer than 3 months and, following transplantation, produced spermatogenesis. These techniques are likely to be applicable to many species, since rat testis cells can be cryopreserved and generate spermatogenesis in the seminiferous tubules of immunodeficient mice.

Brown, K. W., et al. (1989). "Loss of chromosome 11p alleles in cultured cells derived from Wilms' tumours." Br J Cancer **60**(1): 25-29.

Cell cultures have been produced from five Wilms' tumours. All cultures had a finite lifespan and a pattern of antigen expression which indicated that the cells were derived from the differentiated components of the tumours. No cells showed any of the expected characteristics of the putative Wilms' tumour stem cell. Nevertheless, in both cases where the original tumours showed a loss of heterozygosity at chromosome 11p alleles, the cultured cells also demonstrated a loss of heterozygosity. Thus these cell cultures definitely originated from Wilms' tumour tissue. The results demonstrate that cell cultures can be produced from the differentiated tissues present in Wilms' tumours and that these non-immortal cells show no 'transformed' phenotype, even though they possess the genetic changes present in the original tumour.

Bussard, K. M., et al. (2010). "Immortalized, pre-malignant epithelial cell populations contain long-lived, label-retaining cells that asymmetrically divide and retain their template DNA." Breast Cancer Res **12**(5): R86.

INTRODUCTION: During selective segregation of DNA, a cell asymmetrically divides and retains its template DNA. Asymmetric division yields daughter cells whose genome reflects that of the parents', simultaneously protecting the parental cell from genetic errors that may occur during DNA replication. We hypothesized that long-lived epithelial cells are present in immortal, premalignant cell populations, undergo asymmetric division, retain their template DNA strands, and cycle both during allometric growth and during pregnancy. METHODS: The glands of 3-week old immune competent Balb/C female mice were utilized intact or cleared of host epithelium and implanted with ductal-limited, lobule-limited, or alveolar-ductal progenitor cells derived from COMMA-D1 pre-malignant epithelial cells. 5-bromo-2-deoxyuridine (5-BrdU) was administered to identify those cells which retain their template DNA. Nulliparous mice were then either injected with [(3)H]-thymidine ((3)H-TdR) to distinguish 5-BrdU-label retaining cells that enter the cell cycle and euthanized, or mated, injected with (3)H-TdR, and euthanized at various days post-coitus. Sections were stained for estrogen receptor-alpha(ER-alpha) or progesterone receptor (PR) via immunohistochemistry. Cells labelled with both 5-BrdU and (3)H-TdR were indicative of label-retaining epithelial cells (LREC). RESULTS: Cells that retained a 5-BrdU label and cells labelled with [(3)H]-thymidine were found in all mice and were typically detected along the branching epithelium of mature mouse mammary glands. Cells containing double-labelled nuclei (LREC) were found in the intact mammary gland of both pregnant and nulliparous mice, and in mammary glands implanted with pre-malignant cells. Double-labelled cells ((3)H-TdR/5-BrdU) represent a small portion of cells in the mammary gland that cycle and retain their template DNA (5-BrdU). Some label-retaining cells were also ER-alpha or PR positive. LRECs distributed their second label ((3)H-TdR) to daughter cells; and this effect persisted during pregnancy. LRECs, and small focal hyperplasia, were found in all immortalized premalignant mammary implant groups. CONCLUSIONS: The results indicate that a subpopulation of long-lived, label-retaining

epithelial cells (LRECs) is present in immortal premalignant cell populations. These LRECs persist during pregnancy, retain their original DNA, and a small percentage express ER-alpha and PR. We speculate that LRECs in premalignant hyperplasia represent the long-lived (memory) cells that maintain these populations indefinitely.

Cabodi, S. and D. Taverna (2010). "Interfering with inflammation: a new strategy to block breast cancer self-renewal and progression?" Breast Cancer Res **12**(2): 305.

Two recent studies show that epigenetics and inflammation play a relevant role in the regulation of transformation and cancer cell self-renewal in breast tumours, opening up the possibility that cancer progression can be controlled by interfering with inflammation cascades. Struhl's group showed that transient activation of the Src oncoprotein induces transformation and self-renewal of immortal cells via an epigenetic switch involving NF-kappaB, Lin28, Let-7 microRNA and IL-6. Concomitantly, Wicha's laboratory developed a strategy to selectively target cancer stem cells, retarding tumour growth and reducing metastasis by blocking the IL-8 receptor CXCR1 using either an inhibitor, repertaxin or a specific blocking antibody.

Cacci, E., et al. (2007). "Generation of human cortical neurons from a new immortal fetal neural stem cell line." Exp Cell Res **313**(3): 588-601.

Isolation and expansion of neural stem cells (NSCs) of human origin are crucial for successful development of cell therapy approaches in neurodegenerative diseases. Different epigenetic and genetic immortalization strategies have been established for long-term maintenance and expansion of these cells in vitro. Here we report the generation of a new, clonal NSC (hc-NSC) line, derived from human fetal cortical tissue, based on v-myc immortalization. Using immunocytochemistry, we show that these cells retain the characteristics of NSCs after more than 50 passages. Under proliferation conditions, when supplemented with epidermal and basic fibroblast growth factors, the hc-NSCs expressed neural stem/progenitor cell markers like nestin, vimentin and Sox2. When growth factors were withdrawn, proliferation and expression of v-myc and telomerase were dramatically reduced, and the hc-NSCs differentiated into glia and neurons (mostly glutamatergic and GABAergic, as well as tyrosine hydroxylase-positive, presumably dopaminergic neurons). RT-PCR analysis showed that the hc-NSCs retained expression of Pax6, Emx2 and Neurogenin2, which are genes associated with regionalization and cell commitment in cortical precursors during brain development. Our data indicate that this hc-NSC line could be useful for exploring the potential of human NSCs to replace dead or damaged cortical cells in animal models of acute and chronic neurodegenerative diseases. Taking advantage of its clonality and homogeneity, this cell line will also be a valuable experimental tool to study the regulatory role of intrinsic and extrinsic factors in human NSC biology.

Chiodi, I., et al. (2011). "Drug treatment of cancer cell lines: a way to select for cancer stem cells?" Cancers (Basel) **3**(1): 1111-1128.

Tumors are generally composed of different cell types. In recent years, it has been shown that in many types of cancers a subset of cells show peculiar characteristics, such as the ability to induce tumors when engrafted into host animals, self-renew and being immortal, and give rise to a differentiated progeny. These cells have been defined as cancer stem cells (CSCs) or tumor initiating cells. CSCs can be isolated both from tumor specimens and established cancer cell lines on the basis of their ability to exclude fluorescent dyes, express specific cell surface markers or grow in particular culture conditions. A key feature of CSCs is their resistance to chemotherapeutic agents, which could contribute to the remaining of residual cancer cells after therapeutic treatments. It has been shown that CSC-like cells can be isolated after drug treatment of cancer cell lines; in this review, we will describe the strategies so far applied to identify and isolate CSCs. Furthermore, we will discuss the possible use of these selected populations to investigate CSC biology and develop new anticancer drugs.

Chiu, C. P., et al. (1996). "Differential expression of telomerase activity in hematopoietic progenitors from adult human bone marrow." Stem Cells **14**(2): 239-248.

The loss of telomeric DNA may serve as a mitotic clock which signals cell senescence and exit from cell cycle. Telomerase, and enzyme which synthesizes telomeric repeats de novo, is required to maintain telomere lengths. In humans, significant telomerase activity has been found in cells with essentially unlimited replicative potential such as reproductive cells in ovaries and testes, immortal cell lines and cancer tissues, but not in most normal somatic cells or tissues. We have now examined telomerase expression in subpopulations of hematopoietic cells from adult human bone marrow using a sensitive polymerase chain reaction-based telomeric repeat amplification protocol. Telomerase activity was found at low levels in the highly enriched primitive hematopoietic cells (CD34+CD71loCD45RAlo) and was increased transiently when these cells were cultured in the presence of a mixture of cytokines. In contrast, the early progenitors (CD34+CD71+) expressed telomerase activity at a higher level which was subsequently downregulated in response to cytokines. Telomerase activity remained low in the more mature CD34-cells upon exposure to cytokines.

Taken together, our results suggest that telomerase is expressed at a basal level in all hematopoietic cell populations examined, is induced in a primitive subset of hematopoietic progenitor cells and is downregulated upon further proliferation and differentiation of these cells. We have previously observed telomere shortening in cytokine-stimulated primitive hematopoietic cells. The low and transient activation of telomerase activity described here thus appears insufficient to maintain telomere lengths in cultured hematopoietic cells.

Choo, A., et al. (2006). "Immortalized feeders for the scale-up of human embryonic stem cells in feeder and feeder-free conditions." J Biotechnol **122**(1): 130-141.

Human embryonic stem cells (hESC) are pluripotent cells that proliferate indefinitely in culture, whilst retaining their capacity for differentiation into different cell types. However, hESC cultures require culture in direct contact with feeder cells or conditioned medium (CM) from feeder cells. The most common source of feeders has been primary mouse embryonic fibroblast (MEF). In this study, we immortalized a primary MEF line with the E6 and E7 genes from HPV16. The immortal line, DeltaE-MEF, was able to proliferate beyond 7-9 passages and has an extended lifespan beyond 70 passages. When tested for its ability to support hESC growth, it was found that hESC continue to maintain the undifferentiated morphology for >40 passages both in co-culture with DeltaE-MEF and in feeder-free cultures supplemented with CM from DeltaE-MEF. The cultures also continue to express the pluripotent markers, Oct-4, SSEA-4, Tra-1-60, Tra-1-81, alkaline phosphatase and maintain a normal karyotype. In addition, these hESC formed teratomas when injected into SCID mice. Lastly, we demonstrated the feasibility of scaling-up significant quantities of undifferentiated hESC (>10(8) cells) using DeltaE-MEF in cell factories. The results from this study suggest that immortalized feeders can provide a consistent and reproducible source of feeders for hESC expansion and research.

Cianflone, E., et al. (2019). "Adult Cardiac Stem Cell Aging: A Reversible Stochastic Phenomenon?" Oxid Med Cell Longev **2019**: 5813147.

Aging is by far the dominant risk factor for the development of cardiovascular diseases, whose prevalence dramatically increases with increasing age reaching epidemic proportions. In the elderly, pathologic cellular and molecular changes in cardiac tissue homeostasis and response to injury result in progressive deteriorations in the structure and function of the heart. Although the phenotypes of cardiac aging have been the subject of intense study, the recent discovery that cardiac homeostasis during mammalian lifespan is maintained and regulated by regenerative events associated with endogenous cardiac stem cell (CSC) activation has produced a crucial reconsideration of the biology of the adult and aged mammalian myocardium. The classical notion of the adult heart as a static organ, in terms of cell turnover and renewal, has now been replaced by a dynamic model in which cardiac cells continuously die and are then replaced by CSC progeny differentiation. However, CSCs are not immortal. They undergo cellular senescence characterized by increased ROS production and oxidative stress and loss of telomere/telomerase integrity in response to a variety of physiological and pathological demands with aging. Nevertheless, the old myocardium preserves an endogenous functionally competent CSC cohort which appears to be resistant to the senescent phenotype occurring with aging. The latter envisions the phenomenon of CSC ageing as a result of a stochastic and therefore reversible cell autonomous process. However, CSC aging could be a programmed cell cycle-dependent process, which affects all or most of the endogenous CSC population. The latter would infer that the loss of CSC regenerative capacity with aging is an inevitable phenomenon that cannot be rescued by stimulating their growth, which would only speed their progressive exhaustion. The resolution of these two biological views will be crucial to design and develop effective CSC-based interventions to counteract cardiac aging not only improving health span of the elderly but also extending lifespan by delaying cardiovascular disease-related deaths.

De Filippis, L., et al. (2007). "A novel, immortal, and multipotent human neural stem cell line generating functional neurons and oligodendrocytes." Stem Cells **25**(9): 2312-2321.

The discovery and study of neural stem cells have revolutionized our understanding of the neurogenetic process, and their inherent ability to adopt expansive growth behavior in vitro is of paramount importance for the development of novel therapeutics based on neural cell replacement. Recent advances in high-throughput assays for drug development and gene discovery dictate the need for rapid, reproducible, long-term expansion of human neural stem cells (hNSCs). In this view, the complement of wild-type cell lines currently available is insufficient. Here we report the establishment of a stable human neural stem cell line (immortalized human NSCs [IhNSCs]) by v-myc-mediated immortalization of previously derived wild-type hNSCs. These cells demonstrate three- to fourfold faster proliferation than wild-type cells in response to growth factors but retain rather similar properties, including multipotentiality. By molecular biology, biochemistry, immunocytochemistry, fluorescence microscopy, and electrophysiology, we show that upon growth factor removal, IhNSCs completely downregulate v-myc expression, cease proliferation,

and differentiate terminally into three major neural lineages: astrocytes, oligodendrocytes, and neurons. The latter are functional, mature cells displaying clear-cut morphological and physiological features of terminally differentiated neurons, encompassing mostly the GABAergic, glutamatergic, and cholinergic phenotypes. Finally, IhNSCs produce bona fide oligodendrocytes in fractions up to 20% of total cell number. This is in contrast to the negligible propensity of hNSCs to generate oligodendroglia reported so far. Thus, we describe an immortalized hNSC line endowed with the properties of normal hNSCs and suitable for developing the novel, reliable assays and reproducible high-throughput gene and drug screening that are essential in both diagnostics and cell therapy studies.

De Flora, S., et al. (2006). "Induction by 7,12-dimethylbenz(a)anthracene of molecular and biochemical alterations in transformed human mammary epithelial stem cells, and protection by N-acetylcysteine." Int J Oncol **29**(3): 521-529.

Several lines of evidence suggest that stem cells are major targets for carcinogens. A normal human breast epithelial cell type was previously shown to possess stem cell characteristics. Further cell lines were derived following sequential transfection with SV40 large T-antigen (immortal, non-tumorigenic M13SV1 cells), exposure to X-rays (weakly tumorigenic M13SV1R2 cells), and ectopic expression of c-erbB2/neu (highly tumorigenic M13SV1R2N1 cells). We evaluated some characteristics of these cells and their susceptibility to the breast carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). Compared to M13SV1 cells, the two untreated tumorigenic cell lines displayed higher levels of connexin 43 expression and NF-kappaB nuclear translocation, and a higher frequency of fhit loss. The baseline nuclear translocation of AP-1 and pCREB was particularly evident in M13SV1R2N1 cells and was further enhanced by DMBA treatment, indicating an interaction between c-erbB2/neu and DMBA-induced signalling. Treatment with DMBA did neither affect the baseline fhit loss nor p53 mutation, whereas it increased NF-kappaB nuclear translocation, the proportion of apoptotic cells, and the levels of connexin 43, common 4977-bp mitochondrial DNA deletion, and bulky adducts to nuclear DNA. DMBA-treated M13SV1 cells underwent significant oxidative DNA damage and exhibited the highest DNA adduct levels, while they had the lowest apoptotic rate. Co-treatment of cells with N-acetylcysteine (NAC) attenuated DMBA-induced toxicity and DNA alterations, particularly in M13SV1 cells. Thus, the immortal cell line derived from the normal human adult breast stem cell without further tumorigenic progression is the most susceptible both to DMBA-related alterations and to the protective effects of NAC.

de Haan, G. and G. Van Zant (2002). "Stem cells from birth to death: The history and the future." J Am Aging Assoc **25**(2): 79-86.

The concept that adult stem cells, despite their impressive proliferative potential, are immortal has been challenged by experimental studies of hematopoietic stem cells. In this review, we discuss the properties that characterize a stem cell, the growing list of tissues in which stem cells are found, how they can be identified and isolated, how stem cells may transdifferentiate, and the findings that illustrate how age affects the hematopoietic stem cell population. We propose that an aging stem cell population affects tissue and organ homeostasis, particularly in response to environmental stresses, and we hypothesize that through this mechanism the functional status of stem cells affects the longevity of the organism.

De Marzo, A. M., et al. (1998). "Stem cell features of benign and malignant prostate epithelial cells." J Urol **160**(6 Pt 2): 2381-2392.

PURPOSE: We present a new hypothesis suggesting that the different malignant potential of benign prostatic hyperplasia (BPH) and high grade prostatic intraepithelial neoplasia may be explained by distinct alterations in stem cell-like properties. MATERIALS AND METHODS: We used our results and the recent literature to develop this hypothesis in the context of an updated prostate stem cell model. RESULTS: While high grade prostatic intraepithelial neoplasia is a likely precursor lesion to many prostatic adenocarcinomas, BPH rarely if ever progresses directly to carcinoma. Prostate epithelium contains basal and secretory compartments. Secretory cells appear to differentiate from basal cells. Thus, prostatic stem cells most likely reside in the basal compartment. In BPH there is a slight increase in epithelial proliferation, yet most replicating epithelial cells within BPH maintain their normal restriction to the basal compartment. In high grade prostatic intraepithelial neoplasia there is a marked increase in cell proliferation. In contrast to BPH, the majority of proliferating cells in high grade prostatic intraepithelial neoplasia reside in the secretory compartment. The biological significance of this topographic infidelity of proliferation in high grade prostatic intraepithelial neoplasia remains unclear but may relate mechanistically to down regulation of the cyclin dependent kinase inhibitor, p27kip1. Normal basal cells express GSTP1, an enzyme that inactivates reactive electrophiles and organic hydroperoxides, and that may protect cells from deoxyribonucleic acid damaging agents. In contrast, normal secretory cells and high grade prostatic intraepithelial neoplasia cells do not express this enzyme. CONCLUSIONS: We propose that topographic infidelity of proliferation produces a

population of secretory cells replicating in the absence of key genome protective mechanisms, thus setting the stage for an accumulation of genomic alterations and instability in high grade prostatic intraepithelial neoplasia. This action occurs along with activation of telomerase, resulting in an immortal clone capable of developing into invasive carcinoma. The model predicts that genome protection remains intact in BPH, minimizing its malignant potential.

De Miguel, M. P., et al. (2010). "Pluripotent stem cells: origin, maintenance and induction." Stem Cell Rev Rep **6**(4): 633-649.

Pluripotency is defined as the potential of a cell to differentiate into cells of the three germ layers: endoderm, mesoderm and ectoderm. In vivo, the presence of pluripotent stem cells is transient during the very early embryo. However, immortal cell lines with the same properties can be obtained in vitro and grown indefinitely in laboratories under specific conditions. These cells can be induced to differentiate into all the cell types of the organism through different assays, thereby proving their functional pluripotency. This review focuses on the pluripotent stem cells of mammals, giving special attention to the comparison between mouse and human. In particular, embryonic stem cells, epiblast-derived stem cells, primordial germ cells, embryonic germ cells, very small embryonic-like cells and induced pluripotent stem cells will be compared in terms of the following: in vivo specification and location; surface and intracellular markers; in vitro dependence on growth factors; signal transduction pathways; epigenetic characteristics; and pluripotency genes and functional assays.

Denham, M., et al. (2006). "Embryonic stem cells form glandular structures and express surfactant protein C following culture with dissociated fetal respiratory tissue." Am J Physiol Lung Cell Mol Physiol **290**(6): L1210-1215.

Mouse embryonic stem cells (MESCs) are pluripotent, theoretically immortal cells derived from the inner cell mass of developing blastocysts. The respiratory epithelium develops from the primitive foregut endoderm as a result of inductive morphogenetic interactions with the surrounding visceral mesoderm. After dissociation of the explanted fetal lung into single cells, these morphogenetic signaling pathways instruct reconstitution of the developing lung according to a process known as organotypic regeneration. Data presented here demonstrate that such fetal lung morphogenetic cues induce MESC derivatives to incorporate into the reforming pseudoglandular-like tubular ducts, display pan-keratin and surfactant protein C (Sftpc) immunoreactivity, and express Sftpc transcripts while displaying a normal diploid karyotype in coculture. The Sftpc inductive capacity of dissociated fetal lung tissue shows stage specificity with 24% of all MESC derivatives displaying Sftpc immunoreactivity after coculture with embryonic day 11.5 (E11.5) lung buds compared with 6% and 0.02% following coculture with E12.5 and E13.5 lung buds, respectively. MESC derivative Sftpc immunoreactivity follows a spatial and temporal specific maturation profile with an initially ubiquitous cellular Sftpc immunostaining pattern becoming apically polarized with time. Directing differentiation of MESCs into respiratory lineages has important implications for cell replacement therapeutics aimed at treating respiratory-specific diseases such as cystic fibrosis and idiopathic pulmonary fibrosis.

Dennis, J. E. and A. I. Caplan (1996). "Analysis of the developmental potential of conditionally immortal marrow-derived mesenchymal progenitor cells isolated from the H-2Kb-tsA58 transgenic mouse." Connect Tissue Res **35**(1-4): 93-99.

All nucleated cells from the adult transgenic mouse H-2Kb-tsA58 harbor the temperature-sensitive mutant gene for SV40 large T-antigen. Bone marrow cells from this transgenic mouse were isolated, expanded and cloned in vitro under conditions permissive to the expression of stable T-antigen. Clones of marrow-derived mesenchymal progenitor cells were tested in vitro and in vivo for their capacity to differentiate into mature mesenchymal phenotypes of bone, cartilage, muscle, adipose, and hematopoietic support cells (termed "stromacytes"). Mono-, bi-, and tri-potential clones were identified that were able to differentiate into bone, adipose and stromacyte phenotypes. A mixed population of cells showed some chondrocytic potential in vivo, however, no evidence of cartilage matrix production was detected in vitro for any of the immortomouse clones. These results support the hypothesis that marrow contains multiple progenitor cells that are part of a mesenchymal lineage.

Dikmen, Z. G., et al. (2009). "Targeting critical steps of cancer metastasis and recurrence using telomerase template antagonists." Biochim Biophys Acta **1792**(4): 240-247.

Metastasis, tumor relapse, and drug resistance remain major obstacles in the treatment of cancer. Therefore, more research on the mechanisms of these processes in disease is warranted for improved treatment options. Recent evidence suggests that the capability to sustain tumor growth and metastasis resides in a subpopulation of cells, termed cancer stem cells or tumor-initiating cells. Continuous proliferation and self-renewal are characteristics of stem/progenitor cells. Telomerase and the maintenance of telomeres are key players in the ability of stem and cancer cells to bypass senescence and be immortal. Therefore, telomerase inhibitors have the therapeutic potential for reducing tumor relapse by targeting cancer stem cells

and other processes involved in metastasis. Herein we review the role of telomerase in the immortal phenotype of cancer and cancer stem cells, targeting telomerase in cancer, and discuss other opportunities for telomerase inhibitors to target critical steps in cancer metastasis and recurrence.

Doonan, J. H. and R. Sablowski (2010). "Walls around tumours - why plants do not develop cancer." Nat Rev Cancer **10**(11): 794-802.

In plants, as in animals, most cells that constitute the organism limit their reproductive potential in order to provide collective support for the immortal germ line. And, as in animals, the mechanisms that restrict the proliferation of somatic cells in plants can fail, leading to tumours. There are intriguing similarities in tumorigenesis between plants and animals, including the involvement of the retinoblastoma pathway as well as overlap with mechanisms that are used for stem cell maintenance. However, plant tumours are less frequent and are not as lethal as those in animals. We argue that fundamental differences between plant and animal development make it much more difficult for individual plant cells to escape communal controls.

Dou, J., et al. (2009). "Identification of tumor stem-like cells in a mouse myeloma cell line." Cell Mol Biol (Noisy-le-grand) **55 Suppl**: OL1151-1160.

We used colony formation assay in the soft agar media or the serum-free media, the methods of identifying BrdU-label-retaining cells and the SP cells as well as the tumorigenicity test in BALB/c mice, respectively, to analyze tumor stem like cells in the SP2/0 cell line. The results showed that a few SP2/0 cells were capable of forming colonies in the soft agar media, contained BrdU-label-immortal strand in the SP2/0 cell line. The SP2/0 cells in the serum-free media gained higher tumorigenicity in the BALB/c mice than the SP2/0 cells cultivated in the complete media did. Overall, only a few of the SP2/0 cells were found to possess the characteristics of tumor stem-like cells, such as high proliferative potency, more self-renewal and stronger tumorigenesis, or greater similarity to the tumor stem cells (TSCs) traits. The biology of tumor stem-like cells contributes to the identification of molecular targets important for future tumor therapy.

Drpic, D., et al. (2013). "Selective tracking of template DNA strands after induction of mitosis with unreplicated genomes (MUGs) in Drosophila S2 cells." Chromosome Res **21**(3): 329-337.

According to the "immortal" DNA strand hypothesis (Cairns Nature 255:197-200, 1975), stem cells would keep their template strands in order to prevent the accumulation of mutations, which could occur during DNA replication. Despite the growing number of studies that attempt to test this hypothesis, the conclusions remain highly controversial. In the base of this controversy lie the current limitations of available methodology to selectively and faithfully track the fate of template DNA strands throughout and upon cell division. Here, we developed a method that allows the unequivocal tracking of single chromatids containing template DNA strands in Drosophila S2 cells in culture. This method consists in the induction of mitosis with unreplicated genomes (MUGs) in which cells are allowed to enter mitosis without prior DNA replication. This is achieved by RNAi-mediated knockdown of Double parked, a conserved protein required for the initiation of DNA replication and post-replication checkpoint response. The advantages of this system when compared with MUGs generated in mammalian cells is the preservation of chromatid morphology, the ease of loss-of-function studies and the possibility of in vivo applications. Altogether, this approach allows for the readily visualization and tracking of template DNA strands by simply monitoring cells stably expressing GFP-fusions with either Histone H2B or the centromeric Histone variant CID/CENP-A by time-lapse fluorescence microscopy. This might be useful for the dissection of the molecular mechanism behind asymmetric DNA strand segregation.

Fibach, E. (2019). "Erythropoiesis In Vitro-A Research and Therapeutic Tool in Thalassemia." J Clin Med **8**(12).

Thalassemia (thal) is a hereditary chronic hemolytic anemia due to a partial or complete deficiency in the production of globin chains, in most cases, alpha or beta, which compose, together with the iron-containing porphyrins (hemes), the hemoglobin molecules in red blood cells (RBC). The major clinical symptom of beta-thal is severe chronic anemia-a decrease in RBC number and their hemoglobin content. In spite of the improvement in therapy, thal still severely affects the quality of life of the patients and their families and imposes a substantial financial burden on the community. These considerations position beta-thal, among other hemoglobinopathies, as a major health and social problem that deserves increased efforts in research and its clinical application. These efforts are based on clinical studies, experiments in animal models and the use of erythroid cells grown in culture. The latter include immortal cell lines and cultures initiated by erythroid progenitor and stem cells derived from the blood and RBC producing (erythropoietic) sites of normal and thal donors, embryonic stem cells, and recently, "induced pluripotent stem cells" generated by manipulation of differentiated somatic cells. The present review summarizes the use of erythroid cultures, their technological aspects and their contribution to the research and its clinical application

in thal. The former includes deciphering of the normal and pathological biology of the erythroid cell development, and the latter-their role in developing innovative therapeutics-drugs and methods of gene therapy, as well as providing an alternative source of RBC that may complement or substitute blood transfusions.

Filion, T. M., et al. (2009). "Survival responses of human embryonic stem cells to DNA damage." J Cell Physiol **220**(3): 586-592.

Pluripotent human embryonic stem (hES) cells require mechanisms to maintain genomic integrity in response to DNA damage that could compromise competency for lineage-commitment, development, and tissue-renewal. The mechanisms that protect the genome in rapidly proliferating hES cells are minimally understood. Human ES cells have an abbreviated cell cycle with a very brief G1 period suggesting that mechanisms mediating responsiveness to DNA damage may deviate from those in somatic cells. Here, we investigated how hES cells react to DNA damage induced by ionizing radiation (IR) and whether genomic insult evokes DNA repair pathways and/or cell death. We find that hES cells respond to DNA damage by rapidly inducing Caspase-3 and -8, phospho-H2AX foci, phosphorylation of p53 on Ser15 and p21 mRNA levels, as well as concomitant cell cycle arrest in G2 based on Ki67 staining and FACS analysis. Unlike normal somatic cells, hES cells and cancer cells robustly express the anti-apoptotic protein Survivin, consistent with the immortal growth phenotype. SiRNA depletion of Survivin diminishes hES survival post-irradiation. Thus, our findings provide insight into pathways and processes that are activated in human embryonic stem cells upon DNA insult to support development and tissue regeneration.

Forster, P., et al. (2015). "Elevated germline mutation rate in teenage fathers." Proc Biol Sci **282**(1803): 20142898.

Men age and die, while cells in their germline are programmed to be immortal. To elucidate how germ cells maintain viable DNA despite increasing parental age, we analysed DNA from 24 097 parents and their children, from Europe, the Middle East and Africa. We chose repetitive microsatellite DNA that mutates (unlike point mutations) only as a result of cellular replication, providing us with a natural 'cell-cycle counter'. We observe, as expected, that the overall mutation rate for fathers is seven times higher than for mothers. Also as expected, mothers have a low and lifelong constant DNA mutation rate. Surprisingly, however, we discover that (i) teenage fathers already set out from a much higher mutation rate than teenage mothers (potentially equivalent to 77-196 male germline cell divisions by puberty); and (ii) ageing men maintain sperm DNA quality similar to that of teenagers, presumably by using fresh batches of stem cells known as 'A-dark spermatogonia'.

Fukutomi, M., et al. (2001). "Telomerase activity is repressed during differentiation along the hepatocytic and biliary epithelial lineages: verification on immortal cell lines from the same origin." Cell Biochem Funct **19**(1): 65-68.

Recent investigations indicate that telomerase activity regulates the life span of cells by compensating for telomere shortening during DNA replication. In addition, as differentiation progresses, telomerase activity is reduced in several different cell lineages. These findings lend support to the theory that more immature cells have greater remaining proliferative capacity and longer life span. However, it has not been directly demonstrated that the differentiation along a hepatocytic or a bile ductal lineage is accompanied by reduction of telomerase activity. In this study, we present direct evidence that telomerase activity is reduced during hepatocytic and biliary epithelial differentiation by using our unique cell lines including a stem-like cell line, ETK-1. When hepatocytic differentiation was induced in ETK-1 by 5-azacytidine, telomerase activity decreased significantly. Similarly, when we compared the telomerase activity on SSP-25 and RBE cell lines from the same origin but representing different maturation stages of cholangiocarcinoma, more mature cells were found to possess significantly lower activity. These results indicate that the generally accepted relationship between telomerase activity and differentiation stage also applies in the hepatocytic and biliary epithelial lineages.

Galitskii, V. A. (2009). "[Epigenetic nature of ageing]." Tsitologiia **51**(5): 388-397.

The idea proposed in this article is that a specific set of microRNAs expressing in stem cells can restore the initial profile of their epigenetic markers through RNAi-directed DNA methylation, and owing to that the pluripotent immortal status of these cells is supported unlimitedly and possibly minimum level of the mobile genomic elements activity is achieved. However, cell differentiation, starting with the earliest stages, must be accompanied with repression of genes of some microRNAs out of the primary set, otherwise these microRNAs would prevent expression of genes participating in the differentiation processes. Eventually, it results in that the cells slowly lose the repressive chromatin markers and this, sooner or later, will cause derepression of silent transposons and other mobile elements. This, accordingly, leads to the increase in DNA damage induced by these elements, and to following activation of cell systems of the DNA repair including mechanisms based on homologous recombination. In our opinion, these mechanisms cause not only DNA repair, but also unauthorized

recombination on telomere capping structures, since they are pre-recombination structures. It is also possible that transposases in itself can initiate such recombination. As a result, the T-loops converse into rings and, accordingly, telomeres are shortened for the length of the lost circled DNA. This process can cause quick exhaustion of one or more cell telomeres and, therefore, subsequent senescence, cell cycle arrest and apoptosis of the cells, in which the illegitimate activation of recombination process becomes apparent. Apparently, large quantity of organism cells reaches with age the threshold of illegitimate activation of silent mobile genomic elements; subsequent apoptosis of most of these cells causes ageing as a biological phenomenon.

Gauchier, M., et al. (2019). "SETDB1-dependent heterochromatin stimulates alternative lengthening of telomeres." Sci Adv **5**(5): eaav3673.

Alternative lengthening of telomeres, or ALT, is a recombination-based process that maintains telomeres to render some cancer cells immortal. The prevailing view is that ALT is inhibited by heterochromatin because heterochromatin prevents recombination. To test this model, we used telomere-specific quantitative proteomics on cells with heterochromatin deficiencies. In contrast to expectations, we found that ALT does not result from a lack of heterochromatin; rather, ALT is a consequence of heterochromatin formation at telomeres, which is seeded by the histone methyltransferase SETDB1. Heterochromatin stimulates transcriptional elongation at telomeres together with the recruitment of recombination factors, while disrupting heterochromatin had the opposite effect. Consistently, loss of SETDB1, disrupts telomeric heterochromatin and abrogates ALT. Thus, inhibiting telomeric heterochromatin formation in ALT cells might offer a new therapeutic approach to cancer treatment.

Geijsen, N. and D. L. Jones (2008). "Seminal discoveries in regenerative medicine: contributions of the male germ line to understanding pluripotency." Hum Mol Genet **17**(R1): R16-22.

Germ cells are highly specialized cells that form gametes (sperm and eggs), and they are the only cells within an organism that contribute genes to offspring. Due to the fact that the genetic information contained within germ cells is passed from generation to generation, the germ line is often thought of as immortal. Studies have revealed that germ cells are remarkably similar to pluripotent embryonic stem cells (ESCs). For example, there is a significant overlap in the gene expression profile between ESCs and primordial germ cells (PGCs), the founders of the germ cell lineage. In addition, pluripotent embryonic germ (EG) cell lines have been derived from mammalian PGCs. Secondly, a subset of testicular germ cell tumors, known as non-seminomas, often contain differentiated cells representative of all three germ layers, a definitive test of pluripotency. Lastly, recent results have demonstrated the ability of spermatogonial stem cells (SSCs) to de-differentiate into pluripotent ES-like cells, underscoring a unique relationship between the germ line and pluripotent cells that are present during the earliest stages of embryonic development. Here, we will review the factors that regulate the self-renewal and maintenance of male germline stem cells (GSCs) and discuss how these factors may allow us to manipulate the germ line to create pluripotent cells that could serve as a critical tool in cell replacement therapies and regenerative medicine.

Gordon, P. V., et al. (2005). "A methodology for distinguishing divergent cell fates within a common progenitor population: adenoma- and neuroendocrine-like cells are confounders of rat ileal epithelial cell (IEC-18) culture." BMC Cell Biol **6**(1): 2.

BACKGROUND: IEC-18 cells are a non-transformed, immortal cell line derived from juvenile rat ileal crypt cells. They may have experimental advantages over tumor-derived gastrointestinal lineages, including preservation of phenotype, normal endocrine responses and retention of differentiation potential. However, their proclivity for spontaneous differentiation/transformation may be stereotypical and could represent a more profound experimental confounder than previously realized. We hypothesized that IEC-18 cells spontaneously diverge towards a uniform mixture of epigenetic fates, with corresponding phenotypes, rather than persist as a single progenitor lineage. RESULTS: IEC-18 cells were cultured for 72 hours in serum free media (SFM), with and without various insulin-like growth factor agonists to differentially boost the basal rate of proliferation. A strategy was employed to identify constitutive genes as markers of divergent fates through gene array analysis by cross-referencing fold-change trends for individual genes against crypt cell abundance in each treatment. We then confirmed the cell-specific phenotype by immunolocalization of proteins corresponding to those genes. The majority of IEC-18 cells in SFM alone had a loss in expression of the adenomatous polyposis coli (APC) gene at the mRNA and protein levels, consistent with adenoma-like transformation. In addition, a small subset of cells expressed the serotonin receptor 2A gene and had neuroendocrine-like morphology. CONCLUSIONS: IEC-18 cells commonly undergo a change in cell fate prior to reaching confluence. The most common fate switch that we were able to detect correlates with a down regulation of the APC gene and transformation into an adenoma-like phenotype.

Goto, K., et al. (2012). "High-resolution multi-isotope imaging mass spectrometry enables visualisation of stem cell division and metabolism." Chembiochem **13**(8): 1103-1106.

MIMS visualises metabolism: A recent publication by Steinhauser and co-workers presents a novel application of multi-isotope mass spectrometry (MIMS) to visualise physiological metabolism in live mammalian organisms, and validate the "immortal strand hypothesis" of asymmetric chromosomal division of stem cells in the small intestine.

Grigoryan, G. A., et al. (2000). "Conditionally immortal neuroepithelial stem cell grafts restore spatial learning in rats with lesions at the source of cholinergic forebrain projections cholinergic forebrain projections Conditionally immortal neuroepithelial stem cell grafts restore spatial leaming in rats with lesions at the source of cholinergic forebrain projections." Restor Neurol Neurosci **17**(4): 1.

Purpose: Loss of cholinergic projections from the basal forebrain (BF) to the cortex and from the medial septal area (MSA) to tbe hippocampus is a reliable correlate of cognitive deficits in aging and Alzheimer's disease (AD). We assessed the capacity of grafts of the conditionally immortal MHP36 clonal stem cell line to improve spatial learning in rats showing profound deficits after lesions to these projections. Methods: Rats were lesioned by infusions of S-AMPA unilaterally into BF or bilaterally into both BF and MSA. MHP36 cells were implanted ipsilaterally in cortex or basal forebrain two weeks after unilateral BF lesions, and in cortex and hippocampus bilaterally six months after bilateral BF-MSA lesions. Intact and lesion-only controls received vehicle. Six weeks later rats were assessed in spatial learning and memory tasks in the water maze, and then perfused for identification of grafted cells by beta-galactosidase immunohistocheniistry. Results: Lesioned rats with MHP36 grafts, whether implanted two weeks or six months after lesioning, learned to find a submerged platform in the water maze as rapidly as intact controls, and showed a strong preference for the platform quadrant on probe trials, whereas lesioned controls were impaired in all measures. Grafted cells of both neuronal and glial morphologies, migrated away from cortical implantation sites in BF Lesioned rats to the striatum, thalamus and basal forebrain lesion area. Cells implanted in basal forebrain showed a similar distribution. In rats with bilateral BF-MSA lesions, grafts implanted in the hippocampus migrated widely through all layers but cortical grafts largely escaped up the needle tract into the meninges. Conclusions: Although MHP36 grafts were functionally effective in both lesion models, the site and age of lesions and site of implantation influenced the pattern of engraftment. This flexibility encourages the development of conditionally immortal human stem cell lines with similar capacities for functional repair of variable neuronal degeneration in AD or aging.

Groves, A. K., et al. (1993). "The characterization of astrocyte cell lines that display properties of glial scar tissue." Dev Biol **159**(1): 87-104.

The glial scar has been proposed to be a major impediment to regeneration in the adult CNS. Analysis of glial scars in vivo is complicated, however, by the large number of cell types present in such lesions. We have attempted to simplify analysis of the glial scar environment by deriving a series of conditionally immortal astrocyte cell lines that display several properties expressed by glial scar tissue in vitro. The astrocyte lines, which were derived from H-2KbtsA58 transgenic mice, expressed macromolecules associated with glial scars in vivo and were significantly less effective than neonatal astrocytes at promoting neurite outgrowth from postnatal central and peripheral neurons. The astrocyte lines also inhibited migration of oligodendrocyte type-2 astrocyte progenitor cells in vitro. We propose that certain properties shown previously to be expressed by glial scars may be reconstituted in vitro by astrocytes alone.

Gu, Y., et al. (2005). "Androgen and androgen receptor antagonist responsive primary African-American benign prostate epithelial cell line." Anticancer Res **25**(1A): 1-8.

The generation of suitable in vitro models is critical for understanding the process associated with the development and progression of prostate cancer in high-risk African-American men. However, the generation of long-term human prostate epithelial cell lines derived from primary human prostate epithelium have been unsuccessful due to the absence of in vitro immortalization. We have successfully established an immortal human prostate epithelial cell line from primary benign tissues of African-American prostate cancer patients by using telomerase. The actively proliferating secondary African-American prostate epithelial RC-165N cells, derived from benign prostate tissue of a radical prostatectomy specimen, were transduced through infection with a retrovirus vector expressing the human telomerase catalytic subunit (hTERT). A high level of telomerase activity was detected in RC-165N/hTERT cells but not in RC-165N cells. RC-165N/hTERT cells are currently growing well at passage 50 whereas RC-165N cells senesced within passage 3. RC-165N/hTERT cells exhibit epithelial morphology. These immortalized cells showed no cell growth in soft agar, and no tumor formation in SCID mice. The RC-165N/hTERT cells express androgen-regulated prostate-specific homobox gene. NKX 3.1 and epithelial cell specific cytokeratin 8, androgen receptor (AR), prostate stem cell antigen

and p16, but not PSA. AR protein was detected by Western blot analysis.

Guerra, C., et al. (2003). "Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context." Cancer Cell **4**(2): 111-120.

We have targeted a K-ras allele in mouse embryonic stem (ES) cells to express a K-Ras(V12) oncoprotein along with a marker protein (beta-geo) from a single bicistronic transcript. Expression of this oncogenic allele requires removal of a knocked in STOP transcriptional cassette by Cre recombinase. Primary mouse embryonic fibroblasts expressing this K-ras(V12) allele do not undergo proliferative senescence and proliferate as immortal cells. In mice, expression of K-ras(V12) throughout the body fails to induce unscheduled proliferation or other growth abnormalities for up to eight months. Only a percentage of K-ras(V12)-expressing lung bronchiolo-alveolar cells undergo malignant transformation leading to the formation of multiple adenomas and adenocarcinomas. These results indicate that neoplastic growth induced by an endogenous K-ras oncogene depends upon cellular context.

Gurevich, D. B., et al. (2016). "Asymmetric division of clonal muscle stem cells coordinates muscle regeneration in vivo." Science **353**(6295): aad9969.

Skeletal muscle is an example of a tissue that deploys a self-renewing stem cell, the satellite cell, to effect regeneration. Recent in vitro studies have highlighted a role for asymmetric divisions in renewing rare "immortal" stem cells and generating a clonal population of differentiation-competent myoblasts. However, this model currently lacks in vivo validation. We define a zebrafish muscle stem cell population analogous to the mammalian satellite cell and image the entire process of muscle regeneration from injury to fiber replacement in vivo. This analysis reveals complex interactions between satellite cells and both injured and uninjured fibers and provides in vivo evidence for the asymmetric division of satellite cells driving both self-renewal and regeneration via a clonally restricted progenitor pool.

Hamdan, L., et al. (2013). "Alpha cyano-4-hydroxy-3-methoxycinnamic acid inhibits proliferation and induces apoptosis in human breast cancer cells." PLoS One **8**(9): e72953.

This study investigated the underlying mechanism of 4-hydroxy-3-methoxycinnamic acid (ACCA), on the growth of breast cancer cells and normal immortal epithelial cells, and compared their cytotoxic effects responses. Treatment of breast cancer cells (MCF-7, T47D, and MDA-231) with ACCA resulted in dose- and time-dependent decrease of cell proliferation, viability in colony formation assay, and programmed cell death (apoptosis) with minimal effects on non-tumoral cells. The ability of ACCA to suppress growth in cancer cells not expressing or containing defects in p53 gene indicates a lack of involvement of this critical tumor suppressor element in mediating ACCA-induced growth inhibition. Induction of apoptosis correlated with an increase in Bax protein, an established inducer of programmed cell death, and the ratio of Bax to Bcl-2, an established inhibitor of apoptosis. We also documented the ability of ACCA to inhibit the migration and invasion of MDA-231 cells with ACCA in vitro. Additionally, tumor growth of MDA-231 breast cancer cells in vivo was dramatically affected with ACCA. On the basis of its selective anticancer inhibitory activity on tumor cells, ACCA may represent a promising therapeutic drug that should be further evaluated as a chemotherapeutic agent for human breast cancer.

Hao, Y., et al. (2009). "Porcine skin-derived stem cells can serve as donor cells for nuclear transfer." Cloning Stem Cells **11**(1): 101-110.

Although transgenic animal production through somatic cell nuclear transfer (SCNT) has been successful, the process is still inefficient. One major limitation is the use of somatic donor cells that have a finite life span. Identification and isolation of a cell type capable of rapid proliferation while possessing immortal or prolonged life span in culture and is capable of being genetically modified would be very valuable for utilization in the production of genetically modified pigs. Here we report the birth of live piglets after cloning by using porcine skin-derived stem cells (SSC) as a donor cell type. In the present study, cell cycle analysis indicates that the porcine SSC proliferate rapidly in vitro. The porcine SSC are capable of producing live offspring and can be genetically modified with positive selection. Utilization of porcine SSC may prove to be an excellent cell type for genetic modification followed by nuclear transfer for the production of transgenic pigs.

Harman, B. C., et al. (2014). "Antibody discovery from immune competent and immune transplanted mice." Curr Drug Discov Technol **11**(1): 65-73.

Since Kohler and Milstein developed the process of generating hybridomas by fusing antibody secreting B cells with an immortal myeloma cell line, the techniques used to develop monoclonal antibodies for use as human therapeutics have progressed significantly. Here, we will briefly review hybridoma technology and the evolution of therapeutic antibodies for the treatment of human disease. We will focus on the evolution of humanized mouse models for the generation of therapeutic human antibodies, comparing the early models, such as severe combined immunodeficient (SCID) mice which do not engraft human leukocytes well due to residual innate immunity, to the more recently developed models such as non-obese diabetic (NOD)/SCID IL-2Rgamma-

deficient mice in which numerous human hematopoietic lineages can be cultivated. Building on the identification of suitable host strains for the reconstitution of human immune cells, focus has now shifted onto humanizing the murine microenvironment in order to support human immune cell function. Although several recent studies have shown that the provision of human soluble factors can support maturation and function of human immune cells, particularly within the myeloid compartment, this does not appear to impact antibody production significantly. Moreover, models in which grafting of human tissues is performed to provide human microenvironments which support human leukocyte maturation do show improved humoral immune function, but require several surgical manipulations for generation of the model. Ultimately the most desirable scenario is to generate transgenic models that can be bred efficiently and express a sufficient number of human molecules to support functional human immune cells and several groups have made progress in making this idea a reality. These studies in the context of the generation of human antibodies will be discussed.

Hassani, S. N., et al. (2019). "Transition of inner cell mass to embryonic stem cells: mechanisms, facts, and hypotheses." Cell Mol Life Sci **76**(5): 873-892.

Embryonic stem cells (ESCs) are immortal stem cells that own multi-lineage differentiation potential. ESCs are commonly derived from the inner cell mass (ICM) of pre-implantation embryos. Due to their tremendous developmental capacity and unlimited self-renewal, ESCs have diverse biomedical applications. Different culture media have been developed to procure and maintain ESCs in a state of naive pluripotency, and to preserve a stable genome and epigenome during serial passaging. Chromatin modifications such as DNA methylation and histone modifications along with microRNA activity and different signaling pathways dynamically contribute to the regulation of the ESC gene regulatory network (GRN). Such modifications undergo remarkable changes in different ESC media and determine the quality and developmental potential of ESCs. In this review, we discuss the current approaches for derivation and maintenance of ESCs, and examine how differences in culture media impact on the characteristics of pluripotency via modulation of GRN during the course of ICM outgrowth into ESCs. We also summarize the current hypotheses concerning the origin of ESCs and provide a perspective about the relationship of these cells to their in vivo counterparts (early embryonic cells around the time of implantation). Finally, we discuss generation of ESCs from human embryos and domesticated animals, and offer suggestions to further advance this fascinating field.

He, C., et al. (2021). "Microvesicles - promising tiny players' of cancer stem cells targeted liver cancer treatments: The interesting interactions and therapeutic aspects." Pharmacol Res **169**: 105609.

Liver cancer is one of the most malignant cancers worldwide with poor prognosis. Intracellular mediators like microvesicles (MVs) and cancer stem cells (CSCs) are considered as potential candidates in liver cancer progression. CSCs receive stimuli from the tumor microenvironment to initiate tumor formation in which it's secreted MVs play a noteworthy role. The phenotypic conversion of tumor cells during epithelial-to-mesenchymal transition (EMT) is a key step in tumor invasion and metastasis which indicates that the diverse cell populations within the primary tumor are in a dynamic balance and can be regulated by cell to cell communication via secreted microvesicles. Thus, in this review, we aim to highlight the evidences that suggest CSCs are crucial for liver cancer development where the microvesicles plays an important part in the maintenance of its stemness properties. In addition, we summarize the existing evidences that support the concept of microvesicles, the tiny particles have a big role behind the rare immortal CSCs which controls the tumor initiation, propagation and metastasis in liver cancer. Identifying interactions between CSCs and microvesicles may offer new insights into precise anti-cancer therapies in the future.

He, J., et al. (2021). "Treatment Pattern and Outcomes in Newly Diagnosed Multiple Myeloma Patients Who Did Not Receive Autologous Stem Cell Transplantation: A Real-World Observational Study : Treatment pattern and outcomes in patients with multiple myeloma." Adv Ther **38**(1): 640-659.

INTRODUCTION: The objective of this study was to describe the treatment patterns among patients with newly diagnosed multiple myeloma (MM) who had not received autologous stem cell transplantation (ASCT). It further compares the safety and clinical outcomes across different frontline regimens as well as explores whether treatment duration predicts outcomes. METHODS: Patients with MM (> 45 years) who had not received ASCT were retrospectively identified from the US SEER-Medicare (Jan 2007-Dec 2016) and Optum (Jan 2007-Sep 2018) databases. Cox proportional hazard models were used to compare overall survival (OS) among bortezomib + lenalidomide + dexamethasone regimen (VRd), lenalidomide + dexamethasone regimen (Rd), cyclophosphamide + bortezomib + dexamethasone regimen (CyBorD), bortezomib + dexamethasone regimen (Vd), and other bortezomib-containing therapies based on propensity score matching. To address immortal time bias, time-fixed and time-dependent Cox models were employed to estimate the

association of longer frontline treatment exposure with outcomes. RESULTS: Mean (standard deviation; SD) age was 71 (9.8) years; and 49.51% were women. Bortezomib and lenalidomide-based combinations were the most common treatment modalities. After matching, the HR (95% CI) of OS by frontline therapies comparing VRd with Vd was 0.76 (0.66, 0.86), CyBorD was 0.87 (0.75, 1.05), for other bortezomib-based therapies was 0.56 (0.49, 0.64), Rd was 0.83 (0.73, 0.95), and for other therapies was 0.70 (0.61, 0.80). Longer frontline treatment duration was associated with better OS for overall frontline [HR (95% CI) 0.86 (0.82, 0.90)]; Vd [0.81 (0.74, 0.89)]; CyBorD [0.79 (0.64, 0.98)] and Rd [0.86 (0.78, 0.95)]. CONCLUSION: Results demonstrated that the frontline therapies prescribed to most patients who did not receive ASCT for MM in the United States were consistent with the NCCN guideline recommendations. Longer frontline treatment duration was associated with improved OS.

Heil, C. (2019). "Hedgehog pathway permissive conditions allow generation of immortal cell lines from granule cells derived from cancerous and non-cancerous cerebellum." Open Biol **9**(1): 180145.

Cerebellar granule cell progenitors (GCPs) undergo proliferation in the post-natal cerebellum that is dependent on sonic hedgehog (SHH) signalling. Deregulated SHH signalling leads to type 2 medulloblastoma (MB). In this work, a novel cell culture protocol is described, which is suitable for the establishment and long-term maintenance of GCP-derived cells. This method is first applied to SHH pathway active MB cells from Atoh1-cre; Ptch1(FL/FL) tumours, which leads to the generation of neurosphere-like cell lines expressing GCP markers and an active SHH signalling pathway. These cells also show high sensitivity to the Smoothened inhibitor vismodegib, therefore recapitulating the SHH pathway requirement for survival shown by type 2 MB. Analysis of culture supplements reveals that bFGF and fetal bovine serum act as inhibitors of the SHH pathway and therefore preclude generation of cell lines that are relevant to the study of the SHH pathway. Consequently, these insights are transferred from the context of MB to non-transformed, post-natal day 7 cerebellum-derived cellular explants. In contrast to other, previously used methods, these GCP cultures proliferate indefinitely and depend on SHH pathway activation, either by means of the small molecule SAG or through genetic ablation of Ptch1. This culture method therefore leads to the generation of immortal neurosphere-like cell lines, that are named murine SAG-dependent spheres (mSS). Despite long-term culture, mSS cells remain dependent on continuous stimulation of the SHH pathway. Further, mSS cells maintain their lineage after extensive periods in vitro, as demonstrated by their differentiation towards the neural lineage. Herein a simple method for the generation of immortal cell lines from murine cerebella is defined. These lines can be maintained indefinitely through hedgehog pathway activation and maintain the GCP lineage.

Hertzog, R. G. (2006). "Ancestral telomere shortening: a countdown that will increase mean life span?" Med Hypotheses **67**(1): 157-160.

Like cells, all mammals have a limited life span. Among cells there are a few exceptions (e.g., immortal cells), among mammals not, even if some of them live longer. Many in vitro and in vivo studies support the consensus that telomere length is strongly correlated with life span. At the somatic cellular level, long telomeres have been associated with longer life span. A different situation can be seen in immortal cells, such as cancer, germ and stem cells, where telomeres are maintained by telomerase, a specialized reverse transcriptase that is involved in synthesis of telomeres. Irrespective of telomere length, if telomerase is active, telomeres can be maintained at a sufficient length to ensure cell survival. To the contrary, telomeres shorten progressively with each cell division and when a critical telomere length (Hayflick limit) is reached, the cells undergo senescence and subsequently apoptosis. In mammals, those with the longest telomeres (e.g., mice) have the shortest life span. Furthermore, the shorter the mean telomere length, the longer the mean life span, as observed in humans (10-14 kpb) and bowhead-whales (undetermined telomere length), which have the longest mean life span among mammals. Over the past centuries, human average life span has increased. The hypothesis presented here suggests that this continual increase in the mean life span could be due to a decrease of mean telomere length over the last hundreds years. Actually, the life span is not directly influenced by length of telomeres, but rather by telomere length - dependent gene expression pattern. According to Greider, "rather than average telomere length, it is the shortest telomere length that makes the biggest difference to a cell". In the context of fast-growing global elderly population due to increase in life expectancy, it also seem to be an age related increase in cancer incidence. Nevertheless, extending healthy life span could depend on how good cells achieve, during the prenatal period and few years after birth, the equilibrium between telomere length and telomerase activity, as seen in germ cells. After all, I suggest that decrease in mean telomere length might result in, on the one hand, an increased life span and, on the other, a higher risk of tumorigenesis.

Hiragun, T., et al. (2014). "Establishment of a mast cell line, NCL-2, without Kit mutation, derived from NC mouse bone marrow." FEBS Open Bio **4**: 342-346.

Immortal mast cell lines, such as RBL-2H3 and HMC-1 cells, are commonly utilized to investigate the function of mast cells. However, they are tumor cells carrying a gain-of-function mutation of Kit. We established an immortal mast cell line without Kit mutation, NCL-2, derived from NC mouse bone marrow. NCL-2 cells could be maintained without additional growth factors and thus could respond to exogenous growth signals. Moreover, NCL-2 cells expressed FcepsilonRI and KIT, and release histamine and LTB4 in response to antigen stimulation. This cell line could be a useful tool to analyze proliferation, differentiation, and function of normal mast cells.

Hofbauer, R. and D. T. Denhardt (1991). "Cell cycle-regulated and proliferation stimulus-responsive genes." Crit Rev Eukaryot Gene Expr **1**(4): 247-300.

We have reviewed here genes whose expression may vary during the "cell cycle" and we discuss the underlying regulatory mechanisms. Given a correlation between the cell cycle and expression of a particular gene, the question arises whether that gene regulates the cycle, whether the cycle regulates that gene, or whether the correlation is simply the consequence that both the cell cycle and that gene respond to the same signal(s). Gene expression is regulated at diverse levels, and the relative importance of regulation at these different levels depends on which version of the cell cycle one has in mind; depending upon the context, the concept of the (higher eukaryote) cell cycle has a number of different operational meanings. Thus the first few divisions of the fertilized egg consist of successive S and M phases, with insignificant G1 and G2 phases, regulated entirely at the translational and post-translational level by the phosphorylation/dephosphorylation of p34cdc2 and the synthesis/degradation of one or more cyclins-keyed perhaps to the cytoplasm/nucleoplasm ratio and the completion of DNA replication. In contrast, cells stimulated to exit quiescence, (G0), require new gene transcription and changes in the post-transcriptional control of gene expression. Cells proliferating in a constant environment proceed directly from mitosis into G1 and are less dependent on (but not independent of) new transcription; here controls at the post-transcriptional and post-translational levels are more pronounced. In addition to regulation by p34cdc2, input from cell-specific growth factors or other extracellular signals is essential for most untransformed cells to continue through the cycle. Many transformed cells in contrast do not require exogenous signals and are altered in the way that key regulatory genes (e.g., p53, RB) are controlled. While cells of many lower eukaryotes appear capable of an indefinite number of cell cycles, the typical higher eukaryotic cell appears to have a limit on this number--untransformed, nonestablished vertebrate cells are usually mortal. For unknown reasons, established cell lines and certain embryonic or stem cells under the right conditions, are immortal and capable of indefinite proliferation. Apparently, the price paid to construct a differentiated multicellular organism is a limit on the number of cell divisions that the constituent somatic cells are capable of undergoing.

Hoggarth, Z. E., et al. (2019). "Enrichment of genes associated with squamous differentiation in cancer initiating cells isolated from urothelial cells transformed by the environmental toxicant arsenite." Toxicol Appl Pharmacol **374**: 41-52.

Arsenic is an environmental toxicant with long-term exposure associated with the development of urothelial carcinomas. Our lab has developed an in-vitro model of urothelial carcinoma by exposing the immortal, but non-tumorigenic bladder cell line, the UROtsa, to arsenite (As(3+)). These transformed cells form tumors in immune-compromised mice, which resemble urothelial carcinomas with components of the tumor exhibiting squamous differentiation. The goal of the present study was to determine the differences in global gene expression patterns between the As(3+)-transformed UROtsa cells and the urospheres (spheroids containing putative cancer initiating cells) isolated from these cell lines and to determine if the genes involved in the development of squamous differentiation were enriched in the urospheres. The results obtained in this study show an enrichment of genes such as KRT1, KRT5, KRT6A, KRT6B, KRT6C, KRT14 and KRT16 associated with squamous differentiation, a characteristic feature seen in aggressive basal subtypes of urothelial cell carcinoma (UCC) in the urospheres isolated from As(3+)-transformed UROtsa cells. In addition, there is increased expression of several of the small proline-rich proteins (SPRR) in the urospheres and overexpression of these genes occur in UCC's displaying squamous differentiation. In conclusion, the cancer initiating cells present in the urospheres are enriched with genes associated with squamous differentiation.

Holt, S. E., et al. (1996). "Regulation of telomerase activity in immortal cell lines." Mol Cell Biol **16**(6): 2932-2939.

Telomerase is a ribonucleoprotein whose activity has been detected in germ line cells, immortal cells, and most cancer cells. Except in stem cells, which have a low level of telomerase activity, its activity is absent from normal somatic tissues. Understanding the regulation of telomerase activity is critical for the development of potential tools for the diagnosis and treatment of cancer. Using the telomeric repeat amplification protocol, we found that immortal, telomerase-positive, pseudodiploid human cells (HT1080 and HL60 cells) sorted by flow repressed in

quiescent cells. This was true whether quiescence was induced by contact inhibition (NIH 3T3 mouse cells), growth factor removal (bromodeoxyuridine-blocked mouse myoblasts), reexpression of cellular senescence (the reversibly immortalized IDH4 cells), or irreversible cell differentiation (HL60 promyelocytic leukemia cells and C2C12 mouse myoblasts). Taken together, these results indicate that telomerase is active throughout the cell in dividing, immortal cells but that its activity is repressed in cells that exit the cell cycle. This suggests that quiescent stem cells that have the potential to express telomerase may remain unaffected by potential antitelomerase cancer therapies.

Holt, S. E., et al. (1997). "Multiple pathways for the regulation of telomerase activity." Eur J Cancer **33**(5): 761-766.

The ends of vertebrate chromosome are composed of large tracts of a repeated sequence, TTAGGG, which are known as telomeres. Normal somatic cells progressively lose telomeric repeats with each successive cell division due to incomplete replication. Immortal and cancer cells compensate for telomeric loss by expressing the enzyme telomerase, an RNA-dependent DNA polymerase that maintains telomere length. Telomerase activity has been detected in almost 90% of all human cancers. Telomerase activity is generally absent in normal somatic tissues but is detected in adult testes, activated lymphocytes, and lower levels are expressed in proliferative cells of renewal tissues. Telomerase activity is downregulated in cells that exit the cell cycle via either terminal differentiation or (reversible) quiescence. Inhibition of telomerase activity in tumour cells may provide an effective way to treat cancer by potentially reducing the recurrence of tumours due to occult micro-metastases. An understanding of the pathways involved in telomerase regulation will be important for determining the most practical means of inhibiting its activity.

Hou, L. and B. Yao (2007). "[Update of the researches on the niche for spermatogonial stem cells]." Zhonghua Nan Ke Xue **13**(11): 1028-1031.

Spermatogonial stem cells are a population of immortal cells, capable of self-renewal and multi-directional differentiation. The theory of the "stem cell niche" was originally proposed for the hematopoietic system, and niches also exist in testicular tissues. The niche for spermatogonial stem cells is a semi-isolation system, characteristic of specific number regulation and changing with the age. The self-renewal of spermatogonial stem cells is regulated by two endogenous factors, nanos2 and Plzf, as well as by the niche cell-excreted factor, glial-derived neurotrophic factor (GDNF).

Huang, G. T. (2010). "Induced Pluripotent Stem Cells-A New Foundation in Medicine." J Exp Clin Med **2**(5): 202-217.

Generation of induced pluripotent stem (iPS) cells using defined factors has been considered a ground-breaking step towards establishing patient-specific pluripotent stem cells for various applications. The isolation of human embryonic stem (ES) cells set the standard that pluripotent stem cells are attainable as potentially immortal cells for regeneration of many types of tissues. Different approaches have been tested to obtain pluripotent stem cells by circumventing the need for embryos. iPS cells appear to be an ideal substitute for ES cells. Since the first demonstration of creating iPS cells in 2006, tremendous efforts have been made into improving iPS cell generation methods and understanding the reprogramming mechanism as well as the nature of iPS cells. To improve iPS cell generation, several approaches have been taken: (1) eliminate the viral vector integration after delivering the defined factors; (2) select different cell types that more effectively give rise to iPS cells; (3) use of chemicals to facilitate reprogramming; (4) use of protein factors to reprogram cells. The iPS cells are also being rigorously characterized in comparison to ES cells. All these efforts are made for the purpose of making iPS cells closer to clinical applications. This article will give an overview of the following areas: (1) mechanisms of iPS cell derivation; (2) characterization of iPS cells; (3) iPS cells for cell-based therapy; and (4) iPS cells for studying disease mechanism. Questions as to what aspects of iPS cells require further understanding before they may be put to clinical use are also discussed.

Huang, G. T., et al. (2006). "Formation of odontoblast-like cells from cultured human dental pulp cells on dentin in vitro." J Endod **32**(11): 1066-1073.

Recent characterization of human dental pulp stem cells has shed new light on the understanding of the odontoblastic lineage. The purpose of the study was to characterize human adult dental pulp cells isolated and cultured in vitro and to examine the cell differentiation potential grown on dentin. We observed that some pulp cells isolated with an enzyme-digestion approach proliferated at a similar rate as the immortal cell line NIH 3T3. Population doubling time (PDt) for pulp cells at passage 3 was 22.6 +/- 0.5 hours and for NIH 3T3 was 23.1 +/- 2.3 hours. The pulp cells formed mineral nodules stimulated with dexamethasone or dexamethasone plus 1,25-dihydroxyvitamin D3. Pulp cells, after being seeded onto mechanically and chemically treated dentin surface, appeared to establish an odontoblast-like morphology with a cytoplasmic process extending into a dentinal tubule revealed by scanning electron microscopy analysis. Our data demonstrated the formation of cells with odontoblastic morphologies on existing dentin, suggesting that isolated human pulp stem cells may differentiate into odontoblasts on dentin in vitro.

Huang, Y., et al. (2007). "Development of a rabbit monoclonal antibody group against Smads and immunocytochemical study of human and mouse embryonic stem cells." Hybridoma (Larchmt) **26**(6): 387-391.

We report here the development of a group of rabbit monoclonal antibodies against Smad1, Smad2, Smad3, and Smad5, and the immunocytochemistry (ICC) staining of human embryonic stem cells (hESC) and mouse embryonic stem cells (mESC). Eight New Zealand rabbits were immunized with synthesized peptides linked to KLH, and splenocytes from these rabbits were fused with rabbit immortal B cell 240E-W2. Resulting hybridomas producing anti-Smad monoclonal antibodies were screened by enzyme-linked immunosorbent assay (ELISA) with BSA-linked peptides. Clones were chosen for antibody production based on their activities in Western blotting and on paraffin-embedded human tissues, and the capacity of the antibodies in immunocytochemistry was demonstrated. Using these antibodies, we performed ICC staining on routinely cultured human and mouse embryonic stem cells, and showed that both cell types strongly express these genes. We propose that both hESCs and mESCs have the ability to transduce signals from both BMPs and TGF-b/Activin.

Huh, Y. H., et al. (2011). "SACK-expanded hair follicle stem cells display asymmetric nuclear Lgr5 expression with non-random sister chromatid segregation." Sci Rep **1**: 176.

We investigated the properties of clonally-expanded mouse hair follicle stem cells (HF-SCs) in culture. The expansion method, suppression of asymmetric cell kinetics (SACK), is non-toxic and reversible, allowing evaluation of the cells' asymmetric production of differentiating progeny cells. A tight association was discovered between non-random sister chromatid segregation, a unique property of distributed stem cells (DSCs), like HF-SCs, and a recently described biomarker, Lgr5. We found that nuclear Lgr5 expression was limited to the HF-SC sister of asymmetric self-renewal divisions that retained non-randomly co-segregated chromosomes, which contain the oldest cellular DNA strands, called immortal DNA strands. This pattern-specific Lgr5 association poses a potential highly specific new biomarker for delineation of DSCs. The expanded HF-SCs also maintained the ability to make differentiated hair follicle cells spontaneously, as well as under conditions that induced cell differentiation. In future human cell studies, this capability would improve skin grafts and hair replacement therapies.

Huh, Y. H. and J. L. Sherley (2011). "Molecular cloaking of H2A.Z on mortal DNA chromosomes during nonrandom segregation." Stem Cells **29**(10): 1620-1627.

Although nonrandom sister chromatid segregation is a singular property of distributed stem cells (DSCs) that are responsible for renewing and repairing mature vertebrate tissues, both its cellular function and its molecular mechanism remain unknown. This situation persists in part because of the lack of facile methods for detecting and quantifying nonrandom segregating cells and for identifying chromosomes with immortal DNA strands, the cellular molecules that signify nonrandom segregation. During nonrandom segregation, at each mitosis, asymmetrically self-renewing DSCs continuously cosegregate to themselves the set of chromosomes that contain immortal DNA strands, which are the oldest DNA strands. Here, we report the discovery of a molecular asymmetry between segregating sets of immortal chromosomes and opposed mortal chromosomes (i.e., containing the younger set of DNA template strands) that constitutes a new convenient biomarker for detection of cells undergoing nonrandom segregation and direct delineation of chromosomes that bear immortal DNA strands. In both cells engineered with DSC-specific properties and ex vivo-expanded mouse hair follicle stem cells, the histone H2A variant H2A.Z shows specific immunodetection on immortal DNA chromosomes. Cell fixation analyses indicate that H2A.Z is present on mortal chromosomes as well but is cloaked from immunodetection, and the cloaking entity is acid labile. The H2A.Z chromosomal asymmetry produced by molecular cloaking provides a first direct assay for nonrandom segregation and for chromosomes with immortal DNA strands. It also seems likely to manifest an important aspect of the underlying mechanism(s) responsible for nonrandom sister chromatid segregation in DSCs.

Huh, Y. H. and J. L. Sherley (2014). "Decreased H3K27 and H3K4 trimethylation on mortal chromosomes in distributed stem cells." Cell Death Dis **5**: e1554.

The role of immortal DNA strands that co-segregate during mitosis of asymmetrically self-renewing distributed stem cells (DSCs) is unknown. Previously, investigation of immortal DNA strand function and molecular mechanisms responsible for their nonrandom co-segregation was precluded by difficulty in identifying DSCs and immortal DNA strands. Here, we report the use of two technological innovations, selective DSC expansion and establishment of H2A.Z chromosomal asymmetry as a specific marker of 'immortal chromosomes,' to investigate molecular properties of immortal chromosomes and opposing 'mortal chromosomes' in cultured mouse hair follicle DSCs. Although detection of the respective suppressive and activating H3K27me3

and H3K4me3 epigenetic marks on immortal chromosomes was similar to randomly segregated chromosomes, detection of both was lower on mortal chromosomes destined for lineage-committed sister cells. This global epigenomic feature of nonrandom co-segregation may reveal a mechanism that maintains an epigenome-wide 'poised' transcription state, which preserves DSC identity, while simultaneously activating sister chromosomes for differentiation.

Hyun, I. (2019). "Informed Consent Issues for Cell Donors." Methods Mol Biol **2005**: 67-74.

Stem cell-based chimera research depends on the free and voluntary provision of human biomaterials necessary for the derivation of pluripotent stem cell lines. Informed consent requirements for the procurement of human embryos, gametes, and somatic cells must take into account unique features of biomedical research involving the use of immortal cell lines that carry their donors' genetic information. The extent and basis for donors' rights, including the right to withdraw from research, are explored here in detail.

Iglesias, M., et al. (2019). "Downregulation of mTOR Signaling Increases Stem Cell Population Telomere Length during Starvation of Immortal Planarians." Stem Cell Reports **13**(2): 405-418.

Reduction of caloric intake delays and prevents age-associated diseases and extends the life span in many organisms. It may be that these benefits are due to positive effects of caloric restriction on stem cell function. We use the planarian model Schmidtea mediterranea, an immortal animal that adapts to long periods of starvation by shrinking in size, to investigate the effects of starvation on telomere length. We show that the longest telomeres are a general signature of planarian adult stem cells. We also observe that starvation leads to an enrichment of stem cells with the longest telomeres and that this enrichment is dependent on mTOR signaling. We propose that one important effect of starvation for the rejuvenation of the adult stem cell pool is through increasing the median telomere length in somatic stem cells. Such a mechanism has broad implications for how dietary effects on aging are mediated at the whole-organism level.

Iglesias, T., et al. (1994). "c-erbA and v-erbA modulate growth and gene expression of a mouse glial precursor cell line." Cell Growth Differ **5**(7): 697-704.

The c-erbA alpha protooncogene coding for the thyroid hormone (T3) receptor (TR alpha 1) and the viral, mutated v-erbA oncogene were expressed in an immortal mouse glial cell line (B3.1) using retroviral vectors. c-erbA alpha expression led to a decrease in cell proliferation in high and low serum conditions, both in the presence and in the absence of T3. In serum-free medium, c-erbA-expressing cells (B3.1 + TR alpha 1) were completely arrested, whereas cells expressing v-erbA (B3.1 + v-erbA) showed a higher DNA synthesis rate than normal B3.1 cells. Although proliferation of all three cell types was stimulated by platelet-derived growth factor and basic fibroblast growth factor, differences were also observed in the response to these agents. B3.1 + TR alpha 1 cells were more sensitive to platelet-derived growth factor than B3.1 and B3.1 + v-erbA cells. In contrast, B3.1 cells responded to basic fibroblast growth factor better than B3.1 + TR alpha 1 or B3.1 + v-erbA cells. Insulin-like growth factor I potentiated the action of platelet-derived growth factor and basic fibroblast growth factor. Again, different responses to treatment with insulin-like growth factor I alone were observed; B3.1 + TR alpha 1 cells did not respond to it, whereas B3.1 + v-erbA cells showed a dramatic stimulation by this agent. Interestingly, in the presence of T3, the blockade in B3.1 + TR alpha 1 cell proliferation was accompanied by the down-regulation of the typical astrocytic genes, glial fibrillary acidic protein and vimentin. These hormone effects were not found in v-erbA-expressing cells. In addition, v-erbA inhibited the basal expression of the cyclic nucleotide phosphodiesterase gene, an oligodendrocytic marker.(ABSTRACT TRUNCATED AT 250 WORDS)

Intartaglia, M., et al. (2018). "Immunohistochemistry for Cancer Stem Cells Detection: Principles and Methods." Methods Mol Biol **1692**: 195-211.

Cancer stem cells (CSCs) are rare immortal cells within a tumor that can self-renew and drive tumorigenesis. CSCs play a pivotal role in the tumor development, progression and relapse, as well as in the resistance of anticancer therapy. Different tools could help in the analysis of CSCs, especially Immunohistochemistry (IHC) represents a useful technique able to identify several specific CSC markers. The main aims of this chapter are the description of the explain immunohistochemical methods used in the characterization of CSCs. Furthermore, focus on the most common troubleshooting in CSCs IHC is provided, especially the pitfalls of the CSCs markers IHC on tissue microarrays.

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

Fusion of sperm and egg generates a totipotent zygote that develops into a whole organism. Accordingly, the "immortal" germline transmits genetic and epigenetic information to subsequent generations with consequences for human health and disease. In mammals, primordial germ cells (PGCs) originate from peri-gastrulation embryos. While early human embryos are inaccessible for research, in vitro model systems using pluripotent stem cells have provided critical

insights into human PGC specification, which differs from that in mice. This might stem from significant differences in early embryogenesis at the morphological and molecular levels, including pluripotency networks. Here, we discuss recent advances and experimental systems used to study mammalian germ cell development. We also highlight key aspects of germ cell disorders, as well as mitochondrial and potentially epigenetic inheritance in humans.

Islam, M. Q., et al. (2006). "Polyethylene glycol-mediated fusion between primary mouse mesenchymal stem cells and mouse fibroblasts generates hybrid cells with increased proliferation and altered differentiation." Stem Cells Dev **15**(6): 905-919.

Bone marrow-derived mesenchymal stem cells (MSCs) can differentiate into different cell lineages with the appropriate stimulation in vitro. Transplantation of MSCs in human and other animal models was found to repair tissues through the fusion of transplanted MSCs with indigenous cells. We have generated mouseamouse hybrid cell lines in vitro by polyethylene glycol-mediated fusion of primary mouse MSCs with mouse fibroblasts to investigate the characteristics of hybrid cells, including their potentials for proliferation and differentiation. Similar to the parental MSCs, hybrid cells are positive for the cell-surface markers CD29, CD44, CD49e, and Sca-1, and negative for Gr-1, CD11b, CD13, CD18, CD31, CD43, CD45, CD49d, CD90.2, CD445R/B220, and CD117 markers. The hybrid cells also produce a high level of tissue nonspecific alkaline phosphatase compared to the parental cells. Conditioned medium of hybrid cells contain biologically active factors that are capable of stimulating proliferation of other cells. Although the parental MSCs can differentiate into adipogenic and osteogenic lineages, hybrid cells held disparate differentiation capacity. Hybrid cell lines in general have increased proliferative capacity than the primary MSCs. Our study demonstrates that proliferative hybrid cell lines can be generated in vitro by induced fusion of both immortal and primary somatic cells with primary MSCs.

Islam, M. Q., et al. (2007). "Generation of somatic cell hybrids for the production of biologically active factors that stimulate proliferation of other cells." Cell Prolif **40**(1): 91-105.

OBJECTIVE: Some normal somatic cells in culture divide a limited number of times before entering a non-dividing state called replicative senescence and fusion of normal cells with immortal cells claimed to produce hybrid cells of limited proliferation. We reinvestigated the proliferative capacity of hybrid cells between normal cell and immortal cell. MATERIALS AND METHODS: Normal pig fibroblast cells and cells of immortal mouse fibroblast cell line F7, a derivative of GM05267, were fused by polyethylene glycol treatment and subsequently the fused cells were cultured in a selective medium containing hypoxanthine-aminopterin-thymidine in order to enrich the hybrid cells. The hybrid cells were then monitored for chromosome content and proliferation. RESULTS: Cytogenetic analysis revealed that the hybrid cells contained polyploidy chromosomes derived from normal pig fibroblasts. These hybrid cells exhibit no sign of replicative senescence after more than 190 population doublings in vitro. Instead, these hybrid cells have an accelerated growth and proliferate even in the complete absence of glutamine. In addition, these hybrids produce biologically active factors in the conditioned media, which not only can accelerate their own proliferation but also can reinitiate mitotic activity in the senescent-like normal fibroblast cells. CONCLUSIONS: Our results question the validity of cellular senescence as a dominant trait. Additionally, the generation of hybrid cells using the specific mouse cell line can be applied to the generation of hybrids with other normal cell types and can be used to produce tissue-specific growth-factor(s) to extend the lifespan and/or improve the proliferation of various normal cells, including adult stem cells.

Islam, M. Q., et al. (2006). "Functional characterization of cell hybrids generated by induced fusion of primary porcine mesenchymal stem cells with an immortal murine cell line." Cell Tissue Res **326**(1): 123-137.

Bone marrow mesenchymal stem cells (MSC) integrate into various organs and contribute to the regeneration of diverse tissues. However, the mechanistic basis of the plasticity of MSC is not fully understood. The change of cell fate has been suggested to occur through cell fusion. We have generated hybrid cell lines by polyethylene-glycol-mediated cell fusion of primary porcine MSC with the immortal murine fibroblast cell line F7, a derivative of the GM05267 cell line. The hybrid cell lines display fibroblastic morphology and proliferate like immortal cells. They contain tetraploid to hexaploid porcine chromosomes accompanied by hypo-diploid murine chromosomes. Interestingly, many hybrid cell lines also express high levels of tissue-nonspecific alkaline phosphatase, which is considered to be a marker of undifferentiated embryonic stem cells. All tested hybrid cell lines retain osteogenic differentiation, a few of them also retain adipogenic potential, but none retain chondrogenic differentiation. Conditioned media from hybrid cells enhance the proliferation of both early-passage and late-passage porcine MSC, indicating that the hybrid cells secrete diffusible growth stimulatory factors. Murine F7 cells thus have the unique property of generating immortal cell hybrids containing unusually high numbers of chromosomes derived from normal

cells. These hybrid cells can be employed in various studies to improve our understanding of regenerative biology. This is the first report, to our knowledge, describing the generation of experimentally induced cell hybrids by using normal primary MSC.

Isman, O., et al. (2008). "Adenovirus-based targeting in myoblasts is hampered by nonhomologous vector integration." Hum Gene Ther **19**(10): 1000-1008.

Chromosomal correction of dystrophin gene mutations is a most desirable therapeutic solution for Duchenne muscular dystrophy, as it allows production of the full-length dystrophin under the control of locus-specific promoters. Here we explored gene targeting in conditionally immortal mouse dystrophin-deficient myoblasts. We constructed an adenoviral vector for the correction of the mdx mutation, containing 6.0 kb of sequence homologous to the target locus (partial intron 21 through to exon 24 with the normal sequence of exon 23) and a neomycin expression cassette inserted in intron 23. Adenovirus-based gene targeting was previously reported to be beneficial in mouse embryonic stem cells, resulting in one targeted integration per three integration events. However, we found no targeted integration events among 144 stably transduced G418-resistant myoblast clones, reflecting efficient random integration of the adenoviral vector in myogenic cells. We found that mouse myoblasts are capable of integrating recombinant adenoviral DNA with an efficiency approaching 1%. Interestingly, dermal fibroblasts integrate adenoviral DNA up to 100 times less efficiently than myoblasts from the same mice. We also show that the efficiency of recombinant adenoviral DNA integration is influenced by preinfection cell density, possibly indicating the importance of cellular DNA replication for adenoviral integration.

Ito, M., et al. (2004). "Establishment by an original single-cell cloning method and characterization of an immortal mouse melanoblast cell line (NCCmelb4)." Pigment Cell Res **17**(6): 643-650.

We devised a unique new single-cell cloning method which uses microscope cover glasses and established a melanoblast cell line derived from mouse neural crest cells. A microscope cover glass was nicked and broken into small pieces and put on a dish. Culture medium and a suspension of 20-30 cells/ml were dropped in the dish. After 1-3 d, a piece of glass to which only one cell was adhered was picked up and transferred to another dish containing culture medium. The greatest advantage of this method is that the derivation of a colony from a single cell can be directly confirmed by microscopy and there is no risk of migratory cells being contaminated by other colonies. Using this single-cell cloning method, in this study we established a cell line derived from a neural crest cell line (NCC-S4.1) and designated it as NCCmelb4. When the culture medium was supplemented with stem cell factor (SCF) alone, NCCmelb4 cells were KIT-positive and tyrosinase-negative melanocyte precursors; they remained at an immature and undifferentiated stage. When the medium was supplemented with phorbol 12-o-tetradecanoyl-13-acetate (TPA) + cholera toxin (CT), the cell morphology changed and became L-3,4-dihydroxyphenylalanine (DOPA)-positive. This observation indicates that the NCCmelb4 cells are capable of further differentiation with suitable stimulation. NCCmelb4 cells derived from the mouse neural crest has characteristics of melanocyte precursors (melanoblasts), and is a cell line which can be utilized to study differentiation-inducing factors and growth factors without the effects of feeder cells.

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.

Kajstura, J., et al. (2012). "Tracking chromatid segregation to identify human cardiac stem cells that regenerate extensively the infarcted myocardium." Circ Res **111**(7): 894-906.

RATIONALE: According to the immortal DNA strand hypothesis, dividing stem cells selectively segregate chromosomes carrying the old template DNA, opposing accumulation of mutations resulting from nonrepaired replication errors and attenuating telomere shortening. OBJECTIVE: Based on the premise of the immortal DNA strand hypothesis, we propose that stem cells retaining the old DNA would represent the most powerful cells for myocardial regeneration. METHODS AND RESULTS: Division of human cardiac stem cells (hCSCs) by nonrandom and random segregation of chromatids was documented

by clonal assay of bromodeoxyuridine-tagged hCSCs. Additionally, their growth properties were determined by a series of in vitro and in vivo studies. We report that a small class of hCSCs retain during replication the mother DNA and generate 2 daughter cells, which carry the old and new DNA, respectively. hCSCs with immortal DNA form a pool of nonsenescent cells with longer telomeres and higher proliferative capacity. The self-renewal and long-term repopulating ability of these cells was shown in serial-transplantation assays in the infarcted heart; these cells created a chimeric organ, composed of spared rat and regenerated human cardiomyocytes and coronary vessels, leading to a remarkable restoration of cardiac structure and function. The documentation that hCSCs divide by asymmetrical and symmetrical chromatid segregation supports the view that the human heart is a self-renewing organ regulated by a compartment of resident hCSCs. CONCLUSIONS: The impressive recovery in ventricular hemodynamics and anatomy mediated by clonal hCSCs carrying the "mother" DNA underscores the clinical relevance of this stem cell class for the management of heart failure in humans.

Kamada, M., et al. (2016). "Reversible transformation and de-differentiation of human cells derived from induced pluripotent stem cell teratomas." Hum Cell **29**(1): 1-9.

We first aimed to generate transformed cell lines from a human induced pluripotent stem cell (hiPSC)-teratoma, and then examined the tumorigenic risks of the differentiated cells from hiPSC explant, because hiPSC-derivatives give rise to tumors in immune-deficient mice when transplanted. The colonies isolated from sparse cultures of hiPSC-teratoma cells expressed NANOG and OCT3/4 strongly, and telomerase reverse transcriptase (TERT) weakly. However, soft agar assay demonstrated that only one of them generated colonies in the gel, though hiPSCs, hTERT-transfected immortal cells, and its oncogene-transfected cells did not form any colonies. Furthermore, none of colonies isolated from the soft agar gel on primary culture (passage 0) of teratoma cells, expressed NANOG and OCT3/4 in the expanded cultures. The second soft agar assay on the colony-derived cells was unexpectedly negative. The cumulative growth curve, telomere shortening, and senescence-associated beta-galactosidase (SA beta-gal) staining confirmed the mortality of these cells, suggesting their reversible transformation. By using medium for embryonic stem cell (ESC medium) after MCDB 131 (MCDB) medium, the differentiated culture cells derived from hiPSC-teratoma converted into the cells expressing undifferentiated marker proteins, which lost afterwords even in ESC medium with feeder SNL76/7. The reversibility of transformation and de-differentiation suggest that tumorigenic risks of differentiated cells arise when they are exposed to suitable niches in vivo. Thus, removal of only the undifferentiated cells from iPSC-derivatives before transplantation does not solve the problem. Elucidation of mechanisms of reversibility and control of epigenetic changes is discussed as a safety bottleneck for hiPSC therapy.

Kanatsu-Shinohara, M., et al. (2005). "Genetic and epigenetic properties of mouse male germline stem cells during long-term culture." Development **132**(18): 4155-4163.

Although stem cells are believed to divide infinitely by self-renewal division, there is little evidence that demonstrates their infinite replicative potential. Spermatogonial stem cells are the founder cell population for spermatogenesis. Recently, in vitro culture of spermatogonial stem cells was described. Spermatogonial stem cells can be expanded in vitro in the presence of glial cell line-derived neurotrophic factor (GDNF), maintaining the capacity to produce spermatogenesis after transplantation into testis. Here, we examined the stability and proliferative capacity of spermatogonial stem cells using cultured cells. Spermatogonial stem cells were cultured over 2 years and achieved approximately 10(85)-fold expansion. Unlike other germline cells that often acquire genetic and epigenetic changes in vitro, spermatogonial stem cells retained the euploid karyotype and androgenetic imprint during the 2-year experimental period, and produced normal spermatogenesis and fertile offspring. However, the telomeres in spermatogonial stem cells gradually shortened during culture, suggesting that they are not immortal. Nevertheless, the remarkable stability and proliferative potential of spermatogonial stem cells suggest that they have a unique machinery to prevent transmission of genetic and epigenetic damages to the offspring, and these characteristics make them an attractive target for germline modification.

Kanatsu-Shinohara, M., et al. (2019). "Aging of spermatogonial stem cells by Jnk-mediated glycolysis activation." Proc Natl Acad Sci U S A **116**(33): 16404-16409.

Because spermatogonial stem cells (SSCs) are immortal by serial transplantation, SSC aging in intact testes is considered to be caused by a deteriorated microenvironment. Here, we report a cell-intrinsic mode of SSC aging by glycolysis activation. Using cultured SSCs, we found that aged SSCs proliferated more actively than young SSCs and showed enhanced glycolytic activity. Moreover, they remained euploid and exhibited stable androgenetic imprinting patterns with robust SSC activity despite having shortened telomeres. Aged SSCs showed increased Wnt7b expression, which was associated with decreased Polycomb complex 2 activity. Our results suggest that aberrant Wnt7b expression activated c-jun N-terminal

kinase (JNK), which down-regulated mitochondria numbers by suppressing Ppargc1a Down-regulation of Ppargc1a probably decreased reactive oxygen species and enhanced glycolysis. Analyses of the Klotho-deficient aging mouse model and 2-y-old aged rats confirmed JNK hyperactivation and increased glycolysis. Therefore, not only microenvironment but also intrinsic activation of JNK-mediated glycolysis contributes to SSC aging.

Kang, K. S., et al. (1998). "Involvement of tyrosine phosphorylation of p185(c-erbB2/neu) in tumorigenicity induced by X-rays and the neu oncogene in human breast epithelial cells." Mol Carcinog **21**(4): 225-233.

Ionizing radiation is the exogenous agent best proven to induce breast cancer. c-erbB2/neu amplification and overexpression are known to occur in breast cancer and are correlated with aggressive tumor growth and poor prognosis. We have developed simian virus 40-immortalized cell lines from normal human breast epithelial cells (HBECs) with luminal and stem-cell characteristics. In this study, we examined whether x-rays and a mutated neu oncogene are capable of inducing tumorigenicity in these cells. The results indicated that x-rays were effective in converting immortal non-tumorigenic HBECs to weakly tumorigenic cells that then could be transformed to highly tumorigenic cells by the neu oncogene. The in vitro growth of these tumorigenic cells was significantly faster than that of the parental non-tumorigenic cells in growth factor- and hormone-supplemented or -depleted media. The neu oncogene, however, had no tumorigenic effect on immortal non-tumorigenic cells. The expression of p185(c-erb82/neu) was elevated in neu-transduced immortal or weakly tumorigenic cell lines. However, only in the latter was p185(c-erbB2/neu) found to be phosphorylated at tyrosine residues. Thus, x-rays appear to induce a genetic alteration that confers weak tumorigenicity on immortal HBECs and interacts with p185(c-erbB2/neu) directly or indirectly to give rise to fast-growing tumors.

Kang, S. K., et al. (2004). "Expression of telomerase extends the lifespan and enhances osteogenic differentiation of adipose tissue-derived stromal cells." Stem Cells **22**(7): 1356-1372.

Expression of TERT, the catalytic protein subunit of the telomerase complex, can be used to generate cell lines that expand indefinitely and retain multilineage potential. We have created immortal adipose stromal cell lines (ATSCs) by stably transducing nonhuman primate-derived ATSCs with a retroviral vector expressing TERT. Transduced cells (ATSC-TERT) had an increased level of telomerase activity and increased mean telomere length in the absence of malignant cellular transformation. Long-term culture of the ATSC-TERT cells demonstrated that the cells retain the ability to undergo differentiation along multiple lineages such as adipogenic, chondrogenic, and neurogenic. Untransduced cells demonstrated markedly reduced multilineage and self-renewal potentials after 12 passages in vitro. To determine the functional role of telomerase during osteogenesis, we examined osteogenic differentiation potential of ATSC-TERT cells in vitro. Compared with naive ATSCs, which typically begin to accumulate calcium after 3-4 weeks of induction by osteogenic differentiation medium, ATSC-TERT cells were found to accumulate significant amounts of calcium after only 1 week of culture in osteogenic induction medium. The cells have increased production of osteoblastic markers, such as AP2, osteoblast-specific factor 2, chondroitin sulfate proteoglycan 4, and the tumor necrosis factor receptor superfamily, compared with control ATSCs, indicating that telomerase expression may aid in maintaining the osteogenic stem cell pool during in vitro expansion. These results show that ectopic expression of the telomerase gene in nonhuman primate ATSCs prevents senescence-associated impairment of osteoblast functions and that telomerase therapy may be a useful strategy for bone regeneration and repair.

Kanjee, U., et al. (2017). "CRISPR/Cas9 knockouts reveal genetic interaction between strain-transcendent erythrocyte determinants of Plasmodium falciparum invasion." Proc Natl Acad Sci U S A **114**(44): E9356-E9365.

During malaria blood-stage infections, Plasmodium parasites interact with the RBC surface to enable invasion followed by intracellular proliferation. Critical factors involved in invasion have been identified using biochemical and genetic approaches including specific knockdowns of genes of interest from primary CD34(+) hematopoietic stem cells (cRBCs). Here we report the development of a robust in vitro culture system to produce RBCs that allow the generation of gene knockouts via CRISPR/Cas9 using the immortal JK-1 erythroleukemia line. JK-1 cells spontaneously differentiate, generating cells at different stages of erythropoiesis, including terminally differentiated nucleated RBCs that we term "jkRBCs." A screen of small-molecule epigenetic regulators identified several bromodomain-specific inhibitors that promote differentiation and enable production of synchronous populations of jkRBCs. Global surface proteomic profiling revealed that jkRBCs express all known Pfalciparum host receptors in a similar fashion to cRBCs and that multiple Pfalciparum strains invade jkRBCs at comparable levels to cRBCs and RBCs. Using CRISPR/Cas9, we deleted two host factors, basigin (BSG) and CD44, for which no natural nulls exist. BSG interacts with the parasite ligand Rh5, a

prominent vaccine candidate. A BSG knockout was completely refractory to parasite invasion in a strain-transcendent manner, confirming the essential role for BSG during invasion. CD44 was recently identified in an RNAi screen of blood group genes as a host factor for invasion, and we show that CD44 knockout results in strain-transcendent reduction in invasion. Furthermore, we demonstrate a functional interaction between these two determinants in mediating Pfalciparum erythrocyte invasion.

Kaplan, F. and F. Teksen (2016). "Apoptotic effects of salinomycin on human ovarian cancer cell line (OVCAR-3)." Tumour Biol **37**(3): 3897-3903.

In this study, we studied the apoptotic and cytotoxic effects of salinomycin on human ovarian cancer cell line (OVCAR-3) as salinomycin is known as a selectively cancer stem cell killer agent. We used immortal human ovarian epithelial cell line (IHOEC) as control group. Ovarian cancer cells and ovarian epithelial cells were treated by different concentrations of salinomycin such as 0.1, 1, and 40 muM and incubated for 24, 48, and 72 h. Dimethylthiazol (MTT) cell viability assay was performed to determine cell viability and toxicity. On the other hand, the expression levels of some of the apoptosis-related genes, namely anti-apoptotic Bcl-2, apoptotic Bax, and Caspase-3 were determined by quantitative real-time polymerase chain reaction (qRT-PCR). Additionally, Caspase-3 protein level was also determined. As a result, we concluded that incubation of human OVCAR-3 by 0.1 muM concentration of salinomycin for 24 h killed 40 % of the cancer cells by activating apoptosis but had no effect on normal cells. The apoptotic Bax gene expression was upregulated but anti-apoptotic Bcl-2 gene expression was downregulated. Active Caspase-3 protein level was increased significantly ($p < 0.05$).

Karpowicz, P., et al. (2005). "Support for the immortal strand hypothesis: neural stem cells partition DNA asymmetrically in vitro." J Cell Biol **170**(5): 721-732.

The immortal strand hypothesis proposes that asymmetrically dividing stem cells (SCs) selectively segregate chromosomes that bear the oldest DNA templates. We investigated cosegregation in neural stem cells (NSCs). After exposure to the thymidine analogue 5-bromo-2-deoxyuridine (BrdU), which labels newly synthesized DNA, a subset of neural precursor cells were shown to retain BrdU signal. It was confirmed that some BrdU-retaining cells divided actively, and that these cells exhibited some characteristics of SCs. This asymmetric partitioning of DNA then was demonstrated during mitosis, and these results were further supported by real time imaging of SC clones, in which older and newly synthesized DNA templates were distributed asymmetrically after DNA synthesis. We demonstrate that NSCs are unique among precursor cells in the uneven partitioning of genetic material during cell divisions.

Karpowicz, P., et al. (2009). "The germline stem cells of Drosophila melanogaster partition DNA non-randomly." Eur J Cell Biol **88**(7): 397-408.

The Immortal Strand Hypothesis proposes that asymmetrically dividing stem cells cosegregate chromatids to retain ancestral DNA templates. Using both pulse-chase and label retention assays, we show that non-random partitioning of DNA occurs in germline stem cells (GSCs) in the Drosophila ovary as these divide asymmetrically to generate a new GSC and a differentiating cystoblast. This process is disrupted when GSCs are forced to differentiate through the overexpression of Bag of Marbles, a factor that impels the terminal differentiation of cystoblasts. When Decapentaplegic, a ligand which maintains the undifferentiated state of GSCs, is expressed ectopically the non-random partitioning of DNA is similarly disrupted. Our data suggest asymmetric chromatid segregation is coupled to mechanisms specifying cellular differentiation via asymmetric stem cell division.

Kars, M. D. and G. Yildirim (2019). "Determination of the target proteins in chemotherapy resistant breast cancer stem cell-like cells by protein array." Eur J Pharmacol **848**: 23-29.

Breast cancer comes second among the causes of cancer deaths of women. Although new generation hormone therapy is a promising strategy, re-occurrence or emergence of drug resistance limits the success. According to the theory of cancer stem cells (CSCs); CSCs are immortal, tumor inducing and self renewing pluripotent cells and multiply as chemotherapy proceeds, making the chemotherapy inefficient. Emerging scientific reports indicate that the mechanisms of drug resistance are the main features that CSCs gain actually. Due to this fact, cancer stem cell markers should be clarified to target CSCs and this will play important role to reverse drug resistance. In this study, MCF-7/Pac, a cell line resistant to microtubule inhibitor paclitaxel and multiple drugs permanently, was used as a reference cell line for drug resistant mammary cancer. It has some properties that breast cancer stem cells possess so it is considerable to isolate breast cancer stem cell-like cells from MCF-7/Pac population. The chemotherapy resistant breast cancer stem-like (BCSC-like) cells were sorted from MCF-7/Pac population by using markers CD44, CD24 and ALDH. At the next step the proteins that are up-regulated in BCSC-like cells were determined by protein array analysis. Additionally the effect of paclitaxel on BCSC-like cell proliferation was determined. The MCF-7/Pac population contains 12.4% BCSC-like cells. The cells bearing BCSC-like cell phenotype exhibited resistance to paclitaxel. The

over-expressed growth factors, MMP proteins, Frizzled proteins and IL-23 were found to be related to the BCSC-like cell proliferation. These results will guide both basic science and medical science.

Kawa, Y., et al. (2000). "Stem cell factor and/or endothelin-3 dependent immortal melanoblast and melanocyte populations derived from mouse neural crest cells." Pigment Cell Res **13 Suppl 8**: 73-80.

Stem cell factor (SCF) and endothelin-3 (ET3) are both necessary for melanocyte development. In order to obtain immortal cell populations of melanoblasts that can survive without feeder cells, we first obtained an immortal cell population of neural crest cells (NCCs) from Sl/+ and +/+ mice of strain WB by incubating with a culture medium supplemented with SCF and ET3, and then we designated them as NCC-SE3 cells. NCC-SE3 cells were bipolar, polygonal, or round in shape and possessed melanosomes of stages I-III (mainly stage I). They were positive to dihydroxyphenylalanine (DOPA) reaction and expressed KIT (a receptor tyrosine kinase), tyrosinase, tyrosinase-related protein-1 (TRP1), tyrosinase-related protein-2 (TRP2), and endothelin-B receptor (ETRB) as determined by immunostaining. We next cultured NCC-SE3 cells by changing culture medium from the one supplemented with SCF + ET3 to the one supplemented with SCF or ET3. NCC-SE3 cells cultured with ET3 alone, designated as NCC-E3 cells, were bipolar in shape and had mainly stage II melanosomes and expressed the same proteins as did NCC-SE3 cells. However, NCC-SE3 cells cultured with SCF alone, designated as NCC-S4.1 cells, were polygonal in shape and had mainly stage I melanosomes. They are thought to be more immature because they were positive to KIT, TRP1, and TRP2, but not to ETR(B), tyrosinase, and DOPA reaction. When 12-O-tetradecanoylphorbol 13-acetate and cholera toxin were added to the culture medium, NCC-S4.1 cells changed shape from polygonal to bipolar and became DOPA-positive. This suggests that NCC-S4.1 cells are melanoblasts that have the potential to differentiate into melanocytes. These cell populations will be extremely useful to study factors that affect melanocyte development and melanogenesis.

Kawa, Y., et al. (2005). "Establishment of a kit-negative cell line of melanocyte precursors from mouse neural crest cells." Pigment Cell Res **18**(3): 188-195.

We previously established a mouse neural crest cell line named NCCmelb4, which is positive for Kit and negative for tyrosinase. NCCmelb4 cells were useful to study the effects of extrinsic factors such as retinoic acids and vitamin D(3) on melanocyte differentiation, but in order to study the development of melanocytes from multipotent neural crest cells, cell lines of melanocyte progenitors in earlier developmental stages are needed. In the present study, we established an immortal cell line named NCCmelb4M5 that was derived from NCCmelb4 cells. NCCmelb4M5 cells do not express Kit and are immortal and stable in the absence of Kit ligand. They are positive for melanocyte markers such as tyrosinase-related protein 1 and DOPAchrome tautomerase and they contain stage I melanosomes. Interestingly, glial fibrillary acidic protein, which is a marker for glial cells, is also positive in NCCmelb4M5 cells, while NCCmelb4 cells are negative for this protein. Immunostaining and a cell ELISA assay revealed that 12-O-tetradecanoylphorbol 13-acetate (TPA) and cholera toxin (CT) induce Kit expression in NCCmelb4M5 cells. Real-time polymerase chain reaction analysis also demonstrated the induction of Kit mRNA by TPA and CT. Microphthalmia-associated transcription factor mRNA is simultaneously enhanced by the same treatment. Kit induced by TPA/CT in NCCmelb4M5 cells disappeared after the cells were subcultured and incubated without TPA/CT. These findings show that NCCmelb4M5 cells have the potential to differentiate into Kit-positive melanocyte precursors and may be useful to study mechanisms of development and differentiation of melanocytes in mouse neural crest cells.

Kawakami, T., et al. (2008). "BMP-4 upregulates Kit expression in mouse melanoblasts prior to the Kit-dependent cycle of melanogenesis." J Invest Dermatol **128**(5): 1220-1226.

Genes encoding Kit and the Kit ligand (KL) play essential roles in the differentiation of melanoblasts. We previously established three immortal but distinct cell populations of mouse neural crest (NC) cells. NCCmelb4M5 cells do not express Kit and grow independently of KL; they have the potential to differentiate into NCCmelb4 cells, which are Kit-positive melanocyte precursors. NCCmelan5 cells show the characteristics of differentiated melanocytes. All three cell lines demonstrated bone morphogenetic protein (BMP) receptor expression. BMP-4 upregulated Kit protein and mRNA expression in most immature NCCmelb4M5 cells. Noggin, a BMP-4 antagonist, dramatically decreased the Kit expression induced by BMP-4. Western blot analysis revealed that extrinsic BMP-4 leads to the phosphorylation of Smads in NCCmelb4M5 cells. Using transfected Kit-promoter reporter, we showed BMP-4 could activate Kit promoter in transfected NCCmelb4M5 cells. We conclude that BMP-4 is active and is involved in the regulation of Kit expression on most immature melanocyte precursors. We further investigated the influence of BMP-4 in vitro using primary NC cells cultured from wild-type mice. Addition of BMP-4 to the medium increased the number of Kit-positive cells compared to diluent-

treated controls. We have identified BMP-4 as an important factor for prenatal Kit-negative melanoblasts just prior to entering the Kit-dependent cycle of melanogenesis.

Kawakami, T., et al. (2002). "Transforming growth factor beta1 regulates melanocyte proliferation and differentiation in mouse neural crest cells via stem cell factor/KIT signaling." J Invest Dermatol **118**(3): 471-478.

Stem cell factor is essential to the migration and differentiation of melanocytes during embryogenesis based on the observation that mutations in either the stem cell factor gene, or its ligand, KIT, result in defects in coat pigmentation in mice. Stem cell factor is also required for the survival of melanocyte precursors while they are migrating towards the skin. Transforming growth factor beta1 has been implicated in the regulation of both cellular proliferation and differentiation. NCC-melb4, an immortal cloned cell line, was cloned from a mouse neural crest cell. NCC-melb4 cells provide a model to study the specific stage of differentiation and proliferation of melanocytes. They also express KIT as a melanoblast marker. Using the NCC-melb4 cell line, we investigated the effect of transforming growth factor beta1 on the differentiation and proliferation of immature melanocyte precursors. Immunohistochemically, NCC-melb4 cells showed transforming growth factor beta1 expression. The anti-transforming growth factor beta1 antibody inhibited the cell growth, and downregulated the KIT protein and mRNA expression. To investigate further the activation of autocrine transforming growth factor beta1, NCC-melb4 cells were incubated in nonexogenous transforming growth factor beta1 culture medium. KIT protein decreased with anti-transforming growth factor beta1 antibody concentration in a concentration-dependent manner. We concluded that in NCC-melb4 cells, transforming growth factor beta1 promotes melanocyte precursor proliferation in autocrine and/or paracrine regulation. We further investigated the influence of transforming growth factor beta1 in vitro using a neural crest cell primary culture system from wild-type mice. Anti-transforming growth factor beta1 antibody decreased the number of KIT positive neural crest cell. In addition, the anti-transforming growth factor beta1 antibody supplied within the wild-type neural crest explants abolished the growth of the neural crest cell. These results indicate that transforming growth factor beta1 affect melanocyte precursor proliferation and differentiation in the presence of stem cell factor/KIT in an autocrine/paracrine manner.

Khorashadizadeh, M., et al. (2015). "Bypassing the need for pre-sensitization of cancer cells for anticancer TRAIL therapy with secretion of novel cell penetrable form of Smac from hA-MSCs as cellular delivery vehicle." Tumour Biol **36**(6): 4213-4221.

TNF-related apoptosis inducing ligand (TRAIL) is a novel anticancer agent with selective apoptosis-inducing activity on cancer cells. However, many malignant tumors still remain unresponsive. Although cells can bypass apoptosis by different functions, the defect in the blocking role of second mitochondria-derived activator of caspase (Smac) on X-linked inhibitor of apoptosis protein (XIAP) is known to be an important hub for immortal characteristic of malignant cells. Actually, XIAP is known as an apoptosis inhibitor. To date, the sensitization of cancerous cells to TRAIL was successfully performed with different protocols, mainly through blocking XIAP with Smac administration. However, all these sensitization methodologies need to be performed prior to TRAIL administration on cancerous cells which in turn limit their practical application in clinics. Therefore, we hypothesized that concurrent expression of Smac and TRAIL on human adipose-derived mesenchymal stem cells (hA-MSC-ST) could both sensitize and destroy cancerous cells. To this aim, we generated hA-MSC-ST, secreting a novel cell penetrable form of Smac and a trimeric form of TRAIL. Indeed, the cell penetrable form of Smac obviates the need for any pretreatment of cancerous cells. Our data depicted that individual overexpression of TRAIL or Smac in different breast cancer cell types induced limited or no apoptosis, respectively. Conversely, their concomitant overexpression markedly increased cell death even for a resistant type of breast cancer cells, MCF-7. Notably, we observed no cytotoxicity of our methodology on normal cells. In summary, this is the first demonstration that a dual approach using simultaneous overexpression of a cell penetrable form of Smac and TRAIL sensitize and promote apoptotic process even in resistant breast cancer cells.

Kiatpongsan, S., et al. (2006). "Future cancer management with stem cell knowledge and technology." J Med Assoc Thai **89**(8): 1322-1332.

Cancer has been proposed as a result of abnormal control of growth and development of stem cells for more than century. This is the "cancer stem cell hypothesis". Both cancer and stem cells share many common especial properties. They are immortal and have good differentiation potential. In addition, organogenesis and carcinogenesis are very similar processes. Recently, more evidence and convincing data from stem cell biology research are supporting this concept. Furthermore, the research provides new promising approaches for cancer diagnosis and treatment based on stem cell knowledge and technology. Upcoming data and evidence may revolutionize cancer management, making it more effective and safer.

Kiel, M. J., et al. (2007). "Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU." Nature **449**(7159): 238-242.

Stem cells are proposed to segregate chromosomes asymmetrically during self-renewing divisions so that older ('immortal') DNA strands are retained in daughter stem cells whereas newly synthesized strands segregate to differentiating cells. Stem cells are also proposed to retain DNA labels, such as 5-bromo-2-deoxyuridine (BrdU), either because they segregate chromosomes asymmetrically or because they divide slowly. However, the purity of stem cells among BrdU-label-retaining cells has not been documented in any tissue, and the 'immortal strand hypothesis' has not been tested in a system with definitive stem cell markers. Here we tested these hypotheses in haematopoietic stem cells (HSCs), which can be highly purified using well characterized markers. We administered BrdU to newborn mice, mice treated with cyclophosphamide and granulocyte colony-stimulating factor, and normal adult mice for 4 to 10 days, followed by 70 days without BrdU. In each case, less than 6% of HSCs retained BrdU and less than 0.5% of all BrdU-retaining haematopoietic cells were HSCs, revealing that BrdU has poor specificity and poor sensitivity as an HSC marker. Sequential administration of 5-chloro-2-deoxyuridine and 5-iodo-2-deoxyuridine indicated that all HSCs segregate their chromosomes randomly. Division of individual HSCs in culture revealed no asymmetric segregation of the label. Thus, HSCs cannot be identified on the basis of BrdU-label retention and do not retain older DNA strands during division, indicating that these are not general properties of stem cells.

Kim, G. L., et al. (2011). "Generation of immortal cell lines from the adult pituitary: role of cAMP on differentiation of SOX2-expressing progenitor cells to mature gonadotropes." PLoS One **6**(11): e27799.

The pituitary is a complex endocrine tissue composed of a number of unique cell types distinguished by the expression and secretion of specific hormones, which in turn control critical components of overall physiology. The basic function of these cells is understood; however, the molecular events involved in their hormonal regulation are not yet fully defined. While previously established cell lines have provided much insight into these regulatory mechanisms, the availability of representative cell lines from each cell lineage is limited, and currently none are derived from adult pituitary. We have therefore used retroviral transfer of SV40 T-antigen to mass immortalize primary pituitary cell culture from an adult mouse. We have generated 19 mixed cell cultures that contain cells from pituitary cell lineages, as determined by RT-PCR analysis and immunocytochemistry for specific hormones. Some lines expressed markers associated with multipotent adult progenitor cells or transit-amplifying cells, including SOX2, nestin, S100, and SOX9. The progenitor lines were exposed to an adenylate cyclase activator, forskolin, over 7 days and were induced to differentiate to a more mature gonadotrope cell, expressing significant levels of alpha-subunit, LHbeta, and FSHbeta mRNAs. Additionally, clonal populations of differentiated gonadotropes were exposed to 30 nM gonadotropin-releasing hormone and responded appropriately with a significant increase in alpha-subunit and LHbeta transcription. Further, exposure of the lines to a pulse paradigm of GnRH, in combination with 17beta-estradiol and dexamethasone, significantly increased GnRH receptor mRNA levels. This array of adult-derived pituitary cell models will be valuable for both studies of progenitor cell characteristics and modulation, and the molecular analysis of individual pituitary cell lineages.

Kobayashi, N. (2009). "Spreading the wings of organ biology further." Cell Transplant **18**(5): 489-490.

Kobayashi, N., et al. (2004). "Partial hepatectomy and subsequent radiation facilitates engraftment of mouse embryonic stem cells in the liver." Transplant Proc **36**(8): 2352-2354.

For liver-targeted regenerative medicine, embryonic stem (ES) cell-derived hepatocyte-like cells proffer great expectation. In vitro exposure to a combination of various growth factors, such as hepatocyte growth factor and fibroblast growth factor-4, as well as cytokines, leads to differentiation of ES cells into hepatocyte-like cells. We sought to determine the in vivo environment that allowed engraftment of ES cells transplanted to the liver. Thus, we examined the effect of partial hepatectomy (50%) (PHT) and subsequent radiation (RT) of the male Balb/c mouse host liver on ES cell engraftment. ES cells (5 x 10(6)) derived from 129Sv mice were transplanted into the residual liver. The controls were ES cells transplanted into a normal liver. Bromo-deoxy-residine (BrdU)-uptake was performed to evaluate the effect of hepatectomy and RT on hepatocyte regeneration. Mouse ES cells engrafted, forming teratomas in the normal liver without showing any mononuclear infiltration. A liver modified by PHT and RT facilitated engraftment of mouse ES cells compared with a normal liver. Hepatic RT significantly suppressed hepatocytic uptake of BrdU.

Komada, Y. and M. Sakurai (1994). "Cytokines and cytokine receptors in acute lymphoblastic leukemia expressing myeloid markers--role in growth regulation." Leuk Lymphoma **15**(5-6): 411-418.

There is no evidence that cancer cells including leukemic cells are immortal. It has been clearly indicated that certain cytokines can significantly stimulate leukemic cell proliferation in vitro, and

sustain the circuit of autocrine or paracrine stimulation. The biological roles of cytokines and cytokine receptors have been intensively investigated in acute leukemia. Recently coexpression of both lymphoid and myeloid features on a single leukemic cell has been well recognized using a flowcytometric technique. Studies of ALL cells expressing myeloid markers (My+ ALL) have indicated that the profiles of cytokines and cytokine receptors expressed by My+ ALL show both similarities and differences to those in My- ALL or acute myelogenous leukemia (AML), suggesting that My+ ALL cells may originate from uncommitted hematopoietic precursor cells coexpressing features of both lymphoid and myeloid lineages. The exact assessment of cytokine response of leukemic cells would provide an important tool for phenotyping acute leukemia based on the growth properties of the cells (cytokine phenotyping), in addition to the morphologic classification and immunological surface phenotyping. Additionally alteration of sensitivity to cytotoxic anticancer drugs by cytokine stimulation may be the new strategy for biologic therapy of acute leukemia.

Kondoh, H., et al. (2007). "A high glycolytic flux supports the proliferative potential of murine embryonic stem cells." Antioxid Redox Signal **9**(3): 293-299.

Embryonic stem (ES) cells are immortal and present the ability to self-renew while retaining their ability to differentiate. In contrast, most primary cells possess a limited proliferative potential, and when this is exhausted, undergo an irreversible growth arrest termed senescence. In primary cells, senescence can be also triggered by a variety of stress to which ES cells are highly refractory. Here the authors report that the proliferative capacity of murine ES cells closely correlates with high activity of different glycolytic enzymes, elevated glycolytic flux, and low mitochondrial oxygen consumption. The direct relation between glycolytic flux and the ability of ES cells to proliferate is further remarked in experiments where glycolysis or ES cell self-renewal was specifically inhibited. It was previously reported that the upregulation of glycolysis in primary cells results in life span extension. The authors hypothesize that the naturally high glycolytic flux observed in murine ES cells can be responsible for their unlimited proliferative potential.

Kono, K., et al. (2015). "Characterization of the cell growth analysis for detection of immortal cellular impurities in human mesenchymal stem cells." Biologicals **43**(2): 146-149.

The analysis of in vitro cell senescence/growth after serial passaging can be one of ways to show the absence of immortalized cells, which are frequently tumorigenic, in human cell-processed therapeutic products (hCTPs). However, the performance of the cell growth analysis for detection of the immortalized cellular impurities has never been evaluated. In the present study, we examined the growth rates of human mesenchymal stem cells (hMSCs, passage 5 (P = 5)) contaminated with various doses of HeLa cells, and compared with that of hMSCs alone. The growth rates of the contaminated hMSCs were comparable to that of hMSCs alone at P = 5, but significantly increased at P = 6 (0.1% and 0.01% HeLa) or P = 7 (0.001% HeLa) within 30 days. These findings suggest that the cell growth analysis is a simple and sensitive method to detect immortalized cellular impurities in hCTPs derived from human somatic cells.

Kono, K., et al. (2017). "Corrigendum to "Characterization of the cell growth analysis for detection of immortal cellular impurities in human mesenchymal stem cells" [Biologicals 43 (2) (March 2015) 146-149]." Biologicals **45**: 106.

Konrad, L., et al. (2005). "Rat Sertoli cells express epithelial but also mesenchymal genes after immortalization with SV40." Biochim Biophys Acta **1722**(1): 6-14.

A new immortal Sertoli cell line from pubertal rat testis was established and characterized. We have generated the clonal line SCIT-C8 expressing established markers for Sertoli cells (SC) like transferrin, clusterin and steel factor/stem cell factor (SCF). Additionally, the immortalized cells express afadin, a protein which is a member of tight and adherens junctions, therefore the cells may be useful for studies of the blood-testis barrier (BTB) in vitro. In contrast to primary SC, the immortalized cells lost expression of androgen receptor and responsiveness to androgens and follicle-stimulating hormone. Surprisingly, we found mRNA expression and protein secretion of the mesenchymal markers, fibronectin and entactin-1, which we also observed for the immortalized SC lines, ASC-17D and 93RS2. In comparison to primary SC, the immortalized cells demonstrated enhanced adhesion in vitro. This correlated with the expression of entactin-1 because adhesion was strongly reduced by antibody perturbation experiments. Additionally, we found the alternatively spliced and primarily muscle cell-specific long variant of TGF-beta2 not only in peritubular cells (PC), but also in the primary and immortalized SC. Furthermore, all immortalized cell lines secreted higher amounts of TGF-beta2 than primary SC. In conclusion, the immortalized SC lines from different developmental stages showed a similar pattern of epithelial and mesenchymal markers.

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

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

Kraemer, P. M., et al. (1986). "Spontaneous immortalization rate of cultured Chinese hamster cells." J Natl Cancer Inst **76**(4): 703-709.

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.

Krones-Herzig, A., et al. (2003). "Early growth response 1 protein, an upstream gatekeeper of the p53 tumor suppressor, controls replicative senescence." Proc Natl Acad Sci U S A **100**(6): 3233-3238.

The proliferation of most primary cells in culture is limited by replicative senescence and crisis, p53-dependent events. However, the regulation of p53 itself has not been defined. We find that deletion of the early growth response 1 (EGR1) transcription factor leads to a striking phenotype, including complete bypass of senescence and apparent immortal growth consistent with loss of a suppressor gene. EGR1-null mouse embryo fibroblasts (MEFs) exhibit decreased expression of p53, p21(Cip1/Waf1), and other p53 "marker" proteins. Precrisis WT but not EGR1-null cells exhibit irradiation-induced arrest. WT MEFs that emerge from crisis exhibit a mutated p53 (sequence confirmed), colony formation, and tumorigenicity. In contrast, high-passage EGR1-null MEFs retain the WT p53 sequence but with much reduced expression, remain untransformed, and grow continuously. An EGR1-expressing retrovirus restores p53 expression and sencescence to EGR1-null but not p53-null MEFs or postcrisis WT cells. Taken together, the results establish EGR1 as a major regulator of cell senescence and previously undescribed upstream "gatekeeper" of the p53 tumor suppressor pathway.

Kuroki, T. and Y. Murakami (1989). "Random segregation of DNA strands in epidermal basal cells." Jpn J Cancer Res **80**(7): 637-642.

According to the hypothesis proposed by Cairns, stem cells retain the older of the two parental DNA strands, whereas differentiating daughter cells receive the newly synthesized strand, so that a set of "immortal strands" persists in stem cells through successive cell divisions. To test this hypothesis, five successive divisions were induced in basal epidermal cells in vivo by two injections of cholera toxin into mouse skin and cells labeled with [3H]thymidine at the first cell cycle were chased for 50 days. If selective segregation occurs, the labeled strand should be transferred into a non-stem daughter cell after the second division and labeled cells would eventually be eliminated from the epidermis. However, the results suggest random segregation of DNA strands in epidermal basal cells. Labeled basal cells were

persistently present throughout the whole epidermis for 50 days. Furthermore, labeled mitotic cells were found after the third division and their numbers of grains decreased exponentially through 5 cycles of divisions.

Kusuma, G. D., et al. (2017). "Decellularized extracellular matrices produced from immortal cell lines derived from different parts of the placenta support primary mesenchymal stem cell expansion." PLoS One **12**(2): e0171488.

Mesenchymal stem/stromal cells (MSCs) exhibit undesired phenotypic changes during ex vivo expansion, limiting production of the large quantities of high quality primary MSCs needed for both basic research and cell therapies. Primary MSCs retain many desired MSC properties including proliferative capacity and differentiation potential when expanded on decellularized extracellular matrix (dECM) prepared from primary MSCs. However, the need to use low passage number primary MSCs (passage 3 or lower) to produce the dECM drastically limits the utility and impact of this technology. Here, we report that primary MSCs expanded on dECM prepared from high passage number (passage 25) human telomerase reverse transcriptase (hTERT) transduced immortal MSC cell lines also exhibit increased proliferation and osteogenic differentiation. Two hTERT-transduced placenta-derived MSC cell lines, CMSC29 and DMSC23 [derived from placental chorionic villi (CMSCs) and decidua basalis (DMSCs), respectively], were used to prepare dECM-coated substrates. These dECM substrates showed structural and biochemical differences. Primary DMSCs cultured on dECM-DMSC23 showed a three-fold increase in cell number after 14 days expansion in culture and increased osteogenic differentiation compared with controls. Primary CMSCs cultured on the dECM-DMSC23 exhibited a two-fold increase in cell number and increased osteogenic differentiation. We conclude that immortal MSC cell lines derived from different parts of the placenta produce dECM with varying abilities for supporting increased primary MSC expansion while maintaining important primary MSC properties. Additionally, this is the first demonstration of using high passage number cells to produce dECM that can promote primary MSC expansion, and this advancement greatly increases the feasibility and applicability of dECM-based technologies.

Lamb, R., et al. (2015). "Dissecting tumor metabolic heterogeneity: Telomerase and large cell size metabolically define a sub-population of stem-like, mitochondrial-rich, cancer cells." Oncotarget **6**(26): 21892-21905.

Tumor cell metabolic heterogeneity is thought to contribute to tumor recurrence, distant metastasis and chemo-resistance in cancer patients, driving poor clinical outcome. To better understand tumor metabolic heterogeneity, here we used the MCF7 breast cancer line as a model system to metabolically fractionate a cancer cell population. First, MCF7 cells were stably transfected with an hTERT-promoter construct driving GFP expression, as a surrogate marker of telomerase transcriptional activity. To enrich for immortal stem-like cancer cells, MCF7 cells expressing the highest levels of GFP (top 5%) were then isolated by FACS analysis. Notably, hTERT-GFP(+) MCF7 cells were significantly more efficient at forming mammospheres (i.e., stem cell activity) and showed increased mitochondrial mass and mitochondrial functional activity, all relative to hTERT-GFP(-) cells. Unbiased proteomics analysis of hTERT-GFP(+) MCF7 cells directly demonstrated the over-expression of 33 key mitochondrial proteins, 17 glycolytic enzymes, 34 ribosome-related proteins and 17 EMT markers, consistent with an anabolic cancer stem-like phenotype. Interestingly, MT-CO2 (cytochrome c oxidase subunit 2; Complex IV) expression was increased by >20-fold. As MT-CO2 is encoded by mt-DNA, this finding is indicative of increased mitochondrial biogenesis in hTERT-GFP(+) MCF7 cells. Importantly, most of these candidate biomarkers were transcriptionally over-expressed in human breast cancer epithelial cells in vivo. Similar results were obtained using cell size (forward/side scatter) to fractionate MCF7 cells. Larger stem-like cells also showed increased hTERT-GFP levels, as well as increased mitochondrial mass and function. Thus, this simple and rapid approach for the enrichment of immortal anabolic stem-like cancer cells will allow us and others to develop new prognostic biomarkers and novel anti-cancer therapies, by specifically and selectively targeting this metabolic sub-population of aggressive cancer cells. Based on our proteomics and functional analysis, FDA-approved inhibitors of protein synthesis and/or mitochondrial biogenesis, may represent novel treatment options for targeting these anabolic stem-like cancer cells.

Landskron, L., et al. (2018). "The asymmetrically segregating lncRNA cherub is required for transforming stem cells into malignant cells." Elife **7**.

Tumor cells display features that are not found in healthy cells. How they become immortal and how their specific features can be exploited to combat tumorigenesis are key questions in tumor biology. Here we describe the long non-coding RNA cherub that is critically required for the development of brain tumors in Drosophila but is dispensable for normal development. In mitotic Drosophila neural stem cells, cherub localizes to the cell periphery and segregates into the differentiating daughter cell. During tumorigenesis, de-differentiation of cherub-high cells leads to the formation of tumorigenic stem cells that accumulate abnormally high cherub levels. We show

that cherub establishes a molecular link between the RNA-binding proteins Staufen and Syncrip. As Syncrip is part of the molecular machinery specifying temporal identity in neural stem cells, we propose that tumor cells proliferate indefinitely, because cherub accumulation no longer allows them to complete their temporal neurogenesis program.

Lansdorp, P. M. (1995). "Developmental changes in the function of hematopoietic stem cells." Exp Hematol **23**(3): 187-191.

The observation that colony-forming unit-spleen (CFU-S) cells can be found in the yolk sac, fetal liver, and various hematopoietic tissues in adult mice has contributed to the concept that "stem cells" represent a single type of immortal cell that is present at variable numbers in tissues at different stages of development. However, more recent studies have shown dramatic changes in various functional properties of purified "candidate" stem cells during ontogeny. Together with previous studies documenting developmental changes in early hematopoietic cells and observations showing a decrease in the mean telomere length of chromosomes in hematopoietic cells during ontogeny, these findings are difficult to reconcile with the concept of a stem cell as an immortal and invariable cell type. In this minireview, some developmental aspects of stem cell biology are discussed in the context of stem cell definitions and transplantation biology.

Lansdorp, P. M. (2007). "Immortal strands? Give me a break." Cell **129**(7): 1244-1247.

The "immortal strand" hypothesis proposes that asymmetrically dividing stem cells selectively retain chromosomes containing "old" DNA to prevent accumulation of mutations. As I describe in this Essay, such a possibility seems unlikely. An alternative explanation is that asymmetric cell divisions and cell fate are codirected by epigenetic differences between sister chromatids.

Lawoko-Kerali, G., et al. (2004). "Ventral otic cell lines as developmental models of auditory epithelial and neural precursors." Dev Dyn **231**(4): 801-814.

Conditionally immortal cell lines were established from the ventral otocyst of the Immortomouse at embryonic day 10.5 and selected to represent precursors of auditory sensory neural and epithelial cells. Selection was based upon dissection, tissue-specific markers, and expression of the transcription factor GATA3. Two cell lines expressed GATA3 but possessed intrinsically different genetic programs under differentiating conditions. US/VOT-E36 represented epithelial progenitors with potential to differentiate into sensory and nonsensory epithelial cells. US/VOT-N33 represented migrating neuroblasts. Under differentiating conditions in vitro the cell lines expressed very different gene expression profiles. Expression of several cell- and tissue-specific markers, including the transcription factors Pax2, GATA3, and NeuroD, differed between the cell lines in a pattern consistent with that observed between their counterparts in vivo. We suggest that these and other conditionally immortal cell lines can be used to study transient events in development against different backgrounds of cell competence.

Lebkowski, J. S., et al. (2001). "Human embryonic stem cells: culture, differentiation, and genetic modification for regenerative medicine applications." Cancer J **7 Suppl 2**: S83-93.

Human embryonic stem (hES) cells can proliferate extensively in culture and can differentiate into representatives of all three embryonic germ layers in vitro and in vivo. The undifferentiated hES cells have now been cultured for more than 50 passages in vitro, yet maintain a normal karyotype. The hES cells express a series of specific surface antigens, as well as OCT-4 and human telomerase, proteins associated with a pluripotent and immortal phenotype. On differentiation, OCT-4 and human telomerase expression decreases with the emergence of a maturing population of cells. During hES cell differentiation, modulation of the expression of many genes has been evaluated using microarray analysis. To improve the ease, reproducibility, and scalability of hES culture, methods have been developed to propagate the cells in the absence of mouse embryonic cell feeders. hES cells maintained in culture using extracellular matrix factors together with mouse embryonic cell conditioned medium proliferate indefinitely while maintaining a normal karyotype, proliferation rate, and complement of undifferentiated cell markers. hES cells cultured without feeder layers retain their capacity to differentiate into cells of all three germ layers in vitro and in teratomas. The hES cells can also be genetically modified transiently or stably using both plasmid and viral gene transfer agents. These analyses and technological developments will aid in the realization of the full potential of hES cells for both research and therapeutic applications.

Lee, H., et al. (2011). "Stem-cell-triggered immunity through CLV3p-FLS2 signalling." Nature **473**(7347): 376-379.

Stem cells in the shoot apical meristem (SAM) of plants are the self-renewable reservoir for leaf, stem and flower organogenesis. In nature, disease-free plants can be regenerated from SAM despite infections elsewhere, which underlies a horticultural practice for decades. However, the molecular basis of the SAM immunity remains unclear. Here we show that the CLAVATA3 peptide (CLV3p), expressed and secreted from stem cells and functioning as a key regulator of stem-cell homeostasis in the SAM of

Arabidopsis, can trigger immune signalling and pathogen resistance via the flagellin receptor kinase FLS2 (refs 5, 6). CLV3p-FLS2 signalling acts independently from the stem-cell signalling pathway mediated through CLV1 and CLV2 receptors, and is uncoupled from FLS2-mediated growth suppression. Endogenous CLV3p perception in the SAM by a pattern recognition receptor for bacterial flagellin, FLS2, breaks the previously defined self and non-self discrimination in innate immunity. The dual perception of CLV3p illustrates co-evolution of plant peptide and receptor kinase signalling for both development and immunity. The enhanced immunity in SAM or germ lines may represent a common strategy towards immortal fate in plants and animals.

Lee, W. C., et al. (2014). "High-throughput synchronization of mammalian cell cultures by spiral microfluidics." Methods Mol Biol **1104**: 3-13.

The development of mammalian cell cycle synchronization techniques has greatly advanced our understanding of many cellular regulatory events and mechanisms specific to different phases of the cell cycle. In this chapter, we describe a high-throughput microfluidic-based approach for cell cycle synchronization. By exploiting the relationship between cell size and its phase in the cell cycle, large numbers of synchronized cells can be obtained by size fractionation in a spiral microfluidic channel. Protocols for the synchronization of primary cells such as mesenchymal stem cells, and immortal cell lines such as Chinese hamster ovarian cells (CHO-CD36) and HeLa cells are provided as examples.

Ludes-Meyers, J. H., et al. (2001). "AP-1 blockade inhibits the growth of normal and malignant breast cells." Oncogene **20**(22): 2771-2780.

We have previously demonstrated that basal AP-1 transcriptional activity is high in normal human mammary epithelial cells, intermediate in immortal breast cells, and relatively low in breast cancer cells. In this study we investigated whether differences in AP-1 transcriptional activity reflect differences in breast cells' dependence on AP-1 for proliferation. The cJun dominant negative, TAM-67, was used to determine the effect of AP-1 blockade on the growth of normal, immortal and malignant breast cells. We first showed that TAM-67 inhibits AP-1 activity in normal and malignant breast cells. We then determined whether this AP-1 inhibitor affected colony forming efficiency of the immortalized and malignant breast cells. The AP-1 inhibitor reduced colony formation of immortal breast cells by over 50% (by 58% in 184B5 cells and 62% in MCF10A cells), and reduced colony formation in the breast cancer cell line MCF7 by 43%, but did not reduce colony formation in the other breast cancer cell lines (T47D, MDA MB231 and MDA MB 435). We also determined the effect of AP-1 blockade on the growth of normal breast cells using a single cell proliferation assay. Using this assay, the growth of normal breast cells was extremely sensitive to AP-1 blockade, while immortal breast cells were moderately sensitive. We next directly tested the effect of TAM-67 expression on the growth of MCF7 breast cancer cells, using cells stably transfected with TAM-67 under the control of a doxycycline-inducible promoter. Upon induction, TAM-67 was expressed and AP-1 activity was inhibited in these cells. We then measured the growth of these cells in the presence or absence of TAM-67. The results of these studies show that the growth of MCF7 cells was suppressed by the AP-1 inhibitor, TAM-67. These results demonstrate that normal and immortalized breast cells, and some breast cancer cells (such as MCF7), require AP-1 to transduce proliferative signals, while other breast cancer cells (such as T47D, MDA MB 231 and MDA MB 435) do not. These studies suggest that the AP-1 transcription factor is a potential target for future agents for the prevention or treatment of breast cancer.

Macingova, Z. and S. Filip (2008). "Cancer stem cells--new approach to cancerogenensis and treatment." Acta Medica (Hradec Kralove) **51**(3): 139-144.

Recently, there is an increasing evidence supporting the theory of cancer stem cells not only in leukemia but also in solid cancer. To date, the existence of cancer stem cells has been proven in acute and chronic myeloid leukemia, in breast cancer, in brain tumors, in lung cancer and gastrointestinal tumors. This review is focusing on the recent discovery of stem cells in leukemia, human brain tumors and breast cancer. A small population of cells in the tumor (less than 1%) shows the potential to give rise to the tumor and its growth. These cells have a substantial characteristic of stem cells--ability for self-renewal without loss of proliferation capacity with each cell division. Furthermore they are immortal, rather resistant to treatment and express typical markers of stem cells. The origin of these resident cancer stem cells is not clear. Whether the cancer stem cells originate from normal stem cells in consequence of genetic and epigenetic changes and/or redifferentiation from somatic tumor cells to the stem-like cells remains to be investigated. We propose the idea of the relation between normal tissue stem cells and cancer stem cells and their populations--progenitor cells. Based on this we highlight one of the major characteristic of stem cell--plasticity, which is equally important in the physiological regeneration process as well as carcinogenesis. Furthermore, we consider the microenvironment as a limiting factor for tumor genesis in AML, breast cancer and brain tumors. Thus the biological properties of cancer stem cells are just beginning to be revealed, the continuation of these

studies should lead to the development of cancer stem cells target therapies for cancer treatment.

Maiato, H. and Y. Barral (2013). "Unbiased about chromosome segregation: give me a mechanism and I will make you "immortal"." Chromosome Res **21**(3): 189-191.

Makena, M. R., et al. (2020). "Cancer stem cells: Road to therapeutic resistance and strategies to overcome resistance." Biochim Biophys Acta Mol Basis Dis **1866**(4): 165339.

Unlike other normal cells, a subpopulation of cells often termed as "stem cells" are long-lived and generate cellular progeny throughout life. Cancer stem cells (CSCs) are rare immortal cells within a tumor that can both self-renew by dividing and giving rise to many cell types that constitute the tumor. CSCs also have been shown to be involved in fundamental processes of cell proliferation and metastatic dissemination. CSCs are generally resistant to chemotherapy and radiotherapy, a subset of remaining CSCs after therapy can survive and promote cancer relapse and resistance to therapies. Understanding the biological characteristics of CSCs, the pathways leading to their sustainability and proliferation, and the CSCs role in drug resistance is crucial for establishing novel tumor diagnostic and therapeutic strategies. In this review, we address the pathways that regulate CSCs, the role of CSCs in the resistance to therapy, and strategies to overcome therapeutic resistance.

Malumbres, M., et al. (2004). "Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6." Cell **118**(4): 493-504.

Cdk4 and Cdk6 are thought to be essential for initiation of the cell cycle in response to mitogenic stimuli. Previous studies have shown that Cdk4 is dispensable for proliferation in most cell types, an observation attributed to a putative compensatory role by Cdk6. Cdk6-null mice are viable and develop normally although hematopoiesis is slightly impaired. Embryos defective for Cdk4 and Cdk6 die during the late stages of embryonic development due to severe anemia. However, these embryos display normal organogenesis and most cell types proliferate normally. In vitro, embryonic fibroblasts lacking Cdk4 and Cdk6 proliferate and become immortal upon serial passage. Moreover, quiescent Cdk4/Cdk6-null cells respond to serum stimulation and enter S phase with normal kinetics although with lower efficiency. These results indicate that D-type cyclin-dependent kinases are not essential for cell cycle entry and suggest the existence of alternative mechanisms to initiate cell proliferation upon mitogenic stimulation.

Marhaba, R., et al. (2008). "CD44 and EpCAM: cancer-initiating cell markers." Curr Mol Med **8**(8): 784-804.

Embryonic stem cells are immortal, can self renew, and differentiate into all cells of the body. The adult organism maintains adult stem cells in regenerative organs that can differentiate into all cells of the respective organ. Virchow's hypothesis that cancer may arise from embryonic-like cells has received strong support, as it was demonstrated that tumors contain few cells, known as cancer stem or cancer-initiating cells (CIC), that account for primary and metastatic tumor growth. CIC are mostly defined by expression of CIC-markers that are associated and correlated with the potential of CIC to grow in xenogeneic mice. CIC marker profiles have been elaborated for many tumors, with several markers as CD24, CD44, CD133, CD166, EpCAM, and some integrins, being expressed by tumors of different histological type. Their function in promoting CIC maintenance and activity is largely unknown. The fate of stem cells, determined by their position, is minutely regulated by few adjacent cells creating a niche. CIC also require a niche, mostly for settlement and growth in distant organs. This so called pre-metastatic niche is initiated by the primary tumor before metastasizing cell arrival. How do CIC prepare the pre-metastatic niche? Cancer cells secrete a matrix that serves a cross-talk with surrounding tissues. Additionally, cancer cells can abundantly deliver exosomes, which function as long-distance intercellular communicators. Studies on a rat pancreatic adenocarcinoma support our hypothesis that tumor-derived matrix and exosomes are the main actors in forming the pre-metastatic niche with CIC markers being engaged in matrix preparation and/or exosome delivery.

Mazzaro, G., et al. (1999). "The role of wild-type p53 in the differentiation of primary hemopoietic and muscle cells." Oncogene **18**(42): 5831-5835.

Experiments previously performed on 32D and C2C12 cell lines indicated that wild type p53 (wtp53) protein has a role in granulocyte and myotube differentiation. Since these are immortal cells, we asked whether the inhibition of differentiation induced by the expression of dominant-negative p53 (dnp53) proteins was dependent on the immortalization-determined microenvironment. Thus, we evaluated the effects produced by interfering with the endogenous p53 gene in murine primary hemopoietic and muscle cells. Expression of dnp53 protein reduced the differentiation of bone marrow cells into granulocytes and macrophages. Moreover, p53 activation was measurable during the differentiation process of primary myoblasts, while interference with this activation led to a consistent slow down of terminal differentiation. Since the impairment of the differentiation was not accompanied by alterations in the cell cycle withdrawal and in the rate of apoptosis which are coupled with these types of differentiation,

the data here reported support a specific role for p53 in the differentiation process. However, the difference in the intensity of inhibition between immortal and primary cells, i. e., complete versus slow down, respectively, suggests that the immortalization process might render the cells more sensitive to the loss of wtp53 activity for the differentiation process.

McKay, R., et al. (1988). "Reconstructing the brain from immortal cell lines." Prog Brain Res **78**: 647-649.

McKay, R., et al. (1993). "Immortalized stem cells from the central nervous system." C R Acad Sci III **316**(12): 1452-1457.

A remarkable feature of the early development of the mammalian central nervous system (CNS) is the precise and rapid generation of large numbers of many different neuronal types [1]. We have identified the major neuronal precursor cell and shown that this cell can be immortalized by oncogenes. The immortal precursor cell can be grown in culture and can differentiate when transplanted into the developing brain. The implanted neurons are integrated into the synaptic circuitry of the host brain. These results suggest that implanting cultured precursor cells will provide a powerful strategy to uncover the signals that control the differentiation of this multipotential cell into the many cell types of the adult brain.

McKay, R., et al. (1990). "Mechanisms regulating cell number and type in the mammalian central nervous system." Cold Spring Harb Symp Quant Biol **55**: 291-301.

In the developing brain, neurons are derived from multipotential precursor cells in a precise sequence, where the time when a neuron becomes postmitotic is closely linked to the differentiated function of the neuron. Work from our group (1) identifies the Nes+ neuronal precursor state, (2) defines growth factors that regulate the proliferation of Nes+ precursor cells and their differentiation into neurons, and (3) establishes immortal cell lines that may be useful models for the terminal differentiation of Nes+ cells into neurons and astrocytes. These results suggest that the basic technical requirements are now in place to define the signals that control the transition from a precursor cell to a postmitotic neuron in the mammalian CNS. Control of this terminal differentiation is a key step in generating the large numbers and types of cells in the CNS.

Meeker, A. K., et al. (1996). "Telomerase is activated in the prostate and seminal vesicles of the castrated rat." Endocrinology **137**(12): 5743-5746.

Telomeres, the repetitive non-coding DNA sequences found at the ends of all eukaryotic chromosomes, shorten with each cell division. It has been proposed that telomere shortening may be the counting element of a mitotic clock that keeps track of cell divisions; with shortening to a critical length acting as a senescence signal underlying cellular aging. The enzyme telomerase functions to maintain telomere length, thus allowing unlimited cell division, and has been associated with cellular immortalization and cancer. Stem cells have large, perhaps unlimited, replicative capacities. Since these cells are potentially immortal, we reasoned that they might posses active telomerase. We therefore assayed for telomerase activity in the stem cell enriched pools of the androgen-depleted sex accessory tissues in the castrated male rat. Following castration, the ventral prostate and seminal vesicles of the rat involute, losing approximately 90% of their cells by 21 days. These residual glands persist, and are enriched for stem cells, being capable of fully regenerating these glands if testosterone is re-introduced into the animal. We assayed telomerase activity in extracts from normal, involuted, and regenerating ventral prostate and seminal vesicles. Normal glands were found to be telomerase negative, whereas telomerase activity appeared as these glands involuted following castration. Conversely, telomerase activity disappeared during testosterone-induced regeneration of these residual glands. These results provide strong evidence for the ability of androgen to negatively-regulate telomerase activity in stem cell populations of the rat ventral prostate and seminal vesicles. and represent the first in vivo model system for the modulation of telomerase activity.

Meena, J. K., et al. (2015). "Telomerase abrogates aneuploidy-induced telomere replication stress, senescence and cell depletion." EMBO J **34**(10): 1371-1384.

The causal role of aneuploidy in cancer initiation remains under debate since mutations of euploidy-controlling genes reduce cell fitness but aneuploidy strongly associates with human cancers. Telomerase activation allows immortal growth by stabilizing telomere length, but its role in aneuploidy survival has not been characterized. Here, we analyze the response of primary human cells and murine hematopoietic stem cells (HSCs) to aneuploidy induction and the role of telomeres and the telomerase in this process. The study shows that aneuploidy induces replication stress at telomeres leading to telomeric DNA damage and p53 activation. This results in p53/Rb-dependent, premature senescence of human fibroblast, and in the depletion of hematopoietic cells in telomerase-deficient mice. Endogenous telomerase expression in HSCs and enforced expression of telomerase in human fibroblasts are sufficient to abrogate aneuploidy-induced replication stress at telomeres and the consequent induction of premature senescence and hematopoietic cell depletion. Together, these results identify telomerase as an aneuploidy

survival factor in mammalian cells based on its capacity to alleviate telomere replication stress in response to aneuploidy induction.

Menendez, J. A. and J. Joven (2014). "Energy metabolism and metabolic sensors in stem cells: the metabostem crossroads of aging and cancer." Adv Exp Med Biol **824**: 117-140.

We are as old as our adult stem cells are; therefore, stem cell exhaustion is considered a hallmark of aging. Our tumors are as aggressive as the number of cancer stem cells (CSCs) they bear because CSCs can survive treatments with hormones, radiation, chemotherapy, and molecularly targeted drugs, thus increasing the difficulty of curing cancer. Not surprisingly, interest in stem cell research has never been greater among members of the public, politicians, and scientists. But how can we slow the rate at which our adult stem cells decline over our lifetime, reducing the regenerative potential of tissues, while efficiently eliminating the aberrant, life-threatening activity of "selfish", immortal, and migrating CSCs? Frustrated by the gene-centric limitations of conventional approaches to aging diseases, our group and other groups have begun to appreciate that bioenergetic metabolism, i.e., the production of fuel & building blocks for growth and division, and autophagy/mitophagy, i.e., the quality-control, self-cannibalistic system responsible for "cleaning house" and "recycling the trash", can govern the genetic and epigenetic networks that facilitate stem cell behaviors. Indeed, it is reasonable to suggest the existence of a "metabostem" infrastructure that operates as a shared hallmark of aging and cancer, thus making it physiologically plausible to maintain or even increase the functionality of adult stem cells while reducing the incidence of cancer and extending the lifespan. This "metabostemness" property could lead to the discovery of new drugs that reprogram cell metabotypes to increase the structural and functional integrity of adult stem cells and positively influence their lineage determination, while preventing the development and aberrant function of stem cells in cancer tissues. While it is obvious that the antifungal antibiotic rapamycin, the polyphenol resveratrol, and the biguanide metformin already belong to this new family of metabostemness-targeting drugs, we can expect a rapid identification of new drug candidates (e.g., polyphenolic xenohormetins) that reverse or postpone "geroncogenesis", i.e., aging-induced metabolic decline as a driver of tumorigenesis, at the stem cell level.

Menendez, J. A., et al. (2013). "Xenohormetic and anti-aging activity of secoiridoid polyphenols present in extra virgin olive oil: a new family of gerosuppressant agents." Cell Cycle **12**(4): 555-578.

Aging can be viewed as a quasi-programmed phenomenon driven by the overactivation of the nutrient-sensing mTOR gerogene. mTOR-driven aging can be triggered or accelerated by a decline or loss of responsiveness to activation of the energy-sensing protein AMPK, a critical gerosuppressor of mTOR. The occurrence of age-related diseases, therefore, reflects the synergistic interaction between our evolutionary path to sedentarism, which chronically increases a number of mTOR activating gero-promoters (e.g., food, growth factors, cytokines and insulin) and the "defective design" of central metabolic integrators such as mTOR and AMPK. Our laboratories at the Bioactive Food Component Platform in Spain have initiated a systematic approach to molecularly elucidate and clinically explore whether the "xenohormesis hypothesis," which states that stress-induced synthesis of plant polyphenols and many other phytochemicals provides an environmental chemical signature that upregulates stress-resistance pathways in plant consumers, can be explained in terms of the reactivity of the AMPK/mTOR-axis to so-called xenohormetins. Here, we explore the AMPK/mTOR-xenohormetic nature of complex polyphenols naturally present in extra virgin olive oil (EVOO), a pivotal component of the Mediterranean style diet that has been repeatedly associated with a reduction in age-related morbid conditions and longer life expectancy. Using crude EVOO phenolic extracts highly enriched in the secoiridoids oleuropein aglycon and decarboxymethyl oleuropein aglycon, we show for the first time that (1) the anticancer activity of EVOO secoiridoids is related to the activation of anti-aging/cellular stress-like gene signatures, including endoplasmic reticulum (ER) stress and the unfolded protein response, spermidine and polyamine metabolism, sirtuin-1 (SIRT1) and NRF2 signaling; (2) EVOO secoiridoids activate AMPK and suppress crucial genes involved in the Warburg effect and the self-renewal capacity of "immortal" cancer stem cells; (3) EVOO secoiridoids prevent age-related changes in the cell size, morphological heterogeneity, arrayed cell arrangement and senescence-associated beta-galactosidase staining of normal diploid human fibroblasts at the end of their proliferative lifespans. EVOO secoiridoids, which provide an effective defense against plant attack by herbivores and pathogens, are bona fide xenohormetins that are able to activate the gerosuppressor AMPK and trigger numerous resveratrol-like anti-aging transcriptomic signatures. As such, EVOO secoiridoids constitute a new family of plant-produced gerosuppressant agents that molecularly "repair" the aimless (and harmful) AMPK/mTOR-driven quasi-program that leads to aging and aging-related diseases, including cancer.

Merok, J. R., et al. (2002). "Cosegregation of chromosomes containing immortal DNA strands in

cells that cycle with asymmetric stem cell kinetics." Cancer Res **62**(23): 6791-6795.

A long-standing intriguing hypothesis in cancer biology is that adult stem cells avoid mutations from DNA replication errors by a unique pattern of chromosome segregation. At each asymmetric cell division, adult stem cells have been postulated to selectively retain a set of chromosomes that contain old template DNA strands (i.e., "immortal DNA strands"). Using cultured cells that cycle with asymmetric cell kinetics, we confirmed both the existence of immortal DNA strands and the cosegregation of chromosomes that bear them. Our findings also lead us to propose a role for immortal DNA strands in tissue aging as well as cancer.

Micallef, S. J., et al. (2007). "Pancreas differentiation of mouse ES cells." Curr Protoc Stem Cell Biol **Chapter 1**: Unit 1G 2.

This unit describes the derivation of pancreatic cells from mouse embryonic stem cells (ESCs). Mouse ESCs are pluripotent immortal cells derived from the inner cell mass of pre-implantation blastocyst-stage embryos that possess the ability to differentiate into any cell type within the adult animal. In vitro, ESCs can be differentiated into a variety of cell types representing derivatives of the three embryonic germ layers, mesoderm, endoderm, and ectoderm. Successfully differentiating ES cells to pancreatic cells has the potential to provide an alternative to cadaver-derived cells for treatment of type I diabetes. This unit outlines a method for the differentiation of ESCs toward pancreatic endoderm in serum-free medium from embryoid bodies (EBs) formed in suspension or spin EBs. In addition there is a protocol for maintaining ESC.

Mihara, K., et al. (2003). "Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase." Br J Haematol **120**(5): 846-849.

To create immortal mesenchymal cell lines, we transduced primary human bone marrow mesenchymal cells with telomerase reverse transcriptase (TERT). TERT+ mesenchymal cells continued to grow for > 2 years; parallel TERT- cultures underwent senescence after 15 weeks. TERT+ mesenchymal cells did not form foci in soft agar, had a normal karyotype and could differentiate into osteoblasts and chondrocytes. Their capacity to support leukaemic lymphoblasts and normal CD34+ haematopoietic cells was equal to or greater than that of primary cells; 42 TERT+ mesenchymal cell clones varied in their supporting capacity. Immortalized mesenchymal cells offer a promising tool for identifying molecules that regulate human haematopoiesis.

Miura, T., et al. (2004). "Cellular lifespan and senescence signaling in embryonic stem cells." Aging Cell **3**(6): 333-343.

Most mammalian cells when placed in culture will undergo a limited number of cell divisions before entering an unresponsive non-proliferating state termed senescence. However, several pathways that are activated singly or in concert can allow cells to bypass senescence at least for limited periods. These include the telomerase pathway required to maintain telomere ends, the p53 and Rb pathways required to direct senescence in response to DNA damage, telomere shortening and mitogenic signals, and the insulin-like growth factor--Akt pathway that may regulate lifespan and cell proliferation. In this review, we summarize recent findings related to these pathways in embryonic stem (ES) cells and suggest that ES cells are immortal because these pathways are tightly regulated.

Miyazaki, M., et al. (1993). "Immortalization of epithelial-like cells from human liver tissue with SV40 T-antigen gene." Exp Cell Res **206**(1): 27-35.

The cells derived from the human embryo liver tissue were transfected with a plasmid pSV3neo containing both the large and small T-antigen gene of the early region of simian virus 40 (SV40), and two cell strains, OUMS-21 and -22, were obtained. OUMS-22 cells, to date, have reached over 100 population doublings through a culture crisis and are considered to have become an immortal cell line. However, OUMS-21 cells failed to become an immortal cell line. Both OUMS-21 and -22 cells were SV40 T-antigen-positive, epithelial-like, and immunoreactive against an anti-keratin 18 monoclonal antibody but against neither an anti-vimentin nor an anti-von Willebrandt factor VIII monoclonal antibody. The staining pattern of cytokeratin in these cells was similar to that in the differentiated human hepatoblastoma and hepatocellular carcinoma cell lines but not to that in the human cholangiocellular carcinoma cell lines. OUMS-21 and -22 cells expressed neither alpha-fetoprotein nor albumin mRNAs. These cells showed no tyrosine aminotransferase activity. However, both OUMS-21 and -22 cells were sensitive to cytotoxicity of aflatoxin B1, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, and benzo[a]pyrene, whereas human embryo lung fibroblasts were insensitive to the cytotoxicity of these carcinogens. These findings suggest that OUMS-21 and -22 cells may arise from undifferentiated liver stem cells or from hepatocytes that lost their ability to express the liver-specific functions prior to immortalization. Both OUMS-21 and -22 cells expressed glutathione S-transferase pi (GST-pi) mRNA. The expression of GST-pi mRNA highly increased in OUMS-22 cells with their immortalization. Karyotypic analysis showed that numerical and structural aberrations of the

chromosomes were profound, but neither specific events nor marker chromosomes were found in OUMS-21 and -22 cells. Both OUMS-21 and -22 cells could grow in soft agar, but they were not tumorigenic when transplanted into nude mice.

Mizoguchi, M. (2004). "Melanocyte development: with a message of encouragement to young women scientists." Pigment Cell Res **17**(5): 533-544.

This is a semi-biographical review describing my research on melanocyte development and related personal experiences. Having been educated and trained as a dermatologist, I have been involved in many clinically-oriented studies, however, what has always interested me the most is pigment cell biology. Since I started working at St Marianna University in 1991, I have been undertaking research on melanocyte development and relevant growth factors using mice as models. My research in this field was inspired by my collaborations with various scientists, mostly from the field of biology. Many of these specialists I have met at meetings of the Societies of Pigment Cell Research (PCR). Stem cell factor (SCF, Kitl) and endothelin 3 (EDN3) have been identified as indispensable factors regulating the development of melanocytes. Mice mutant at loci encoding those factors (or their receptors) such as Sl/Sl (receptors W/W) and ls/ls (receptors s/s) have white coat colors and white patches, respectively. Our murine neural crest cell (NCC) primary cultures derived from Sl/Sl embryos showed that EDN3 cannot develop melanocyte precursors without SCF and that EDN3 can elicit proliferation and differentiation in the presence of SCF. These results suggest that without EDN3 and the endothelin type B receptor (EDNRB), melanocytes can not fully increase in number, which could well be the cause of the partial white coat color of ls/ls and s/s mice. Contamination with factors derived from the serum in medium or in feeder cells sometimes causes experimental errors, and therefore we established three immortal cell lines derived from NCC in different developmental stages and designated them as NCCmelb4, NCCmelb4M5 and NCCmelan5, all of which can survive without feeder cells. Using these cell lines and NCC primary cultures, we studied the effect of many factors related to melanocyte development. From the results, it has become evident that Vitamin D3 induces EDNRB expression by NCCmelb4 cells. In addition to the International Pigment Cell Conference (IPCC), I have also taken part in many annual meetings of the Japanese Society for Pigment Cell Research (JSPCR), Pan American Society for Pigment Cell Research (PASPCR) and European Society for Pigment Cell Research (ESPCR). Not only have I learned a great deal, I have enjoyed myself immensely at those meetings. Moreover, I have made many good friends there, some of whom I have collaborated with in my research. To conclude, I would like to give my message 'be ambitious' to young scientists, especially young women.

Moccetti, T., et al. (2015). "A Novel Class of Human Cardiac Stem Cells." Cardiol Rev **23**(4): 189-200.

Following the recognition that hematopoietic stem cells improve the outcome of myocardial infarction in animal models, bone marrow mononuclear cells, CD34-positive cells, and mesenchymal stromal cells have been introduced clinically. The intracoronary or intramyocardial injection of these cell classes has been shown to be safe and to produce a modest but significant enhancement in systolic function. However, the identification of resident cardiac stem cells in the human heart (hCSCs) has created great expectation concerning the potential implementation of this category of autologous cells for the management of the human disease. Although phase 1 clinical trials have been conducted with encouraging results, the search for the most powerful hCSC for myocardial regeneration is in its infancy. This manuscript discusses the efforts performed in our laboratory to characterize the critical biological variables that define the growth reserve of hCSCs. Based on the theory of the immortal DNA template, we propose that stem cells retaining the old DNA represent 1 of the most powerful cells for myocardial regeneration. Similarly, the expression of insulin-like growth factor-1 receptors in hCSCs recognizes a cell phenotype with superior replicating reserve. However, the impressive recovery in ventricular hemodynamics and anatomy mediated by clonal hCSCs carrying the "mother" DNA underscores the clinical relevance of this hCSC class for the treatment of human heart failure.

Modo, M., et al. (2003). "Transplantation of neural stem cells modulates apolipoprotein E expression in a rat model of stroke." Exp Neurol **183**(2): 320-329.

The expression of apolipoprotein E (apoE) after ischemic brain damage has been associated with plasticity involved in promoting functional recovery. We therefore examined the expression and distribution of apoE in rats that received intraparenchymal grafts of the conditionally immortal stem cell line MHP36 either ipsilateral or contralateral to the lesion or intraventricular grafts 4 months after transplantation. ApoE immunoreactivity was highly expressed in the striatum, somatosensory cortex, and thalamus of the lesioned hemisphere in all rats subjected to middle cerebral artery occlusion. Only in rats with intraparenchymal grafts, apoE was significantly upregulated in the contralateral hemisphere, whereas levels and distribution in rats with intraventricular grafts resembled those of ischemic controls. In ischemic rats, apoE was seen in both astrocytes and neurons on the lesioned side, and in grafted rats, apoE was present in host and transplanted neurons and

astrocytes. Previously we have shown that intraparenchymal grafts reduced sensorimotor asymmetry, whereas intraventricular grafts improved cognitive dysfunction, with transplanted cells being widely distributed in cortex, striatum, and corpus callosum on both sides of the brain in all grafted groups. Thus, stem cells grafted in the parenchyma are not only capable of limited expression of apoE in the host brain but also trigger a robust increase on the side contralateral to stroke damage where this does not normally occur. Findings that parenchymal, but not ventricular, grafts facilitated sensorimotor recovery suggests that apoE might contribute to plastic changes in relevant pathways, possibly on both sides of the brain. In contrast, no evidence was found for an association between apoE and recovery of cognitive function in rats with intraventricular grafts.

Moltzahn, F. R., et al. (2008). ""Cancer stem cells"-lessons from Hercules to fight the Hydra." Urol Oncol **26**(6): 581-589.

Following the initial identification of hematopoietic tumor stem cells, such cells were also found in several solid tumor types. In urology, cancer stem cells have only been found in prostate tumors so far. The concept and detection of tumor stem cells rely heavily on findings derived from stem cell research. Therefore, in addition to identifying and characterizing urologic tumor stem cells, research in uro-oncology should also aim at better understanding the stem-cell biology of urologic organs. Insights in similarities and differences gleaned from these studies could be used to develop strategies for targeted destruction of tumor stem cells while sparing the physiological stem cells. The main target of future curative therapies in uro-oncology must therefore be the central, immortal head of the Hydra, the tumor stem cell.

Monk, M. and C. Holding (2001). "Human embryonic genes re-expressed in cancer cells." Oncogene **20**(56): 8085-8091.

Human preimplantation embryonic cells are similar in phenotype to cancer cells. Both types of cell undergo deprogramming to a proliferative stem cell state and become potentially immortal and invasive. To investigate the hypothesis that embryonic genes are re-expressed in cancer cells, we prepare amplified cDNA from human individual preimplantation embryos and isolate embryo-specific sequences. We show that three novel embryonic genes, and also the known gene, OCT4, are expressed in human tumours but not expressed in normal somatic tissues. Genes specific to this unique phase of the human life cycle and not expressed in somatic cells may have greater potential for targeting in cancer treatment.

Monti, M., et al. (2012). "Stem cells: sources and therapies." Biol Res **45**(3): 207-214.

The historical, lexical and conceptual issues embedded in stem cell biology are reviewed from technical, ethical, philosophical, judicial, clinical, economic and biopolitical perspectives. The mechanisms assigning the simultaneous capacity to self-renew and to differentiate to stem cells (immortal template DNA and asymmetric division) are evaluated in the light of the niche hypothesis for the stemness state. The induction of cell pluripotency and the different stem cells sources are presented (embryonic, adult and cord blood). We highlight the embryonic and adult stem cell properties and possible therapies while we emphasize the particular scientific and social values of cord blood donation to set up cord blood banks. The current scientific and legal frameworks of cord blood banks are reviewed at an international level as well as allogenic, dedicated and autologous donations. The expectations and the challenges in relation to present-day targeted diseases like diabetes mellitus type I, Parkinson's disease and myocardial infarction are evaluated in the light of the cellular therapies for regenerative medicine.

Moore, H. and B. Aflatoonian (2007). "From stem cells to spermatozoa and back." Soc Reprod Fertil Suppl **65**: 19-32.

As a strategy for survival, the metazoa segregated immortal germ cells from the mortal soma by an early differentiation process and thereby reproduced asymmetrically in a specialised tissue. The legacy of this process is seen in the generation of mammalian gametes which are derived from a founder population of primordial germ cells (PGCs) that are determined early in embryogenesis and set aside for unique development. Understanding the mechanisms of PGC determination and differentiation is important for elucidating causes of infertility and how endocrine disrupting chemicals may increase susceptibility to congenital reproductive abnormalities and conditions such as testicular cancer in adulthood (testicular dysgenesis syndrome). Primordial germ cells are closely related to embryonic stem (ESCs) cells and embryonic germ (EG) cells and comparisons between these various cell types are providing new information about pluripotency and epigenetic processes. Murine embryonic stem cells can differentiate to PGCs, gametes and even blastocysts. Recently live mouse pups were born from sperm generated from ESCs. Although investigations are still preliminary, human embryonic stem cells (hESCs) apparently display a similar developmental capacity to generate PGCs and subsequently gametes. The findings indicate hESCs have the potential to differentiate to PGCs (as determined with surface markers and gene expression profiles) although these phenotypes represented a small proportion of the total cell population (approximately 0.1%). Postmeiotic spermatids have been identified

using specific markers, while primordial follicular structures have been observed in culture although not characterised definitely. Germ cells will enter meiosis autonomously and develop as oocytes unless meiosis is blocked and cells are induced into a spermatogenic pathway. During hESC differentiation both pathways seem to occur, regardless of the sex karyotype. Exactly how gamete-like cells are generated during stem cell culture remains unclear especially as conditions are currently ill defined. The findings are discussed in relation to the mechanisms of human PGC and gamete development and the biotechnology of hESCs and hEG cells.

Moore, K. D., et al. (1996). "In vitro properties of a newly established medulloblastoma cell line, MCD-1." Mol Chem Neuropathol **29**(2-3): 107-126.

Medulloblastomas are poorly differentiated brain tumors believed to arise from primitive pleuripotential stem cells, and tend to express mixed neuronal and glial properties. In the present study, we examined immunohistochemical and neurotransmitter phenotypic properties in a newly established medulloblastoma cell line, MCD-1. MCD-1 cells were immortal, not contact-inhibited, but did not grow in soft agar. Immunohistochemical studies showed positive staining for neurofilament protein (NF), neuron-specific enolase (NSE), synaptophysin, MAP 2, tau, NCAM 180, vimentin, and S-100 protein. The cells expressed specific uptake of glutamate, serotonin, and choline, but not GABA or dopamine. A significant increase in process extension was seen in response to agents that enhance intracellular cyclic AMP, especially 3-isobutyl-1-methylxanthine (IBMX). Process formation induced by IBMX was associated with a decrease in cell proliferation as evidenced by a reduction in numbers of cells incorporating 5-bromo-2-deoxyuridine (BrdU). No increase in process extension was observed following exposure to NGF or retinoic acid. MCD-1 cells were shown to produce transforming growth factor beta (TGF beta), and were immunopositive for mutant p53. Transfection assays with the PG13-Luc reporter plasmid, which contains a p53-responsive enhancer element and a luciferase reporter gene, suggested MCD-1 cells are deficient in wild-type p53 and do not activate p53 on treatment with the anticancer agent adriamycin. The MCD-1 cell line is suggested to represent an abnormally differentiated cell type, which has some properties consistent with a multipotent neuronal phenotype while retaining some properties of immature cells of a glial lineage. The MCD-1 cell line can be used to provide a model of a medulloblastoma cell line that is resistant to growth-controlling and anticancer agents.

Moore, K. J. and M. W. Freeman (1999). "Embryonal Stem (ES) Cell-Derived Macrophages : A Cellular System that Facilitates the Genetic Dissection of Macrophage Function." Methods Mol Med **30**: 343-355.

The monocyte/macrophage (Mo) contributes to atherosclerotic lesion initiation and progression through a variety of interactions with cells of the artery wall that depend on the elucidation of a host of cytokines and growth factors by cells residing in the intima. The number and complexity of these interactions make it difficult to determine which cellular functions are contributing to the progression of atherosclerosis and which might be exploited to interrupt that progression. Studies of macrophage functions in atherosclerosis have been hindered by the limitations of available macrophage cell lines and primary cultures, including poor transfectability and the transformed state of imMortal cell lines. Recent studies have demonstrated that pluripotential mouse embryonic stem cells can be differentiated down specific hematopoietic lineages in vitro, including lines that give rise to macrophages (1). This technique provides a genetically tractable cellular system for studying myeloid cell function. Macrophages arising from this differentiation system demonstrate cell surface presentation of classic macrophage markers and macrophage functions including phagocytosis and responses to inflammatory stimuli. There are several important advantages inherent in using embryonic stem (ES) cell derived macrophages as a cell culture system for studying Mo function. As the cells are not transformed, and the progenitor cells arising from ES cells are capable of reconstituting the entire hematopoietic compartment of a mouse, they represent a cell culture system that appears to retain the physiologic regulation on growth and differentiation that is absent from transformed myelomonocytic cell lines.

Morelli, C., et al. (2002). "Cloning and characterization of the common fragile site FRA6F harboring a replicative senescence gene and frequently deleted in human tumors." Oncogene **21**(47): 7266-7276.

The common fragile site FRA6F, located at 6q21, is an extended region of about 1200 kb, with two hot spots of breakage each spanning about 200 kb. Transcription mapping of the FRA6F region identified 19 known genes, 10 within the FRA6F interval and nine in a proximal or distal position. The nucleotide sequence of FRA6F is rich in repetitive elements (LINE1 and LINE2, Alu, MIR, MER and endogenous retroviral sequences) as well as in matrix attachment regions (MARs), and shows several DNA segments with increased helix flexibility. We found that tight clusters of stem-loop structures were localized exclusively in the two regions with greater frequency of breakage. Chromosomal instability at FRA6F probably depends on a complex interaction of different factors, involving regions of greater DNA flexibility

and MARs. We propose an additional mechanism of fragility at FRA6F, based on stem-loop structures which may cause delay or arrest in DNA replication. A senescence gene likely maps within FRA6F, as suggested by detection of deletion and translocation breakpoints involving this fragile site in immortal human-mouse cell hybrids and in SV40-immortalized human fibroblasts containing a human chromosome 6 deleted at q21. Deletion breakpoints within FRA6F are common in several types of human leukemias and solid tumors, suggesting the presence of a tumor suppressor gene in the region. Moreover, a gene associated to hereditary schizophrenia maps within FRA6F. Therefore, FRA6F may represent a landmark for the identification and cloning of genes involved in senescence, leukemia, cancer and schizophrenia.

Morgan, J. E., et al. (2002). "Myogenic cell proliferation and generation of a reversible tumorigenic phenotype are triggered by preirradiation of the recipient site." J Cell Biol **157**(4): 693-702.

Environmental influences have profound yet reversible effects on the behavior of resident cells. Earlier data have indicated that the amount of muscle formed from implanted myogenic cells is greatly augmented by prior irradiation (18 Gy) of the host mouse muscle. Here we confirm this phenomenon, showing that it varies between host mouse strains. However, it is unclear whether it is due to secretion of proliferative factors or reduction of antiproliferative agents. To investigate this further, we have exploited the observation that the immortal myogenic C2 C12 cell line forms tumors far more rapidly in irradiated than in nonirradiated host muscle. We show that the effect of preirradiation on tumor formation is persistent and dose dependent. However, C2 C12 cells are not irreversibly compelled to form undifferentiated tumor cells by the irradiated muscle environment and are still capable of forming large amounts of muscle when reimplanted into a nonirradiated muscle. In a clonal analysis of this effect, we discovered that C2 C12 cells have a bimodal propensity to form tumors; some clones form no tumors even after extensive periods in irradiated graft sites, whereas others rapidly form extensive tumors. This illustrates the subtle interplay between the phenotype of implanted cells and the factors in the muscle environment.

Morrison, S. J., et al. (1996). "Telomerase activity in hematopoietic cells is associated with self-renewal potential." Immunity **5**(3): 207-216.

It has been proposed that the biological clock underlying the limited division potential of eukaryotic cells is telomere length. We assayed telomerase activity in single cells of the hematopoietic and immune systems. We examined hematopoietic stem cells at four stages of differentiation, lineage-committed progenitors, and mature myeloid and lymphoid cells. The frequency of telomerase-expressing cells within each population was proportional to the frequency of cells thought to have self-renewal potential. Among bone marrow hematopoietic stem cells, 70% exhibited detectable telomerase activity. The telomerase-expressing somatic cells observed in this study are not thought to be immortal, and expression was not correlated with cell cycle distribution or differentiation state. This study demonstrates that the developmental characteristic most consistently associated with telomerase expression is self-renewal potential.

Mull, J. L. and A. Asakura (2012). "A New Look at an Immortal DNA Hypothesis for Stem Cell Self-Renewal." J Stem Cell Res Ther **2**(1).

Mumcuoglu, M., et al. (2010). "The ability to generate senescent progeny as a mechanism underlying breast cancer cell heterogeneity." PLoS One **5**(6): e11288.

BACKGROUND: Breast cancer is a remarkably heterogeneous disease. Luminal, basal-like, "normal-like", and ERBB2+ subgroups were identified and were shown to have different prognoses. The mechanisms underlying this heterogeneity are poorly understood. In our study, we explored the role of cellular differentiation and senescence as a potential cause of heterogeneity. METHODOLOGY/PRINCIPAL FINDINGS: A panel of breast cancer cell lines, isogenic clones, and breast tumors were used. Based on their ability to generate senescent progeny under low-density clonogenic conditions, we classified breast cancer cell lines as senescent cell progenitor (SCP) and immortal cell progenitor (ICP) subtypes. All SCP cell lines expressed estrogen receptor (ER). Loss of ER expression combined with the accumulation of p21(Cip1) correlated with senescence in these cell lines. p21(Cip1) knockdown, estrogen-mediated ER activation or ectopic ER overexpression protected cells against senescence. In contrast, tamoxifen triggered a robust senescence response. As ER expression has been linked to luminal differentiation, we compared the differentiation status of SCP and ICP cell lines using stem/progenitor, luminal, and myoepithelial markers. The SCP cells produced CD24+ or ER+ luminal-like and ASMA+ myoepithelial-like progeny, in addition to CD44+ stem/progenitor-like cells. In contrast, ICP cell lines acted as differentiation-defective stem/progenitor cells. Some ICP cell lines generated only CD44+/CD24-/ER-/ASMA- progenitor/stem-like cells, and others also produced CD24+/ER- luminal-like, but not ASMA+ myoepithelial-like cells. Furthermore, gene expression profiles clustered SCP cell lines with luminal A and "normal-like" tumors, and ICP cell lines with luminal B and basal-like tumors. The ICP cells displayed higher tumorigenicity in immunodeficient mice. CONCLUSIONS/SIGNIFICANCE: Luminal A and "normal-like" breast cancer cell lines were able to

generate luminal-like and myoepithelial-like progeny undergoing senescence arrest. In contrast, luminal B/basal-like cell lines acted as stem/progenitor cells with defective differentiation capacities. Our findings suggest that the malignancy of breast tumors is directly correlated with stem/progenitor phenotypes and poor differentiation potential.

Muses, S., et al. (2011). "A new extensively characterised conditionally immortal muscle cell-line for investigating therapeutic strategies in muscular dystrophies." PLoS One **6**(9): e24826.

A new conditionally immortal satellite cell-derived cell-line, H2K 2B4, was generated from the H2K(b)-tsA58 immortomouse. Under permissive conditions H2K 2B4 cells terminally differentiate in vitro to form uniform myotubes with a myogenic protein profile comparable with freshly isolated satellite cells. Following engraftment into immunodeficient dystrophin-deficient mice, H2K 2B4 cells regenerated host muscle with donor derived myofibres that persisted for at least 24 weeks, without forming tumours. These cells were readily transfectable using both retrovirus and the non-viral transfection methods and importantly upon transplantation, were able to reconstitute the satellite cell niche with functional donor derived satellite cells. Finally using the Class II DNA transposon, Sleeping Beauty, we successfully integrated a reporter plasmid into the genome of H2K 2B4 cells without hindering the myogenic differentiation. Overall, these data suggest that H2K 2B4 cells represent a readily transfectable stable cell-line in which to investigate future stem cell based therapies for muscle disease.

Mustata, R. C., et al. (2013). "Identification of Lgr5-independent spheroid-generating progenitors of the mouse fetal intestinal epithelium." Cell Rep **5**(2): 421-432.

Immortal spheroids were generated from fetal mouse intestine using the culture system initially developed to culture organoids from adult intestinal epithelium. Spheroid proportion progressively decreases from fetal to postnatal period, with a corresponding increase in production of organoids. Like organoids, spheroids show Wnt-dependent indefinite self-renewing properties but display a poorly differentiated phenotype reminiscent of incompletely caudalized progenitors. The spheroid transcriptome is strikingly different from that of adult intestinal stem cells, with minimal overlap of Wnt target gene expression. The receptor LGR4, but not LGR5, is essential for their growth. Trop2/Tacstd2 and Cnx43/Gja1, two markers highly enriched in spheroids, are expressed throughout the embryonic-day-14 intestinal epithelium. Comparison of in utero and neonatal lineage tracing using Cnx43-CreER and Lgr5-CreERT2 mice identified spheroid-generating cells as developmental progenitors involved in generation of the prenatal intestinal epithelium. Ex vivo, spheroid cells have the potential to differentiate into organoids, qualifying as a fetal type of intestinal stem cell.

Nagano, M. and R. L. Brinster (1998). "Spermatogonial transplantation and reconstitution of donor cell spermatogenesis in recipient mice." APMIS **106**(1): 47-55; discussion 56-47.

Recently, we described a method to transplant testicular cells from a fertile donor mouse to the seminiferous tubules of an infertile recipient male, where the donor cells generate spermatogenesis. Spermatozoa produced by the transplanted cells in the recipient testis will fertilize eggs and transmit the donor haplotype to the resulting animals. In the most successful transplantations, up to 80 per cent of progeny sired by the recipient male arise from donor cell-derived spermatozoa. The cell responsible for generation of spermatogenesis following transplantation clearly is a spermatogonial stem cell, since the repopulation of recipient testes extends for periods exceeding 12 months. In the past year, experiments have shown that rat testicular cells transplanted to the seminiferous tubules of immunodeficient mice will generate rat spermatogenesis and produce normal appearing rat spermatozoa, opening the possibility of xenogeneic testicular cell transplantation for other species. In addition, it has proved possible to cryopreserve spermatogonial stem cells for long periods, following which they will produce normal spermatogenesis when transplanted to a recipient. The ability to cryopreserve testicular stem cells makes individual male germ lines immortal. In current work, we are focusing on techniques to increase the efficiency of spermatogonial transplantation and methods to culture the stem cells.

Nagle, P. W. and R. P. Coppes (2020). "Current and Future Perspectives of the Use of Organoids in Radiobiology." Cells **9**(12).

The majority of cancer patients will be treated with radiotherapy, either alone or together with chemotherapy and/or surgery. Optimising the balance between tumour control and the probability of normal tissue side effects is the primary goal of radiation treatment. Therefore, it is imperative to understand the effects that irradiation will have on both normal and cancer tissue. The more classical lab models of immortal cell lines and in vivo animal models have been fundamental to radiobiological studies to date. However, each of these comes with their own limitations and new complementary models are required to fill the gaps left by these traditional models. In this review, we discuss how organoids, three-dimensional tissue-resembling structures derived from tissue-resident, embryonic or induced pluripotent stem cells, overcome the limitations of these models and

thus have a growing importance in the field of radiation biology research. The roles of organoids in understanding radiation-induced tissue responses and in moving towards precision medicine are examined. Finally, the limitations of organoids in radiobiology and the steps being made to overcome these limitations are considered.

Napolitano, A. P., et al. (2007). "Scaffold-free three-dimensional cell culture utilizing micromolded nonadhesive hydrogels." Biotechniques **43**(4): 494, 496-500.

Techniques that allow cells to self-assemble into three-dimensional (3-D) spheroid microtissues provide powerful in vitro models that are becoming increasingly popular--especially in fields such as stem cell research, tissue engineering, and cancer biology. Unfortunately, caveats involving scale, expense, geometry, and practicality have hindered the widespread adoption of these techniques. We present an easy-to-use, inexpensive, and scalable technology for production of complex-shaped, 3-D microtissues. Various primary cells and immortal cell lines were utilized to demonstrate that this technique is applicable to many cell types and highlight differences in their self-assembly phenomena. When seeded onto micromolded, nonadhesive agarose gels, cells settle into recesses, the architectures of which optimize the requisite cell-to-cell interactions for spontaneous self-assembly. With one pipeting step, we were able to create hundreds of uniform spheroids whose size was determined by seeding density. Multicellular tumor spheroids (MCTS) were assembled or grown from single cells, and their proliferation was quantified using a modified 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1) assay. Complex-shaped (e.g., honeycomb) microtissues of homogeneous or mixed cell populations can be easily produced, opening new possibilities for 3-D tissue culture.

Paradis, V., et al. (1999). "Expression of the RNA component of human telomerase (hTR) in prostate cancer, prostatic intraepithelial neoplasia, and normal prostate tissue." J Pathol **189**(2): 213-218.

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA on chromosomal ends. While telomerase is undetectable in most normal somatic tissues, telomerase activation has been detected by a polymerase chain reaction (PCR)-based assay (TRAP) in many immortal cell lines and various cancers, including prostate cancers. To investigate the role of telomerase in prostate cancer at the cellular level, the expression of one of the ribonucleoprotein complexes, the RNA component of human telomerase (hTR), was studied in normal, preneoplastic, and cancerous prostate tissues using a non-radioactive in situ hybridization procedure. Nine human prostates resected at the time of radical prostatectomy were studied. In each case, archival paraffin-embedded samples from normal tissue, prostatic intraepithelial neoplasia (PIN) lesions, the putative precancerous lesion, and prostate carcinomas were selected for in situ hybridization. hTR mRNA expression was detected in carcinomatous glands of seven out of the nine cancers (75 per cent). Furthermore, in seven out of the eight cases showing PIN lesions, the epithelial cells of PIN foci also expressed hTR mRNA. By contrast, in normal tissue, epithelial cells were negative, whereas hTR mRNA expression was detected in the basal cells. The detection of hTR mRNA in PIN lesions clearly strengthens the link between PIN and carcinomatous glands and suggests that telomerase expression occurs early in prostate carcinogenesis. Furthermore, this study confirms previous experimental data suggesting that the basal cell layer is the stem cell compartment in prostate.

Park, I. H., et al. (2008). "Disease-specific induced pluripotent stem cells." Cell **134**(5): 877-886.

Tissue culture of immortal cell strains from diseased patients is an invaluable resource for medical research but is largely limited to tumor cell lines or transformed derivatives of native tissues. Here we describe the generation of induced pluripotent stem (iPS) cells from patients with a variety of genetic diseases with either Mendelian or complex inheritance; these diseases include adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD) and Becker muscular dystrophy (BMD), Parkinson disease (PD), Huntington disease (HD), juvenile-onset, type 1 diabetes mellitus (JDM), Down syndrome (DS)/trisomy 21, and the carrier state of Lesch-Nyhan syndrome. Such disease-specific stem cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation in vitro, thereby enabling disease investigation and drug development.

Park, J. P., et al. (2013). "Differential gene expression in nuclear label-retaining cells in the developing mouse mammary gland." Stem Cells Dev **22**(8): 1297-1306.

The immortal strand theory postulates stem cells protect themselves from DNA replication-associated mutations and subsequent cancer risk through selective segregation of template DNA strands. Stem cells self-renew by asymmetric cellular division. During asymmetric division, stem cells maintain their template DNA strands, while the newly synthesized DNA strands segregate to newly formed daughter cells. Previous studies have demonstrated that self-renewing mammary stem cells originate in the expanding mammary ducts during puberty-associated allometric growth. In this study, we labeled newly forming

mammary stem cells with the thymidine analog 5-ethynl-2'-deoxyuridine for 2 weeks during allometric ductal expansion. Cells that incorporate and retain the nuclear label following extended chase periods are termed label-retaining cells (LRCs). A second nuclear label, 5-bromodeoxyuridine, was administered before euthanasia to identify cells traversing the cell cycle. Mammary cells collected following euthanasia were sorted based on nuclear label retention. Members of the Notch and Wnt signaling pathways were found differentially expressed by mammary LRCs. These pathways are involved in the regulation of stem cells in the mouse mammary gland. Upon further analysis, we found that in contrast to non-LRCs, Notch1 and Notch2 are expressed and localized in the nuclei of the LRCs. Expression of Notch-inducible genes, Hes1 and Hey2, was elevated in LRCs. Inhibition of Notch1 by shRNA reduced colony forming potential and label retention by mammary epithelial cells in vitro. These results indicate that genes are differentially regulated in the LRC population of mammary glands and Notch1 mediates asymmetric cell division of mammary progenitor cells.

Park, S. P., et al. (2004). "Establishment of human embryonic stem cell lines from frozen-thawed blastocysts using STO cell feeder layers." Hum Reprod **19**(3): 676-684.

BACKGROUND: Recently, human embryonic stem (hES) cells have become very important resources for basic research on cell replacement therapy and other medical applications. The purpose of this study was to test whether pluripotent hES cell lines could be successfully derived from frozen-thawed embryos that were destined to be discarded after 5 years in a routine human IVF-embryo transfer programme and whether an STO cell feeder layer can be used for the culture of hES cells. METHODS: Donated frozen embryos (blastocysts or pronuclear) were thawed, and recovered or in vitro developed blastocysts were immunosurgically treated. All inner cell masses were cultured continuously on an STO cell feeder layer and then presumed hES cell colonies were characterized. RESULTS: Seven and two cell lines were established from frozen-thawed blastocysts (7/20, 35.0%) and pronuclear stage embryos (2/20, 10.0%), respectively. The doubling time of hES cells on the immortal STO cell feeder layer was approximately 36 h, similar to that of cells grown using fresh mouse embryonic fibroblast (MEF) feeder conditions. Subcultured hES cell colonies showed strong positive immunostaining for alkaline phosphatase, stage-specific embryonic antigen-4 (SSEA-4) and tumour rejection antigen 1-60 (TRA1-60) cell surface markers. Also, the hES colonies retained normal karyotypes and Oct-4 expression in prolonged subculture. When in vitro differentiation of hES cells was induced by retinoic acid, three embryonic germ layer cells were identified by RT-PCR or indirect immunocytochemistry. CONCLUSIONS: This study indicates that establishment of hES cells from frozen-thawed blastocysts minimizes the ethical problem associated with the use of human embryos in research and that the STO cell feeder layer can be used for the culture of hES cells.

Park, W. C., et al. (2005). "Deregulation of estrogen induced telomerase activity in tamoxifen-resistant breast cancer cells." Int J Oncol **27**(5): 1459-1466.

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.

Parker, G. C. (2006). "Embryonic stem cell research: the state of the union of "those who create the lines" and "those who draw the lines"." Stem Cells Dev **15**(5): 623-629.

Recent events have ostensibly removed the primacy of the United States in embryonic stem (ES) cell research. George Walker Bush was the first U.S. President to allow federal funding of ES cell research under guidelines influenced by statements from pre-

eminent stem cell scientists. The guidelines delimiting federal funding of ES cell research put forth by President Bush were endorsed by the public, the National Institutes of Health (NIH) and by, at least by silent assent, the scientific community. At the time, the President was assured by eminent scientists and advisors that ES cells were immortal and stable, many viable lines existed, and they could be used for years for research and therapy. These premises have now been publicly challenged and individual states and other countries have announced plans to proceed with research that would be considered outside of the President's guidelines. All have thus far discovered that removal of U.S. federal support and oversight not only blunts science, but also abdicates responsible stewardship of the scientific process as well as the resulting technology. So while California, Canada, New Jersey, and Britain engage in ES research, suddenly the process stops; and no advances are made. Below I present a historical account of the seminal events and statements of legislators and ES cell researchers leading to and from that decision. The Journal welcomes our readers' comments on the issue at hand.

Patananan, A. N., et al. (2020). "Pressure-Driven Mitochondrial Transfer Pipeline Generates Mammalian Cells of Desired Genetic Combinations and Fates." Cell Rep **33**(13): 108562.

Generating mammalian cells with desired mitochondrial DNA (mtDNA) sequences is enabling for studies of mitochondria, disease modeling, and potential regenerative therapies. MitoPunch, a high-throughput mitochondrial transfer device, produces cells with specific mtDNA-nuclear DNA (nDNA) combinations by transferring isolated mitochondria from mouse or human cells into primary or immortal mtDNA-deficient (rho0) cells. Stable isolated mitochondrial recipient (SIMR) cells isolated in restrictive media permanently retain donor mtDNA and reacquire respiration. However, SIMR fibroblasts maintain a rho0-like cell metabolome and transcriptome despite growth in restrictive media. We reprogrammed non-immortal SIMR fibroblasts into induced pluripotent stem cells (iPSCs) with subsequent differentiation into diverse functional cell types, including mesenchymal stem cells (MSCs), adipocytes, osteoblasts, and chondrocytes. Remarkably, after reprogramming and differentiation, SIMR fibroblasts molecularly and phenotypically resemble unmanipulated control fibroblasts carried through the same protocol. Thus, our MitoPunch "pipeline" enables the production of SIMR cells with unique mtDNA-nDNA combinations for additional studies and applications in multiple cell types.

Pate, S. A. and P. Rameshwar (2011). "Exploring a stem cell basis to identify novel treatment for human malignancies." J Stem Cells **6**(4): 233-243.

Research investigations on various sources of stem cells have been conducted for potential to exert tissue regeneration, reverse immune-enhancement, and protect against tissue insult. At a more distant goal, it is likely that stem cells could be applied to medicine via organogenesis. However, the field of stem cells is not new since immune replacement via bone marrow transplantation is considered a successful form of cell therapy. There is evidence that stem cell therapies are close for several disorders such as neurodegeneration, immune hyperactivity, and functional insufficiencies such as Type I diabetes mellitus. The field of stem cell biology is gaining a strong foothold in science and medicine as the molecular mechanisms underlying stem cell behavior are gradually being unraveled. Although stem cells have tremendous therapeutic applicability in the aforementioned conditions, their uniqueness may also confer adverse properties, rendering them a double-edged sword. The discovery that stem cells have immortal and resilient characteristics has shed insight into the link between stem cells and tumorigenesis. Specifically, recent advancements in cancer research have implicated that a stem cell may be responsible for the refractoriness of cancers to conventional treatment such as chemotherapy and radiation. Here, we summarize the recent advancements in the cancer stem cell hypothesis and present the challenges associated with targeting resistant cancers in the context of stem cell microenvironments.

Pawelec, G. (2000). "Human T-cell clones." Methods Mol Med **38**: 53-61.

Techniques for generating human T-cell clones (TCCs) were first described nearly two decades ago (1, 2). This was a direct consequence of the discovery of T-cell growth factor and the subsequent ability to propagate T-cells over extended periods (3). Early on, numerous publications in immunology indicated an apparently unlimited growth potential of normal mammalian T lymphocyte cultures; however, even at this time, other investigators challenged this conclusion (4, 5). Nonetheless, the possibility remained that at least some TCCs represented an exception to the rule of the Hayflick Limit for growth of normal somatic cells. If this were the case, the real relevance of replicative senescence as a universal phenomenon would be highly questionable. On the other hand, if those T-cells surviving apparently indefinitely were endowed with the properties of stem cells rather than differentiated cells, this quandary would be resolved. However, as far as could be judged, the apparently immortal TCCs described in the literature seemed to possess all the attributes of normal T-cells, not stem

cells. Several explanations for this apparent paradox have been proposed, the most likely of which may be that such immortal lines are in fact abnormal. Few clones were tested for karyotypic or other abnormalities. Such analyses, when performed, often revealed genetic aberrations in human as well as murine clones (6, 7). In the case of murine cells, continuous cultures often transform spontaneously in culture, but in humans this is rare or absent.

Peehl, D. M. (2005). "Primary cell cultures as models of prostate cancer development." Endocr Relat Cancer **12**(1): 19-47.

This review focuses on primary cultures of human prostatic epithelial cells and their applications as models of normal and malignant biological behavior. Current abilities to culture cells from normal tissues, from premalignant dysplastic lesions (prostatic intraepithelial neoplasia), from primary adenocarcinomas, and from metastases are described. Evidence for representation of the interrelated cells of the normal prostatic epithelium--stem cells, basal epithelial cells, secretory epithelial cells, transit amplifying cells and neuroendocrine cells--in primary cultures is presented. Comparisons between normal and cancer-derived primary cultures are made regarding biological activities relevant to carcinogenesis, such as proliferation, apoptosis, differentiation, senescence, adhesion, migration, invasion, steroid hormone metabolism, other metabolic pathways and angiogenesis. Analyses of tumor suppressor activity, differential gene expression and cytogenetics in primary cultures have revealed changes relevant to prostate cancer progression. Preclinical studies with primary cultures have provided information useful for designing new strategies for chemoprevention, chemotherapy, cytotoxin therapy, radiation therapy, gene therapy and imaging. While the behavior of normal primary cultures is often used as a basis for comparison with established, immortal prostate cancer cell lines, the most informative studies are performed with donor-matched pairs of normal and malignant primary cultures, grown under identical conditions. Challenges that remain to be addressed if the full potential of primary cultures as a model system is to be realized include isolation, culture and characterization of stem cells, improved methodology to induce or maintain a fully differentiated, androgen-responsive phenotype, and identification of cell surface antigens or other markers with which to purify pure populations of live cancer or premalignant cells apart from non-malignant epithelial cells prior to culture.

Pine, S. R. and W. Liu (2014). "Asymmetric cell division and template DNA co-segregation in cancer stem cells." Front Oncol **4**: 226.

During tissue homeostasis, normal stem cells self-renew and repopulate the diverse cell types found within the tissue via a series of carefully controlled symmetric and asymmetric cell divisions (ACDs). The notion that solid tumors comprise a subset of cancer stem cells (CSCs) with dysregulated self-renewal and excessive symmetric cell divisions has led to numerous studies aimed to elucidate the mechanisms regulating ACD under steady-state conditions, during stem-cell expansion and in cancer. In this perspective, we focus on a type of asymmetry that can be established during ACD, called non-random co-segregation of template DNA, which has been identified across numerous species, cell types, and cancers. We discuss the role of p53 loss in maintaining self-renewal in both normal and malignant cells. We then review our current knowledge of the mechanisms underlying co-segregation of template DNA strands and the stem-cell pathways associated with it in normal and CSCs.

Pineiro-Ramil, M., et al. (2019). "Usefulness of Mesenchymal Cell Lines for Bone and Cartilage Regeneration Research." Int J Mol Sci **20**(24).

The unavailability of sufficient numbers of human primary cells is a major roadblock for in vitro repair of bone and/or cartilage, and for performing disease modelling experiments. Immortalized mesenchymal stromal cells (iMSCs) may be employed as a research tool for avoiding these problems. The purpose of this review was to revise the available literature on the characteristics of the iMSC lines, paying special attention to the maintenance of the phenotype of the primary cells from which they were derived, and whether they are effectively useful for in vitro disease modeling and cell therapy purposes. This review was performed by searching on Web of Science, Scopus, and PubMed databases from 1 January 2015 to 30 September 2019. The keywords used were ALL = (mesenchymal AND ("cell line" OR immortal*) AND (cartilage OR chondrogenesis OR bone OR osteogenesis) AND human). Only original research studies in which a human iMSC line was employed for osteogenesis or chondrogenesis experiments were included. After describing the success of the immortalization protocol, we focused on the iMSCs maintenance of the parental phenotype and multipotency. According to the literature revised, it seems that the maintenance of these characteristics is not guaranteed by immortalization, and that careful selection and validation of clones with particular characteristics is necessary for taking advantage of the full potential of iMSC to be employed in bone and cartilage-related research.

Plass, M., et al. (2018). "Cell type atlas and lineage tree of a whole complex animal by single-cell transcriptomics." Science **360**(6391).

Flatworms of the species Schmidtea mediterranea are immortal-adult animals contain a large pool of pluripotent stem cells that continuously differentiate into all adult cell types. Therefore, single-cell transcriptome profiling of adult animals should reveal mature and progenitor cells. By combining perturbation experiments, gene expression analysis, a computational method that predicts future cell states from transcriptional changes, and a lineage reconstruction method, we placed all major cell types onto a single lineage tree that connects all cells to a single stem cell compartment. We characterized gene expression changes during differentiation and discovered cell types important for regeneration. Our results demonstrate the importance of single-cell transcriptome analysis for mapping and reconstructing fundamental processes of developmental and regenerative biology at high resolution.

Pollock, K., et al. (2006). "A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke." Exp Neurol **199**(1): 143-155.

Transplantation of neural stem cells into the brain is a novel approach to the treatment of chronic stroke disability. For clinical application, safety and efficacy of defined, stable cell lines produced under GMP conditions are required. To this end, a human neural stem cell line, CTX0E03, was derived from human somatic stem cells following genetic modification with a conditional immortalizing gene, c-mycER(TAM). This transgene generates a fusion protein that stimulates cell proliferation in the presence of a synthetic drug 4-hydroxy-tamoxifen (4-OHT). The cell line is clonal, expands rapidly in culture (doubling time 50-60 h) and has a normal human karyotype (46 XY). In the absence of growth factors and 4-OHT, the cells undergo growth arrest and differentiate into neurons and astrocytes. Transplantation of CTX0E03 in a rat model of stroke (MCAo) caused statistically significant improvements in both sensorimotor function and gross motor asymmetry at 6-12 weeks post-grafting. In addition, cell migration and long-term survival in vivo were not associated with significant cell proliferation. These data indicate that CTX0E03 has the appropriate biological and manufacturing characteristics necessary for development as a therapeutic cell line.

Popov, L. S. and L. I. Korochkin (2004). "[Strategy of production and utilization of immortal cells]." Ontogenez **35**(1): 5-15.

The current methods of production of conditionally immortal cells in vivo and in vitro have been considered, including the method based on transgenesis of animals. Examples are given for utilization of conditionally immortal cells obtained in vivo from tissues of transgenic mice and rats carrying the gene of mutant T-antigen tsA58 SV40. The recent studies were analyzed, which concern the investigation and utilization of embryonic and regional stem cells, as well as immortal cells obtained through transfection of the recombinant construct of telomerase gene into human cells. The main problems of cell biotechnology are discussed.

Power, C. and J. E. Rasko (2008). "Whither prometheus' liver? Greek myth and the science of regeneration." Ann Intern Med **149**(6): 421-426.

Stem-cell biologists and those involved in regenerative medicine are fascinated by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle. This myth invariably provokes the question: Did the ancient Greeks know about the liver's amazing capacity for self-repair? The authors address this question by exploring the origins of Greek myth and medicine, adopting a 2-fold strategy. First, the authors consider what opportunities the ancient Greeks had to learn about the liver's structure and function. This involves a discussion of early battlefield surgery, the beginnings of anatomical research, and the ancient art of liver augury. In addition, the authors consider how the Greeks understood Prometheus' immortal liver. Not only do the authors examine the general theme of regeneration in Greek mythology, they survey several scholarly interpretations of Prometheus' torture.

Prager, P., et al. (2019). "Characterization of human telomerase reverse transcriptase immortalized anterior cruciate ligament cell lines." Biomed J **42**(6): 371-380.

BACKGROUND: The anterior-cruciate-ligament (ACL) contains mesenchymal stem cells (ACL-MSCs), suggesting the feasibility of regenerative treatments of this tissue. The immortalization of isolated cells results in cell-lines applicable to develop cell-based therapies. Immortal cell lines eliminate the need for frequent cell isolation from donor tissues. The objective of this study was to characterize cell lines that were generated from isolated ACL-MSCs using TERT gene transfer. METHODS: We isolated ACL-MSCs from human ACLs derived at the time of ACL reconstruction surgery or total knee arthroplasty. We generated cell lines and compared them to non-immortalized ACL-MSCs. We assessed the cellular morphology and we detected surface antigen expression. The resistance to senescence was inferred using the beta galactosidase activity. Histology, immunohistochemistry, and reverse transcriptase polymerase chain reaction (RT-PCR) were used to evaluate the multilineage differentiation capacity. RESULTS: The morphology of hTERT-ACL-MSCs was similar to ACL up to the last assessment at passage eight. We detected a strong surface expression of CD44, CD90, CD105, and STRO-1 in hTERT-ACL-MSCs. No substantial reduction in the ATP activity

was observed in hTERT-ACL-MSCs. CONCLUSION: Cell lines generated from ACL-MSCs maintain their morphology, surface antigen expression profile, and proliferative capacity; while markers of senescence appear to be reduced. These cell-lines maintained their multilineage differentiation capacity. The demonstrated model systems can be used for further development of new cell-based regenerative approaches in anterior cruciate ligament research, which may lead to new therapeutic strategies in the future.

Price, J. E. and D. Tarin (1989). "Low incidence of tumourigenicity in agarose colonies from spontaneous murine mammary tumours." Differentiation **41**(3): 202-207.

In these experiments individual colonies growing in agarose seeded with monocellular suspensions from freshly disaggregated naturally-occurring mouse mammary tumours, induced by the murine mammary tumour virus (MMTV), were reimplanted into mammary fat pads of virus-free mice. It was found that only a small proportion of these colonies generated tumours and that the implantation of multiple colonies in each site did not result in disproportionate, synergistic, increase in tumour takes. It was also observed that the proportion of colonies which were tumourigenic on reimplantation differed for each donor tumour and represented only a small fraction of the total cell population (0.001%-0.1%). However, this value was significantly higher in tumours which produced large numbers of deposits in lung colony assays following i.v. injections, than in tumours of low pulmonary colonisation potential. A point of particular interest was that tumours derived from agarose colonies of spontaneously metastatic donor tumours were substantially more spontaneously metastatic themselves than those from nonmetastatic donors, indicating that this property is heritable through numerous cell divisions, manipulations in vitro and transplantation procedures. From these results it is concluded that measurement of clonogenicity in agar is useful as an index of the capability of a tumour to propagate itself and to colonise new sites, but that individual agarose colonies are not all the progeny of potentially immortal stem cells.

Proenca, A. M., et al. (2019). "Cell aging preserves cellular immortality in the presence of lethal levels of damage." PLoS Biol **17**(5): e3000266.

Cellular aging, a progressive functional decline driven by damage accumulation, often culminates in the mortality of a cell lineage. Certain lineages, however, are able to sustain long-lasting immortality, as prominently exemplified by stem cells. Here, we show that Escherichia coli cell lineages exhibit comparable patterns of mortality and immortality. Through single-cell microscopy and microfluidic techniques, we find that these patterns are explained by the dynamics of damage accumulation and asymmetric partitioning between daughter cells. At low damage accumulation rates, both aging and rejuvenating lineages retain immortality by reaching their respective states of physiological equilibrium. We show that both asymmetry and equilibrium are present in repair mutants lacking certain repair chaperones, suggesting that intact repair capacity is not essential for immortal proliferation. We show that this growth equilibrium, however, is displaced by extrinsic damage in a dosage-dependent response. Moreover, we demonstrate that aging lineages become mortal when damage accumulation rates surpass a threshold, whereas rejuvenating lineages within the same population remain immortal. Thus, the processes of damage accumulation and partitioning through asymmetric cell division are essential in the determination of proliferative mortality and immortality in bacterial populations. This study provides further evidence for the characterization of cellular aging as a general process, affecting prokaryotes and eukaryotes alike and according to similar evolutionary constraints.

Reinhardt, P., et al. (2013). "Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling." PLoS One **8**(3): e59252.

Phenotypic drug discovery requires billions of cells for high-throughput screening (HTS) campaigns. Because up to several million different small molecules will be tested in a single HTS campaign, even small variability within the cell populations for screening could easily invalidate an entire campaign. Neurodegenerative assays are particularly challenging because neurons are post-mitotic and cannot be expanded for implementation in HTS. Therefore, HTS for neuroprotective compounds requires a cell type that is robustly expandable and able to differentiate into all of the neuronal subtypes involved in disease pathogenesis. Here, we report the derivation and propagation using only small molecules of human neural progenitor cells (small molecule neural precursor cells; smNPCs). smNPCs are robust, exhibit immortal expansion, and do not require cumbersome manual culture and selection steps. We demonstrate that smNPCs have the potential to clonally and efficiently differentiate into neural tube lineages, including motor neurons (MNs) and midbrain dopaminergic neurons (mDANs) as well as neural crest lineages, including peripheral neurons and mesenchymal cells. These properties are so far only matched by pluripotent stem cells. Finally, to demonstrate the usefulness of smNPCs we show that mDANs differentiated from smNPCs with LRRK2 G2019S are more susceptible to apoptosis in the

presence of oxidative stress compared to wild-type. Therefore, smNPCs are a powerful biological tool with properties that are optimal for large-scale disease modeling, phenotypic screening, and studies of early human development.

Reizel, Y., et al. (2011). "Colon stem cell and crypt dynamics exposed by cell lineage reconstruction." PLoS Genet **7**(7): e1002192.

Stem cell dynamics in vivo are often being studied by lineage tracing methods. Our laboratory has previously developed a retrospective method for reconstructing cell lineage trees from somatic mutations accumulated in microsatellites. This method was applied here to explore different aspects of stem cell dynamics in the mouse colon without the use of stem cell markers. We first demonstrated the reliability of our method for the study of stem cells by confirming previously established facts, and then we addressed open questions. Our findings confirmed that colon crypts are monoclonal and that, throughout adulthood, the process of monoclonal conversion plays a major role in the maintenance of crypts. The absence of immortal strand mechanism in crypts stem cells was validated by the age-dependent accumulation of microsatellite mutations. In addition, we confirmed the positive correlation between physical and lineage proximity of crypts, by showing that the colon is separated into small domains that share a common ancestor. We gained new data demonstrating that colon epithelium is clustered separately from hematopoietic and other cell types, indicating that the colon is constituted of few progenitors and ruling out significant renewal of colonic epithelium from hematopoietic cells during adulthood. Overall, our study demonstrates the reliability of cell lineage reconstruction for the study of stem cell dynamics, and it further addresses open questions in colon stem cells. In addition, this method can be applied to study stem cell dynamics in other systems.

Rinkevich, B. (2011). "Cell cultures from marine invertebrates: new insights for capturing endless stemness." Mar Biotechnol (NY) **13**(3): 345-354.

Despite several decades of extensive research efforts, there is yet no single permanent cell line available from marine invertebrates as these cells stop dividing in vitro within 24-72 h after their isolation, starting cellular quiescence. This ubiquitous quiescent state should be modified in a way that at least some of the quiescent cells will become pluripotent, so they will have the ability to divide and become immortal. Following the above need, this essay introduces the rationale that the discipline of marine invertebrates' cell culture should gain from applying of two research routes, relevant to mammalian systems but less explored in the marine arena. The first is the use of adult stem cells (ASC) from marine organisms. Many marine invertebrate taxa maintain large pools of ASC in adulthood. Ample evidence attests that these cells from sponges, cnidarians, flatworms, crustaceans, mollusks, echinoderms, and ascidians play important roles in maintenance, regeneration, and asexual cloning, actively proliferating in vivo, resembling the vertebrates' cancer stem cells features. The second route is to target resting somatic cell constituents, manipulating them in the same way as has recently been performed on mammalian induced pluripotent stem (iPS) cells. While "iPS cells" are the outcome of an experimental manipulation, ASC are natural and rather frequent in a number of marine invertebrates. Above two cell categories reveal that there are more than a few types of seeds (cells) waiting to be sowed in the right soil (in vitro environmental conditions) for acquiring stemness and immortality. This rationale carries the potential to revolutionize the discipline of marine invertebrate cell cultures. When cultured "correctly," ASC and "iPS cells" from marine invertebrates may stay in their primitive stage and proliferate without differentiating into cells lineages, harnessing the stem cell's inherent abilities of self-replication versus differentiated progenies, toward the development of immortal cell lines.

Rivolta, M. N., et al. (1998). "Auditory hair cell precursors immortalized from the mammalian inner ear." Proc Biol Sci **265**(1406): 1595-1603.

Mammalian auditory hair cells are few in number, experimentally inaccessible, and do not proliferate postnatally or in vitro. Immortal cell lines with the potential to differentiate into auditory hair cells would substantially facilitate auditory research, drug development, and the isolation of critical molecules involved in hair cell biology. We have established two conditionally immortal cell lines that express at least five characteristic hair cell markers. These markers are the transcription factor Brn3.1, the alpha 9 subunit of the acetylcholine receptor, the stereociliary protein fimbrin and the myosins VI and VIIA. These hair cell precursors permit functional studies of cochlear genes and in the longer term they will provide the means to explore therapeutic methods of stimulating auditory hair cell regeneration.

Rivolta, M. N. and M. C. Holley (2002). "Asymmetric segregation of mitochondria and mortalin correlates with the multi-lineage potential of inner ear sensory cell progenitors in vitro." Brain Res Dev Brain Res **133**(1): 49-56.

The sensory epithelia of the inner ear include hair cells and supporting cells that share a common precursor. One possible mechanism involved in the genesis of these cell types is through asymmetric cell division. In this work we have studied asymmetric division of inner ear sensory cell progenitors in vitro in an attempt to understand how the different cell

phenotypes are generated. In the search for molecules that will segregate asymmetrically we have found that mitochondria in general, and a mitochondrial protein named mortalin in particular, are asymmetrically segregated during certain cell divisions. In one conditionally immortal cell line (UB/OC-1), which represents a population of committed hair cell precursors, mortalin is uniformly distributed in the cytoplasm and shared equally between sibling cells during division. In another cell line (UB/UE-1), which represents a bipotent, vestibular supporting cell that can produce both neonatal hair cells as well as supporting cells, mortalin segregates asymmetrically. In UB/UE-1, approximately 12% of the cells display an asymmetric distribution of mortalin and mitochondria. The proportion of asymmetric cells increases immediately after the release of the immortalizing gene and before the onset of differentiation. The asymmetric segregation of mortalin in the bipotent cell line and its uniform distribution in a committed, lineage-restricted cell line raises the possibility that it may play a role in cell fate determination.

Rocheteau, P. and S. Tajbakhsh (2008). "[Immortal DNA or epigenetic signature ?]." Med Sci (Paris) **24**(10): 847-852.

During mitosis each daughter cell inherits a full copy of the maternal genomic material. DNA replication, however, is an imprecise process, thus errors can arise resulting in potentially deleterious mutations over extended rounds of cell division and these may lead to cancinogenesis. Over thirty years ago, J. Cairns proposed that a cell could avoid the accumulation of mutations arising from DNA replication if all template DNA strands are inherited in one daughter cell during cell division, thus giving rise to the notion of << immortal >> DNA strands. In this model the stem cells would retain the template DNA (older) strands. Proving or disproving this notion experimentally has been challenging. Further, it has recently become apparent that epigenetic regulation of gene expression plays a critical role in governing cell states, self-renewal and differentiation. In light of these data, can the phenomenon on template DNA strand segregation also reflect this epigenetic signature? In this review we explore these notions, discuss the evidence in support of this theory, the implications, and some of the mechanisms which could explain this phenomenon.

Rodriguez-Brenes, I. A. and C. S. Peskin (2010). "Quantitative theory of telomere length regulation and cellular senescence." Proc Natl Acad Sci U S A **107**(12): 5387-5392.

In normal somatic cells, telomere length shortens with each cell replication. This progressive shortening is associated with cellular senescence and apoptosis. Germ cells, stem cells, and the majority of cancer cells express telomerase, an enzyme that extends telomere length and, when expressed at sufficient levels, can immortalize or extend the life span of a cell line. It is believed that telomeres switch between two states: capped and uncapped. The telomere state determines its accessibility to telomerase and also the onset of senescence. One hypothesis is that the t loop, a large lariat-like structure, represents the capped state. In this paper we model a telomere state on the basis of the biophysics of t-loop formation, allowing us to develop a single mathematical model that accounts for two processes: telomere length regulation for telomerase positive cells and cellular senescence in somatic cells. The model predicts the steady-state length distribution for telomerase positive cells, describes the time evolution of telomere length, and computes the life span of a cell line on the basis of the levels of TRF2 and telomerase expression. The model reproduces a wide range of experimental behavior and fits data from immortal cell lines (HeLa S3 and 293T) and somatic cells (human diploid fibroblasts) well. We conclude that the t loop as the capped state is a quantitatively reasonable model of telomere length regulation and cellular senescence.

Rosenberger, R. F. (1995). "The initiation of senescence and its relationship to embryonic cell differentiation." Bioessays **17**(3): 257-260.

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.

Rossant, J. (2001). "Stem cells from the Mammalian blastocyst." Stem Cells **19**(6): 477-482.

Early differentiation of the mammalian embryo leads to the development of two distinct lineages-the inner cell mass (ICM) and the trophectoderm. Cells of the ICM are pluripotent and give rise to all tissues of the fetus, while trophectoderm cells are restricted in their potential to the trophoblast

cell layers of the placenta. In the mouse, apparently immortal stem cell lines can be obtained from both cell types. These cell lines, embryonic stem (ES) cells and trophoblast stem (TS) cells, are morphologically and molecularly distinct and depend on different signaling pathways for their maintenance. They also show different cell fates when introduced into early embryos to generate chimeras. However, a change in the levels of expression of a key regulator of pluripotency, Oct4, can push ES cells towards the TS phenotype, when grown in TS cell conditions. Stem cell potential in the early embryo thus appears to depend on a combination of the levels of expression of key intrinsic regulators and the appropriate extrinsic environmental factors. Manipulation of both intrinsic and extrinsic regulators may be needed to reveal the full potential of stem cells from other stages of development and the adult.

Rota Nodari, L., et al. (2010). "Long-term survival of human neural stem cells in the ischemic rat brain upon transient immunosuppression." PLoS One **5**(11): e14035.

Understanding the physiology of human neural stem cells (hNSCs) in the context of cell therapy for neurodegenerative disorders is of paramount importance, yet large-scale studies are hampered by the slow-expansion rate of these cells. To overcome this issue, we previously established immortal, non-transformed, telencephalic-diencephalic hNSCs (IhNSCs) from the fetal brain. Here, we investigated the fate of these IhNSC's immediate progeny (i.e. neural progenitors; IhNSC-Ps) upon unilateral implantation into the corpus callosum or the hippocampal fissure of adult rat brain, 3 days after global ischemic injury. One month after grafting, approximately one fifth of the IhNSC-Ps had survived and migrated through the corpus callosum, into the cortex or throughout the dentate gyrus of the hippocampus. By the fourth month, they had reached the ipsilateral subventricular zone, CA1-3 hippocampal layers and the controlateral hemisphere. Notably, these results could be accomplished using transient immunosuppression, i.e administering cyclosporine for 15 days following the ischemic event. Furthermore, a concomitant reduction of reactive microglia (Iba1+ cells) and of glial, GFAP+ cells was also observed in the ipsilateral hemisphere as compared to the controlateral one. IhNSC-Ps were not tumorigenic and, upon in vivo engraftment, underwent differentiation into GFAP+ astrocytes, and beta-tubulinIII+ or MAP2+ neurons, which displayed GABAergic and GLUTAmatergic markers. Electron microscopy analysis pointed to the formation of mature synaptic contacts between host and donor-derived neurons, showing the full maturation of the IhNSC-P-derived neurons and their likely functional integration into the host tissue. Thus, IhNSC-Ps possess long-term survival and engraftment capacity upon transplantation into the globally injured ischemic brain, into which they can integrate and mature into neurons, even under mild, transient immunosuppressive conditions. Most notably, transplanted IhNSC-P can significantly dampen the inflammatory response in the lesioned host brain. This work further supports hNSCs as a reliable and safe source of cells for transplantation therapy in neurodegenerative disorders.

Ruedl, C., et al. (2008). "Manipulation of immune system via immortal bone marrow stem cells." Int Immunol **20**(9): 1211-1218.

Extensive amplification of hematopoietic stem cells (HSCs) and their multipotent primitive progenitors (MPPs) in culture would greatly benefit not only clinical transplantation but also provide a potential tool to manipulate all cellular lineages derived from these cells for gene therapy and experimental purposes. Here, we demonstrate that mouse bone marrow cultures containing cells engineered to over-express NUP98-HOXB4 fusion protein support self-renewal of physiologically normal HSC and MPP for several weeks leading practically to their unlimited expansion. This allows time consuming and cumulative in vitro experimental manipulations without sacrificing their ability to differentiate in vivo or in vitro to any hematopoietic lineage.

Sabour, D. and H. R. Scholer (2012). "Reprogramming and the mammalian germline: the Weismann barrier revisited." Curr Opin Cell Biol **24**(6): 716-723.

The germline represents a unique cell type that can transmit genetic material to the next generation. During early embryonic development, somatic cells give rise to a small population of cells known as germ cells, which eventually differentiate into mature gametes. Germ cells undergo a process of removing and resetting relevant epigenetic information, mainly by DNA demethylation. This extensive epigenetic reprogramming leads to the conversion of germ cells into immortal cells that can pass on the genome to the next generation. In the absence of germline-specific reprogramming, germ cells would preserve the old, parental epigenetic memory, which would prevent the transfer of heritable information to the offspring. On the contrary, somatic cells cannot reset epigenetic information by preserving the full methylation pattern on imprinting genes. In this review, we focus on the capacity of germ cells and somatic cells (soma) to transfer genetic information to the next generation, and thus revisit the Weismann theory of heredity.

Sachs, L. and J. Lotem (1993). "Control of programmed cell death in normal and leukemic cells: new implications for therapy." Blood **82**(1): 15-21.

Programmed cell death (apoptosis) is a normal process by which cells are eliminated during normal embryonic development and in adult life. Disruption of this normal process resulting in illegitimate cell survival can cause developmental abnormalities and facilitate cancer development. Normal cells require certain viability factors and undergo programmed cell death when these factors are withdrawn. The viability factors are required throughout the differentiation process from immature to mature cells. Although many viability factors are also growth factors, viability and growth are separately regulated. Viability factors can have clinical value in decreasing the loss of normal cells including the loss that occurs after irradiation, exposure to other cytotoxic agents or virus infection including AIDS. There is no evidence that occurs after irradiation, exposure to other cytotoxic agents or virus infection including AIDS. There is no evidence that cancer cells are immortal. Programmed cell death can be induced in leukemic cells by removal of viability factors, by cytotoxic therapeutic agents, or by the tumor-suppressor gene wild-type p53. All these forms of induction of programmed cell death in leukemic cells can be suppressed by the same viability factors that suppress programmed cell death in normal cells. A tumor-promoting phorbol ester can also suppress this death program. The induction of programmed cell death can be enhanced by deregulated expression of the gene c-myc and suppressed by the gene bcl-2. Mutant p53 and bcl-2 suppress the enhancing effect on cell death of deregulated c-myc, and thus allow induction of cell proliferation and inhibition of differentiation which are other functions of deregulated c-myc. The suppression of cell death by mutant p53 and bcl-2 increases the probability of developing cancer. The suppression of programmed cell death in cancer cells by viability factors suggests that decreasing the level of these factors may increase the effectiveness of cytotoxic cancer therapy. Treatments that downregulate the expression or activity of mutant p53 and bcl-2 in cancer cells should also be useful for therapy.

Safinia, N. and S. L. Minger (2009). "Generation of hepatocytes from human embryonic stem cells." Methods Mol Biol **481**: 169-180.

Use of human hepatocytes for therapeutic and drug discovery applications is hampered by limited tissue source and the inability of hepatocytes to proliferate and maintain function long-term in vitro. Human embryonic stem (hES) cells are immortal and pluripotent and may provide a cell source for functional human hepatocytes (1) Here we have outlined some of the protocols currently in use for the generation of hepatocytes from hES cells.

Sahu, S., et al. (2017). "Secrets from immortal worms: What can we learn about biological ageing from the planarian model system?" Semin Cell Dev Biol **70**: 108-121.

Understanding how some animals are immortal and avoid the ageing process is important. We currently know very little about how they achieve this. Research with genetic model systems has revealed the existence of conserved genetic pathways and molecular processes that affect longevity. Most of these established model organisms have relatively short lifespans. Here we consider the use of planarians, with an immortal life-history that is able to entirely avoid the ageing process. These animals are capable of profound feats of regeneration fueled by a population of adult stem cells called neoblasts. These cells are capable of indefinite self-renewal that has underpinned the evolution of animals that reproduce only by fission, having disposed of the germline, and must therefore be somatically immortal and avoid the ageing process. How they do this is only now starting to be understood. Here we suggest that the evidence so far supports the hypothesis that the lack of ageing is an emergent property of both being highly regenerative and the evolution of highly effective mechanisms for ensuring genome stability in the neoblast stem cell population. The details of these mechanisms could prove to be very informative in understanding how the causes of ageing can be avoided, slowed or even reversed.

Sakellariou, P., et al. (2016). "Neuromuscular electrical stimulation promotes development in mice of mature human muscle from immortalized human myoblasts." Skelet Muscle **6**: 4.

BACKGROUND: Studies of the pathogenic mechanisms underlying human myopathies and muscular dystrophies often require animal models, but models of some human diseases are not yet available. Methods to promote the engraftment and development of myogenic cells from individuals with such diseases in mice would accelerate such studies and also provide a useful tool for testing therapeutics. Here, we investigate the ability of immortalized human myogenic precursor cells (hMPCs) to form mature human myofibers following implantation into the hindlimbs of non-obese diabetic-Rag1 (null) IL2rgamma (null) (NOD-Rag)-immunodeficient mice. RESULTS: We report that hindlimbs of NOD-Rag mice that are X-irradiated, treated with cardiotoxin, and then injected with immortalized control hMPCs or hMPCs from an individual with facioscapulohumeral muscular dystrophy (FSHD) develop mature human myofibers. Furthermore, intermittent neuromuscular electrical stimulation (iNMES) of the peroneal nerve of the engrafted limb enhances the development of mature fibers in the grafts formed by both immortal cell lines. With control cells, iNMES increases the number and size of the human myofibers that form and promotes closer fiber-to-fiber packing. The human myofibers in

the graft are innervated, fully differentiated, and minimally contaminated with murine myonuclei. CONCLUSIONS: Our results indicate that control and FSHD human myofibers can form in mice engrafted with hMPCs and that iNMES enhances engraftment and subsequent development of mature human muscle.

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

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

Sanchez, J. F., et al. (2006). "GABAergic lineage differentiation of AF5 neural progenitor cells in vitro." Cell Tissue Res **324**(1): 1-8.

We have previously described an immortal rat central-nervous-system progenitor cell line, AF5, which is able to exit the cell cycle and assume a differentiated state with neuronal properties. The phenotypic specification of differentiated AF5 cells, however, is not known. In the present study, when induced to differentiate by serum starvation in Neurobasal medium, AF5 cells down-regulate glial fibrillary acidic protein and up-regulate expression of beta-III-tubulin, medium-molecular-weight neurofilament protein, and neuronal growth-associated protein 43. Expression of the gamma-aminobutyric acid (GABA) lineage marker, glutamic acid decarboxylase 67 (GAD67), increases during differentiation, suggesting that AF5 cells adopt a GABAergic lineage. Time-course analysis of the GABAergic neuron specification transcription factor, Pitx2, by reverse transcription/polymerase chain reaction, has shown an increase in the Pitx2 transcript 48 h after initiation of differentiation. In differentiated AF5 cells, expression of the Pitx2 target gene products GAD65 and GABA transporter-1 increases. Cellular GABA levels in differentiated AF5 cells increase by about 26-fold, and GABA release into the medium is 150-fold higher compared with that of undifferentiated cells. Therefore, AF5 cells can be induced to differentiate to a neuronal phenotype with a GABAergic lineage.

Sandmaier, S. E., et al. (2015). "Generation of induced pluripotent stem cells from domestic goats." Mol Reprod Dev **82**(9): 709-721.

The creation of genetically modified goats provides a powerful approach for improving animal health, enhancing production traits, animal pharming, and for ensuring food safety all of which are high-priority goals for animal agriculture. The availability of goat embryonic stem cells (ESCs) that are characteristically immortal in culture would be of enormous benefit for developing genetically modified animals. As an alternative to long-sought goat ESCs, we generated induced pluripotent stem cells (iPSC) by forced expression of bovine POU5F1, SOX2, MYC, KLF4, LIN-28, and NANOG reprogramming factors in combination with a MIR302/367 cluster, delivered by lentiviral vectors. In order to minimize integrations, the reprogramming factor coding sequences were assembled with porcine teschovirus-1 2A (P2A) self-cleaving peptides that allowed for tri-cistronic expression from each vector. The lentiviral-transduced cells were cultured on irradiated mouse feeder cells in a semi-defined, serum-free medium containing fibroblast growth factor (FGF) and/or leukemia inhibitory factor (LIF). The resulting goat iPSC exhibit cell and colony morphology typical of human and mouse ESCs-that is, well-defined borders, a high nuclear-to-cytoplasmic ratio, a short cell-cycle interval, alkaline phosphatase expression, and the ability to generate teratomas in vivo. Additionally, these goat iPSC demonstrated the ability to differentiate into directed lineages in vitro. These results constitute the first steps in establishing integration and footprint-free iPSC from ruminants. Mol. Reprod. Dev. 82: 709-721, 2015. (c) 2015 Wiley Periodicals, Inc.

Santoro, G., et al. (2009). "The anatomic location of the soul from the heart, through the brain, to the whole body, and beyond: a journey through Western history, science, and philosophy." Neurosurgery **65**(4): 633-643; discussion 643.

OBJECTIVE: To describe representative Western philosophical, theological, and scientific ideas regarding the nature and location of the soul from the Egyptians to the contemporary period; and to determine the principal themes that have structured the

history of the development of the concept of the soul and the implications of the concept of the soul for medical theory and practice. METHODS: We surveyed the ancient Egyptian, Greek, and Roman periods, the early, Medieval, and late Christian eras, as well as the Renaissance, Enlightenment, and Modern periods to determine the most salient ideas regarding the nature and location of the soul. RESULTS: In the history of Western theological, philosophical, and scientific/medical thought, there exist 2 dominant and, in many respects, incompatible concepts of the soul: one that understands the soul to be spiritual and immortal, and another that understands the soul to be material and mortal. In both cases, the soul has been described as being located in a specific organ or anatomic structure or as pan-corporeal, pervading the entire body, and, in some instances, trans-human and even pan-cosmological. Moreover, efforts to discern the nature and location of the soul have, throughout Western history, stimulated physiological exploration as well as theoretical understanding of human anatomy. The search for the soul has, in other words, led to a deepening of our scientific knowledge regarding the physiological and, in particular, cardiovascular and neurological nature of human beings. In addition, in virtually every period, the concept of the soul has shaped how societies thought about, evaluated, and understood the moral legitimacy of scientific and medical procedures: from performing abortions and autopsies to engaging in stem cell research and genetic engineering. CONCLUSION: Our work enriches our shared understanding of the soul by describing some of the key formulations regarding the nature and location of the soul by philosophers, theologians, and physicians. In doing so, we are better able to appreciate the significant role that the concept of the soul has played in the development of Western scientific, medical, and spiritual life. Although ideas about the soul have changed significantly throughout Western history, the idea of the soul as being real and essential to one's personhood has been, and remains, pervasive throughout every period of Western history.

Sato, S., et al. (2016). "Single-cell lineage tracking analysis reveals that an established cell line comprises putative cancer stem cells and their heterogeneous progeny." Sci Rep **6**: 23328.

Mammalian cell culture has been used in many biological studies on the assumption that a cell line comprises putatively homogeneous clonal cells, thereby sharing similar phenotypic features. This fundamental assumption has not yet been fully tested; therefore, we developed a method for the chronological analysis of individual HeLa cells. The analysis was performed by live cell imaging, tracking of every single cell recorded on imaging videos, and determining the fates of individual cells. We found that cell fate varied significantly, indicating that, in contrast to the assumption, the HeLa cell line is composed of highly heterogeneous cells. Furthermore, our results reveal that only a limited number of cells are immortal and renew themselves, giving rise to the remaining cells. These cells have reduced reproductive ability, creating a functionally heterogeneous cell population. Hence, the HeLa cell line is maintained by the limited number of immortal cells, which could be putative cancer stem cells.

Schepers, A. G., et al. (2011). "Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes." EMBO J **30**(6): 1104-1109.

Somatic cells have been proposed to be limited in the number of cell divisions they can undergo. This is thought to be a mechanism by which stem cells retain their integrity preventing disease. However, we have recently discovered intestinal crypt stem cells that persist for the lifetime of a mouse, yet divide every day. We now demonstrate biochemically that primary isolated Lgr5+ve stem cells contain significant telomerase activity. Telomerase activity rapidly decreases in the undifferentiated progeny of these stem cells and is entirely lost in differentiated villus cells. Conversely, asymmetric segregation of chromosomes has been proposed as a mechanism for stem cells to protect their genomes against damage. We determined the average cell cycle length of Lgr5+ve stem cells at 21.5 h and find that Lgr5+ve intestinal stem cells randomly segregate newly synthesized DNA strands, opposing the 'immortal strand' hypothesis.

Shen, Q., et al. (2018). "Overexpression of the 14-3-3gamma protein in uterine leiomyoma cells results in growth retardation and increased apoptosis." Cell Signal **45**: 43-53.

Protein 14-3-3gamma was significantly reduced in human uterine leiomyoma compared to the adjacent normal myometrium tissue. To investigate the possible link between the reduced 14-3-3gamma expression and uterine leiomyoma growth, we have overexpressed 14-3-3gamma protein in uterine leiomyomal cells and its effects on cell proliferation and apoptosis were analyzed. Over-expression of 14-3-3gamma was achieved by transducing into two types of uterine leiomyoma cells (primary culture cells and immortal stem cells) with a 14-3-3gamma expressing adenovirus vector. Differentially expressed proteins were screened by the proteomics tool (TMT-LCTMS), followed by PANTHER database analysis to single out specifically modified signaling pathway proteins, which were confirmed by Phospho-MAPK Antibody Array and Western blots analysis. The results showed that increase in 14-3-3gamma expression in both two types of human uterine leiomyoma cells inhibited cell

proliferation and induced apoptosis. Proteomic screening has found 42 proteins, among 5846, that were significantly affected. PANTHER database and GeneMANIA analysis of the differentially expressed proteins have found that proteins involved in apoptosis signaling and cytoskeletal/adhesion were among the ones affected the most. Further analysis of the key signaling pathways have found that over-expression of 14-3-3gamma resulted in reductions in the phosphorylations of multiple signaling molecules, including AKT, pan, ERK1/2, GSK-3 alpha/beta, MEK1/2, Foxo1 and Vimentin. In conclusion, the loss of 14-3-3gamma may have causal effects on the growth of uterine leiomyoma, which may function through modifying multiple signaling pathways, including AKT-Foxo and/or MEK1/2-ERK1/2.

Sherley, J. L. (2002). "Asymmetric cell kinetics genes: the key to expansion of adult stem cells in culture." ScientificWorldJournal **2**: 1906-1921.

A singular challenge in stem cell research today is the expansion and propagation of functional adult stem cells. Unlike embryonic stem cells, which are immortal in culture, adult stem cells are notorious for the difficulty encountered when attempts are made to expand them in culture. One overlooked reason for this difficulty may be the inherent asymmetric cell kinetics of stem cells in postnatal somatic tissues. Senescence is the expected fate of a culture whose growth depends on adult stem cells that divide with asymmetric cell kinetics. Therefore, the bioengineering of strategies to expand adult stem cells in culture requires knowledge of cellular mechanisms that control asymmetric cell kinetics. The properties of several genes recently implicated to function in a cellular pathway(s) that regulates asymmetric cell kinetics are discussed. Understanding the function of these genes in asymmetric cell kinetics mechanisms may be the key that unlocks the adult stem cell expansion problem.

Sherley, J. L. (2002). "Asymmetric cell kinetics genes: the key to expansion of adult stem cells in culture." Stem Cells **20**(6): 561-572.

A singular challenge in stem cell research today is the expansion and propagation of functional adult stem cells. Unlike embryonic stem cells, which are immortal in culture, adult stem cells are notorious for the difficulty encountered when attempts are made to expand them in culture. One overlooked reason for this difficulty may be the inherent asymmetric cell kinetics of stem cells in postnatal somatic tissues. Senescence is the expected fate of a culture whose growth depends on adult stem cells that divide with asymmetric cell kinetics. Therefore, the bioengineering of strategies to expand adult stem cells in culture requires knowledge of cellular mechanisms that control asymmetric cell kinetics. The properties of several genes recently implicated to function in a cellular pathway(s) that regulates asymmetric cell kinetics are discussed. Understanding the function of these genes in asymmetric cell kinetics mechanisms may be the key that unlocks the adult stem cell expansion problem.

Sherley, J. L. (2008). "A new mechanism for aging: chemical "age spots" in immortal DNA strands in distributed stem cells." Breast Dis **29**: 37-46.

The existence of immortal DNA strands (IDSs) in distributed stem cells (DSCs) of adult human tissues was first inferred by Cairns. Cairns deduced the existence of IDSs by connecting two seemingly disparate observations - one his own and the other belonging to Lark. Cairns noted a mathematical discrepancy between predicted human tissue cell mutation rates and human cancer incidence. He integrated this insight with Lark's earlier discovery of non-random mitotic chromosome segregation in both plant root tip cells and mouse fetal fibroblast cultures to predict the existence of IDSs as the essential elements of a mutation-defense mechanism in DSCs. Since Cairns' seminal prediction, several laboratories have identified IDSs in diverse mammalian cells with DSC properties. Past studies focused on the potential roles of IDSs as originally envisioned in DSC genetic fidelity or in the maintenance of the DSC phenotype. Another possible consequence of IDSs, aging, has received little attention. Herein, the potential for cumulative chemical modifications and decompositions (i.e., "age spots") of IDSs in DSCs to act as a major determinant of human aging is considered. If accrued chemical alterations of IDSs prove to be essential determinants of aging, then a means to restore IDSs may yield new strategies for tissue rejuvenation.

Sherley, J. L. (2015). "Stem cell genetic fidelity." Front Genet **6**: 51.

Shervington, A., et al. (2009). "Telomerase downregulation in cancer brain stem cell." Mol Cell Biochem **331**(1-2): 153-159.

Cancer stem cells (CSCs) are a minute sub-population of self-renewing, immortal cells, which can be responsible for chemoresistance observed in the treatment of cancer. CSCs are similar to cancer cells requiring telomerase activity or alternative mechanisms for their proliferation and regeneration. This study explored the correlation between CD133 (stem cell marker) and telomerase expression using CD133+ cells isolated from the glioma GOS-3 cell line with magnetic affinity cell sorting (MACS). GOS-3 CD133+ showed a transcription downregulation of hTERT (approximately 100-fold decrease) compared with CD133- cells. In order to further substantiate the novel finding, serum deprivation was adopted to enrich CD133 expression in GOS-3 cells. A pronounced upregulation of cd133 and downregulation of telomerase expression were produced as a consequence of decreasing serum supplement levels in GOS-3 cells.

These findings showed for the first time that telomerase is downregulated in brain cancer stem cells compared to cancer cells.

Shimoni-Sebag, A., et al. (2013). "RRM1 domain of the splicing oncoprotein SRSF1 is required for MEK1-MAPK-ERK activation and cellular transformation." Carcinogenesis **34**(11): 2498-2504.

Alternative splicing regulators have emerged as new players in cancer development, modulating the activities of many tumor suppressors and oncogenes and regulating the signaling pathways. However, little is known about the mechanisms by which these oncogenic splicing factors lead to cellular transformation. We have shown previously that the splicing factor serine and arginine splicing factor 1 (SRSF1; SF2/ASF) is a proto-oncogene, which is amplified in breast cancer and transforms immortal cells when overexpressed. In this study, we performed a structure-function analysis of SRSF1 and found that the RNA recognition motif 1 (RRM1) domain is required for its oncogenic activity. Deletion of RRM1 eliminated the splicing activity of SRSF1 on some of its endogenous targets. Moreover, we found that SRSF1 elevates the expression of B-Raf and activates the mitogen-activated protein kinase kinase (MEK) extracellular signal-regulated kinase (ERK) pathway and that RRM1 is required for this activation as well. B-Raf-MEK-ERK activation by SRSF1 contributes to transformation as pharmacological inhibition of MEK1 inhibits SRSF1-mediated transformation. In conclusion, RRM1 of SRSF1 is both required (and when tethered to the RS domain) also sufficient to activate the Raf-MEK-ERK pathway and to promote cellular transformation.

Shinin, V., et al. (2006). "Asymmetric division and cosegregation of template DNA strands in adult muscle satellite cells." Nat Cell Biol **8**(7): 677-687.

Satellite cells assure postnatal skeletal muscle growth and repair. Despite extensive studies, their stem cell character remains largely undefined. Using pulse-chase labelling with BrdU to mark the putative stem cell niche, we identify a subpopulation of label-retaining satellite cells during growth and after injury. Strikingly, some of these cells display selective template-DNA strand segregation during mitosis in the muscle fibre in vivo, as well as in culture independent of their niche, indicating that genomic DNA strands are nonequivalent. Furthermore, we demonstrate that the asymmetric cell-fate determinant Numb segregates selectively to one daughter cell during mitosis and before differentiation, suggesting that Numb is associated with self-renewal. Finally, we show that template DNA cosegregates with Numb in label-retaining cells that express the self-renewal marker Pax7. The cosegregation of 'immortal' template DNA strands and their link with the asymmetry apparatus has important implications for stem cell biology and cancer.

Shrestha, S., et al. (2019). "Characterization and determination of cadmium resistance of CD133(+)/CD24(+) and CD133(-)/CD24(+) cells isolated from the immortalized human proximal tubule cell line, RPTEC/TERT1." Toxicol Appl Pharmacol **375**: 5-16.

Stem/progenitor cells are involved in the regeneration of the renal tubules after damage due to a toxic insult. However, the mechanism involved in the regeneration of the tubules by the stem cells is not well understood due to the lack of immortal cell lines that represent the stem/progenitor cells of the kidney. A previous study from our laboratory has shown that the immortalized cell line RPTEC/TERT1 contains two populations of cells, one co-expressing CD24 and CD133, the other expressing CD24 only. The goal of the present study was to determine if both these populations could be sorted into separate independent cultures and if so, determine their characteristic features and response to the nephrotoxicant cadmium. The results of our study show that both the populations of cells could grow as independent cultures and maintain their phenotype after extended sub-culture. The CD133(+)/CD24(+) co-expressing cells formed multicellular spheroids (nephrospheres), a characteristic feature of stem/progenitor cells, and formed branched tubule-like structures when grown on the surface of matrigel, whereas the CD133(-)/CD24(+) cells were unable to form these structures. The CD133(+)/CD24(+) cells were able to grow and undergo neurogenic, adipogenic, osteogenic, and tubulogenic differentiation, whereas the CD133(-)/CD24(+) cells expressed some of the differentiation markers but were unable to grow in some of the specialized growth media. The CD133(+)/ CD24(+) co-expressing cells had a shorter doubling time compared to the cells that expressed only CD24, and were more resistant to the toxic effects of the heavy metal, cadmium. In conclusion, the isolation and characterization of these two cell populations form the RPTEC/TERT1 cell line will facilitate the development of studies that determine the mechanisms involved in tubular damage and regeneration particularly after a toxic insult.

Sinden, J. D., et al. (2000). "Functional repair with neural stem cells." Novartis Found Symp **231**: 270-283; discussion 283-278, 302-276.

Approval to commence phase I/II clinical trials with neural stem cells requires proof of concept in well-accepted animal models of human neurological disease or injury. We initially showed that the conditionally immortal MHP36 line of hippocampal origin (derived from the H-2Kb-tsA58 transgenic mouse) was effective in repopulating CA1 neurons in

models of global ischaemia and repairing cognitive function, and have now shown that this line is multifunctional. MHP36 cells are effective in restoring spatial memory deficits in rats after excitotoxic lesions of the cholinergic projections to cortex and hippocampus and in rats showing cognitive impairments due to normal ageing. Moreover, grafts of MHP36 cells are effective in reversing sensory and motor deficits and reducing lesion volume as a consequence of occlusion of the middle cerebral artery, the major cause of stroke. In contrast, MHP36 cell grafts were unable to repair motor asymmetries in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal dopamine system, the prototype rodent model of Parkinson's disease. These data show that conditionally immortal neuroepithelial stem cells are multifunctional, being able to repair diverse types of brain damage. However, there are limitations to this multifunctionality, suggesting that lines from different regions of the developing brain will be required to treat different brain diseases. ReNeuron is currently developing human neuroepithelial stem cell lines from different brain regions and with similar reparative properties to our murine lines.

Slape, C., et al. (2007). "Mouse embryonic stem cells that express a NUP98-HOXD13 fusion protein are impaired in their ability to differentiate and can be complemented by BCR-ABL." Leukemia **21**(6): 1239-1248.

NUP98-HOXD13 (NHD13) fusions have been identified in patients with myelodysplastic syndrome, acute myelogenous leukemia and chronic myeloid leukemia blast crisis. We generated 'knock-in' mouse embryonic stem (ES) cells that express a NHD13 fusion gene from the endogenous murine NUP98 promoter, and used an in vitro differentiation system to differentiate the ES cells to hematopoietic colonies. Replating assays demonstrated that the partially differentiated NHD13 ES cells were immortal, and two of these cultures were transferred to liquid culture. These cell lines are partially differentiated immature hematopoietic cells, as determined by morphology, immunophenotype and gene expression profile. Despite these characteristics, they were unable to differentiate when exposed to high concentrations of erythropoietin (Epo), granulocyte colony-stimulating factor or macrophage colony-stimulating factor. The cell lines are incompletely transformed, as evidenced by their dependence on interleukin 3 (IL-3), and their failure to initiate tumors when injected into immunodeficient mice. We attempted genetic complementation of the NHD13 gene using IL-3 independence and tumorigenicity in immunodeficient mice as markers of transformation, and found that BCR-ABL successfully transformed the cell lines. These findings support the hypothesis that expression of a NHD13 fusion gene impairs hematopoietic differentiation, and that these cell lines present a model system to study the nature of this impaired differentiation.

Snoeck, H. W. (2015). "Can Metabolic Mechanisms of Stem Cell Maintenance Explain Aging and the Immortal Germline?" Cell Stem Cell **16**(6): 582-584.

The mechanisms underlying the aging process are not understood. Even tissues endowed with somatic stem cells age while the germline appears immortal. I propose that this paradox may be explained by the pervasive use of glycolysis by somatic stem cells as opposed to the predominance of mitochondrial respiration in gametes.

Soltysova, A., et al. (2005). "Cancer stem cells." Neoplasma **52**(6): 435-440.

There is an increasing evidence supporting the cancer stem cell hypothesis. Normal stem cells in the adult organism are responsible for tissue renewal and repair of aged or damaged tissue. A substantial characteristic of stem cells is their ability for self-renewal without loss of proliferation capacity with each cell division. The stem cells are immortal, and rather resistant to action of drugs. They are able to differentiate and form specific types of tissue due to the influence of microenvironmental and some other factors. Stem cells divide asymmetrically producing two daughter cells -- one is a new stem cell and the second is progenitor cell, which has the ability for differentiation and proliferation, but not the capability for self-renewal. Cancer stem cells are in many aspects similar to the stem cells. It has been proven that tumor cells are heterogeneous comprising rare tumor initiating cells and abundant non-tumor initiating cells. Tumor initiating cells -- cancer stem cells have the ability of self-renewal and proliferation, are resistant to drugs, and express typical markers of stem cells. It is not clear whether cancer stem cells originate from normal stem cells in consequence of genetic and epigenetic changes and/or by redifferentiation from somatic tumor cells to the stem-like cells. Probably both mechanisms are involved in the origin of cancer stem cells. Dysregulation of stem cell self-renewal is a likely requirement for the development of cancer. Isolation and identification of cancer stem cells in human tumors and in tumor cell lines has been successful. To date, the existence of cancer stem cells has been proven in acute and chronic myeloid leukemia, in breast cancer, in brain tumors, in lung cancer and gastrointestinal tumors. Cancer stem cell model is also consistent with some clinical observations. Although standard chemotherapy kills most cells in a tumor, cancer stem cells remain viable. Despite the small number of such cells, they might be the cause of tumor recurrence, sometimes many years after the "successful" treatment of primary tumor. Growth of metastases in distinct areas of body and their

cellular heterogeneity might be consequence of cancer stem cell differentiation and/or dedifferentiation and asymmetric division of cancer stem cells. Further characterization of cancer stem cells is needed in order to find ways to destroy them, which might contribute significantly to the therapeutic management of malignant tumors.

Sotillo, R., et al. (2001). "Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors." EMBO J **20**(23): 6637-6647.

We have introduced a point mutation in the first coding exon of the locus encoding the cyclin-dependent kinase 4 (Cdk4) by homologous recombination in embryonic stem cells. This mutation (replacement of Arg24 by Cys) was first found in patients with hereditary melanoma and renders Cdk4 insensitive to INK4 inhibitors. Here, we report that primary embryonic fibroblasts expressing the mutant Cdk4R24C kinase are immortal and susceptible to transformation by Ras oncogenes. Moreover, homozygous Cdk4(R24C/R24C) mutant mice develop multiple tumors with almost complete penetrance. The most common neoplasia (endocrine tumors and hemangiosarcomas) are similar to those found in pRb(+/-) and p53(-/-) mice. This Cdk4 mutation cooperates with p53 and p27(Kip1) deficiencies in decreasing tumor latency and favoring development of specific tumor types. These results provide experimental evidence for a central role of Cdk4 regulation in cancer and provide a valuable model for testing the potential anti-tumor effect of Cdk4 inhibitors in vivo.

Sotiropoulou, P. A., et al. (2008). "The majority of multipotent epidermal stem cells do not protect their genome by asymmetrical chromosome segregation." Stem Cells **26**(11): 2964-2973.

The maintenance of genome integrity in stem cells (SCs) is critical for preventing cancer formation and cellular senescence. The immortal strand hypothesis postulates that SCs protect their genome by keeping the same DNA strand throughout life by asymmetrical cell divisions, thus avoiding accumulation of mutations that can arise during DNA replication. The in vivo relevance of this model remains to date a matter of intense debate. In this study, we revisited this long-standing hypothesis, by analyzing how multipotent hair follicle (HF) SCs segregate their DNA strands during morphogenesis, skin homeostasis, and SC activation. We used three different in vivo approaches to determine how HF SCs segregate their DNA strand during cell divisions. Double-labeling studies using pulse-chase experiments during morphogenesis and the first adult hair cycle showed that HF SCs incorporate two different nucleotide analogs, contradictory to the immortal strand hypothesis. The co-segregation of DNA and chromatin labeling during pulse-chase experiments demonstrated that label retention in HF SCs is rather a mark of relative quiescence. Moreover, DNA labeling of adult SCs, similar to labeling during morphogenesis, also resulted in label retention in HF SCs, indicating that chromosome segregation occurs randomly in most of these cells. Altogether, our results demonstrate that DNA strand segregation occurs randomly in the majority of HF SCs during development, tissue homeostasis, and following SC activation. Disclosure of potential conflicts of interest is found at the end of this article.

Southam, K. A., et al. (2013). "Microfluidic primary culture model of the lower motor neuron-neuromuscular junction circuit." J Neurosci Methods **218**(2): 164-169.

Modelling the complex process of neuromuscular signalling is key to understanding not only normal circuit function but also importantly the mechanisms underpinning a range of degenerative diseases. We describe a novel in vitro model of the lower motor neuron-neuromuscular junction circuit, incorporating primary spinal motor neurons, supporting glia and skeletal muscle. This culture model is designed to spatially mimic the unique anatomical and cellular interactions of this circuit in compartmented microfluidic devices, such that the glial cells are located with motor neuron cell bodies in the cell body chamber and motor neuron axons extend to a distal chamber containing skeletal muscle cells whilst simultaneously allowing targeted intervention. This model is suitable for use in conjunction with a range of downstream experimental approaches and could also be modified to utilise other cellular sources including appropriate immortal cell lines, cells derived from transgenic models of disease and also patient derived stem cells.

Sparks, R. L., et al. (1986). "Differentiation, dedifferentiation, and transdifferentiation of BALB/c 3T3 T mesenchymal stem cells: potential significance in metaplasia and neoplasia." Cancer Res **46**(10): 5312-5319.

The expression of defects in the control of cellular differentiation is thought to be of etiological significance in the early stages of carcinogenesis. This possibility is supported by a variety of experimental studies including those that have established that metaplastic changes in cells can represent preneoplastic lesions in vivo. To evaluate this question in greater detail, we have used 3T3 T mesenchymal stem cells as a model system. These cells express certain characteristics of preneoplastic cells even though they can regulate their proliferation and even though they can undergo nonterminal and terminal differentiation into adipocytes. For example, they are immortal and aneuploid, and they show a proclivity to undergo

spontaneous or induced neoplastic transformation compared to normal human cells. The question we sought to answer in the current experiments concerns whether predifferentiation growth arrest and/or nonterminal differentiation in such preneoplastic cells is completely reversible or whether these processes induce the expression of the new stable program that limits the cells' proliferative potential and reduces the cells' subsequent differentiation potential in a manner comparable to that which is thought to occur in normal stem cells. The results show that arrest at both the predifferentiation state and at the nonterminal differentiation state is a completely reversible phenomenon that does not limit the cells' subsequent growth or differentiation potential. In fact, the results show that, when nonterminally differentiated 3T3 T adipocytes are induced to dedifferentiate, they can subsequently redifferentiate into macrophages. We therefore suggest that preneoplasia as expressed in 3T3 T mesenchymal stem cells is associated with the expression of defects in the ability to integrally control cellular differentiation and proliferation. As a result, the data suggest that such cells express an increased proclivity to undergo metaplastic change and complete neoplastic transformation.

Steinhauser, M. L., et al. (2012). "Multi-isotope imaging mass spectrometry quantifies stem cell division and metabolism." Nature **481**(7382): 516-519.

Mass spectrometry with stable isotope labels has been seminal in discovering the dynamic state of living matter, but is limited to bulk tissues or cells. We developed multi-isotope imaging mass spectrometry (MIMS) that allowed us to view and measure stable isotope incorporation with submicrometre resolution. Here we apply MIMS to diverse organisms, including Drosophila, mice and humans. We test the 'immortal strand hypothesis', which predicts that during asymmetric stem cell division chromosomes containing older template DNA are segregated to the daughter destined to remain a stem cell, thus insuring lifetime genetic stability. After labelling mice with (15)N-thymidine from gestation until post-natal week 8, we find no (15)N label retention by dividing small intestinal crypt cells after a four-week chase. In adult mice administered (15)N-thymidine pulse-chase, we find that proliferating crypt cells dilute the (15)N label, consistent with random strand segregation. We demonstrate the broad utility of MIMS with proof-of-principle studies of lipid turnover in Drosophila and translation to the human haematopoietic system. These studies show that MIMS provides high-resolution quantification of stable isotope labels that cannot be obtained using other techniques and that is broadly applicable to biological and medical research.

Stratford, E. W., et al. (2012). "Characterization of liposarcoma cell lines for preclinical and biological studies." Sarcoma **2012**: 148614.

Liposarcoma cell lines represent in vitro models for studying disease mechanisms at the cellular level and for preclinical evaluation of novel drugs. To date there are a limited number of well-characterized models available. In this study, nine immortal liposarcoma cell lines were evaluated for tumor-forming ability, stem cell- and differentiation potential, and metastatic potential, with the aim to generate a well-characterized liposarcoma cell line panel. Detailed stem cell and differentiation marker analyses were also performed. Five of the liposarcoma cell lines were tumorigenic, forming tumors in mice. Interestingly, tumor-forming ability correlated with high proliferative capacity in vitro. All the cell lines underwent adipocytic differentiation, but the degree varied. Surprisingly, the expression of stem cell and differentiation markers did not correlate well with function. Overall, the panel contains cell lines suited for in vivo analyses (LPS141, SA-4, T778, SW872, and LISA-2), for testing novel drugs targeting cancer stem cells (LPS141) and for studying tumor progression and metastasis (T449 and T778).

Strom, S. C. and R. Gramignoli (2016). "Human amnion epithelial cells expressing HLA-G as novel cell-based treatment for liver disease." Hum Immunol **77**(9): 734-739.

Despite routine liver transplantation and supporting medical therapies, thousands of patients currently wait for an organ and there is an unmet need for more refined and widely available regenerative strategies to treat liver diseases. Cell transplants attempt to maximize the potential for repair and/or regeneration in liver and other organs. Over 40years of laboratory pre-clinical research and 25years of clinical procedures have shown that certain liver diseases can be treated by the infusion of isolated cells (hepatocyte transplant). However, like organ transplants, hepatocyte transplant suffers from a paucity of tissues useful for cell production. Alternative sources have been investigated, yet with limited success. The tumorigenic potential of pluripotent stem cells together with their primitive level of hepatic differentiation, have limited the use of stem cell populations. Stem cell sources from human placenta, and the amnion tissue in particular are receiving renewed interest in the field of regenerative medicine. Unlike pluripotent stem cells, human amnion epithelial (AE) cells are easily available without ethical or religious concerns; they do not express telomerase and are not immortal or tumorigenic when transplanted. In addition, AE cells have been reported to express genes normally expressed in mature liver, when transplanted into the liver. Moreover, because of the possibility of an

immune-privileged status related to their expression of HLA-G, it might be possible to transplant human AE cells without immunosuppression of the recipient.

Suda, Y., et al. (1987). "Mouse embryonic stem cells exhibit indefinite proliferative potential." J Cell Physiol **133**(1): 197-201.

The proliferative potential of embryonic stem cells was examined. In contrast to the current concept of the finite life-span being the hallmark of normal cells, we have been able to maintain these embryonic stem cells in vitro up to about 250 cumulative doublings with no indication of "crisis" or transformation. These cells could be considered normal on the basis of: (1) their apparently normal diploid karyotype, (2) their ability to extensively colonize embryos without causing tumors and developmental anomalies, and (3) their ability to form normal gametes when differentiated into the germ-line. These results suggest that embryonic stem cells prior to differentiation into germ and somatic cells are indeed immortal.

Sun, W., et al. (1999). "High susceptibility of a human breast epithelial cell type with stem cell characteristics to telomerase activation and immortalization." Cancer Res **59**(24): 6118-6123.

We have recently characterized two types of normal human breast epithelial cells (HBECs) from reduction mammoplasty. Type I cells express estrogen receptor, luminal epithelial cell markers, and stem cell characteristics (i.e., the ability to differentiate into other cell types and to form budding/ductal structures on Matrigel), whereas Type II cells show basal epithelial cell phenotypes. In this study, we have examined whether Type I HBECs are more susceptible to telomerase activation and immortalization after transfection with SV40 large T-antigen. The results show that both types of cells acquire extended life span [(EL); i.e., bypassing senescence] at a comparable frequency. However, they differ significantly in the ability to become immortal in continuous culture, ie., 11 of 11 Type I EL clones became immortal compared with 1 of 10 Type II EL clones. Both parental Type I and Type II cells as well as their transformed EL clones at early passages [approximately 30 cumulative population doubling level (cpdl)] showed a low level of telomerase activity as measured by the telomeric repeat amplification protocol assay. For all 11 of the Type I EL clones and the single Type II EL clone that became immortal, telomerase activities were invariably activated at middle passages (approximately 60 cpdl) or late passages (approximately 100 cpdl). For the four Type II EL clones randomly selected from the nine Type II clones that did not become immortal, the telomerase activities were found to be further diminished at mid-passage, before the end of the life span. Thus, normal HBECs do have a low level of telomerase activity, and Type I HBECs with stem cell characteristics are more susceptible to telomerase activation and immortalization, a basis on which they may be major target cells for breast carcinogenesis.

Sundararaman, B., et al. (2012). "Asymmetric chromatid segregation in cardiac progenitor cells is enhanced by Pim-1 kinase." Circ Res **110**(9): 1169-1173.

RATIONALE: Cardiac progenitor cells (CPCs) in the adult heart are used for cell-based treatment of myocardial damage, but factors determining stemness, self-renewal, and lineage commitment are poorly understood. Immortal DNA strands inherited through asymmetric chromatid segregation correlate with self-renewal of adult stem cells, but the capacity of CPCs for asymmetric segregation to retain immortal strands is unknown. Cardioprotective kinase Pim-1 increases asymmetric cell division in vivo, but the ability of Pim-1 to enhance asymmetric chromatid segregation is unknown. OBJECTIVE: We aimed to demonstrate immortal strand segregation in CPCs and the enhancement of asymmetric chromatid distribution by Pim-1 kinase. METHODS AND RESULTS: Asymmetric segregation is tracked by incorporation of bromodeoxyuridine. The CPC DNA was labeled for several generations and then blocked in second cytokinesis during chase to determine distribution of immortal versus newly synthesized strands. Intensity ratios of binucleated CPCs with bromodeoxyuridine of >/=70:30 between daughter nuclei indicative of asymmetric chromatid segregation occur with a frequency of 4.57, and asymmetric chromatid segregation is demonstrated at late mitotic phases. Asymmetric chromatid segregation is significantly enhanced by Pim-1 overexpression in CPCs (9.19 versus 4.79 in eGFP-expressing cells; P=0.006). CONCLUSIONS: Asymmetric segregation of chromatids in CPCs is increased nearly two-fold with Pim-1 kinase overexpression, indicating that Pim-1 promotes self-renewal of stem cells.

Sviderskaya, E. V., et al. (1998). "Impaired growth and differentiation of diploid but not immortal melanoblasts from endothelin receptor B mutant (piebald) mice." Dev Dyn **213**(4): 452-463.

Endothelin 3 (Edn3) and its preferred receptor, endothelin receptor B (Ednrb), are implicated in development, especially that of two neural-crest-derived cell lineages: melanocytes and enteric ganglion cells. Mice and humans with a null mutation at either locus can show major deficiencies in both cell types: congenital white spotting and aganglionic megacolon (Hirschsprung disease in human). Numbers of early (migrating) embryonic melanoblasts are low in Ednrb(ls) mutant mice, while added Edn3 appears to promote the growth of melanocyte precursors in neural

crest cultures. However, it is hard to assess cell differentiation in these mixed cultures, and it is not known whether Ednrb has any role in the postnatal melanocytic lineage. We have therefore studied primary cultures of neonatal melanoblasts homozygous for the piebald (Ednrb(s)) mutation. These mutant melanoblasts showed severe impairment of both net cell growth and differentiation compared to wild-type melanoblasts. They were also unresponsive to stimulation of growth by cholera toxin. We have established three immortal lines of melanoblasts and one of melanocytes homozygous for Ednrb(s). These immortal lines, however, had no detectable deficiency of growth or differentiation as judged by cell counts, induced pigmentation and immunocytochemistry for melanocytic markers. Consistent with this, neither Ednrb nor Edn3 mRNA was detected in 3/3 tested immortal lines of mouse melanoblasts and 5/5 lines of melanocytes, of various genotypes. We also report for the first time a method to grow immortal melanoblasts in pure culture, without feeder cells.

Sviderskaya, E. V., et al. (2009). "Functional neurons and melanocytes induced from immortal lines of postnatal neural crest-like stem cells." FASEB J **23**(9): 3179-3192.

Stem cells, that is, cells that can both reproduce themselves and differentiate into functional cell types, attract much interest as potential aids to healing and disease therapy. Embryonic neural crest is pluripotent and generates the peripheral nervous system, melanocytes, and some connective tissues. Neural-crest-related stem cells have been reported previously in postnatal skin: committed melanocytic stem cells in the hair follicle, and pluripotent cell types from the hair follicle and papilla that can produce various sets of lineages. Here we describe novel pluripotent neural crest-like stem cells from neonatal mouse epidermis, with different potencies, isolated as 3 independent immortal lines. Using alternative regulatory factors, they could be converted to large numbers of either Schwann precursor cells, pigmented melanocytes, chondrocytes, or functional sensory neurons showing voltage-gated sodium channels. Some of the neurons displayed abundant active TRPV1 and TRPA1 receptors. Such functional neurons have previously been obtained in culture only with difficulty, by explantation. The system was also used to generate comparative gene expression data for the stem cells, melanocytes, and melanoblasts that sufficiently explain the lack of pigment in melanoblasts and provide a rationale for some genes expressed apparently ectopically in melanomas, such as ephrin receptors.

Sviderskaya, E. V., et al. (2001). "Agouti signaling protein and other factors modulating differentiation and proliferation of immortal melanoblasts." Dev Dyn **221**(4): 373-379.

The melanocyte lineage potentially forms an attractive model system for studies in cell differentiation, developmental genetics, cell signaling, and melanoma, because differentiated cells produce the visible pigment melanin. Immortal lines of murine melanoblasts (melanocyte precursors) have been described previously, but induction of differentiation involved a complex culture system with keratinocyte feeder cells. Here we describe conditions for both growth and induced differentiation of the melanoblast line melb-a, without feeder cells, and analyze factors that directly control proliferation and differentiation of these pure melanoblasts. Several active factors are products of developmental and other coat color genes, including stem cell factor (SCF), melanocyte-stimulating hormone (alphaMSH), and agouti signaling protein (ASP), a natural antagonist at the MSH receptor (melanocortin 1 receptor, MC1R) encoded by the agouti gene. A stable analog of alphaMSH (NDP-MSH) stimulated differentiation and inhibited growth. ASP in excess inhibited both effects of NDP-MSH, that is, ASP could inhibit pigmentation and stimulate growth. These effects provide an explanation for the interactions in mice of melanocyte developmental mutations with yellow agouti and Mc1r alleles, and a role for embryonic expression patterns of ASP.

Sviderskaya, E. V., et al. (1995). "A cloned, immortal line of murine melanoblasts inducible to differentiate to melanocytes." Development **121**(5): 1547-1557.

Cultures of differentiated melanocytes can readily be grown from the dissociated epidermis of neonatal mice, and immortal cell lines often develop from these. However, the first cells that grow and transiently dominate the cultures, while similar to melanocytes, are unpigmented. These have been shown to be precursors of melanocytes and may be termed melanoblasts. Under our previous standard culture conditions, involving the use of keratinocyte feeder cells, foetal calf serum, the phorbol ester 12-O-tetradecanoyl phorbol acetate (TPA) and cholera toxin, all the melanoblasts spontaneously differentiated to pigmented melanocytes within about 3 weeks. We now describe some factors affecting the proliferation and differentiation of diploid murine melanoblasts in the presence of serum. Murine stem cell factor/steel factor (SCF), basic fibroblast growth factor (bFGF) and murine leukaemia inhibitory factor/differentiation-inhibiting activity (LIF/DIA) all increased melanoblast numbers. SCF and LIF also slightly inhibited melanoblast differentiation, while cholera toxin and TPA promoted differentiation. Using some of these findings, and by regular replacement of keratinocyte or fibroblastoid feeder cells, we have established a clonal line of immortal murine melanoblasts, 'melb-a'. These

cells express tyrosinase-related protein-2 but not, in general, tyrosinase. They can be induced to differentiate irreversibly to functional melanocytes (also proliferative and immortal) by plating in the absence of feeder cells. Thus a new immortal melanocyte line, 'melan-a2', has also been produced.

Sykes, D. B. and M. P. Kamps (2004). "E2a/Pbx1 induces the rapid proliferation of stem cell factor-dependent murine pro-T cells that cause acute T-lymphoid or myeloid leukemias in mice." Mol Cell Biol **24**(3): 1256-1269.

Oncoprotein E2a/Pbx1 is produced by the t(1;19) chromosomal translocation of human pre-B acute lymphoblastic leukemia. E2a/Pbx1 blocks differentiation of primary myeloid progenitors but, paradoxically, induces apoptosis in established pre-B-cell lines, and no transforming function of E2a/Pbx1 has been reported in cultured lymphoid progenitors. Here, we demonstrate that E2a/Pbx1 induces immortal proliferation of stem cell factor (SCF)-dependent pro-T thymocytes by a mechanism dependent upon both its transactivation and DNA-binding functions. E2a-Pbx1 cooperated with cytokines or activated signaling oncoproteins to induce cell division, as inactivation of conditional E2a/Pbx1 in either factor-dependent pro-T cells or pro-T cells made factor independent by expression of Bcr/Abl resulted in pro-T-cell quiescence, while reactivation of E2a/Pbx1 restored cell division. Infusion of E2a/Pbx1 pro-T cells in mice caused T lymphoblastic leukemia and, unexpectedly, acute myeloid leukemia. The acute lymphoblastic leukemia did not evidence further maturation, suggesting that E2a/Pbx1 establishes an early block in pro-T-cell development that cannot be overcome by marrow or thymic microenvironments. In an E2a/Pbx1 pro-T thymocyte clone that induced only pro-T acute lymphoblastic leukemia, coexpression of Bcr/Abl expanded its leukemic phenotype to include acute myeloid leukemia, suggesting that unique functions of cooperating signaling oncoproteins can influence the lymphoid versus myeloid character of E2a/Pbx1 leukemia and may cooperate with E2a/Pbx1 to dictate the pre-B-cell phenotype of human leukemia containing t(1;19).

Tada, Y., et al. (2015). "The stem cell transcription factor ZFP57 induces IGF2 expression to promote anchorage-independent growth in cancer cells." Oncogene **34**(6): 752-760.

Several common biological properties between cancer cells and embryonic stem (ES) cells suggest the possibility that some genes expressed in ES cells might have important roles in cancer cell growth. The transcription factor ZFP57 is expressed in self-renewing ES cells and its expression level decreases during ES cell differentiation. This study showed that ZFP57 is involved in the anchorage-independent growth of human fibrosarcoma HT1080 cells in soft agar. ZFP57 overexpression enhanced, whereas knockdown suppressed, HT1080 tumor formation in nude mice. Furthermore, ZFP57 regulates the expression of insulin-like growth factor 2 (IGF2), which has a critical role in ZFP57-induced anchorage-independent growth. ZFP57 also promotes anchorage-independent growth in ES cells and immortal fibroblasts. Finally, immunohistochemical analysis revealed that ZFP57 is overexpressed in human cancer clinical specimens. Taken together, these results suggest that the ES-specific transcription factor ZFP57 is a novel oncogene.

Tajbakhsh, S. (2008). "Stem cell identity and template DNA strand segregation." Curr Opin Cell Biol **20**(6): 716-722.

The quest for stem cell properties to distinguish their identity from that of committed daughters has led to a re-investigation of the notion that DNA strands are not equivalent, and 'immortal' DNA strands are retained in stem cells whereas newly replicated DNA strands segregate to the differentiating daughter cell during mitosis. Whether this process occurs only in stem cells, and also in all tissues, remains unclear. That individual chromosomes can be also partitioned non-randomly raises the question if this phenomenon is related to the immortal DNA hypothesis, and it underscores the need for high-resolution techniques to observe these events empirically. Although initially postulated as a mechanism to avoid DNA replication errors, alternative views including epigenetic regulation and sister chromatid silencing may provide insights into this process.

Tajbakhsh, S., et al. (2009). "Asymmetric cell divisions and asymmetric cell fates." Annu Rev Cell Dev Biol **25**: 671-699.

The regulation of self-renewal, cell diversity, and differentiation can occur by modulating symmetric and asymmetric cell divisions. Remarkably, asymmetric cell divisions can arise through multiple processes in which molecules in the cytoplasm and nucleus, as well as template "immortal" DNA strands, can segregate to one daughter cell during cell division. Explaining how these events direct distinct daughter cell fates is a major challenge to understanding how the organism is assembled and maintained for a lifetime. Numerous technical issues that are associated with assessing how distinct cell fates are executed in vivo have resulted in divergent interpretations of experimental findings. This review addresses some of these points and considers different developmental model systems that attempt to investigate how cell fate decisions are determined, as well as the molecules that guide these choices.

Tanaka, S. (2006). "Derivation and culture of mouse trophoblast stem cells in vitro." Methods Mol Biol **329**: 35-44.

In the mouse preimplantation embryo, the first cell fate determination segregates two morphologically and functionally distinct cell lineages. One is the inner cell mass, and the other is the trophectoderm. A subset of the trophectoderm maintains a proliferative capacity and forms the extraembryonic ectoderm, the ectoplacental cone, and the secondary giant cells of the early conceptus after implantation. A stem cell population of the trophectoderm lineage can be isolated and maintained in vitro under the presence of fibroblast growth factor 4, heparin, and a feeder layer of mouse embryonic fibroblast cells. Such apparently immortal stem cells, trophoblast stem (TS) cells, exhibit the potential to differentiate to multiple cell types in vitro. TS cells also have the ability to contribute to normal development in chimeras. However, TS cells exclusively contribute to the trophoblastic component of the placenta and of the parietal yolk sac, making a striking contrast with embryonic stem cells, which never contribute to these tissues in chimeras. In this chapter, detailed protocols for the isolation and establishment of TS cell lines from blastocysts and their maintenance are described.

Tannenbaum, E., et al. (2005). "Evolutionary dynamics of adult stem cells: comparison of random and immortal-strand segregation mechanisms." Phys Rev E Stat Nonlin Soft Matter Phys **71**(4 Pt 1): 041914.

This paper develops a point-mutation model describing the evolutionary dynamics of a population of adult stem cells. Such a model may prove useful for quantitative studies of tissue aging and the emergence of cancer. We consider two modes of chromosome segregation: (1) random segregation, where the daughter chromosomes of a given parent chromosome segregate randomly into the stem cell and its differentiating sister cell and (2) "immortal DNA strand" co-segregation, for which the stem cell retains the daughter chromosomes with the oldest parent strands. Immortal strand co-segregation is a mechanism, originally proposed by [Cairns Nature (London) 255, 197 (1975)], by which stem cells preserve the integrity of their genomes. For random segregation, we develop an ordered strand pair formulation of the dynamics, analogous to the ordered strand pair formalism developed for quasispecies dynamics involving semiconservative replication with imperfect lesion repair (in this context, lesion repair is taken to mean repair of postreplication base-pair mismatches). Interestingly, a similar formulation is possible with immortal strand co-segregation, despite the fact that this segregation mechanism is age dependent. From our model we are able to mathematically show that, when lesion repair is imperfect, then immortal strand co-segregation leads to better preservation of the stem cell lineage than random chromosome segregation. Furthermore, our model allows us to estimate the optimal lesion repair efficiency for preserving an adult stem cell population for a given period of time. For human stem cells, we obtain that mispaired bases still present after replication and cell division should be left untouched, to avoid potentially fixing a mutation in both DNA strands.

Terskikh, V. V., et al. (2012). "Label retaining cells and cutaneous stem cells." Stem Cell Rev Rep **8**(2): 414-425.

This is a comprehensive review on label retaining cells (LRC) in epidermal development and homeostasis. The precise in vivo identification and location of epidermal stem cells is a crucial issue in cutaneous biology. We discuss here the following problems: (1) Identification and location of LRC in the interfollicular epithelium and hair follicle; (2) The proliferative potential of LRC and their role in cutaneous homeostasis (3); LRC phenomenon and the Immortal Strand Hypothesis, which suggests an alternative mechanism for retention of genetic information; (4) Significance of LRC studies for development of stem cell concept. Now, it seems evident that LRC are a frequent feature of stem cell niches and revealing highly dormant LRC may be used for identification of stem cell niches in different tissues. LRC were used for screening specific markers of epidermal stem cells. Within a given tissue stem cells have different proliferative characteristics. There are more frequently cycling stem cells which function primarily in homeostasis, while LRC form a reserve of dormant, may be ultimate, stem cells, which are set aside for regeneration of injury or unforeseen need. The authors suggest that LRC dormancy described in Mammalia has much in common with developmental quiescence found in some other animals. For example in C. elegans reproductive system, vulval precursor cells have developmentally programmed cell-cycle arrest in the first larval stage, and then undergo an extended period of quiescence before resuming proliferation. Another example of developmental quiescence is the diapause, a widespread phenomenon exhibited by animals ranging from nematodes to mammals, often occurring at genetically predetermined life history stage.

Thansa, K., et al. (2021). "Establishment of hematopoietic tissue primary cell cultures from the giant freshwater prawn Macrobrachium rosenbergii." Cytotechnology **73**(2): 141-157.

The giant freshwater prawn Macrobrachium rosenbergii is one of the most important aquaculture species in Southeast Asia. In this study, in vitro culture of its hematopoietic tissue cells was achieved and

characterized for use as a tool to study its pathogens that cause major farm losses. By transmission electron microscopy, the ultrastructure of the primary culture cells was similar to that of cells lining intact hematopoietic tissue lobes. Proliferating cell nuclear antigen (PCNA) (a marker for hematopoietic stem cell proliferation) was detected in some of the cultured cells by polymerase chain reaction (PCR) testing and flow cytometry. Using a specific staining method to detect phenoloxidase activity and using PCR to detect expression markers for semigranular and granular hemocytes (e.g., prophenoloxidase activating enzyme and prophenoloxidase) revealed that some of the primary cells were able to differentiate into mature hemocytes within 24 h. These results showed that some cells in the cultures were hematopoietic stem cells that could be used to study other interesting research topics (e.g. host pathogen interactions and development of an immortal hematopoietic stem cell line).

Tilly, J. L., et al. (2009). "The current status of evidence for and against postnatal oogenesis in mammals: a case of ovarian optimism versus pessimism?" Biol Reprod **80**(1): 2-12.

Whether or not oogenesis continues in the ovaries of mammalian females during postnatal life was heavily debated from the late 1800s through the mid-1900s. However, in 1951 Lord Solomon Zuckerman published what many consider to be a landmark paper summarizing his personal views of data existing at the time for and against the possibility of postnatal oogenesis. In Zuckerman's opinion, none of the evidence he considered was inconsistent with Waldeyer's initial proposal in 1870 that female mammals cease production of oocytes at or shortly after birth. This conclusion rapidly became dogma, and remained essentially unchallenged until just recently, despite the fact that Zuckerman did not offer a single experiment proving that adult female mammals are incapable of oogenesis. Instead, 20 years later he reemphasized that his conclusion was based solely on an absence of data he felt would be inconsistent with the idea of a nonrenewable oocyte pool provided at birth. However, in the immortal words of Carl Sagan, an "absence of evidence is not evidence of absence." Indeed, building on the efforts of a few scientists who continued to question this dogma after Zuckerman's treatise in 1951, we reported several data sets in 2004 that were very much inconsistent with the widely held belief that germ cell production in female mammals ceases at birth. Perhaps not surprisingly, given the magnitude of the paradigm shift being proposed, this work reignited a vigorous debate that first began more than a century ago. Our purpose here is to review the experimental evidence offered in recent studies arguing support for and against the possibility that adult mammalian females replenish their oocyte reserve.

"Never discourage anyone who continually makes progress, no matter how slow."-Plato (427-347 BC).

Tkemaladze, J. V. and K. N. Chichinadze (2005). "Centriolar mechanisms of differentiation and replicative aging of higher animal cells." Biochemistry (Mosc) **70**(11): 1288-1303.

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

Toh, W. S., et al. (2011). "Potential of human embryonic stem cells in cartilage tissue engineering and regenerative medicine." Stem Cell Rev Rep **7**(3): 544-559.

The current surgical intervention of using autologous chondrocyte implantation (ACI) for cartilage repair is associated with several problems such as donor site morbidity, de-differentiation upon expansion and fibrocartilage repair following transplantation. This has led to exploration of the use of stem cells as a model for chondrogenic differentiation as well as a potential source of chondrogenic cells for cartilage tissue engineering and repair. Embryonic stem cells (ESCs) are advantageous, due to their unlimited self-renewal and pluripotency, thus representing an immortal cell source that could potentially provide an unlimited supply of chondrogenic cells for both cell and tissue-based therapies and replacements. This review aims to present an overview of emerging trends of using ESCs in cartilage tissue engineering and regenerative medicine. In particular, we will be focusing on ESCs as a promising cell source for cartilage regeneration, the various strategies and approaches employed in chondrogenic differentiation and tissue engineering, the associated outcomes from

animal studies, and the challenges that need to be overcome before clinical application is possible.

Tokuzawa, Y., et al. (2003). "Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development." Mol Cell Biol **23**(8): 2699-2708.

Embryonic stem (ES) cells are immortal and pluripotent cells derived from early mammalian embryos. Transcription factor Oct3/4 is essential for self-renewal of ES cells and early mouse development. However, only a few Oct3/4 target genes have been identified. In this study, we found that F-box-containing protein Fbx15 was expressed predominantly in mouse undifferentiated ES cells. Inactivation of Oct3/4 in ES cells led to rapid extinction of Fbx15 expression. Reporter gene analyses demonstrated that this ES cell-specific expression required an 18-bp enhancer element located approximately 500 nucleotides upstream from the transcription initiation site. The enhancer contained an octamer-like motif and an adjacent Sox-binding motif. Deletion or point mutation of either motif abolished the enhancer activity. The 18-bp fragment became active in NIH 3T3 cells when Oct3/4 and Sox2 were coexpressed. A gel mobility shift assay demonstrated cooperative binding of Oct3/4 and Sox2 to the enhancer sequence. In mice having a beta-galactosidase gene knocked into the Fbx15 locus, 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside staining was detected in ES cells, early embryos (two-cell to blastocyst stages), and testis tissue. Despite such specific expression of Fbx15, homozygous mutant mice showed no gross developmental defects and were fertile. Fbx15-null ES cells were normal in morphology, proliferation, and differentiation. These data demonstrate that Fbx15 is a novel target of Oct3/4 but is dispensable for ES cell self-renewal, development, and fertility.

Tomasetti, C. and I. Bozic (2015). "The (not so) immortal strand hypothesis." Stem Cell Res **14**(2): 238-241.

BACKGROUND: Non-random segregation of DNA strands during stem cell replication has been proposed as a mechanism to minimize accumulated genetic errors in stem cells of rapidly dividing tissues. According to this hypothesis, an "immortal" DNA strand is passed to the stem cell daughter and not the more differentiated cell, keeping the stem cell lineage replication error-free. After it was introduced, experimental evidence both in favor and against the hypothesis has been presented. PRINCIPAL FINDINGS: Using a novel methodology that utilizes cancer sequencing data we are able to estimate the rate of accumulation of mutations in healthy stem cells of the colon, blood and head and neck tissues. We find that in these tissues mutations in stem cells accumulate at rates strikingly similar to those expected without the protection from the immortal strand mechanism. SIGNIFICANCE: Utilizing an approach that is fundamentally different from previous efforts to confirm or refute the immortal strand hypothesis, we provide evidence against non-random segregation of DNA during stem cell replication. Our results strongly suggest that parental DNA is passed randomly to stem cell daughters and provides new insight into the mechanism of DNA replication in stem cells.

Tommasi, S., et al. (2013). "Mammalian cells acquire epigenetic hallmarks of human cancer during immortalization." Nucleic Acids Res **41**(1): 182-195.

Progression to malignancy requires that cells overcome senescence and switch to an immortal phenotype. Thus, exploring the genetic and epigenetic changes that occur during senescence/immortalization may help elucidate crucial events that lead to cell transformation. In the present study, we have globally profiled DNA methylation in relation to gene expression in primary, senescent and immortalized mouse embryonic fibroblasts. Using a high-resolution genome-wide mapping technique, followed by extensive locus-specific validation assays, we have identified 24 CpG islands that display significantly higher levels of CpG methylation in immortalized cell lines as compared to primary murine fibroblasts. Several of these hypermethylated CpG islands are associated with genes involved in the MEK-ERK pathway, one of the most frequently disrupted pathways in cancer. Approximately half of the hypermethylated targets are developmental regulators, and bind to the repressive Polycomb group (PcG) proteins, often in the context of bivalent chromatin in mouse embryonic stem cells. Because PcG-associated aberrant DNA methylation is a hallmark of several human malignancies, our methylation data suggest that epigenetic reprogramming of pluripotency genes may initiate cell immortalization. Consistent with methylome alterations, global gene expression analysis reveals that the vast majority of genes dysregulated during cell immortalization belongs to gene families that converge into the MEK-ERK pathway. Additionally, several dysregulated members of the MAP kinase network show concomitant hypermethylation of CpG islands. Unlocking alternative epigenetic routes for cell immortalization will be paramount for understanding crucial events leading to cell transformation. Unlike genetic alterations, epigenetic changes are reversible events, and as such, can be amenable to pharmacological interventions, which makes them appealing targets for cancer therapy when genetic approaches prove inadequate.

Torii, D., et al. (2015). "Cementogenic potential of multipotential mesenchymal stem cells purified from

the human periodontal ligament." Odontology **103**(1): 27-35.

The periodontal ligament (PDL) consists of a group of specialized connective tissue fibers embedded in the alveolar bone and cementum that are believed to contain progenitors for mineralized tissue-forming cell lineages. These progenitors may contribute to regenerative cell therapy or tissue engineering methods aimed at recovery of tissue formation and functions lost in periodontal degenerative changes. Some reports using immortal clonal cell lines of cementoblasts, which are cells containing mineralized tissue-forming cell lineages, have shown that their phenotypic alteration and gene expression are associated with mineralization. Immortal, multipotential PDL-derived cell lines may be useful biological tools for evaluating differentiation-inducing agents. In this study, we confirmed the gene expression and mineralization potential of primary and immortal human PDL cells and characterized their immunophenotype. Following incubation with mineralization induction medium containing beta-glycerophosphate, ascorbic acid, and dexamethasone, normal human PDL (Pel) cells and an immortal derivative line (Pelt) cells showed higher levels of mineralization compared with cells grown in normal growth medium. Both cell types were positive for putative surface antigens of mesenchymal cells (CD44, CD73, CD90, and CD105). They were also positive for stage-specific embryonic antigen-3, a marker of multipotential stem cells. Furthermore, PDL cells expressed cementum attachment protein and cementum protein 1 when cultured with recombinant human bone morphogenetic protein-2 or -7. The results suggest that normal and immortal human PDL cells contain multipotential mesenchymal stem cells with cementogenic potential.

Torii, D., et al. (2016). "Bone morphogenetic protein 7 induces cementogenic differentiation of human periodontal ligament-derived mesenchymal stem cells." Odontology **104**(1): 1-9.

Bone morphogenetic protein 7 (BMP-7) is a multifunctional differentiation factor that belongs to the transforming growth factor superfamily. BMP-7 induces gene expression of protein tyrosine phosphatase-like, member A/cementum attachment protein (PTPLA/CAP) and cementum protein 1 (CEMP1), both of which are markers of cementoblasts and cementocytes. In the previous study, we reported that BMP-7 treatment enhanced PTPLA/CAP and CEMP1 expression in both normal and immortal human periodontal ligament (PDL) cells. To elucidate the molecular mechanisms of the gene expression of these molecules, in this study, we identified a functional transcription activator binding region in the promoter region of PTPLA/CAP and CEMP1 that is responsive to BMP signals. Here, we report that some short motifs termed GC-rich Smad-binding elements (GC-SBEs) that are located in the human PTPLA/CAP promoter and CEMP1 promoter are BMP-7 responsive as analyzed with luciferase promoter assays. On the other hand, we found that transcription of Sp7/Osterix and PTPLA/CAP was up-regulated after 1 week of BMP-7 treatment on purified normal human PDL cells as a result of gene expression microarray analysis. Furthermore, transcription of Sp7/Osterix, runt-related transcription factor 2 (RUNX2), and alkaline phosphatase (ALP) was up-regulated after 2 weeks of BMP-7 treatment, whereas gene expression of osteo/odontogenic markers such as integrin-binding sialoprotein (IBSP), collagen, type I, alpha 1 (COL1A1), dentin matrix acidic phosphoprotein 1 (DMP1), and dentin sialophosphoprotein (DSPP) was not up-regulated in purified normal or immortal human PDL cells as a result of qRT-PCR. The results suggest that BMP-7 mediates cementogenesis via GC-SBEs in human PDL cells and that its molecular mechanism is different from that for osteo/odontogenesis.

Trosko, J. E. (2003). "Human stem cells as targets for the aging and diseases of aging processes." Med Hypotheses **60**(3): 439-447.

While many theories have been proposed for the aging process, and many debates on the matter of aging and the diseases of aging being either the result of the same or independent processes, most have not considered humans as a hierarchical system made up of cybernetically interacting levels of organization. To understand the aging process and the diseases of aging, one must view the human as the result of the total genomic DNA in the single fertilized egg that proliferates, differentiates and develops into an individual of about 100 trillion cells, organized by different cell types (pluri-potent stem cells, progenitor stem cells, terminally differentiated cells) into multiple tissue, organ and organ systems which interact with each other via endogenous factors and with exogenous factors. Our hypothesis is that both aging and diseases of aging are dependent of the normal functioning of the pluri-potent stem cell pool. Specifically, the concept involves the cybernetic feedback between the 'quantity' of the stem cell pool in each tissue niche with the 'quality' of the stem cells in the pool. The process of gap junctional inter-cellular communication (GJIC), which has been implicated in the evolution from the single cell organism to the multi-cellular organisms, requiring growth control, differentiation, apoptosis, adaptive response capability of differentiated cells and senescence, is speculated to be a shared mechanism in stem cell biology and in many chronic disease processes (teratogenesis; carcinogenesis, atherogenesis, diabetigenesis, etc.). Specifically, stem cells are assumed to be 'immortal' until induced to express their connexin genes and have functional GJIC, at which

time they can differentiate and become 'mortal'. As long as the stem cells are communicating with their differentiated daughters via some extra-cellular soluble negative growth factor, the homeostatic control of their growth and differentiation is maintained for the organism. However, if the stem cell pool is depleted by any process, replacement of tissue due to wear and tear is diminished. The dependence of this tissue/organ to maintain homeostatic control of other organ systems then diminishes, leading to 'systems failure'. In addition, if the stem cells in the pool have been exposed to agents that prevent the normal terminal differentiation of that cell, but whereby these 'initiated' stem cells can be expanded in any tissue, clones of partially differentiated and non-functional appear in the tissue. This diminishes the efficacy of that tissue to function properly and, thereby, also contributes to 'system failure' by contributing to the breakdown of homeostatic organ system control. One clear example, that of carcinogenesis, illustrates this point.

Trosko, J. E. (2006). "Dietary modulation of the multistage, multimechanisms of human carcinogenesis: effects on initiated stem cells and cell-cell communication." Nutr Cancer **54**(1): 102-110.

Diet can influence the risk to cancer in both negative and positive ways. Worldwide, more than 10 million persons develop cancer annually. Diet could prevent many cancers. Carcinogenesis is a multistage, multimechanism process, consisting of "initiation," "promotion," and "progression" phases. Although diet could affect each phase, an efficacious strategy for dietary chemoprevention would be intervention during the promotion phase. The tumor-promotion process requires sustained exposure to agents that stimulate the growth and inhibition of apoptosis of initiated cells in the absence of antipromoters. Chronic inflammation has been associated with the promotion process. The mechanism affecting the promotion process appears to be the inhibition of cell-cell communication between normal and initiated cells. Most, if not all, tumor-promoting agents and conditions, reversibly, inhibit cell-cell communication, whereas antipromoters, antioxidants, and anti-inflammatory agents have been shown to ameliorate the effects of tumor promoters on cell-cell communication. Additionally, adult stem cells are hypothesized to be the target cells for initiating the carcinogenic process. A new paradigm has been presented that postulates the first function of the carcinogenic process is to block the "mortalization" of a normal, "immortal" adult stem cell rather than the induction of "immortalization" of a normal mortal cell.

Trosko, J. E. and C. C. Chang (2001). "Role of stem cells and gap junctional intercellular communication in human carcinogenesis." Radiat Res **155**(1 Pt 2): 175-180.

Epidemiological data, experimental animal bioassays, studies of in vitro neoplastic transformation, and molecular oncology studies have implicated a multistage, multimechanism process in human carcinogenesis. From animal carcinogenesis studies, the operational concept of a single normal cell being irreversibly altered during the first step in carcinogenesis is called initiation. The subsequent interruptible or reversible clonal expansion of these initiated cells by non-cytotoxic mitogenic stimuli, compensatory hyperplasia due to cell death by necrosis, or inhibition of apoptosis is referred to as the promotion phase. Last, when one of these clonally expanded, initiated cells acquires sufficient genetic/epigenetic alterations to become neoplastically transformed and acquire the phenotypes of promoter independence, invasiveness and metastasis, it is referred to as the progression step of carcinogenesis. This report hypothesizes that the single normal cell that is initiated is a pluripotent stem cell. By assuming that the normal pluripotent stem cell is immortal and becomes mortal when induced to terminally differentiate, initiation would be viewed as the irreversible process by which a stable alteration in a finite number of proto-oncogenes and/or tumor suppressor genes could block terminal differentiation or "mortalization". Promotion would involve the reversible inhibition of gap junctional intercellular communication (GJIC) and while progression occurs with the stable down-regulation of GJIC.

Trosko, J. E. and C. C. Chang (2003). "Isolation and characterization of normal adult human epithelial pluripotent stem cells." Oncol Res **13**(6-10): 353-357.

Both reproductive and therapeutic cloning of human stem cells have been made possible with recent technological advances in the isolation of embryonic stem cells and of pluripotent stem cells from adult tissues. We have isolated normal human kidney and human breast epithelial stem cells, as well as having characterized "immortalized" cells from human neuronal and human pancreatic tissue (Trosko et al., Methods 20:245-264, 2000). The isolation was motivated by the stem cell theory of carcinogenesis. Based on the assumption that stem cells would not express connexin genes, nor have functional gap junctional intercellular communication (GJIC), we have demonstrated that the human kidney, breast, neuronal, and pancreatic stem cells can divide either symmetrically or asymmetrically, depending on whether they are grown in microenvironmental conditions that suppress GJIC (the undifferentiated, proliferative state) or induce GJIC (the differentiated state). Normal breast epithelial stem cells appear to be intrinsically "immortal" until induced to express GJIC, at which time, with appropriate substrate and microenvironmental nutrients, they can form three-

dimensional "organoids." expressing markers associated with the mature mammary tissue and forming a physical structure very similar to the in vivo budding, ductal structures. The breast stem cells can be prevented from "mortalizing" and can be converted to neoplastic cells, which maintain many phenotypes of the stem cells.

Trosko, J. E., et al. (2004). "Ignored hallmarks of carcinogenesis: stem cells and cell-cell communication." Ann N Y Acad Sci **1028**: 192-201.

Hanahan and Weinberg (2000, Cell 100: 57-70) listed "hallmarks" of cancer that must be considered in order to understand the underlying determinants of carcinogenesis: (a) self-sufficiency in growth signals; (b) insensitivity to growth-inhibitory (antigrowth) signals; (c) evasion of programmed cell death (apoptosis); (d) limitedless replicative potential; (e) sustained angiogenesis; and (f) tissue invasion and metastasis. While these are important phenotypic markers, important concepts--the role of pluripotent stem cells and gap junctional intercellular communication (GJIC)--must be brought into this analysis of carcinogenesis. Carcinogenesis is a multistage, multimechanism process consisting of a single cell that has been irreversibly blocked from terminal differentiation (the initiation stage). The promotion phase is a potentially reversible or interruptible clonal expansion of the initiated cell by a combination of growth stimulation and inhibition of apoptosis. When the expanded initiated cells accrue sufficient mutations and epigenetic alterations to become growth stimulus independent and resistant to growth inhibitors and apoptosis, to have unlimited replicative potential and invasive and metastatic phenotypes, then the progression phase has been achieved. The hypothesis that integrates these hallmarks is that the stem cell and its early progenitor cell are the target cells for the initiation event. These cells are naturally immortal and become mortal only when they are induced to terminally differentiate and lose their telomerase activity. These two types of initiated cells are suppressed by either secreted negative growth regulators (the stem cells) or GJIC (the early initiated progenitor cells). Promoters inhibit either the secreted growth inhibitor to initiated stem cells or GJIC between the initiated progenitor cells and the normal progenitor cells. When a stable resistance to the secreted negative growth regulator or permanent downregulation of GJIC has occurred, the cell has entered the progression phase. These two new concepts contradict the current paradigm that the first phase of carcinogenesis is the immortalization of a normal cell followed by its neoplastic transformation. Our hypothesis is that the first stage of carcinogenesis must prevent the "mortalization" or terminal differentiation of a naturally immortal cell. Chemoprevention and chemotherapeutic implications suggest that one must induce connexin genes in initiated stem cells and restore GJIC in initiated early progenitor cells.

Trosko, J. E. and K. S. Kang (2012). "Evolution of energy metabolism, stem cells and cancer stem cells: how the warburg and barker hypotheses might be linked." Int J Stem Cells **5**(1): 39-56.

The evolutionary transition from single cells to the metazoan forced the appearance of adult stem cells and a hypoxic niche, when oxygenation of the environment forced the appearance of oxidative phosphorylation from that of glycolysis. The prevailing paradigm in the cancer field is that cancers start from the "immortalization" or "re-programming" of a normal, differentiated cell with many mitochondria, that metabolize via oxidative phosphorylation. This paradigm has been challenged with one that assumes that the target cell for carcinogenesis is the normal, immortal adult stem cell, with few mitochondria. This adult organ-specific stem cell is blocked from "mortalizing" or from "programming" to be terminally differentiated. Two hypotheses have been offered to explain cancers, namely, the "stem cell theory" and the "de-differentiation" or "re-programming" theory. This Commentary postulates that the paleochemistry of the oceans, which, initially, provided conditions for life' s energy to arise via glycolysis, changed to oxidative phosphorylation for life' s processes. In doing so, stem cells evolved, within hypoxic niches, to protect the species germinal and somatic genomes. This Commentary provides support for the "stem cell theory", in that cancer cells, which, unlike differentiated cells, have few mitochondria and metabolize via glycolysis. The major argument against the "de-differentiation theory" is that, if re-programming of a differentiated cell to an "induced pluri-potent stem cell" happened in an adult, teratomas, rather than carcinomas, should be the result.

Trounson, A. O. (2001). "The derivation and potential use of human embryonic stem cells." Reprod Fertil Dev **13**(7-8): 523-532.

Human embryonic stem cells lines can be derived from human blastocysts at high efficiency (>50%) by immunosurgical isolation of the inner cell mass and culture on embryonic fibroblast cell lines. These cells will spontaneously differentiate into all the primary embryonic lineages in vitro and in vivo, but they are unable to form an integrated embryo or body plan by themselves or when combined with trophectoderm cells. They may be directed into a number of specific cell types and this enrichment process requires specific growth factors, cell-surface molecules, matrix molecules and secreted products of other cell types. Embryonic stem (ES) cells are immortal and represent a major potential for cell therapies for regenerative medicine. Their use in

transplantation may depend on the formation of a large bank of suitable human leucocyte antigen (HLA) types or the genetic erasure of their HLA expression. Successful transplantation may also require induction of tolerance in recipients and ongoing immune suppression. Although it is possible to customize ES cells by therapeutic cloning or cytoplasmic transfer, it would appear unlikely that these strategies will be used extensively for producing ES cells compatible for transplantation. Embryonic stem cell research may deliver a new pathway for regenerative medicine.

Tsai, C. L., et al. (2015). "Differentiation of Stem Cells From Human Exfoliated Deciduous Teeth Toward a Phenotype of Corneal Epithelium In Vitro." Cornea **34**(11): 1471-1477.

PURPOSE: The aim of this study was to characterize stem cells from human exfoliated deciduous teeth (SHED) and to investigate the potential of SHED to differentiate toward corneal epithelium-like cells in vitro. METHODS: Mesenchymal and embryonic stem cell markers were analyzed by flow cytometry. The SHED was cocultured in either a transwell noncontact system or in a mixed culture system with immortalized human corneal epithelial (HCE-T) cells to induce the epithelial transdifferentiation. Expression of the mature corneal epithelium-specific marker cytokeratin 3 (CK3) and corneal epithelial progenitor marker cytokeratin 19 (CK19) were detected by immunofluorescence and the reverse transcription-polymerase chain reaction, respectively. RESULTS: SHED strongly expressed a set of mesenchymal stromal cell markers and pluripotency markers including NANOG and OCT-4. Seven days after the transwells were cocultured with HCE-T cells, SHED successfully upregulated epithelial lineage markers CK3 (16.6 +/- 7.9%) and CK19 (10.0 +/- 4.3%) demonstrating the potential for epithelial transdifferentiation, whereas CK3 and CK19 were barely expressed in SHED when cultured alone. Expression of transcript levels of CK3 and CK19 were significantly upregulated when SHED were transwell cocultured or mixed cultured with HCE-T cells by 7, 14, and 21 days. CONCLUSIONS: We have demonstrated that SHED retain the potential for transdifferentiation to corneal epithelium-like cells by in vitro coculture with immortal corneal epithelium cells. Thus, exfoliated teeth may be an alternative tissue resource for providing stem cells for potential clinical applications in ocular surface regeneration.

Tsai, R. Y. (2016). "Balancing self-renewal against genome preservation in stem cells: How do they manage to have the cake and eat it too?" Cell Mol Life Sci **73**(9): 1803-1823.

Stem cells are endowed with the awesome power of self-renewal and multi-lineage differentiation that allows them to be major contributors to tissue homeostasis. Owing to their longevity and self-renewal capacity, they are also faced with a higher risk of genomic damage compared to differentiated cells. Damage on the genome, if not prevented or repaired properly, will threaten the survival of stem cells and culminate in organ failure, premature aging, or cancer formation. It is therefore of paramount importance that stem cells remain genomically stable throughout life. Given their unique biological and functional requirement, stem cells are thought to manage genotoxic stress somewhat differently from non-stem cells. The focus of this article is to review the current knowledge on how stem cells escape the barrage of oxidative and replicative DNA damage to stay in self-renewal. A clear statement on this subject should help us better understand tissue regeneration, aging, and cancer.

Utikal, J., et al. (2009). "Immortalization eliminates a roadblock during cellular reprogramming into iPS cells." Nature **460**(7259): 1145-1148.

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.

Van Gansen, P. and N. Van Lerberghe (1987). "[Possibilities and limitations of fibroblast cultures in the study of animal aging]." Cellule **74**: 317-373.

INTRODUCTION. Aging--the effect of time--occurs in every living organism. Senescence is the last period of the lifespan, leading to death. It happens in all animals, with the exception of a few didermic species (Hydras) having a stock of embryonic cells and being

immortal. The causes of animal senescence are badly known. They depend both on genetic characters (maximal lifespan of a species) and on medium factors (mean expectation of life of the animals of a species). Animal senescence could depend on cell aging: 1) by senescence and death of the differentiated cells, 2) by modified proliferation and differentiation of the stem cells of differentiated tissues, 3) by alterations in the extracellular matrices, 4) by interactions between factors 1) 2) and 3) in each tissue, 5) by interactions between the several tissues of an organism. This complexity badly impedes the experimental study of animal senescence. Normal mammal cells are aging when they are cultivated (in vitro ageing): their phenotype varies and depends on the cell generation (in vitro differentiation); the last cell-generation doesn't divide anymore and declines until death of the culture (in vitro senescence). Analysis of these artificial but well controlled systems allows an experimental approach of the proliferation, differentiation, senescence and death of the cells and of the extracellular matrix functions. Present literature upon in vitro aging of cultivated human cells is essentially made of papers where proliferation and differentiation characteristics are compared between early ("young") and late ("old") cell-generations of the cultures. FIBROBLASTIC CELLS OF THE MOUSE SKIN. This cell type has been studied in our laboratory, using different systems: 1) Primary cultures isolated from peeled skins of 19 day old mouse embryos, 2) Mouse dermis analyzed in the animals, 3) Cultivated explants of skins, 4) Serial sub-cultures of fibroblasts isolated from these explants, 5) Cells cultivated comparably on plane substrates (glass, plastic, collagen films) and on tridimensional matrices (collagen fibres). Systems 2), 3), 4) and 5) have been obtained either from 19 day old embryos or from 6 groups of animals of different ages (from 1/2 till 25 month). In primary cultures (system 1) all the cell generations have been analyzed, including the last one until death of the culture. We have shown that many characters are varying with cell-generation: cell form and cell mass, rate of DNA replication and cell division, rate of RNA transcription, nature of the accumulated and of the synthetized proteins, organization of the cytoskeletal elements, organization of the extracellular matrix, type of cell death.(ABSTRACT TRUNCATED AT 400 WORDS)

Van Gansen, P. and N. Van Lerberghe (1988). "Potential and limitations of cultivated fibroblasts in the study of senescence in animals. A review on the murine skin fibroblasts system." Arch Gerontol Geriatr **7**(1): 31-74.

Senescence is the last period of the life span, leading to death. It happens in all animals, with the exception of a few didermic species (Hydras) having a stock of embryonic cells and being immortal. The causes of animal senescence are badly known. They depend both on genetic characters (maximum life span of a species) and on medium factors (mean expectation of life of the animals of a species). Animal senescence could depend on cell aging: (1) by senescence and death of the differentiated cells, (2) by modified proliferation of the stem cells of differentiated tissues, (3) by alterations in the extracellular matrices, (4) by interactions between factors (1) (2) and (3) in each tissue, and (5) by interactions between the several tissues of an organism. This complexity badly impedes the experimental study of animal senescence. Normal mammal cells are aging when they are cultivated (in vitro aging). Present literature upon in vitro aging of cultivated human fibroblasts consists essentially of papers devoted to proliferation and differentiation characteristics and not to cell senescence. Murine skin fibroblasts have been studied in our laboratory, using different systems: (1) primary cultures isolated from peeled skins of mouse embryos, (2) mouse derms analysed in the animals, (3) cultivated explants of skins, (4) serial sub-cultures of fibroblasts isolated from these explants, (5) cells cultivated comparably on plane substrates (glass, plastic, collagen films) and on three-dimensional matrices (collagen fibres). In primary cultures (system 1) all the cell generations have been analysed, including the last one until death of the culture. We have shown that many characters are varying with cell generation. All the observed variations were: progressive, non-linear and correlated (intracellular feedbacks). We come to the conclusion that the main effects of cell mitotic age are (1) to depress the plasticity of the chromatin, (2) to change the organization of the cytoplasmic filaments, (3) to change the organization of the extracellular matrix. The collagen fibres are also acting upon nucleus and filaments either in the animals or in the cultures. The phenotype of a fibroblastic cell is thus both age- and environment-dependent. Overall data on in vitro cell aging point to the hypothesis that senescent cells are phenotypic variants and not mutant cells. Aging cell cultures are remarkably useful to the studies on cell proliferation decrease and cell cycle lengthening shown by the stem cells in animal tissues. We propose the hypothesis that the fibroblasts of the vertebrates would be homologous to the pluripotent mesenchyme cells of their embryos.

Veizovic, T., et al. (2001). "Resolution of stroke deficits following contralateral grafts of conditionally immortal neuroepithelial stem cells." Stroke **32**(4): 1012-1019.

BACKGROUND AND PURPOSE: Grafts of MHP36 cells have previously been shown to reduce dysfunction after global ischemia in rats. To test their efficacy after focal ischemia, MHP36 cells were grafted 2 to 3 weeks after transient intraluminal middle

cerebral artery occlusion (tMCAO) in rats. METHODS: MHP36 cells were implanted into the hemisphere contralateral to the lesion, with 8 deposits of 3 microL of cell suspension (25 000 cells per microliter). Sham grafted rats received equivalent volumes of vehicle. Three groups, sham-operated controls (n=11), MCAO+sham grafts (n=10), and MCAO+MHP36 grafts (n=11), were compared in 3 behavioral tests. RESULTS: In the bilateral asymmetry test, MCAO+MHP36 grafted rats exhibited neglect before grafting but subsequently showed no significant dysfunction, whereas MCAO+sham grafted rats showed stable sensorimotor deficits over 18 weeks relative to controls. MCAO+sham grafted rats demonstrated spontaneous motor asymmetry and increased rotational bias after injection of dopamine agonists. MCAO+MHP36 and control groups exhibited no bias in either spontaneous or drug-induced rotation. In contrast to motor recovery, MCAO+MHP36 grafted rats showed no improvement relative to MCAO+sham grafted rats in spatial learning and memory in the water maze. MCAO produced large striatal and cortical cavitations in both occluded groups. Lesion volume was significantly reduced ($P<0.05$) in the MCAO+MHP36 grafted group. The majority of MHP36 cells were identified within the intact grafted hemisphere. However, MHP36 cells were also seen in the cortex, striatum, and corpus callosum of the lesioned hemisphere. CONCLUSIONS: MHP36 cells may improve functional outcome after MCAO by assisting spontaneous reorganization in both the damaged and intact hemispheres.

Venu, P., et al. (2010). "Analysis of long-term culture properties and pluripotent character of two sibling human embryonic stem cell lines derived from discarded embryos." In Vitro Cell Dev Biol Anim **46**(3-4): 200-205.

We had earlier reported the derivation and characterization of two new sibling human embryonic stem cell lines BJNhem19 and BJNhem20, from discarded grade III embryos of Indian origin. We report here the characteristics of the two sibling cell lines after long-term continuous culture for over 2 yr during which they have been passaged over 200 times. We show that both cell lines adapt well to culture on various mouse and human feeders as well as in feeder-free conditions. The cells show normal diploid karyotype and continue to express all pluripotency markers. Both cell lines differentiate to derivatives of all three germ layers in vitro. However as reported earlier, BJNhem19 is unable to generate teratomas in nude or SCID mice or differentiate to beating cardiomyocytes when tested over several passages during long-term stable culture. On the other hand, the cardiac differentiation capacity of BJNhem20 is greatly increased, and it can generate beating cardiomyocytes that proliferate when isolated and cultured further. In conclusion, the two cell lines have maintained a stable phenotype for over 2 yr and are indeed immortal. Their derivation from grade III embryos does not seem to have any adverse effect on their long-term phenotype. The cells can be obtained for research purposes from the UK Stem Cell Bank and from the authors.

Verdoodt, F., et al. (2012). "Stem cells propagate their DNA by random segregation in the flatworm Macrostomum lignano." PLoS One **7**(1): e30227.

Adult stem cells are proposed to have acquired special features to prevent an accumulation of DNA-replication errors. Two such mechanisms, frequently suggested to serve this goal are cellular quiescence, and non-random segregation of DNA strands during stem cell division, a theory designated as the immortal strand hypothesis. To date, it has been difficult to test the in vivo relevance of both mechanisms in stem cell systems. It has been shown that in the flatworm Macrostomum lignano pluripotent stem cells (neoblasts) are present in adult animals. We sought to address by which means M. lignano neoblasts protect themselves against the accumulation of genomic errors, by studying the exact mode of DNA-segregation during their division. In this study, we demonstrated four lines of in vivo evidence in favor of cellular quiescence. Firstly, performing BrdU pulse-chase experiments, we localized 'Label-Retaining Cells' (LRCs). Secondly, EDU pulse-chase combined with Vasa labeling demonstrated the presence of neoblasts among the LRCs, while the majority of LRCs were differentiated cells. We showed that stem cells lose their label at a slow rate, indicating cellular quiescence. Thirdly, CldU/IdU- double labeling studies confirmed that label-retaining stem cells showed low proliferative activity. Finally, the use of the actin inhibitor, cytochalasin D, unequivocally demonstrated random segregation of DNA-strands in LRCs. Altogether, our data unambiguously demonstrated that the majority of neoblasts in M. lignano distribute their DNA randomly during cell division, and that label-retention is a direct result of cellular quiescence, rather than a sign of co-segregation of labeled strands.

Vilchez, D., et al. (2012). "Increased proteasome activity in human embryonic stem cells is regulated by PSMD11." Nature **489**(7415): 304-308.

Embryonic stem cells can replicate continuously in the absence of senescence and, therefore, are immortal in culture. Although genome stability is essential for the survival of stem cells, proteome stability may have an equally important role in stem-cell identity and function. Furthermore, with the asymmetric divisions invoked by stem cells, the passage of damaged proteins to daughter cells could potentially destroy the resulting lineage of cells. Therefore, a firm understanding of how stem cells

maintain their proteome is of central importance. Here we show that human embryonic stem cells (hESCs) exhibit high proteasome activity that is correlated with increased levels of the 19S proteasome subunit PSMD11 (known as RPN-6 in Caenorhabditis elegans) and a corresponding increased assembly of the 26S/30S proteasome. Ectopic expression of PSMD11 is sufficient to increase proteasome assembly and activity. FOXO4, an insulin/insulin-like growth factor-I (IGF-I) responsive transcription factor associated with long lifespan in invertebrates, regulates proteasome activity by modulating the expression of PSMD11 in hESCs. Proteasome inhibition in hESCs affects the expression of pluripotency markers and the levels of specific markers of the distinct germ layers. Our results suggest a new regulation of proteostasis in hESCs that links longevity and stress resistance in invertebrates to hESC function and identity.

Villa, A., et al. (2004). "Long-term molecular and cellular stability of human neural stem cell lines." Exp Cell Res **294**(2): 559-570.

Human Neural Stem Cells (hNSCs) are excellent candidates for in vitro and in vivo molecular, cellular, and developmental research, and also for ex-vivo gene transfer and cell therapy in the nervous system. However, hNSCs are mortal somatic cells, and thus invariably enter an irreversible growth arrest after a finite number of cell divisions in culture. It has been proposed that this is due to telomere shortening. Here, we show that long-term cultured (up to 4 years) v-myc perpetuated hNSC lines do preserve short but stable and homogeneous telomeres (TRF and Q-FISH determinations). hNSC lines (but not strains) express high levels of telomerase activity, which is activated by v-myc, as demonstrated here. Telomerase activity is not constitutive, becoming non-detectable after differentiation (in parallel to v-myc down-regulation). hNSC lines also maintain a stable cell cycle length, mitotic potential, differentiation and neuron generation capacity, and do not express senescence-associated beta-galactosidase over years, as studied here. These data, collectively, help to explain the immortal nature of v-myc-perpetuated hNSC lines, and to establish them as excellent research tools for basic and applied neurobiological and translational studies.

von Lindern, M., et al. (2001). "Leukemic transformation of normal murine erythroid progenitors: v- and c-ErbB act through signaling pathways activated by the EpoR and c-Kit in stress erythropoiesis." Oncogene **20**(28): 3651-3664.

Primary erythroid progenitors can be expanded by the synergistic action of erythropoietin (Epo), stem cell factor (SCF) and glucocorticoids. While Epo is required for erythropoiesis in general, glucocorticoids and SCF mainly contribute to stress erythropoiesis in hypoxic mice. This ability of normal erythroid progenitors to undergo expansion under stress conditions is targeted by the avian erythroblastosis virus (AEV), harboring the oncogenes v-ErbB and v-ErbA. We investigated the signaling pathways required for progenitor expansion under stress conditions and in leukemic transformation. Immortal strains of erythroid progenitors, able to undergo normal, terminal differentiation under appropriate conditions, were established from fetal livers of p53-/- mice. Expression and activation of the EGF-receptor (HER-1/c-ErbB) or its mutated oncogenic version (v-ErbB) in these cells abrogated the requirement for Epo and SCF in expansion of these progenitors and blocked terminal differentiation. Upon inhibition of ErbB function, differentiation into erythrocytes occurred. Signal transducing molecules important for renewal induction, i.e. Stat5- and phosphoinositide 3-kinase (PI3K), are utilized by both EpoR/c-Kit and v/c-ErbB. However, while v-ErbB transformed cells and normal progenitors depended on PI3K signaling for renewal, c-ErbB also induces progenitor expansion by PI3K-independent mechanisms.

Waghmare, S. K., et al. (2008). "Quantitative proliferation dynamics and random chromosome segregation of hair follicle stem cells." EMBO J **27**(9): 1309-1320.

Regulation of stem cell (SC) proliferation is central to tissue homoeostasis, injury repair, and cancer development. Accumulation of replication errors in SCs is limited by either infrequent division and/or by chromosome sorting to retain preferentially the oldest 'immortal' DNA strand. The frequency of SC divisions and the chromosome-sorting phenomenon are difficult to examine accurately with existing methods. To address this question, we developed a strategy to count divisions of hair follicle (HF) SCs over time, and provide the first quantitative proliferation history of a tissue SC during its normal homoeostasis. We uncovered an unexpectedly high cellular turnover in the SC compartment in one round of activation. Our study provides quantitative data in support of the long-standing infrequent SC division model, and shows that HF SCs do not retain the older DNA strands or sort their chromosome. This new ability to count divisions in vivo has relevance for obtaining basic knowledge of tissue kinetics.

Waghmare, S. K. and T. Tumbar (2013). "Adult hair follicle stem cells do not retain the older DNA strands in vivo during normal tissue homeostasis." Chromosome Res **21**(3): 203-212.

Tissue stem cells have been proposed to segregate the chromosomes asymmetrically (in a non-random manner), thereby retaining preferentially the older "immortal" DNA strands bearing the stemness characteristics into one daughter cell, whereas the newly synthesized strands are segregated to the other

daughter cell that will commit to differentiation. Moreover, this non-random segregation would protect the stem cell genome from accumulating multiple mutations during repeated DNA replication. This long-standing hypothesis remains an active subject of study due to conflicting results for some systems and lack of consistency among different tissue stem cell populations. In this review, we will focus on work done in the hair follicle, which is one of the best-understood vertebrate tissue stem cell system to date. In cell culture analysis of paired cultured keratinocytes derived from hair follicle, stem cells suggested a non-random segregation of chromosome with respect to the older DNA strand. In vivo, the hair follicle stem cells appear to self-renew and differentiate at different phases of their homeostatic cycle. The fate decisions occur in quiescence when some stem cells migrate out of their niche and commit to differentiation without self-renewal. The stem cells left behind in the niche self-renew symmetrically and randomly segregate the chromosomes at each division, making more stem cells. This model seems to apply to at least a few other vertebrate tissue stem cells in vivo.

Wakeman, J. A., et al. (2012). "The immortal strand hypothesis: still non-randomly segregating opinions." Biomol Concepts **3**(3): 203-211.

Abstract Cairns first suggested a mechanism for protecting the genomes of stem cells (SCs) from replicative errors some 40 years ago when he proposed the immortal strand hypothesis, which argued for the inheritance of a so-called immortal strand by an SC following asymmetric SC divisions. To date, the existence of immortal strands remains contentious with published evidence arguing in favour of and against the retention of an immortal strand by asymmetrically dividing SCs. The conflicting evidence is derived from a diverse array of studies on adult SC types and is predominantly based on following the fate of labelled DNA strands during asymmetric cell division events. Here, we review current data, highlighting limitations of such labelling techniques, and suggest how interpretation of such data may be improved in the future.

Walters, K. (2009). "Colonic stem cell data are consistent with the immortal model of stem cell division under non-random strand segregation." Cell Prolif **42**(3): 339-347.

OBJECTIVES: Colonic stem cells are thought to reside towards the base of crypts of the colon, but their numbers and proliferation mechanisms are not well characterized. A defining property of stem cells is that they are able to divide asymmetrically, but it is not known whether they always divide asymmetrically (immortal model) or whether there are occasional symmetrical divisions (stochastic model). By measuring diversity of methylation patterns in colon crypt samples, a recent study found evidence in favour of the stochastic model, assuming random segregation of stem cell DNA strands during cell division. Here, the effect of preferential segregation of the template strand is considered to be consistent with the 'immortal strand hypothesis', and explore the effect on conclusions of previously published results. MATERIALS AND METHODS: For a sample of crypts, it is shown how, under the immortal model, to calculate mean and variance of the number of unique methylation patterns allowing for non-random strand segregation and compare them with those observed. RESULTS: The calculated mean and variance are consistent with an immortal model that incorporates non-random strand segregation for a range of stem cell numbers and levels of preferential strand segregation. CONCLUSIONS: Allowing for preferential strand segregation considerably alters previously published conclusions relating to stem cell numbers and turnover mechanisms. Evidence in favour of the stochastic model may not be as strong as previously thought.

Walters, K. (2012). "Parameter estimation for an immortal model of colonic stem cell division using approximate Bayesian computation." J Theor Biol **306**: 104-114.

In this paper we use approximate Bayesian computation to estimate the parameters in an immortal model of colonic stem cell division. We base the inferences on the observed DNA methylation patterns of cells sampled from the human colon. Utilising DNA methylation patterns as a form of molecular clock is an emerging area of research and has been used in several studies investigating colonic stem cell turnover. There is much debate concerning the two competing models of stem cell turnover: the symmetric (immortal) and asymmetric models. Early simulation studies concluded that the observed methylation data were not consistent with the immortal model. A later modified version of the immortal model that included preferential strand segregation was subsequently shown to be consistent with the same methylation data. Most of this earlier work assumes site independent methylation models that do not take account of the known processivity of methyltransferases whilst other work does not take into account the methylation errors that occur in differentiated cells. This paper addresses both of these issues for the immortal model and demonstrates that approximate Bayesian computation provides accurate estimates of the parameters in this neighbour-dependent model of methylation error rates. The results indicate that if colonic stem cells divide asymmetrically then colon stem cell niches are maintained by more than 8 stem cells. Results also indicate the possibility of preferential strand segregation and provide clear evidence against a site-independent model for methylation errors. In addition,

algebraic expressions for some of the summary statistics used in the approximate Bayesian computation (that allow for the additional variation arising from cell division in differentiated cells) are derived and their utility discussed.

Wang, D., et al. (2004). "N-acetyl-seryl-aspartyl-lysyl-proline stimulates angiogenesis in vitro and in vivo." Am J Physiol Heart Circ Physiol **287**(5): H2099-2105.

N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), a natural inhibitor of pluripotent hematopoietic stem cell proliferation, has been suggested as capable of promoting an angiogenic response. We studied whether Ac-SDKP stimulates endothelial cell proliferation, migration, and tube formation; enhances angiogenic response in the rat cornea after implantation of a tumor spheroid; and increases capillary density in rat hearts with myocardial infarction (MI). In vitro, an immortal BALB/c mouse aortic endothelial 22106 cell line was used to determine the effects of Ac-SDKP on endothelial cell proliferation and migration and tube formation. In vivo, a 9L-gliosarcoma cell spheroid (250-300 microm in diameter) was implanted in the rat cornea and vehicle or Ac-SDKP (800 microg.kg(-1).day(-1) ip) infused via osmotic minipump. Myocardial capillary density was studied in rats with MI given either vehicle or Ac-SDKP. We found that Ac-SDKP 1) stimulated endothelial cell proliferation and migration and tube formation in a dose-dependent manner, 2) enhanced corneal neovascularization, and 3) increased myocardial capillary density. Endothelial cell proliferation and angiogenesis stimulated by Ac-SDKP could be beneficial in cardiovascular diseases such as hypertension and MI. Furthermore, because Ac-SDKP is mainly cleaved by ACE, it may partially mediate the cardioprotective effect of ACE inhibitors.

Wang, K. H., et al. (2010). "Increasing CD44+/CD24(-) tumor stem cells, and upregulation of COX-2 and HDAC6, as major functions of HER2 in breast tumorigenesis." Mol Cancer **9**: 288.

BACKGROUND: Cancer cells are believed to arise primarily from stem cells. CD44+/CD24(-) have been identified as markers for human breast cancer stem cells. Although, HER2 is a well known breast cancer oncogene, the mechanisms of action of this gene are not completely understood. Previously, we have derived immortal (M13SV1), weakly tumorigenic (M13SV1R2) and highly tumorigenic (M13SV1R2N1) cell lines from a breast epithelial cell type with stem cell phenotypes after successive SV40 large T-antigen transfection, X-ray irradiation and ectopic expression of HER2/C-erbB2/neu. Recently, we found that M13SV1R2 cells became non-tumorigenic after growing in a growth factor/hormone-deprived medium (R2d cells). RESULTS: In this study, we developed M13SV1R2N1 under the same growth factor/hormone-deprived condition (R2N1d cells). This provides an opportunity to analyze HER2 effect on gene expression associated with tumorigenesis by comparative study of R2d and R2N1d cells with homogeneous genetic background except HER2 expression. The results reveal distinct characters of R2N1d cells that can be ascribed to HER2: 1) development of fast-growing tumors; 2) high frequency of CD44+/CD24(-) cells (~50% for R2N1d vs. ~10% for R2d); 3) enhanced expression of COX-2, HDAC6 mediated, respectively, by MAPK and PI3K/Akt pathways, and many genes associated with inflammation, metastasis, and angiogenesis. Furthermore, HER2 expression can be down regulated in non-adhering R2N1d cells. These cells showed longer latent period and lower rate of tumor development compared with adhering cells. CONCLUSIONS: HER2 may induce breast cancer by increasing the frequency of tumor stem cells and upregulating the expression of COX-2 and HDAC6 that play pivotal roles in tumor progression.

Wang, L., et al. (2020). "Establishment of a feeder and serum-free culture system for human embryonic stem cells." Zygote **28**(3): 175-182.

Stem cells are an immortal cell population capable of self-renewal; they are essential for human development and ageing and are a major focus of research in regenerative medicine. Despite considerable progress in differentiation of stem cells in vitro, culture conditions require further optimization to maximize the potential for multicellular differentiation during expansion. The aim of this study was to develop a feeder-free, serum-free culture method for human embryonic stem cells (hESCs), to establish optimal conditions for hESC proliferation, and to determine the biological characteristics of the resulting hESCs. The H9 hESC line was cultured using a homemade serum-free, feeder-free culture system, and growth was observed. The expression of pluripotency proteins (OCT4, NANOG, SOX2, LIN28, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81) in hESCs was determined by immunofluorescence and western blotting. The mRNA expression levels of genes encoding nestin, brachyury and alpha-fetoprotein in differentiated H9 cells were determined by RT-PCR. The newly developed culture system resulted in classical hESC colonies that were round or elliptical in shape, with clear and neat boundaries. The expression of pluripotency proteins was increased, and the genes encoding nestin, brachyury, and alpha-fetoprotein were expressed in H9 cells, suggesting that the cells maintained in vitro differentiation capacity. Our culture system containing a unique set of components, with animal-derived substances, maintained the self-renewal potential and pluripotency of H9 cells for eight passages. Further optimization of this system may expand the clinical application of hESCs.

Wang, Y., et al. (2009). "Rac1 and Rac2 in osteoclastogenesis: a cell immortalization model." Calcif Tissue Int **85**(3): 257-266.

Cell lines generated from primary cells with a particular gene deletion are useful for examining the function of the specific deleted genes and provide the opportunity to genetically rescue the lost genes using standard gene transfection techniques. In the present study, bone marrow monocytes from wild-type (WT), Rac1 null, and Rac2 null mice were primed with macrophage colony-stimulating factor and soluble receptor activator of NF-kappaB ligand to generate preosteoclasts. This was followed by transduction of a retrovirus containing simian virus 40 large T-antigen and a neomycin-resistant cassette. Seven to 19 immortalized cell lines from each genotype were established. Among them, WT2, Rac1 null-D9, and Rac2 null-A2 were characterized to verify that osteoclastogenesis and osteoclast functions were identical to the parental primary cells. Results showed that immortalized WT2 cells were able to differentiate into mature, multinucleated, functional, tartrate-resistant acid phosphatase-positive osteoclasts. Immortal Rac1 null cells, as with their primary cell counterparts, displayed a severe defect in osteoclastogenesis and function. Transfection of the Rac1 gene into Rac1 null cells was sufficient to rescue osteoclastogenesis. We believe this method of generating immortalized preosteoclasts will provide a key tool for studying the signaling mechanisms involved in osteoclastogenesis.

Watabe, H., et al. (2002). "All-trans retinoic acid induces differentiation and apoptosis of murine melanocyte precursors with induction of the microphthalmia-associated transcription factor." J Invest Dermatol **118**(1): 35-42.

The effects of all-trans retinoic acid on the differentiation and proliferation of immature melanocyte precursors were studied. NCC-melb4 cells are an immortal cloned cell line established from mouse neural crest cells using a single-cell cloning method. These cells were positive for tyrosinase-related protein 1, tyrosinase-related protein 2 and KIT, but were negative for tyrosinase and had no dihydroxyphenylalanine reaction. They contained only stage I melanosomes without any melanosomes in more advanced stages. After treatment with all-trans retinoic acid, many of the cells became tyrosinase- and dihydroxyphenylalanine-reaction-positive, changed from polygonal to dendritic in shape, and had stage III to IV melanosomes. These findings indicate that treatment with all-trans retinoic acid induced the differentiation of NCC-melb4 cells. Reverse transcription polymerase chain reaction analysis revealed a marked increase in expression of microphthalmia-associated transcription factor mRNA after all-trans retinoic acid treatment, suggesting that microphthalmia-associated transcription factor may be the key molecule in this event. Enhanced expression of protein kinase Calpha following treatment with all-trans retinoic acid was also demonstrated. The proliferation of NCC-melb4 cells was inhibited by all-trans retinoic acid in a dose-dependent manner. Increased apoptosis after all-trans retinoic acid treatment was observed by electron microscopy, the TUNEL method, DNA fragmentation assay, and flow cytometry. All-trans retinoic acid upregulated caspase-3 and downregulated bcl-2. Electron microscopy showed that apoptotic cells contained melanosomes of advanced stages, suggesting that mature melanocytes may tend to undergo apoptosis after all-trans retinoic acid treatment. This study provides important clues towards understanding the roles and working mechanisms of retinoic acids in melanocyte development and melanogenesis.

Wei, P., et al. (2012). "Reversible immortalization of Nestin-positive precursor cells from pancreas and differentiation into insulin-secreting cells." Biochem Biophys Res Commun **418**(2): 330-335.

Pancreatic stem cells or progenitor cells posses the ability of directed differentiation into pancreatic beta cells. However, these cells usually have limited proliferative capacity and finite lifespan in vitro. In the present study, Nestin-positive progenitor cells (NPPCs) from mouse pancreas that expressed the pancreatic stem cells or progenitor cell marker Nestin were isolated to obtain a sufficient number of differentiated pancreatic beta cells. Tet-on system for SV40 large T-antigen expression in NPPCs was used to achieve reversible immortalization. The reversible immortal Nestin-positive progenitor cells (RINPPCs) can undergo at least 80 population doublings without senescence in vitro while maintaining their biological and genetic characteristics. RINPPCs can be efficiently induced to differentiate into insulin-producing cells that contain a combination of glucagon-like peptide-1 (GLP-1) and sodium butyrate. The results of the present study can be used to explore transplantation therapy of type I diabetes mellitus.

Wen, C. M., et al. (2008). "Isolation and characterization of a neural progenitor cell line from tilapia brain." Comp Biochem Physiol A Mol Integr Physiol **149**(2): 167-180.

Astroglial cell lines have many applications for advancing neural developmental and functional studies. However, few astroglial cell lines have been reported from fish. In this study, we report the characterization of the immortal cell line TB2 isolated from adult tilapia brain tissue. The cell line was established at 25 degrees C in L15 medium supplemented with 15% fetal bovine serum. Most of the cells displayed a fibrous morphology and were

immunoreactive for A2B5 antigen, glial fibrillary acidic protein (GFAP), keratin, vimentin, and the gap junction protein connexin 43 (Cx43). They weakly expressed glutamine synthetase (GS), S100 protein, and the neural stem cell markers Sox2 and brain lipid binding protein (BLBP). In contrast to astroglia in vivo, most TB2 cells also expressed galactocerebroside (GalC), substance P (SP), and tyrosine hydroxylase (TH). By immunoblot and RT-PCR, the cells also expressed myelin basic protein (MBP), proteolipid protein (PLP), and Cx35. On a poly-L-lysine-coated substrate in vitro, TB2 cells showed increases in neuronal dopamine decarboxylase (DDC) and microtubule-associated protein 2 (MAP2), indicating that they can initiate differentiation into neurons. Taken together, the results suggest that TB2 cells are astroglial progenitor cells (neural stem cells) and may develop into oligodendrocytes and neurons in a suitable environment. The present study advances our knowledge of fish astroglia. However, the factors that affect neural development in fish remain unknown, as do the characteristics of the intermediate differentiation stages between stem cells and mature nerve cells. The TB2 cell line will promote these investigations.

Werner, B. and A. Sottoriva (2018). "Variation of mutational burden in healthy human tissues suggests non-random strand segregation and allows measuring somatic mutation rates." PLoS Comput Biol **14**(6): e1006233.

The immortal strand hypothesis poses that stem cells could produce differentiated progeny while conserving the original template strand, thus avoiding accumulating somatic mutations. However, quantitating the extent of non-random DNA strand segregation in human stem cells remains difficult in vivo. Here we show that the change of the mean and variance of the mutational burden with age in healthy human tissues allows estimating strand segregation probabilities and somatic mutation rates. We analysed deep sequencing data from healthy human colon, small intestine, liver, skin and brain. We found highly effective non-random DNA strand segregation in all adult tissues (mean strand segregation probability: 0.98, standard error bounds (0.97,0.99)). In contrast, non-random strand segregation efficiency is reduced to 0.87 (0.78,0.88) in neural tissue during early development, suggesting stem cell pool expansions due to symmetric self-renewal. Healthy somatic mutation rates differed across tissue types, ranging from 3.5 x 10-9/bp/division in small intestine to 1.6 x 10-7/bp/division in skin.

Whitehead, R. E., Jr., et al. (1992). "Detection of multiple tumor suppressor genes for Syrian hamster fibrosarcomas by somatic cell hybridization." Somat Cell Mol Genet **18**(2): 131-142.

Identification of tumor suppressor gene loci in rodent cell culture systems has relied upon the use of somatic cell hybridization studies. Although normal rodent fibroblasts are capable of suppressing the tumorigenicity of a variety of tumor cells, the lack of complementation in tumor cell x tumor cell hybrids has left the possibility that a single tumor suppressor gene may be responsible for tumor suppression in a particular rodent cell culture system. Using this same approach, we found no evidence for complementation resulting in suppression of the transformed phenotype when three viral oncogene-transformed Syrian hamster embryo (SHE) cell lines and one spontaneously transformed baby hamster kidney (BHK) cell line were fused to benzo[a]pyrene-transformed SHE cells (BP6T-M3). However, v-src oncogene-transformed cell line (srcT) x BP6T-M3 hybrids did demonstrate limited suppression of the transformed phenotype, suggesting at least two complementing tumor suppressor genes in this system. We were able to confirm and extend this finding using another experimental approach with preneoplastic hamster cell lines that are immortal in culture but nontumorigenic in nude mice. We propose that fusion of these preneoplastic cells to various tumor cells may reveal tumor suppressor genes not evident in the tumor cell x tumor cell complementation studies. Subclones of two nontumorigenic, immortal hamster cell lines, 10W and DES4, displayed differing abilities to suppress BP6T-M3 cells in somatic cell hybrids, as quantitated by the ability of the hybrid cells to form colonies in soft agar. With a panel of preneoplastic hamster cell x BP6T-M3 hybrids, a distinct pattern of suppression or expression of the transformed phenotype was observed. Marked differences in this pattern were seen when the same 10W and DES4 subclones were fused to other hamster fibrosarcoma cell lines, indicating different tumor suppressing activities of multiple tumor suppressor genes. Analysis of this data suggests that as few as three or as many as six different tumor suppressor genes may be active in the Syrian hamster embryo cell culture system. Thus, this system may provide a useful model for identifying and studying the effects and regulation of a number of different tumor suppressor genes for fibrosarcomas.

Wikswo, J. P. (2014). "The relevance and potential roles of microphysiological systems in biology and medicine." Exp Biol Med (Maywood) **239**(9): 1061-1072.

Microphysiological systems (MPS), consisting of interacting organs-on-chips or tissue-engineered, 3D organ constructs that use human cells, present an opportunity to bring new tools to biology, medicine, pharmacology, physiology, and toxicology. This issue of Experimental Biology and Medicine describes the ongoing development of MPS that can serve as in-vitro models for bone and cartilage, brain, gastrointestinal

tract, lung, liver, microvasculature, reproductive tract, skeletal muscle, and skin. Related topics addressed here are the interconnection of organs-on-chips to support physiologically based pharmacokinetics and drug discovery and screening, and the microscale technologies that regulate stem cell differentiation. The initial motivation for creating MPS was to increase the speed, efficiency, and safety of pharmaceutical development and testing, paying particular regard to the fact that neither monolayer monocultures of immortal or primary cell lines nor animal studies can adequately recapitulate the dynamics of drug-organ, drug-drug, and drug-organ-organ interactions in humans. Other applications include studies of the effect of environmental toxins on humans, identification, characterization, and neutralization of chemical and biological weapons, controlled studies of the microbiome and infectious disease that cannot be conducted in humans, controlled differentiation of induced pluripotent stem cells into specific adult cellular phenotypes, and studies of the dynamics of metabolism and signaling within and between human organs. The technical challenges are being addressed by many investigators, and in the process, it seems highly likely that significant progress will be made toward providing more physiologically realistic alternatives to monolayer monocultures or whole animal studies. The effectiveness of this effort will be determined in part by how easy the constructs are to use, how well they function, how accurately they recapitulate and report human pharmacology and toxicology, whether they can be generated in large numbers to enable parallel studies, and if their use can be standardized consistent with the practices of regulatory science.

Winquist, R. J., et al. (2014). "Evaluating the immortal strand hypothesis in cancer stem cells: symmetric/self-renewal as the relevant surrogate marker of tumorigenicity." Biochem Pharmacol **91**(2): 129-134.

Stem cells subserve repair functions for the lifetime of the organism but, as a consequence of this responsibility, are candidate cells for accumulating numerous genetic and/or epigenetic aberrations leading to malignant transformation. However, given the importance of this guardian role, stem cells likely harbor some process for maintaining their precious genetic code such as non-random segregation of chromatid strands as predicted by the Immortal Strand Hypothesis (ISH). Discerning such non-random chromosomal segregation and asymmetric cell division in normal or cancer stem cells has been complicated by methodological shortcomings but also by differing division kinetics amongst tissues and the likelihood that both asymmetric and symmetric cell divisions, dictated by local extrinsic factors, are operant in these cells. Recent data suggest that cancer stem cells demonstrate a higher incidence of symmetric versus asymmetric cell division with both daughter cells retaining self-renewal characteristics, a profile which may underlie poorly differentiated morphology and marked clonal diversity in tumors. Pathways and targets are beginning to emerge which may provide opportunities for preventing such a predilection in cancer stem cells and that will hopefully translate into new classes of chemotherapeutics in oncology. Thus, although the existence of the ISH remains controversial, the shift of cell division dynamics to symmetric random chromosome segregation/self-renewal, which would negate any likelihood of template strand retention, appears to be a surrogate marker for the presence of highly malignant tumorigenic cell populations.

Wrzesinski, K. and S. J. Fey (2018). "Metabolic Reprogramming and the Recovery of Physiological Functionality in 3D Cultures in Micro-Bioreactors." Bioengineering (Basel) **5**(1).

The recovery of physiological functionality, which is commonly seen in tissue mimetic three-dimensional (3D) cellular aggregates (organoids, spheroids, acini, etc.), has been observed in cells of many origins (primary tissues, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and immortal cell lines). This plurality and plasticity suggest that probably several basic principles promote this recovery process. The aim of this study was to identify these basic principles and describe how they are regulated so that they can be taken in consideration when micro-bioreactors are designed. Here, we provide evidence that one of these basic principles is hypoxia, which is a natural consequence of multicellular structures grown in microgravity cultures. Hypoxia drives a partial metabolic reprogramming to aerobic glycolysis and an increased anabolic synthesis. A second principle is the activation of cytoplasmic glutaminolysis for lipogenesis. Glutaminolysis is activated in the presence of hypo- or normo-glycaemic conditions and in turn is geared to the hexosamine pathway. The reducing power needed is produced in the pentose phosphate pathway, a prime function of glucose metabolism. Cytoskeletal reconstruction, histone modification, and the recovery of the physiological phenotype can all be traced to adaptive changes in the underlying cellular metabolism. These changes are coordinated by mTOR/Akt, p53 and non-canonical Wnt signaling pathways, while myc and NF-kB appear to be relatively inactive. Partial metabolic reprogramming to aerobic glycolysis, originally described by Warburg, is independent of the cell's rate of proliferation, but is interwoven with the cells abilities to execute advanced functionality needed for replicating the tissues physiological performance.

Wrzesinski, K., et al. (2021). "Clinostat 3D Cell Culture: Protocols for the Preparation and Functional Analysis of Highly Reproducible, Large, Uniform Spheroids and Organoids." Methods Mol Biol **2273**: 17-62.

Growing cells as 3D structures need not be difficult. Often, it is not necessary to change cell type, additives or growth media used. All that needs to be changed is the geometry: cells (whether primary, induced pluripotent, transformed or immortal) simply have to be grown in conditions that promote cell-cell adhesion while allowing gas, nutrient, signal, and metabolite exchange. Downstream analysis can become more complicated because many assays (like phase contrast microscopy) cannot be used, but their replacements have been in use for many years. Most importantly, there is a huge gain in value in obtaining data that is more representative of the organism in vivo. It is the goal of the protocols presented here to make the transition to a new dimension as painless as possible. Grown optimally, most biopsy derived organoids will retain patient phenotypes, while cell (both stem cell, induced or otherwise or immortalized) derived organoids or spheroids will recover tissue functionality.

Xu, C., et al. (2004). "Immortalized fibroblast-like cells derived from human embryonic stem cells support undifferentiated cell growth." Stem Cells **22**(6): 972-980.

Human embryonic stem cells (hESCs) have the potential to generate multiple cell types and hold promise for future therapeutic applications. Although undifferentiated hESCs can proliferate indefinitely, hESC derivatives significantly downregulate telomerase and have limited replication potential. In this study we examine whether the replicative lifespan of hESC derivatives can be extended by ectopic expression of human telomerase reverse transcriptase (hTERT), the catalytic component of the telomerase complex. To this end, we have derived HEF1 cells, a fibroblast-like cell type, differentiated from hESCs. Infection of HEF1 cells with a retrovirus expressing hTERT extends their replicative capacity, resulting in immortal human HEF1-hTERT cells. HEF1-hTERT cells can be used to produce conditioned medium (CM) capable of supporting hESC growth under feeder-free conditions. Cultures maintained in HEF1-CM show characteristics similar to mouse embryonic fibroblast CM control cultures, including morphology, surface marker and transcription factor expression, telomerase activity, differentiation, and karyotypic stability. In addition, HEF1-hTERT cells have the capacity to differentiate into cells of the osteogenic lineage. These results suggest that immortalized cell lines can be generated from hESCs and that cells derived from hESCs can be used to support their own growth, creating a genotypically homogeneous system for the culture of hESCs.

Xu, X., et al. (2018). "Development of a primary culture system for haematopoietic tissue cells from Cherax quadricarinatus and an exploration of transfection methods." Dev Comp Immunol **88**: 45-54.

Various known and unknown viral diseases can threaten crustacean aquaculture. To develop prophylactic and therapeutic strategies against viruses, crustacean cell lines are urgently needed for immunology and virology studies. However, there are currently no permanent crustacean cell lines available. In this study, we developed a new method for preparing crayfish plasma (CP) and found that CP enhanced the proliferative capacity of haematopoietic tissue (hpt) cells from Cherax quadricarinatus by an EdU (5-ethynyl-2'-deoxyuridine) assay. The optimal CP concentration for hpt cell culture and the optimal subculture method are discussed. To achieve efficient expression of a foreign gene in hpt cells cultured in vitro, different transfection methods and vectors were analysed. We found that Lipofectamine 2000 could be used to efficiently transfect a foreign vector into hpt cells and exhibited a lower level of cytotoxicity than the other methods tested, and transfection of pEGFP-N1/w249 and pDHsp70-EGFP-FLAG resulted in high EGFP expression. By transmission electron microscopy (TEM) and virus copy number analysis, we found that white spot syndrome virus (WSSV) could infect hpt cells and multiply efficiently. Our results implied that the crayfish hpt cell culture system we improved could be used as a replacement for immortal crustacean cell lines in viral infection studies. Our findings provide a solid foundation for future immortalization and gene function studies in crustacean cells.

Xue, Y., et al. (1998). "Cell kinetics of prostate exocrine and neuroendocrine epithelium and their differential interrelationship: new perspectives." Prostate Suppl **8**: 62-73.

The prostate gland consists of a complex ductal system lined with exocrine basal and luminal cells, and neuroendocrine epithelial cells. This paper reviews the histologic and molecular cell biologic characteristics of these cells, in normal adult tissue, during prostate morphogenesis, and in the development of benign and malignant neoplastic conditions. Expression of differentiation markers, as well as proliferation and apoptosis markers, growth factors and associated receptors, and abnormalities in genes and chromosomes are reviewed. Accumulating data indicate that (1) pluripotent immortal stem cells are located in the basal cell compartment of the prostate; (2) there is a subpopulation of epithelial cells in the prostate gland (intermediate cells) that have both structural and functional characteristics common to basal and luminal cells, which may be identified in

various conditions; and prostate NE cells may have the same common origin as other exocrine cells, and share the same differentiation pathway. A stem cell model is proposed in which both exocrine and endocrine cells are derived from a subpopulation of basal cells (stem cell) that give rise to luminal cells through intermediate cells (pluripotent amplifying cells). These cells are also probably highly implicated in the early development of prostate benign and malignant neoplasia.

Yadlapalli, S., et al. (2011). "Drosophila male germline stem cells do not asymmetrically segregate chromosome strands." J Cell Sci **124**(Pt 6): 933-939.

Adult stem cells continuously supply differentiated cells throughout the life of organisms. This increases the risk of replicative senescence or neoplastic transformation due to mutations that accumulate over many rounds of DNA replication. The immortal strand hypothesis proposes that stem cells reduce the accumulation of replication-induced mutations by retaining the older template DNA strands. Other models have also been proposed in which stem cells asymmetrically segregate chromosome strands for other reasons, such as retention of epigenetic memories. Recently, the idea has emerged that the mother centrosome, which is stereotypically retained within some asymmetrically dividing stem cells, might be utilized as a means of asymmetrically segregating chromosome strands. We have tested this hypothesis in germline stem cells (GSCs) from Drosophila melanogaster testis, which undergo asymmetric divisions marked by the asymmetric segregation of centrosomes and the acquisition of distinct daughter cell fates (stem cell self-renewal versus differentiation). Using 5-bromo-2-deoxyuridine labeling combined with direct visualization of GSC-gonialblast (differentiating daughter) pairs, we directly scored the outcome of chromosome strand segregation. Our data show that, in male GSCs in the Drosophila testis, chromosome strands are not asymmetrically segregated, despite asymmetrically segregating centrosomes. Our data demonstrate that asymmetric centrosome segregation in stem cells does not necessarily lead to asymmetric chromosome strand segregation.

Yadlapalli, S. and Y. M. Yamashita (2013). "DNA asymmetry in stem cells - immortal or mortal?" J Cell Sci **126**(Pt 18): 4069-4076.

The immortal strand hypothesis proposes that stem cells retain a template copy of genomic DNA (i.e. an 'immortal strand') to avoid replication-induced mutations. An alternative hypothesis suggests that certain cells segregate sister chromatids non-randomly to transmit distinct epigenetic information. However, this area of research has been highly controversial, with conflicting data even from the same cell types. Moreover, historically, the same term of 'non-random sister chromatid segregation' or 'biased sister chromatid segregation' has been used to indicate distinct biological processes, generating a confusion in the biological significance and potential mechanism of each phenomenon. Here, we discuss the models of non-random sister chromatid segregation, and we explore the strengths and limitations of the various techniques and experimental model systems used to study this question. We also describe our recent study on Drosophila male germline stem cells, where sister chromatids of X and Y chromosomes are segregated non-randomly during cell division. We aim to integrate the existing evidence to speculate on the underlying mechanisms and biological relevance of this long-standing observation on non-random sister chromatid segregation.

Yamada, O., et al. (2003). "Overexpression of telomerase confers a survival advantage through suppression of TRF1 gene expression while maintaining differentiation characteristics in K562 cells." Cell Transplant **12**(4): 365-377.

Leukemic stem cells that expressed endogenous telomerase activity were induced to show overexpression of exogenous hTERT and were analyzed for biological changes in order to assess the possible influence of telomerase gene therapy on the transplantation of normal hematopoietic stem cells. Introduction of hTERT into K562, a telomerase-positive immortal cell line, resulted in a 2.5-fold elevation of telomerase activity and the lengthening of telomeres by 6 kb to 23 kb. Real-time fluorescent PCR, which could perform quantitative analysis of transcripts, revealed a 175-fold increase in hTERT expression, suggesting the posttranscriptional regulation of telomerase. Ectopic expression of hTERT in K562 cells showed a survival advantage during culture in the absence of serum. Expression of mRNA for the telomeric-repeat binding factor 1 (TRF1) and caspase-3 activity were both decreased in hTERT-transfected K562 cells. Transduced cells retained their usual phenotypic characteristics, differentiation ability, and signal transduction response to TPA. These data suggest that ectopic expression of hTERT by normal hematopoietic stem cells may confer a survival advantage without changing their innate biological characteristics.

Yamashita, Y. M. (2013). "Biased DNA segregation in Drosophila male germline stem cells." Semin Cell Dev Biol **24**(8-9): 618-626.

The immortal strand hypothesis, which emerged four decades ago, proposes that certain cells retain a template copy of chromosomal DNA to protect against replication-induced mutations. As the interest in stem cells rose in recent years, researchers speculated that stem cells, which must maintain proliferative capacity throughout the life of the

organism, may be the population that most needs the strong protection afforded by immortal strand segregation. Alternative hypotheses have also been proposed to explain observed non-random sister chromatid segregation. We recently found that Drosophila male germline stem cells segregate sister chromatids non-randomly, but such bias was limited to the sex chromosomes. Interestingly, the biased segregation does not lead to immortal strand segregation. We will discuss the implications of this observation and molecular mechanisms, which might be applicable to non-random sister chromatid segregation in other systems as well.

Yang, S. R., et al. (2005). "The role of p38 MAP kinase and c-Jun N-terminal protein kinase signaling in the differentiation and apoptosis of immortalized neural stem cells." Mutat Res **579**(1-2): 47-57.

The two distinct members of the mitogen-activated protein (MAP) kinase family c-Jun N-terminal protein kinase (JNK) and p38 MAP kinase, play an important role in central nervous system (CNS) development and differentiation. However, their role and functions are not completely understood in CNS. To facilitate in vitro study, we have established an immortal stem cell line using SV40 from fetal rat embryonic day 17. In these cells, MAP kinase inhibitors (SP600125, SB202190, and PD98059) were treated for 1, 24, 48, and 72 h to examine the roles of protein kinases. Early inhibition of JNK did not alter phenotypic or morphological changes of immortalized cells, however overexpression of Bax and decrease of phosphorylated AKT was observed. The prolonged inhibition of JNK induced polyploidization of immortalized cells, and resulted in differentiation and inhibition of cell proliferation. Moreover, JNK and p38 MAP kinase but not ERK1/2 was activated, and p21, p53, and Bax were overexpressed by prolonged inhibition of JNK. These results indicate that JNK and p38 MAP kinase could play dual roles on cell survival and apoptosis. Furthermore, this established cell line could facilitate study of the role of JNK and p38 MAP kinase on CNS development or differentiation/apoptosis.

Yang, X., et al. (1999). "Functional roles of chromosomes 11 and 17 in the transformation of human breast epithelial cells in vitro." Int J Oncol **15**(4): 629-638.

Genomic alterations in primary breast cancer play a role in the initiation and progression of the disease. We have analyzed the molecular events involved in the initiation and progression of the neoplastic process in an in vitro experimental system. Immortalization of human breast epithelial cells (HBEC) is associated with 3:9 translocation, p53 mutation and microsatellite instability (MSI) of chromosomes 11p13, and 17p. BP1-E cells, derived from the immortalized MCF-10F cells transformed by the carcinogen benzo(a)pyrene (BP), express in vitro growth advantage, anchorage independence, enhanced chemoinvasiveness, loss of ductulogenic capabilities and tumorigenesis in a heterologous host. This neoplastic progression is also associated with mutations and/or amplification of c-H-ras, int-2, c-neu, c-myc and MDM2, MSI at 11q25 and 13q12-q13 and loss of heterozygosity at 17p. In order to test whether chromosomes 11 or 17 play a functional role in the phenotypic expression of transformation of BP1E cells, we utilized microcell-mediated chromosome transfer (MMCT) technique for inserting the corresponding normal chromosomes to these transformed cells. BP1E cells were transfected with PsV2neo plasmid and fused with microcells obtained from the mouse cell line A9, containing a normal chromosome 11 or 17 (A9-11neo and A9-17neo cells, selected in G418 and cloned. Sixteen primary microcell hybrids from each chromosome transfer, designated BP1E-11neo and BP1E-17neo survived selection in G-418 containing medium. A single clone from each group, BP1E-11neo #145 and BP1E-17neo D100, survived subcloning and were utilized for a detailed panel of analyses. The presence of a donor chromosome was confirmed by dual color fluorescence in situ hybridization (FISH), southern blot analysis of the marker vector pSV2neo, and microsatellite polymorphism analysis. The transfer of the normal chromosomes 11 and 17 resulted in a 50% and 90% inhibition of cell growth respectively, and reduced both colony efficiency and colony size. Telomerase activity was significantly reduced only by chromosome 17 insertion, providing a possible explanation for the more significant senescence observed in BP1E-17neo D100 cells. Microsatellite polymorphism analysis revealed that three loci, 11q13-23, 11q23.1, and 11q23.3 (markers D11S911, DRD2, and D11S29) were retained in BP1E-11neo #145 cells, and two, 17q24.2-25.2, 17q25.2 (markers D17S515 and D17S785 were retained in BP1E-17neo D100 cells. We conclude that the specific regions of normal chromosomes 11 and 17 transferred play a functional role in the expression of immortal and transformed phenotypes of HBEC in vitro.

Yang, Y. C., et al. (2009). "A tumorigenic homeobox (HOX) gene expressing human gastric cell line derived from putative gastric stem cell." Eur J Gastroenterol Hepatol **21**(9): 1016-1023.

GOAL: Study the mechanism of gastric tumor development. BACKGROUND: We have generated and characterized a novel human gastric cell line, KMU-CS12 (CS12), from an immortal cell line, KMU-CSN (CSN; formerly named as GI2CS) which was derived from putative human gastric stem cell/progenitor cell clone, KMU-GI2. STUDY: The characterization of the CS12 cell line includes gene

expression by immunocytochemical staining, cell proliferation and differentiation potential, cyotogenetic analysis by Giemsa banding and spectral karyotype analysis (SKY), and tumorigenicity in immune-deficient congenic inbred, nude mice (BALB/cAnN-Foxn1nu/CrlNarl). The Agilent Human 1A oligo-array and RT-PCR were also employed to analyze the expression of homeobox (HOX) genes. RESULTS: The CS12 gastric cell line showed cancer cell phenotypes, i.e. the ability of anchorage-independent growth high frequency (44%) and to the expression of Oct-4, a transcription factor expressed in embryonic stem cells and many types of cancer cells, and tumor development in immune deficient mice. SKY analysis indicated a characteristic duplication of the short arm of chromosome 7 to chromosome 12. Agilent Human 1A oligo-array analysis showed that the expression of 1145 genes was upregulated while that of 890 genes was downregulated in CS12 cells. RT-PCR revealed that homeobox genes (HOXA4, HOXA5, HOXA7, HOXA9, and HOXA13) were highly expressed in CS12 cells in culture, as well as tumor tissues developed by CS12 cells in immunodeficient mice for six to eight weeks. CONCLUSION: Except for the duplication of the short arm of Chromosome 7 on Chromosome12, the karyotype of the tumorigenic CS12 cells is similar to the parental GI2 cells which are non-tumorigenic and normal in karyotype. This chromosomal change could be the cause for the high expression of HOXA genes and tumorigenicity of these cells found in this study. Thus HOXA genes might play an important role in gastric carcinogenesis.

Yaniv, A., et al. (2011). "Establishment of immortal multipotent rat salivary progenitor cell line toward salivary gland regeneration." Tissue Eng Part C Methods **17**(1): 69-78.

Adult salivary gland stem cells are promising candidates for cell therapy and tissue regeneration in cases of irreversible damage to salivary glands in head and neck cancer patients undergoing irradiation therapy. At present, the major restriction in handling such cells is their relatively limited life span during in vitro cultivation, resulting in an inadequate experimental platform to explore the salivary gland-originated stem cells as candidates for future clinical application in therapy. We established a spontaneous immortal integrin alpha6beta1-expressing cell line of adult salivary progenitor cells from rats (rat salivary clone [RSC]) and investigated their ability to sustain cellular properties. This line was able to propagate for more than 400 doublings without loss of differentiation potential. RSC could differentiate in vitro to both acinar- and ductal-like structures and could be further manipulated upon culturing on a 3D scaffolds with different media supplements. Moreover, RSC expressed salivary-specific mRNAs and proteins as well as epithelial stem cell markers, and upon differentiation process their expression was changed. These results suggest RSC as a good model for further studies exploring cellular senescence, differentiation, and in vitro tissue engineering features as a crucial step toward reengineering irradiation-impaired salivary glands.

Yatabe, Y., et al. (2001). "Investigating stem cells in human colon by using methylation patterns." Proc Natl Acad Sci U S A **98**(19): 10839-10844.

The stem cells that maintain human colon crypts are poorly characterized. To better determine stem cell numbers and how they divide, epigenetic patterns were used as cell fate markers. Methylation exhibits somatic inheritance and random changes that potentially record lifelong stem cell division histories as binary strings or tags in adjacent CpG sites. Methylation tag contents of individual crypts were sampled with bisulfite sequencing at three presumably neutral loci. Methylation increased with aging but varied between crypts and was mosaic within single crypts. Some crypts appeared to be quasi-clonal as they contained more unique tags than expected if crypts were maintained by single immortal stem cells. The complex epigenetic patterns were more consistent with a crypt niche model wherein multiple stem cells were present and replaced through periodic symmetric divisions. Methylation tags provide evidence that normal human crypts are long-lived, accumulate random methylation errors, and contain multiple stem cells that go through "bottlenecks" during life.

Yennek, S. and S. Tajbakhsh (2013). "DNA asymmetry and cell fate regulation in stem cells." Semin Cell Dev Biol **24**(8-9): 627-642.

The semi-conservative nature of DNA replication has suggested that identical DNA molecules within chromatids are inherited by daughter cells after cell division. Numerous reports of non-random DNA segregation in prokaryotes and eukaryotes suggest that this is not always the case, and that epigenetic marks on chromatids, if not the individual DNA strands themselves, could have distinct signatures. Their selective distribution to daughter cells provides a novel mechanism for gene and cell fate regulation by segregating chromatids asymmetrically. Here we highlight some examples and potential mechanisms that can regulate this process. We propose that cellular asymmetry is inherently present during each cell division, and that it provides an opportunity during each cell cycle for moderating cell fates.

Yoon, J., et al. (2008). "dSETDB1 and SU(VAR)3-9 sequentially function during germline-stem cell differentiation in Drosophila melanogaster." PLoS One **3**(5): e2234.

Germline-stem cells (GSCs) produce gametes and are thus true "immortal stem cells". In Drosophila

ovaries, GSCs divide asymmetrically to produce daughter GSCs and cystoblasts, and the latter differentiate into germline cysts. Here we show that the histone-lysine methyltransferase dSETDB1, located in pericentric heterochromatin, catalyzes H3-K9 trimethylation in GSCs and their immediate descendants. As germline cysts differentiate into egg chambers, the dSETDB1 function is gradually taken over by another H3-K9-specific methyltransferase, SU(VAR)3-9. Loss-of-function mutations in dsetdb1 or Su(var)3-9 abolish both H3K9me3 and heterochromatin protein-1 (HP1) signals from the anterior germarium and the developing egg chambers, respectively, and cause localization of H3K9me3 away from DNA-dense regions in most posterior germarium cells. These results indicate that dSETDB1 and SU(VAR)3-9 act together with distinct roles during oogenesis, with dsetdb1 being of particular importance due to its GSC-specific function and more severe mutant phenotype.

Yoshida, K., et al. (1997). "Detection of telomerase activity in exfoliated cancer cells in colonic luminal washings and its related clinical implications." Br J Cancer **75**(4): 548-553.

Telomerase is a ribonucleoprotein capable of replacing telomeric DNA sequences that are lost at each cell division. Under normal circumstances, it is active in rapidly dividing embryonic cells and in stem cell populations but not in terminally differentiated somatic cells. Much attention has recently focused on the hypothesis that activity of this enzyme is necessary for cells to become immortal. This predicts that telomerase activity should be detectable in malignant cells and tissues but not in their normal counterparts, which slowly senesce and die. In accordance with this notion, telomerase activity has been reported in a wide range of malignancies, including those of the gastrointestinal tract, breast and lung. In the present study, we used a polymerase chain reaction (PCR)-based assay for telomerase activity, designated the "telomeric repeat amplification protocol (TRAP)', to examine initially 35 colonic carcinomas, their corresponding normal tissues and 12 inflammatory bowel disease (IBD) lesions. We detected strong enzyme activity in 32 (92%) of the 35 colon carcinomas while there was no activity in 30 (86%) of 35 matched normal colonic tissue specimens and only very weak activity in the remainder. Four of seven specimens of ulcerative colitis and two of five Crohn's disease lesions were negative, and the rest were only weakly positive. These results led us to examine whether telomerase could be detected in carcinoma cells exfoliated into the colonic lumen. We assayed lysates of exfoliated cells in luminal washings from colectomy specimens of 15 patients with colon carcinoma and nine with IBD. Telomerase activity was detected in washings from 9 (60%) of the 15 colon carcinoma cases but not in any from cases with IBD, suggesting that it can be a good marker for the detection of colon carcinoma, possibly even in non-invasively obtained samples.

Yoshimatsu, S., et al. (2021). "Establishment of an induced pluripotent stem cell line from a female domestic ferret (Mustela putorius furo) with an X chromosome instability." Stem Cell Res **53**: 102385.

The domestic ferret (ferret; Mustela putorius furo) is an important animal model for neuroscience and preclinical/veterinary medicine owing to its highly developed cerebral cortex and susceptibility to avian influenza and corona viruses. Nevertheless, there is a lack of in vitro ferret models, since immortal cell lines including induced pluripotent stem cells (iPSCs) of ferrets have been scarce. In this study, we established an iPSC line from ferret skin fibroblasts. The established iPSC line, fiPS-1, showed standard characteristics of pluripotency, but its X chromosome was unstable. Collectively, the present study provides a useful resource for in vitro model using the ferret.

Yu, P., et al. (2005). "[Intergration and epression of porcine endogenous retrovinus in the immortal cell line of Banna Minipig Inberd Line-Mesenhymal Stem Cells]." Sichuan Da Xue Xue Bao Yi Xue Ban **36**(6): 770-772.

OBJECTIVE: To detect the integration and expression of porcine endogenous retrovirus (PERV) in the immortal cell line of Banna Minipig Inbred Line-Mesenchymal Stem Cells (BMI-MSCs). METHODS: DNA and total RNA of the immortal cell line of BMI-MSCs were extracted and PCR, RT-PCR were performed to detect PERV-gag, pol and env gene, and the type of PERV was also detected. RESULTS: PERV-gag, pol and env gene were all detected in the primary culture and immortal cell line (passage 150 and passage 180) of BMI-MSCs, and the type of PERV was PERV-A, B. Functional expression of PERV-gag and pol mRNA was also detected. CONCLUSION: In this laboratory, PERV was not lost during the proceeding of pig inbred and since has been in long-term culture of pig cells in vitro. PERV has integrated into the genome of its natural host, and virus mRNA can effectively express. So it is very essential to evaluate the possibility of xenozoonoses in pig-to-human xenotransplantation.

Yuan, C. H., et al. (2003). "[Expansion of bone marrow LTC-ICs in vitro by mouse fetal liver-derived stromal cell lines]." Sheng Wu Gong Cheng Xue Bao **19**(4): 450-455.

As main component of fetal liver hematopoietic microenvironment, different stromal cells may play distinct roles in the regulation of hematopoietic stem cell self-renewal, proliferation and differentiation. It is a unique approach to establish

stromal cell lines for analyzing the interaction of hematopoietic cells with the stroma on the clonal level to dissect the function of hematopoietic microenvironment. In this study two immortal stromal cell lines-A4, B3 were established from mouse embryonic day 12.5 fetal liver by transfection of pSV3 neo plasmid. A4 exhibited a fibroblast-like morphology, 25 hours population doubling time as well as high levels of CD29, CD44, UEA-1 and low levels of CD105 expression. In contrast B3 displayed an epithelium-like morphology, 37 hours population doubling time along with high levels of CD105 and low levels of UEA-1 expression. In addition no or low levels of CD31, CD34, CD45 and CD144 expression were found in the two cell lines. These results indicate that A4, B3 are two discriminating cell lines in terms of morphological characters, growth behaviors and surface molecular expression types. Next functional assays using Limited-Diluted Assay(LDA) and Bulk-LTC-IC were done: both stromal cell lines had similar ability to maintain the survival proportion of inoculated mouse bone marrow-derived Long-Term Culture-Initiating Cells (LTC-ICs), and they could also support LTC-ICs expansion up to 4 weeks by co-culture in vitro. More strikingly, B3 could expand the absolute number of LTC-ICs over 13-fold at week 4 than that of week 0, and the ability of B3 to expand absolute number of LTC-ICs was over 8-fold of that of A4. Proportions of LTC-ICs in proliferating cell populations in two long-term culture systems was similar at week 4, no matter with or without extra cytokines. Further study indicated that the ability of LTC-ICs to yield CFCs was held as the number 6(+/- 1.2) after 4 weeks co-culture. Extra cytokines-SCF + IL-3 + IL-6 + Epo had no influence in the maintenance and expansion of LTC-ICs, but expanded the absolute number of CFCs and proliferating cell populations, and maintained similar proportions of LTC-ICs and CFCs in the end in two culture systems at week 4. Take together, these results implicate that B3 may act as an important functional component in embryonic day 12.5 fetal liver microenvironment to effectively expand primitive hematopoietic cells, yield more committed hematopoietic progenitor cells and mature hematopoietic cells to meet the need of quick development especially in the early phase of embryonic development.

Yun, E. J., et al. (2016). "The evolving landscape of prostate cancer stem cell: Therapeutic implications and future challenges." Asian J Urol **3**(4): 203-210.

Prostate cancer (PCa) is the most common cause of malignancy in males and the second leading cause of cancer mortality in United States. Current treatments for PCa include surgery, radiotherapy, and androgen-deprivation therapy. Eventually, PCa relapses to an advanced castration-resistant PCa (CRPC) that becomes a systematic disease and incurable. Therefore, identifying cellular components and molecular mechanisms that drive aggressive PCa at early stage is critical for disease prognosis and therapeutic intervention. One potential strategy for aggressive PCa is to target cancer stem cells (CSCs) that are identified by several unique characteristics such as immortal, self-renewal, and pluripotency. Also, CSC is believed to be a major factor contributing to resistance to radiotherapy and conventional chemotherapies. Moreover, CSCs are thought to be the critical cause of metastasis, tumor recurrence and cancer-related death of multiple cancer types, including PCa. In this review, we discuss recent progress made in understanding prostate cancer stem cells (PCSCs). We focus on the therapeutic strategies aimed at targeting specific surface markers of CSCs, the key signaling pathways in the maintenance of self-renewal capacity of CSCs, ATP-binding cassette (ABC) transporters that mediate the drug-resistance of CSCs, dysregulated microRNAs expression profiles in CSCs, and immunotherapeutic strategies developed against PCSCs surface markers.

Zajicek, G. (1985). "Congenital neoplasia--a stem cell pathology." Med Hypotheses **16**(3): 303-313.

The theory presented herewith provides a unified explanation for neoplasia and teratogenesis, both regarded here as stem cell disorders. Proliferating cells in the organism form two mutually exclusive classes, immortal stem cells and transitional cells whose life span is limited. Any lasting change in the organism e.g. tissue differentiation or neoplasia, is determined by stem cells. Congenital as well as adult neoplasms exhibit three key features: maturation arrest, stem cell pool expansion and increased cell turnover and they progress through the following states: dysplasia, neoplasia in situ, benign stage (e.g. polyp) and overt neoplasia. The neoplasm is regarded here as an organ with a purpose intended to supply the organism with a missing substance. Besides serving as tissue progenitors, stem cells are postulated here to secrete a substance 'A' necessary for a proper tissue function. Carcinogens interfere with the substance production mainly by depleting stem cells so that less 'A' is produced. The capacity of the adult to replenish the depleted stem cells is limited, and the missing substance has to be formed by an alternative way i.e. by Neoplasia. The 'A' substitute formed by the neoplasm denominated as 'B' is however less efficient than 'A'. Neoplastic growth thus depends upon the relative abundance of substances 'A' and 'B'. Since in the growing organism, stem cells multiply, some of the missing 'A' is replenished by them and the neoplasm may regress.

Zarate-Garcia, L., et al. (2016). "FACS-sorted putative oogonial stem cells from the ovary are neither DDX4-positive nor germ cells." Sci Rep **6**: 27991.

Whether the adult mammalian ovary contains oogonial stem cells (OSCs) is controversial. They have been isolated by a live-cell sorting method using the germ cell marker DDX4, which has previously been assumed to be cytoplasmic, not surface-bound. Furthermore their stem cell and germ cell characteristics remain disputed. Here we show that although OSC-like cells can be isolated from the ovary using an antibody to DDX4, there is no good in silico modelling to support the existence of a surface-bound DDX4. Furthermore these cells when isolated were not expressing DDX4, and did not initially possess germline identity. Despite these unremarkable beginnings, they acquired some pre-meiotic markers in culture, including DDX4, but critically never expressed oocyte-specific markers, and furthermore were not immortal but died after a few months. Our results suggest that freshly isolated OSCs are not germ stem cells, and are not being isolated by their DDX4 expression. However it may be that culture induces some pre-meiotic markers. In summary the present study offers weight to the dogma that the adult ovary is populated by a fixed number of oocytes and that adult de novo production is a rare or insignificant event.

Zeps, N. and C. Hemmings (2011). "Chasing the immortal strand: evidence for nature's way of protecting the breast genome." Breast Cancer Res **13**(1): 101.

Mutations arise during cell division at a predictable rate. Besides DNA repair mechanisms, the existence of cellular hierarchies that originate with a stem cell serve to reduce the number of divisions necessary for normal physiology. In a previous issue, Bussard and colleagues demonstrate that mammary stem cells have an additional remarkable trait; namely the ability to selectively retain a template DNA strand during self renewal. In doing so, they avoid the accumulation of mutations in that so called 'immortal strand'. The implications of this are discussed with reference to the development and treatment of cancer.

Zhang, J. and Z. Ju (2010). "Telomere, DNA damage, and oxidative stress in stem cell aging." Birth Defects Res C Embryo Today **90**(4): 297-307.

"Stem cell aging" is a novel concept that developed together with the advances of stem cell biology, especially the sophisticated prospectively isolation and characterization of multipotent somatic tissue stem cells. Although being immortal in principle, stem cells can also undergo aging processes and potentially contribute to organismal aging. The impact of an age-dependent decline of stem cell function weighs differently in organs with high or low rates of cell turnover. Nonetheless, most of the organ systems undergo age-dependent loss of homeostasis and functionality, and emerging evidence showed that this has to do with the aging of resident stem cells in the organ systems. The mechanisms of stem cell aging and its real contribution to human aging remain to be defined. Many antitumor mechanisms protect potential malignant transformation of stem cell by inducing apoptosis or senescence but simultaneously provoke stem cell aging. In this review, we try to discuss several concept of stem cell aging and summarize recent progression on the molecular mechanisms of stem cell aging.

Zhang, X., et al. (2006). "Successful immortalization of mesenchymal progenitor cells derived from human placenta and the differentiation abilities of immortalized cells." Biochem Biophys Res Commun **351**(4): 853-859.

We reported previously that mesenchymal progenitor cells derived from chorionic villi of the human placenta could differentiate into osteoblasts, adipocytes, and chondrocytes under proper induction conditions and that these cells should be useful for allogeneic regenerative medicine, including cartilage tissue engineering. However, similar to human mesenchymal stem cells (hMSCs), though these placental cells can be isolated easily, they are difficult to study in detail because of their limited life span in vitro. To overcome this problem, we attempted to prolong the life span of human placenta-derived mesenchymal cells (hPDMCs) by modifying hTERT and Bmi-1, and investigated whether these modified hPDMCs retained their differentiation capability and multipotency. Our results indicated that the combination of hTERT and Bmi-1 was highly efficient in prolonging the life span of hPDMCs with differentiation capability to osteogenic, adipogenic, and chondrogenic cells in vitro. Clonal cell lines with directional differentiation ability were established from the immortalized parental hPDMC/hTERT+Bmi-1. Interestingly, hPDMC/Bmi-1 showed extended proliferation after long-term growth arrest and telomerase was activated in the immortal hPDMC/Bmi-1 cells. However, the differentiation potential was lost in these cells. This study reports a method to extend the life span of hPDMCs with hTERT and Bmi-1 that should become a useful tool for the study of mesenchymal stem cells.

Zhao, X., et al. (2011). "A block in lineage differentiation of immortal human mammary stem / progenitor cells by ectopically-expressed oncogenes." J Carcinog **10**: 39.

INTRODUCTION: Emerging evidence suggests a direct role of cancer stem cells (CSCs) in the development of breast cancer. In vitro cellular models that recapitulate properties of CSCs are therefore highly desirable. We have previously shown that normal human mammary epithelial cells (hMECs) immortalized with human telomerase reverse transcriptase (hTERT) possess properties of mammary

stem / progenitor cells. MATERIALS AND METHODS: In the present study, we used this cell system to test the idea that other known hMEC-immortalizing oncogenes (RhoA, HPVE6, HPVE7, p53 mutant, and treatment with gamma-radiation), share with hTERT, the ability to maintain mammary stem / progenitor cells. RESULTS: The results presented here demonstrate that similar to hMECs immortalized with hTERT, all hMEC cell lines immortalized using various oncogenic strategies express stem / progenitor cell markers. Furthermore, analyses using 2D and 3D culture assays demonstrate that all the immortal cell lines retain their ability to self-renew and to differentiate along the luminal lineage. Remarkably, the stem / progenitor cell lines generated using various oncogenic strategies exhibit a block in differentiation along the myoepithelial lineage, a trait that is retained on hTERT-immortalized stem / progenitors. The inability to differentiate along the myoepithelial lineage could be induced by ectopic mutant p53 expression in hTERT-immortalized hMEC. CONCLUSIONS: Our studies demonstrate that stem / progenitor cell characteristics of hMECs are maintained upon immortalization by using various cancer-relevant oncogenic strategies. Oncogene-immortalized hMECs show a block in their ability to differentiate along the myoepithelial lineage.

Zhao, X., et al. (2012). "Derivation of myoepithelial progenitor cells from bipotent mammary stem/progenitor cells." PLoS One **7**(4): e35338.

There is increasing evidence that breast and other cancers originate from and are maintained by a small fraction of stem/progenitor cells with self-renewal properties. Recent molecular profiling has identified six major subtypes of breast cancer: basal-like, ErbB2-overexpressing, normal breast epithelial-like, luminal A and B, and claudin-low subtypes. To help understand the relationship among mammary stem/progenitor cells and breast cancer subtypes, we have recently derived distinct hTERT-immortalized human mammary stem/progenitor cell lines: a K5(+)/K19(-) type, and a K5(+)/K19(+) type. Under specific culture conditions, bipotent K5(+)/K19(-) stem/progenitor cells differentiated into stable clonal populations that were K5(-)/K19(-) and exhibit self-renewal and unipotent myoepithelial differentiation potential in contrast to the parental K5(+)/K19(-) cells which are bipotent. These K5(-)/K19(-) cells function as myoepithelial progenitor cells and constitutively express markers of an epithelial to mesenchymal transition (EMT) and show high invasive and migratory abilities. In addition, these cells express a microarray signature of claudin-low breast cancers. The EMT characteristics of an un-transformed unipotent mammary myoepithelial progenitor cells together with claudin-low signature suggests that the claudin-low breast cancer subtype may arise from myoepithelial lineage committed progenitors. Availability of immortal MPCs should allow a more definitive analysis of their potential to give rise to claudin-low breast cancer subtype and facilitate biological and molecular/biochemical studies of this disease.

Zhao, X., et al. (2010). "Telomerase-immortalized human mammary stem/progenitor cells with ability to self-renew and differentiate." Proc Natl Acad Sci U S A **107**(32): 14146-14151.

There is increasing evidence that breast and other cancers originate from and are maintained by a small fraction of stem/progenitor cells with self-renewal properties. Whether such cancer stem/progenitor cells originate from normal stem cells based on initiation of a de novo stem cell program, by reprogramming of a more differentiated cell type by oncogenic insults, or both remains unresolved. A major hurdle in addressing these issues is lack of immortal human stem/progenitor cells that can be deliberately manipulated in vitro. We present evidence that normal and human telomerase reverse transcriptase (hTERT)-immortalized human mammary epithelial cells (hMECs) isolated and maintained in Dana-Farber Cancer Institute 1 (DFCI-1) medium retain a fraction with progenitor cell properties. These cells coexpress basal (K5, K14, and vimentin), luminal (E-cadherin, K8, K18, or K19), and stem/progenitor (CD49f, CD29, CD44, and p63) cell markers. Clonal derivatives of progenitors coexpressing these markers fall into two distinct types--a K5(+)/K19(-) type and a K5(+)/K19(+) type. We show that both types of progenitor cells have self-renewal and differentiation ability. Microarray analyses confirmed the differential expression of components of stem/progenitor-associated pathways, such as Notch, Wnt, Hedgehog, and LIF, in progenitor cells compared with differentiated cells. Given the emerging evidence that stem/progenitor cells serve as precursors for cancers, these cellular reagents represent a timely and invaluable resource to explore unresolved questions related to stem/progenitor origin of breast cancer.

Zhao, Y., et al. (2009). "[Differentiation of bovine male germ-line stem cells in vitro]." Sheng Wu Gong Cheng Xue Bao **25**(2): 287-291.

Male germ-line stem cells (mGSCs) have the capability of self-renewal and latent capability of differentiation. mGSCs is the unique diploid immortal cell which can transfer genetic information to filial generation. The combination of transgenic technology and mGSCs heterotransplanting will supply new opportunities and paths to cloning animal, transgenic animal and gene therapy of some human hereditary disease. We studied the isolation and cultivation of mGSCs that were isolated and purified from 5-6 month

old bovine fetal testis, new born bovine testis by adopting mixed enzymes digestion and different attaching velocities methods. The results showed that Sertoli cells were indispensable to mGSC's proliferation and differentiation in vitro. The Sertoli cells in logarithmic phase have a significant effect on mGSC's attaching, proliferation and differentiation. Co-culture with Sertoli cells, mGSCs differentiated to long sperm after 16 days. A preliminary system for mGSC's inducing differentiation was established.

Zhou, Q. J., et al. (2005). "[Advances in the research of differentiation of embryonic stem cells into hepatocytes]." Sheng Wu Gong Cheng Xue Bao **21**(2): 171-176.

Orthotopic liver transplantation has proven to be effective in the treatment of a variety of life-threatening liver diseases, however, the limitations of donated organs available and long-term immunosuppression provided an impetus for developing alternative therapies. Cell replacement strategies have been one major effective approach for overcoming the obstacles of organ transplantation in recent years. The exogenous cells should be able to proliferate and differentiate into mature hepatic cells after grafting. Use of mature hepatocytes is also hampered by limited tissue source and inability to proliferate and maintain the function for a long term in vitro. Embryonic stem cells are immortal and pluripotent and may provide a novel cell source for potential cell therapy. This review summarizes the mechanisms of controlling early liver development and hepatic differentiation of visceral endoderm in embryoid bodies, and provides an overview of diverse differentiation systems in vitro and in vivo that were applied to hepatic research in recent years. Several studies have demonstrated that ES cell-derived hepatocytes can incorporate into liver tissue and function in vivo , but a few of them have shown complete restoration of liver function after transplantation into mice with liver diseases. Further studies should be made to exploit efficient methods and clinical applications of hepatocytes derived from ES cells in the future. In addition to clinical transplantation for treatment of liver diseases, ES cells can provide a valuable tool for drug discovery applications and study on of molecular basis of hepatic differentiation.

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References

1. Baidu. http://www.baidu.com. 2021.
2. Cancer Biology. http://www.cancerbio.net. 2021.
3. Google. http://www.google.com. 2021.
4. Journal of American Science. http://www.jofamericanscience.org. 2021.
5. Life Science Journal. http://www.lifesciencesite.com. 2021.
6. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92. doi:10.7537/marsjas010205.14. http://www.jofamericanscience.org/journals/am-sci/0102/14-mahongbao.pdf.
7. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96. doi:10.7537/marsnsj050107.10. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
8. Ma H, Cherng S. Nature of Life. Life Science Journal 2005;2(1):7-15. doi:10.7537/marslsj020105.03. http://www.lifesciencesite.com/lsj/life0201/life-0201-03.pdf.
9. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. doi:10.7537/marsnsj080210.03. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.
10. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11. doi:10.7537/marsnsj010103.01. http://www.sciencepub.net/nature/0101/01-ma.pdf.
11. Marsland Press. http://www.sciencepub.net. 2021; http://www.sciencepub.org. 2021.
12. National Center for Biotechnology Information, U.S. National Library of Medicine. http://www.ncbi.nlm.nih.gov/pubmed. 2021.
13. Nature and Science. http://www.sciencepub.net/nature. 2021.
14. Stem Cell. http://www.sciencepub.net/stem. 2021.
15. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2021.

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