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.
[Mark H. **Stem Cell and Immortality Research Literatures**. 2024;15(4):102-153] ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 2. doi:10.7537/marsscj150324.02

Key words: stem cell; technology; 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 above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing. The following introduces recent reports as references in the related studies.

Ambrosi, D. J., et al. (2007). "Genome-wide reprogramming in hybrids of somatic cells and embryonic stem cells." Stem Cells **25**(5): 1104-1113.

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

Amit, M., et al. (2003). "Human feeder layers for human embryonic stem cells." Biol Reprod **68**(6): 2150-2156.

Human embryonic stem (hES) cells hold great promise for future use in various research areas, such as human developmental biology and cell-based therapies. Traditionally, these cells have been cultured on mouse embryonic fibroblast (MEF) feeder layers, which permit continuous growth in an undifferentiated stage. To use these unique cells in human therapy, an animal-free culture system must be used, which will prevent exposure to mouse retroviruses. Animal-free culture systems for hES cells enjoy three major

advantages in the basic culture conditions: 1). the ability to grow these cells under serum-free conditions, 2). maintenance of the cells in an undifferentiated state on Matrigel matrix with 100% MEF-conditioned medium, and 3). the use of either human embryonic fibroblasts or adult fallopian tube epithelial cells as feeder layers. In the present study, we describe an additional animal-free culture system for hES cells, based on a feeder layer derived from foreskin and a serum-free medium. In this culture condition, hES cells maintain all embryonic stem cell features (i.e., pluripotency, immortality, unlimited undifferentiated proliferation capability, and maintenance of normal karyotypes) after prolonged culture of 70 passages (>250 doublings). The major advantage of foreskin feeders is their ability to be continuously cultured for more than 42 passages, thus enabling proper analysis for foreign agents, genetic modification such as antibiotic resistance, and reduction of the enormous workload involved in the continuous preparation of new feeder lines.

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.

Ashkenazi, R., et al. (2008). "Pathways to tumorigenesis--modeling mutation acquisition in stem cells and their progeny." Neoplasia **10**(11): 1170-1182.

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

Beeraka, N. M., et al. (2020). "The Role of Exosomes in Stemness and Neurodegenerative Diseases-Chemoresistant-Cancer Therapeutics and Phytochemicals." Int J Mol Sci **21**(18).

Exosomes exhibit a wide range of biological properties and functions in the living organisms. They are nanometric vehicles and used for delivering drugs, as they are biocompatible and minimally immunogenic. Exosomal secretions derived from cancer cells contribute to metastasis, immortality, angiogenesis, tissue invasion, stemness and chemo/radio-resistance. Exosome-derived microRNAs (miRNAs) and long non-coding RNAs (lnc RNAs) are involved in the pathophysiology of cancers and neurodegenerative diseases. For instance, exosomes derived from mesenchymal stromal cells, astrocytes, macrophages, and acute myeloid leukemia (AML) cells are involved in the cancer progression and stemness as they induce chemotherapeutic drug resistance in several cancer cells. This review covered the recent research advances in understanding the role of exosomes in cancer progression, metastasis,

angiogenesis, stemness and drug resistance by illustrating the modulatory effects of exosomal cargo (ex. miRNA, lncRNAs, etc.) on cell signaling pathways involved in cancer progression and cancer stem cell growth and development. Recent reports have implicated exosomes even in the treatment of several cancers. For instance, exosomes-loaded with novel anti-cancer drugs such as phytochemicals, tumor-targeting proteins, anticancer peptides, nucleic acids are known to interfere with drug resistance pathways in several cancer cell lines. In addition, this review depicted the need to develop exosome-based novel diagnostic biomarkers for early detection of cancers and neurodegenerative disease. Furthermore, the role of exosomes in stroke and oxidative stress-mediated neurodegenerative diseases including Alzheimer's disease (AD), and Parkinson's disease (PD) is also discussed in this article.

Bell, R. J., et al. (2016). "Understanding TERT Promoter Mutations: A Common Path to Immortality." Mol Cancer Res **14**(4): 315-323.

Telomerase (TERT) activation is a fundamental step in tumorigenesis. By maintaining telomere length, telomerase relieves a main barrier on cellular lifespan, enabling limitless proliferation driven by oncogenes. The recently discovered, highly recurrent mutations in the promoter of TERT are found in over 50 cancer types, and are the most common mutation in many cancers. Transcriptional activation of TERT, via promoter mutation or other mechanisms, is the rate-limiting step in production of active telomerase. Although TERT is expressed in stem cells, it is naturally silenced upon differentiation. Thus, the presence of TERT promoter mutations may shed light on whether a particular tumor arose from a stem cell or more differentiated cell type. It is becoming clear that TERT mutations occur early during cellular transformation, and activate the TERT promoter by recruiting transcription factors that do not normally regulate TERT gene expression. This review highlights the fundamental and widespread role of TERT promoter mutations in tumorigenesis, including recent progress on their mechanism of transcriptional activation. These somatic promoter mutations, along with germline variation in the TERT locus also appear to have significant value as biomarkers of patient outcome. Understanding the precise molecular mechanism of TERT activation by promoter mutation and germline variation may inspire novel cancer cell-specific targeted therapies for a large number of cancer patients.

Bernardi, R. and P. P. Pandolfi (2003). "The nucleolus: at the stem of immortality." Nat Med **9**(1): 24-25.

Bhartiya, D., et al. (2023). "Very Small Embryonic-Like Stem Cells Transform Into Cancer Stem Cells and Are Novel Candidates for Detecting/Monitoring Cancer by a Simple Blood Test." Stem Cells **41**(4): 310-318.

Cancer continues to remain a "Black Box," as there is no consensus on how it initiates, progresses, metastasizes, or recurs. Many imponderables exist about whether somatic mutations initiate cancer, do cancer stem cells (CSCs) exist, and if yes, are they a result of de-differentiation or originate from tissue-resident stem cells; why do cancer cells express embryonic markers, and what leads to metastasis and recurrence. Currently, the detection of multiple solid cancers through liquid biopsy is based on circulating tumor cells (CTCs) or clusters, or circulating tumor DNA (ctDNA). However, quantity of starting material is usually adequate only when the tumor has grown beyond a certain size. We posit that pluripotent, endogenous, tissue-resident, very small embryonic-like stem cells (VSELs) that exist in small numbers in all adult tissues, exit from their quiescent state due to epigenetic changes in response to various insults and transform into CSCs to initiate cancer. VSELs and CSCs share properties like quiescence, pluripotency, self-renewal, immortality, plasticity, enrichment in side-population, mobilization, and resistance to oncotherapy. HrC test, developed by Epigeneres, offers the potential for early detection of cancer using a common set of VSEL/CSC specific bio-markers in peripheral blood. In addition, NGS studies on VSELs/CSCs/tissue-specific progenitors using the All Organ Biopsy (AOB) test provide exomic and transcriptomic information regarding impacted organ(s), cancer type/subtype, germline/somatic mutations, altered gene expressions, and dysregulated pathways. To conclude, HrC and AOB tests can confirm the absence of cancer and categorize the rest of subjects into low/moderate/high risk of cancer, and also monitor response to therapy, remission, and recurrence.

Blagosklonny, M. V. (2006). "Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition." Cell Cycle **5**(18): 2087-2102.

While ruling out programmed aging, evolutionary theory predicts a quasi-program for aging, a continuation of the developmental program that is not turned off, is constantly on, becoming hyper-functional and damaging, causing diseases of aging. Could it be switched off pharmacologically? This would require identification of a molecular target involved in cell senescence, organism aging and diseases of aging. Notably, cell senescence is associated with activation of the TOR (target of rapamycin) nutrient- and mitogen-sensing pathway,

which promotes cell growth, even though cell cycle is blocked. Is TOR involved in organism aging? In fact, in yeast (where the cell is the organism), caloric restriction, rapamycin and mutations that inhibit TOR all slow down aging. In animals from worms to mammals caloric restrictions, life-extending agents, and numerous mutations that increase longevity all converge on the TOR pathway. And, in humans, cell hypertrophy, hyper-function and hyperplasia, typically associated with activation of TOR, contribute to diseases of aging. Theoretical and clinical considerations suggest that rapamycin may be effective against atherosclerosis, hypertension and hyper-coagulation (thus, preventing myocardial infarction and stroke), osteoporosis, cancer, autoimmune diseases and arthritis, obesity, diabetes, macula-degeneration, Alzheimer's and Parkinson's diseases. Finally, I discuss that extended life span will reveal new causes for aging (e.g., ROS, 'wear and tear', Hayflick limit, stem cell exhaustion) that play a limited role now, when quasi-programmed senescence kills us first.

Blagosklonny, M. V. (2007). "Cancer stem cell and cancer stemloids: from biology to therapy." Cancer Biol Ther **6**(11): 1684-1690.

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

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.

Bonuccelli, G., et al. (2017). "Targeting cancer stem cell propagation with palbociclib, a CDK4/6 inhibitor: Telomerase drives tumor cell heterogeneity." Oncotarget **8**(6): 9868-9884.

In this report, we systematically examined the role of telomerase activity in lung and ovarian cancer stem cell (CSC) propagation. For this purpose, we indirectly gauged telomerase activity, by linking the hTERT-promoter to eGFP. Using lung (A549) and ovarian (SKOV3) cancer cells, transduced with the hTERT-GFP reporter, we then employed GFP-expression levels to fractionate these cell lines into GFP-high and GFP-low populations. We functionally compared the phenotype of these GFP-high and GFP-low populations. More specifically, we now show that the cancer cells with higher telomerase activity (GFP-high) are more energetically activated, with increased mitochondrial mass and function, as well as increased glycolytic activity. This was further validated and confirmed by unbiased proteomics analysis. Cells with high telomerase activity also showed an increased capacity for stem cell activity (as measured using the 3D-spheroid assay) and cell migration (as measured using a Boyden chamber approach). These enhanced biological phenotypes were effectively inhibited by classical modulators of energy metabolism, which target either i) mitochondrial metabolism (i.e., oligomycin) or ii) glycolysis (i.e., 2-deoxy-glucose), or iii) by using the FDA-approved antibiotic doxycycline, which inhibits mitochondrial biogenesis.

Finally, the level of telomerase activity also determined the ability of hTERT-high cells to proliferate, as assessed by measuring DNA synthesis via EdU incorporation. Consistent with these observations, treatment with an FDA-approved CDK4/6 inhibitor (PD-0332991/palbociclib) specifically blocked the propagation of both lung and ovarian CSCs. Virtually identical results were obtained with breast CSCs, which were also highly sensitive to palbociclib at concentrations in the nanomolar range. In summary, CSCs with high telomerase activity are among the most energetically activated, migratory and proliferative cell sub-populations. These observations may provide a mechanistic explanation for tumor metabolic heterogeneity, based on telomerase activity. FDA-approved drugs, such as doxycycline and palbociclib, were both effective at curtailing CSC propagation. Thus, these FDA-approved drugs could be used to target telomerase-high proliferative CSCs, in multiple cancer types. Finally, our experiments also allowed us to distinguish two different cellular populations of hTERT-high cells, one that was proliferative (i.e., replicative immortality) and the other that was non-proliferative (i.e., quiescent). We speculate that the non-proliferative population of hTERT-high cells that we identified could be mechanistically involved in tumor dormancy.

Borges, R. M. (2009). "Phenotypic plasticity and longevity in plants and animals: cause and effect?" J Biosci **34**(4): 605-611.

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

Bosch, T. C. (2009). "Hydra and the evolution of stem cells." Bioessays **31**(4): 478-486.

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

Bosch, T. C., et al. (2010). "The Hydra polyp: nothing but an active stem cell community." Dev Growth Differ **52**(1): 15-25.

Hydra is a powerful stem cell model because its potential immortality and extensive regeneration capacity is due to the presence of three distinct stem cell lineages. All three lineages conform to a well-defined spatial distribution across the whole body column of the polyp. Stem cell function in Hydra is controlled by extracellular cues and intrinsic genetic programs. This review focuses on the elusive stem cell niche of the epithelial layers. Based on a comparison of the differences between, and commonalities among, stem cells and stem cell niches in Hydra and other invertebrates and vertebrates, we propose that the whole body column of the polyp may be considered a stem cell "niche" in which stem cell populations are established and signals ensuring the proper balance between stem cells and progenitor cells are integrated. We show that, at over 500 million years old, Hydra offers an early glimpse of the regulatory potential of stem cell niches.

Brown, D. (2009). "The energy body and its functions: immunosurveillance, longevity, and regeneration." Ann N Y Acad Sci **1172**: 312-337.

There are three interrelated levels of a macromolecular energy-information relay system in the human body, each generated by a specific type of semiconductant tissue and each with a specific function. The surface layer of the energy body, generated by fluid connective tissue and known as the ordinary channel system or meridian system in traditional Chinese medicine (TCM), functions in the service of immunosurveillance through detection of distress signals and transmitting energy-information regarding immunoresponse. The middle layer of the energy body, generated by semiconductant hard and spongy bone tissue, known as the extraordinary channel system in TCM, functions in the service of longevity and regeneration, as described in

Bodhidharma's classic, Bone Marrow Washing. The bone marrow energy-information system has direct relevance to modern stem-cell research on the role of stem cells in regeneration of injured tissue. The deepest layer of the energy body generated by semiconductant nervous system tissue notably the vagus nerve and spinal column, functions in the service of awakening consciousness and in immortality. This system is described in the Tibetan Inner Fire meditations as well as in the Taoist shen breathing practices. There is very little scientific understanding of the central channel system.

Carruba, G. and J. E. Trosko (2017). "The Long Evolutionary Journey of Cancer from Ancestor to Modern Humans." Crit Rev Oncog **22**(3-4): 323-352.

In this article, we review various key issues in cancer development and progression that have important implications for both cancer prevention and treatment: (1) evolutionary aspects of cancer appearance; (2) evidence of organ-specific adult stem cells as cancer-initiating cells; (3) the immortality of cancer-initiating cells; (4) cancer cell loss of growth control, contact inhibition, terminal differentiation, and apoptosis; (5) stem-cell versus de-differentiation theory of carcinogenesis; (6) mutations in cancer; (7) oncogenes and tumor suppressor genes; (8) epigenetics as the rate-limiting step in carcinogenesis; (9) the potential role of cultural, lifestyle, and nutritional behaviors in oncology; and (10) changes of commensal microbial community and its metagenome in carcinogenesis and tumor progression. Relevant, combined evidence is discussed from a standpoint whereby cancer is considered a multifaceted disease requiring integrated biomolecular and clinico-pathological information to design and implement strategies for either primary prevention or therapy.

Chandhasin, C., et al. (2023). "TACH101, a first-in-class pan-inhibitor of KDM4 histone demethylase." Anticancer Drugs **34**(10): 1122-1131.

Histone lysine demethylase 4 (KDM4) is an epigenetic regulator that facilitates the transition between transcriptionally silent and active chromatin states by catalyzing the removal of methyl groups on histones H3K9, H3K36, and H1.4K26. KDM4 overamplification or dysregulation has been reported in various cancers and has been shown to drive key processes linked to tumorigenesis, such as replicative immortality, evasion of apoptosis, metastasis, DNA repair deficiency, and genomic instability. KDM4 also plays a role in epigenetic regulation of cancer stem cell renewal and has been linked to more aggressive disease and poorer clinical outcomes. The KDM4 family is composed of four main isoforms (KDM4A-D) that demonstrate functional redundancy and cross-activity; thus, selective inhibition of one isoform appears to be ineffective and pan-inhibition targeting multiple KDM4 isoforms is required. Here, we describe TACH101, a novel, small-molecule pan-inhibitor of KDM4 that selectively targets KDM4A-D with no effect on other KDM families. TACH101 demonstrated potent antiproliferative activity in cancer cell lines and organoid models derived from various histologies, including colorectal, esophageal, gastric, breast, pancreatic, and hematological malignancies. In vivo , potent inhibition of KDM4 led to efficient tumor growth inhibition and regression in several xenograft models. A reduction in the population of tumor-initiating cells was observed following TACH101 treatment. Overall, these observations demonstrate the broad applicability of TACH101 as a potential anticancer agent and support its advancement into clinical trials.

Chao, T. H., et al. (2014). "Pluripotent Stem Cell Therapy in Ischemic Cardiovascular Disease." Acta Cardiol Sin **30**(5): 365-374.

Stem cell therapy has been viewed as a promising therapeutic strategy in ischemic cardiovascular disease for almost a decade. Although many progenitor/stem cells obtained from patients have been investigated, and are alleged to be suitable for autologous transplantation, their therapeutic application has been limited by their inability to yield a sufficient number of stem cells, as well as impaired regeneration capacity from ageing and cardiovascular risk factors. Pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have the capacity for functional multi-lineage differentiation and properties of self-renewal and immortality, and can generate clinically relevant amounts of stem cells. The regeneration capacity of these cells is not affected by ageing. Patient-specific pluripotent stem cells, iPSCs, can be established by epigenetically reprogramming somatic fibroblasts. iPSCs and iPSC-derived stem cells share similar phenotypes and gene expressions of ESCs and ESC-derived stem cells. Transplantation of pluripotent stem cell-derived endothelial cells, mural cells, cardiomyocytes, or cardiovascular progenitor cells contribute to neovascularization and cardiomyogenesis with better limb perfusion and recovery of myocardial contractility in the preclinical studies. Several strategies have been developed to enhance the efficacy of reprogramming and engrafting, and improve graft survival, proliferation, and electromechanical coupling by tissue engineering. However, the therapeutic application of ESCs and derivatives is limited by ethical concerns. Before wide clinical application of these cells in regeneration therapy occurs, substantial effort should be undertaken

to discover the most promising cell type and derivatives, the best protocol regarding cell preparation, reprogramming and differentiation, and the most efficacious methods to avoid adverse effects. KEY WORDS: Embryonic stem cells; Induced pluripotent stem cells; Limb ischemia; Myocardial infarction.

Chen, M., et al. (2010). "Emerging role of the MORF/MRG gene family in various biological processes, including aging." Ann N Y Acad Sci **1197**: 134-141.

Cellular senescence is the dominant phenotype over immortality. In our studies to identify senescence-related genes, we cloned Morf4, which induced senescence in a subset of tumor cells. Morf4 is a member of a family of seven genes, and Morf-related genes (Mrg) on chromosomes 15 (Mrg15) and X (MrgX) are also expressed. In contrast to MORF4, MRG15 and MRGX are positive regulators of cell division. All three proteins interact with histone deacetylases and acetyltransferases, suggesting that they function in regulation of chromatin dynamics. Mrg15 knockout mice are embryonic lethal, and mouse embryonic fibroblasts derived from Mrg15 null embryos proliferate poorly, enter senescence rapidly, and have impaired DNA repair compared to the wild type. Mrg15 null embryonic neural stem and progenitor cells also have a decreased capacity for proliferation and differentiation. Further studies are needed to determine the function of this gene family in various biological processes, including neural stem and progenitor cell aging.

Chen, Y. M., et al. (2013). "[Host glial cell canceration induced by glioma stem cells in GFP/RFP dual fluorescence orthotopic glioma models in nude mice]." Zhonghua Zhong Liu Za Zhi **35**(1): 5-10.

OBJECTIVE: During the process of tissue remodeling in human tumor transplantation models, the roles of the inoculated tumor cells and host tissue in tumor progression is still largely unknown. The aim of this study was to investigate the relationships and interactions between these two sides using GFP-RFP double fluorescence tracing technique. METHODS: Red fluorescence protein (RFP) gene was stably transfected into glioma stem cell line SU3, then SU3-RFP cells were transplanted into the brain of athymic nude mice with green fluorescence protein (GFP) expression. After the intracerebral tumors were formed, the relationship and interaction between GFP cells and RFP cells were analyzed. Highly proliferative GFP cells were screened out, and monocloned with micro-pipetting. DNA content assay, chromosome banding and carcinogenicity test of the GFP cells were performed to observe the GFP cells' cancerous phenotype in nude mice. RESULTS: In the transplantable tumor tissue, besides a great quantity of RFP cells, there were still a proportion of GFP cells and GFP/RFP fusion cells. The proportion of RFP cells, GFP cells and GFP/RFP cells were (88.99 +/- 1.46)%, (5.59 +/- 1.00)%, and (4.11 +/- 1.020)%, respectively. Two monoclonal host GFP cells (H1 and H9) were cloned, which demonstrated the properties of immortality, loss of contact inhibition, and ultra-tetraploid when cultured in vitro. Both H1 and H9 cells expressed CNP, a specific marker of oligodendrocytes. The GFP cells also demonstrated 100% tumorigenic rate and high invasive properties in vivo. CONCLUSIONS: In this glioma transplantation model, the transplanted tumor tissues contained not only transplanted glioma stem cells but also cancerous host GFP cells. Our findings offer important clues to further research on the relationships among different members in the tumor microenvironment.

Chu, F., et al. (2002). "[Expression of telomerase during induction of committed differentiation of human cord blood hematopoietic stem/progenitor cells in vitro]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **10**(4): 281-284.

To investigate the expression of telomerase in cord blood hematopoietic stem/progenitor cells during their committed differentiation in vitro and provide an index of monitoring the proliferating potential of the hematopoietic stem/progenitor cells and security for clinical application. Human CD34 positive cells were isolated from umbilical cord blood by using magnetic cell sorting system (MACS), and were induced to differentiation with hematopoietic growth factors (SCF + IL3 + IL6 + GCSF and SCF + IL3 + IL6 + EPO) in a liquid culture system. The telomerase activity and the cytalytic subunit of telomerase (hTERT) of the cells were analysed during different periods of culture by using TRAP-PCR, TRAP-ELISA, Western blot and RT-PCR techniques, respectively. The results showed that a peak of cell growth was achieved on day 14 - 21 during induction of differentiation in vitro. Total cell number could increase 1006.4 +/- 103.2 times and could not increase there after. Telomerase activity and hTERT expression were low in freshly isolated cord blood CD34(+) cells and increased after about 7 days of culture in addition of cytokine combinations of SCF + IL3 + IL6 + GCSF and SCF + IL3 + IL6 + EPO, respectively. The telomerase activity and hTERT decreased after 14 days of culture and were not detected after 28 days of culture. It was concluded that the hematopoietic stem/progenitor cells can be expanded in large number in vitro and do not have the character of immortality and the telomerase activity could be a useful index in hematopoiesis regulation.

Collins, D. P., et al. (2020). "Development of immortalized human hepatocyte-like hybrid cells by fusion of multi-lineage progenitor cells with primary hepatocytes." PLoS One **15**(6): e0234002.

Human primary hepatocytes (PHs) are critical to studying liver functions, drug metabolism and toxicity. PHs isolated from livers that are unacceptable for transplantation have limited expansion and culture viability in vitro, in addition to rapidly deteriorating enzymatic functions. The unsuitability of immortalized hepato-carcinoma cell lines for this function has prompted studies to develop hepatocyte-like cells from alternative sources like ESC, iPS, and other stem cell types using differentiation protocols. This study describes a novel technique to produce expandable and functional hepatocyte-like cells from the fusion of an immortalized human umbilical cord blood derived cell line (E12 MLPC) to normal human primary hepatocytes. Multi-lineage progenitor cells (MLPC) comprise a small subset of mesenchymal-like cells isolated from human umbilical cord blood. MLPC are distinguishable from other mesenchymal-like cells by their extended expansion capacity (up to 80 cell doublings before senescence) and the ability to be differentiated into cells representative of endo-, meso- and ectodermal origins. Transfection of MLPC with the gene for telomerase reverse transcriptase (TERT) resulted in clonal cell lines that were capable of differentiation to different cellular outcomes while maintaining their functional immortality. A methodology for the development of immortalized hepatocyte-like hybrid cells by the in vitro fusion of human MLPC with normal human primary hepatocytes is reported. The resultant hybrid cells exhibited homology with hepatocytes by morphology, immunohistochemistry, urea and albumin production and gene expression. A medium that allows stable long-term expansion of hepatocyte-like fusion cells is described.

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

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

Danko, M. J., et al. (2015). "Unraveling the non-senescence phenomenon in Hydra." J Theor Biol **382**: 137-149.

Unlike other metazoans, Hydra does not experience the distinctive rise in mortality with age known as senescence, which results from an increasing imbalance between cell damage and cell repair. We propose that the Hydra controls damage accumulation mainly through damage-dependent cell selection and cell sloughing. We examine our hypothesis with a model that combines cellular damage with stem cell renewal, differentiation, and elimination. The Hydra individual can be seen as a large single pool of three types of stem cells with some features of differentiated cells. This large stem cell community prevents "cellular damage drift," which is inevitable in complex conglomerate (differentiated) metazoans with numerous and generally isolated pools of stem cells. The process of cellular damage drift is based on changes in the distribution of damage among cells due to random events, and is thus similar to Muller's ratchet in asexual populations. Events in the model that are sources of randomness include budding, cellular death, and cellular damage and repair. Our results suggest that non-senescence is possible only in simple Hydra-like organisms which have a high proportion and number of stem cells, continuous cell divisions, an effective cell selection mechanism, and stem cells with the ability to undertake some roles of differentiated cells.

de Caralt, S., et al. (2007). "Cell culture from sponges: pluripotency and immortality." Trends Biotechnol **25**(10): 467-471.

Sponges are a source of compounds with

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

Dennis, J. E., et al. (1999). "A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse." J Bone Miner Res **14**(5): 700-709.

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

Dewhirst, M. W., et al. (2016). "The future of biology in driving the field of hyperthermia." Int J Hyperthermia **32**(1): 4-13.

In 2011 Hanahan and Weinberg updated their well-established paper 'The hallmarks of cancer'. The rationale for that review and its predecessor was to produce a conceptual framework for future research in cancer. The original Hallmarks included: cell signalling to enhance tumour cell proliferation, acquisition of ability to evade growth suppressors, developing mechanisms to resist cell death, enabling replicative immortality, initiating angiogenesis and activating processes to enable invasion and metastasis. In the more recent paper, Hanahan and Weinberg added important new features to this composite paradigm. The new features were: (1) altered metabolism, (2) evasion of immune destruction, (3) tumour promoting inflammation, and (4) the cellular microenvironment. These four new features are the main focus of this review. Hanahan and Weinberg did not specifically include the physiological microenvironment which is dominated by hypoxia and acidosis. In this review we will consider these features in addition to the cellular and metabolic components of the microenvironment. The purpose of this review is to present a vision of emerging fields of study in hyperthermia biology over the next decade and beyond. As such, we are focusing our attention on pre-clinical studies, primarily using mice. The application of hyperthermia in human patients has been thoroughly reviewed elsewhere.

Dogan, F. and C. Biray Avci (2018). "Correlation between telomerase and mTOR pathway in cancer stem cells." Gene **641**: 235-239.

Cancer stem cells (CSCs), which are defined as a subset of tumor cells, are able to self-renew, proliferate, differentiate similar to normal stem cells. Therefore, targeting CSCs has been considered as a new approach in cancer therapy. The mammalian target of rapamycin (mTOR) is a receptor tyrosine kinase which plays an important role in regulating cell proliferation, differentiation, cell growth, self-renewal in CSCs. On the other hand, hTERT overactivation provides replicative feature and immortality to CSCs, so the stemness and replicative properties of CSCs depend on telomerase activity. Therefore hTERT/telomerase activity may become a universal biomarker for anticancer therapy and it is an attractive

therapeutic target for CSCs. It is known that mTOR regulates telomerase activity at the translational and post-translational level. Researchers show that mTOR inhibitor rapamycin reduces telomerase activity without changing hTERT mRNA activity. Correlation between mTOR and hTERT is important for survival and immortality of cancer cells. In addition, the PI3K/AKT/mTOR signaling pathway and hTERT up-regulation are related with cancer stemness features and drug resistance. mTOR inhibitor and TERT inhibitor combination may construct a novel strategy in cancer stem cells and it can make a double effect on telomerase enzyme. Consequently, inhibition of PI3K/AKT/mTOR signaling pathway components and hTERT activation may prohibit CSC self-renewal and surpass CSC-mediated resistance in order to develop new cancer therapeutics.

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

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

Drosos, I. and G. Kolios (2013). "Stem cells in liver regeneration and their potential clinical applications." Stem Cell Rev Rep **9**(5): 668-684.

Stem cells constitute a population of "primitive cells" with the ability to divide indefinitely and give rise to specialized cells under special conditions. Because of these two characteristics they have received particular attention in recent decades. These cells are the primarily responsible factors for the regeneration of tissues and organs and for the healing of lesions, a feature that makes them a central key in the development of cell-based medicine, called Regenerative Medicine. The idea of wound and organ repair and body regeneration is as old as the mankind, reflecting the human desire for inhibiting aging and immortality and it is first described in the ancient Greek myth of Prometheus. It is of interest that the myth refers to liver, an organ with remarkable regenerative ability after loss of mass and function caused by liver injury or surgical resection. Over the last decade there has been an important progress in understanding liver physiology and the mechanisms underlying hepatic development and regeneration. As liver transplantation, despite its difficulties, remains the only effective therapy for advanced liver disease so far, scientific interest has nowadays been orientated towards Regenerative Medicine and the use of stem cells to repair damaged liver. This review is focused on the available literature concerning the role of stem cells in liver regeneration. It summarizes the results of studies concerning endogenous liver regeneration and stem cell experimental protocols. Moreover, this review discusses the clinical studies that have been conducted in humans so far.

Duleba, M., et al. (2019). "Unlimited expansion of intestinal stem cells from a wide range of ages." Integr Mol Med **6**(4).

The recent technical advance in cloning and culturing ground-state intestinal stem cells (ISC) provides us an opportunity of accurate assessment of age-related impact on the function of highly proliferative intestinal stem cells. Our ability of indefinitely and robustly expanding single-stem-cell derived pedigrees in vitro allows us to study intestinal stem cells at the clonal level. Interestingly, comparable number of ISC clones was yielded from 1mm endoscopic biopsy of all donors despite the age. They

were passaged in vitro as pedigrees and expanded to 1 billion cells in approximately sixty days without changes in stemness demonstrated by clonogenicity and multipotency. Therefore, our study shows that ISCs from a wide range of ages can be cloned and expanded to unlimited number in vitro with similar efficiency and stability. These patient-derived ISCs harbor intrinsic immortality and are ideal for autologous transplantation, supporting the promise of adult-stem-cell based personalized medicine.

Duleba, M., et al. (2020). "Cloning of ground-state intestinal stem cells from endoscopic biopsy samples." Nat Protoc **15**(5): 1612-1627.

'Adult' or 'somatic' stem cells harbor an intrinsic ability to regenerate tissues. Heterogeneity of such stem cells along the gastrointestinal tract yields the known segmental specificity of this organ and may contribute to the pathology of certain enteric conditions. Here we detail technology for the generation of 'libraries' of clonogenic cells from 1-mm-diamter endoscopic biopsy samples from the human gastrointestinal tract. Each of the 150-300 independent clones in a typical stem cell library can be clonally expanded to billions of cells in a few weeks while maintaining genomic stability and the ability to undergo multipotent differentiation to the specific epithelia from which the sample originated. The key to this methodology is the intrinsic immortality of normal intestinal stem cells (ISCs) and culture systems that maintain them as highly immature, ground-state ISCs marked by a single-cell clonogenicity of 70% and a corresponding 250-fold proliferative advantage over spheroid technologies. Clonal approaches such as this enhance the resolution of molecular genetics, make genome editing easier, and may be useful in regenerative medicine, unravelling heterogeneity in disease, and facilitating drug discovery.

Erenpreisa, J., et al. (2015). "The "virgin birth", polyploidy, and the origin of cancer." Oncoscience **2**(1): 3-14.

Recently, it has become clear that the complexity of cancer biology cannot fully be explained by somatic mutation and clonal selection. Meanwhile, data have accumulated on how cancer stem cells or stemloids bestow immortality on tumour cells and how reversible polyploidy is involved. Most recently, single polyploid tumour cells were shown capable of forming spheroids, releasing EMT-like descendents and inducing tumours in vivo. These data refocus attention on the centuries-old embryological theory of cancer. This review attempts to reconcile seemingly conflicting data by viewing cancer as a pre-programmed phylogenetic life-cycle-like process. This cycle is apparently initiated by a meiosis-like process and driven as an alternative to accelerated senescence at the DNA damage checkpoint, followed by an asexual syngamy event and endopolyploid-type embryonal cleavage to provide germ-cell-like (EMT) cells. This cycle is augmented by genotoxic treatments, explaining why chemotherapy is rarely curative and drives resistance. The logical outcome of this viewpoint is that alternative treatments may be more efficacious - either those that suppress the endopolyploidy-associated 'life cycle' or, those that cause reversion of embryonal malignant cells into benign counterparts. Targets for these opposing strategies are components of the same molecular pathways and interact with regulators of accelerated senescence.

Fahey, M. C. and E. M. Wallace (2011). "Stem cells: research tools and clinical treatments." J Paediatr Child Health **47**(9): 672-675.

The term 'stem cell' most commonly refers to embryonic stem cells, particularly in the lay media; however, it also describes other cell types. A stem cell represents a cell of multi-lineage potential with the ability for self-renewal. It is now clear that the plasticity and immortality of a given stem cell will depend on what type of stem cell it is, whether an embryonic stem cell, a fetal-placental stem cell or an adult stem cell. Stem cells offer great promise as cell-based therapies for the future. With evolving technology, much of the socio-political debate regarding stem cells can now be avoided.

Fahnrich, S., et al. (2024). "Optimized for routine: highly sensitive fluorescent Telomeric Repeat Amplification Protocol (f-TRAP)." Biotechniques: 1-6.

The strict suppression of telomerase activity (TA) in terminally differentiated human cells causes a shortening of the chromosome ends after each cell division. This tumor suppression surveillance mechanism is associated with a limited number of cell divisions known as Hayflick limit. Here we present an optimized protocol for measuring TA that combines a fluorescently labeled bait primer and polymerase chain reaction (PCR) amplification with analytical capillary electrophoresis (CE) to achieve a detection limit of one telomerase-positive cell per ten thousand negative cells. In research laboratories today, a broad panel of TRAP assay protocols enables the assessment of the immortality of newly generated cell lines or the unambiguous evaluation of the reprogramming of induced pluripotent stem cells (iPSCs). The present f-TRAP protocol, optimized for routine use, enables fast ad hoc application for single measurements up to a high throughput of mass samples using a triplicate approach of different lysate concentrations. Final CE

analysis facilitates standardized data processing and storage for documentation of results and could make f-TRAP a useful assay in research and clinical oncology.
Telomerase activity is the most universal of all known tumor markers and a promising target for anti-tumor therapies. The robustness and high sensitivity of f-TRAP can (i) demonstrate early validation of cell line immortality in the establishment of model systems, (ii) lead to rapid detection of successful reprogramming or differentiation of cells in vitro and (iii) be used in high throughput assays in the search for new inhibitors of telomerase activity.
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Fathi, E., et al. (2020). "Interleukin-6, -8, and TGF-beta Secreted from Mesenchymal Stem Cells Show Functional Role in Reduction of Telomerase Activity of Leukemia Cell Via Wnt5a/beta-Catenin and P53 Pathways." Adv Pharm Bull **10**(2): 307-314.

Purpose: The effect of mesenchymal stem cells (MSCs) on the immortality features of malignant cells, such as hematologic cancerous cells, are controversial, and the associated mechanisms are yet to be well understood. The aim of the present study was to investigate the in vitro effect of bone marrow-derived MSCs (BMSCs) on the chronic myeloid leukemia cell line K562 through telomere length measurements, telomerase activity assessments, and hTERT gene expression. The possible signaling pathways involved in this process, including Wnt-5a/beta-catenin and P53, were also evaluated. Methods: Two cell populations (BMSCs and K562 cell line) were co-cultured on transwell plates for 7 days. Next, K562 cells were collected and subjected to quantitative real-time PCR, PCR-ELISA TRAP assay, and the ELISA sandwich technique for telomere length, hTERT gene expression, telomerase activity assay, and cytokine measurement, respectively. Also, the involvement of the mentioned signaling pathways in this process was reported by real-time PCR and Western blotting through gene and protein expression, respectively. Results: The results showed that BMSCs caused significant decreases in telomere length, telomerase activity, and the mRNA level of hTERT as a regulator of telomerase activity. The significant presence of interleukin (IL)-6, IL-8, and transforming growth factor beta (TGF-beta) was obvious in the co-cultured media. Also, BMSCs significantly decreased and increased the gene and protein expression of beta-catenin and P53, respectively. Conclusion: It was concluded that the mentioned effects of IL-6, IL-8, and TGF-beta cytokines secreted from MSCs on K562 cells as therapeutic agents were applied by Wnt-5a/beta-catenin and P53 pathways.

Fernandes, S. G., et al. (2020). "Role of Telomeres and Telomeric Proteins in Human Malignancies and Their Therapeutic Potential." Cancers (Basel) **12**(7).

Telomeres are the ends of linear chromosomes comprised of repetitive nucleotide sequences in humans. Telomeres preserve chromosomal stability and genomic integrity. Telomere length shortens with every cell division in somatic cells, eventually resulting in replicative senescence once telomere length becomes critically short. Telomere shortening can be overcome by telomerase enzyme activity that is undetectable in somatic cells, while being active in germline cells, stem cells, and immune cells. Telomeres are bound by a shelterin complex that regulates telomere lengthening as well as protects them from being identified as DNA damage sites. Telomeres are transcribed by RNA polymerase II, and generate a long noncoding RNA called telomeric repeat-containing RNA (TERRA), which plays a key role in regulating subtelomeric gene expression. Replicative immortality and genome instability are hallmarks of cancer and to attain them cancer cells exploit telomere maintenance and telomere protection mechanisms. Thus, understanding the role of telomeres and their associated proteins in cancer initiation, progression and treatment is very important. The present review highlights the critical role of various telomeric components with recently established functions in cancer. Further, current strategies to target various telomeric components including human telomerase reverse transcriptase (hTERT) as a therapeutic approach in human malignancies are discussed.

Floor, S., et al. (2011). "Cancer cells in epithelial-to-mesenchymal transition and tumor-propagating-cancer stem cells: distinct, overlapping or same populations." Oncogene **30**(46): 4609-4621.

Cell populations of solid cancers and their distant models, the cancer cell lines, have been categorized in sub-populations: cancer stem-tumor-propagating cells (CSC-TPC) versus derived cells, epithelial- versus mesenchymal-type cells, dormant versus actively proliferating cells and so on. CSC-TPC are minimally defined by their operational properties: immortality and the ability to regenerate in vivo or in vitro the whole panel of cancer cells. The epithelial-to-mesenchymal transition (EMT), mostly observed in vitro, generates mesenchymal-type from epithelial-type cells. The converse transition is mesenchymal-to-epithelial transition. In vitro work suggests that CSC-TPC and EMT cell phenotypes overlap. An analysis of the properties of these sub-populations, as studied in vitro, shows that indeed these two phenotypes may be linked to some extent. However, the in vivo counterpart of this relation in human tumors has barely

been investigated. A model in which among the EMT cells released from the tumor only the most competent CSC-TPC will succeed to metastasize is proposed. It is suggested that in the Darwinian evolution of cancer cells, many phenotypes reflecting the expression of various programs, reversible to irreversible, exclusive, overlapping or linked coexist and compete with each other.

Flora, P., et al. (2018). "Transient transcriptional silencing alters the cell cycle to promote germline stem cell differentiation in Drosophila." Dev Biol **434**(1): 84-95.

Transcriptional silencing is a conserved process used by embryonic germ cells to repress somatic fate and maintain totipotency and immortality. In Drosophila, this transcriptional silencing is mediated by polar granule component (pgc). Here, we show that in the adult ovary, pgc is required for timely germline stem cell (GSC) differentiation. Pgc is expressed transiently in the immediate GSC daughter (pre-cystoblast), where it mediates a pulse of transcriptional silencing. This transcriptional silencing mediated by pgc indirectly promotes the accumulation of Cyclin B (CycB) and cell cycle progression into late-G2 phase, when the differentiation factor bag of marbles (bam) is expressed. Pgc mediated accumulation of CycB is also required for heterochromatin deposition, which protects the germ line genome against selfish DNA elements. Our results suggest that transient transcriptional silencing in the pre-cystoblast "re-programs" it away from self-renewal and toward the gamete differentiation program.

Florea, M. (2017). "Aging and immortality in unicellular species." Mech Ageing Dev **167**: 5-15.

It has been historically thought that in conditions that permit growth, most unicellular species do not to age. This was particularly thought to be the case for symmetrically dividing species, as such species lack a clear distinction between the soma and the germline. Despite this, studies of the symmetrically dividing species Escherichia coli and Schizosaccharomyces pombe have recently started to challenge this notion. They indicate that E. coli and S. pombe do age, but only when subjected to environmental stress. If true, this suggests that aging may be widespread among microbial species in general, and that studying aging in microbes may inform other long-standing questions in aging. This review examines the recent evidence for and against replicative aging in symmetrically dividing unicellular organisms, the mechanisms that underlie aging, why aging evolved in these species, and how microbial aging fits into the context of other questions in aging.

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'.

Franzese, O., et al. (1998). "Effect of prostaglandin A1 on proliferation and telomerase activity of human melanoma cells in vitro." Melanoma Res **8**(4): 323-328.

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

Fuller, M. T. and A. C. Spradling (2007). "Male and

female Drosophila germline stem cells: two versions of immortality." Science **316**(5823): 402-404.

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

Furuhashi, H. and W. G. Kelly (2010). "The epigenetics of germ-line immortality: lessons from an elegant model system." Dev Growth Differ **52**(6): 527-532.

Epigenetic mechanisms are thought to help regulate the unique transcription program that is established in germ cell development. During the germline cycle of many organisms, the epigenome undergoes waves of extensive resetting events, while a part of epigenetic modification remains faithful to specific loci. Little is known about the mechanisms underlying these events, how loci are selected for, or avoid, reprogramming, or even why these events are required. In particular, although the significance of genomic imprinting phenomena involving DNA methylation in mammals is now well accepted, the role of histone modification as a transgenerational epigenetic mechanism has been the subject of debate. Such epigenetic mechanisms may help regulate transcription programs and/or the pluripotent status conferred on germ cells, and contribute to germ line continuity across generations. Recent studies provide new evidence for heritability of histone modifications through germ line cells and its potential effects on transcription regulation both in the soma and germ line of subsequent generations. Unraveling transgenerational epigenetic mechanisms involving highly conserved histone modifications in elegant model systems will accelerate the generation of new paradigms and inspire research in a wide variety of fields, including basic developmental studies and clinical stem cell research.

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

Stem cells are the closest relatives of the totipotent primordial cell, which is able to spawn millions of daughter cells and hundreds of cell types in multicellular organisms. Stem cells are involved in tissue homeostasis and regeneration, and may play a major role in cancer development. Among animals, planarians host a model stem cell type, called the neoblast, which essentially confers immortality. Gaining insights into the global transcriptional landscape of these exceptional cells takes an unprecedented turn with the advent of Next Generation Sequencing methods. Two Digital Gene Expression transcriptomes of Schmidtea mediterranea planarians, with or without neoblasts lost through irradiation, were produced and analyzed. Twenty one bp NlaIII tags were mapped to transcripts in the Schmidtea and Dugesia taxids. Differential representation of tags in normal versus irradiated animals reflects differential gene expression. Canonical and non-canonical tags were included in the analysis, and comparative studies with human orthologs were conducted. Transcripts fell into 3 categories: invariant (including housekeeping genes), absent in irradiated animals (potential neoblast-specific genes, IRDOWN) and induced in irradiated animals (potential cellular stress response, IRUP). Different mRNA variants and gene family members were recovered. In the IR-DOWN class, almost all of the neoblast-specific genes previously described were found. In irradiated animals, a larger number of genes were induced rather than lost. A significant fraction of IRUP genes behaved as if transcript versions of different lengths were produced. Several novel potential neoblast-specific genes have been identified that varied in relative abundance, including highly conserved as well as novel proteins without predicted orthologs. Evidence for a large body of antisense transcripts, for example regulated antisense for the Smed-piwil1 gene, and evidence for RNA shortening in irradiated animals is presented. Novel neoblast-specific candidates include a peroxiredoxin protein that appears to be preferentially expressed in human embryonic stem cells.

Gilgenkrantz, H. and A. Collin de l'Hortet (2011). "New insights into liver regeneration." Clin Res Hepatol Gastroenterol **35**(10): 623-629.

Even if the Greeks probably anticipated rather than discovered the extraordinary regenerative capacity of the liver with the Prometheus myth, this phenomenon still fascinates scientists nowadays with the same enthusiasm. There are good reasons to decipher this process other than to find an answer to our fantasy of immortality: it could indeed help patients needing large liver resections or living-donor liver transplantation, it could increase our understanding of liver pathology and finally it could enable novel cell-therapy approaches. For decades, most of our knowledge about the mechanisms involved in liver regeneration came from the classic two-thirds partial hepatectomy (PH) model. In this scenario, hepatocytes play the leading role, which

raises the question of the simple existence of a stem cell population. Recently however, hepatic progenitor cells come again under the limelight, seeming to play a role in liver physiology and in various liver diseases such as steatosis or cirrhosis. Excellent reviews have recently addressed liver regeneration. Our goal is therefore to focus on recent improvements in the field, highlighting data mostly published in the last two years in order to draw a putative picture of what the future research axes on liver regeneration might look like.

Goding, C. R. (2011). "Commentary. A picture of Mitf in melanoma immortality." Oncogene **30**(20): 2304-2306.

The Mitf gene has a key role in melanocytes and melanoma by regulating cell cycle progression, survival and differentiation. Two papers in this issue of Oncogene (Cheli et al., 2011; Strub et al., 2011) reveal that low-Mitf cells can initiate tumors with high efficiency, and that Mitf blocks senescence by regulating genes implicated in S-phase progression and mitosis.

Goding, C. R., et al. (2014). "Cancer: pathological nuclear reprogramming?" Nat Rev Cancer **14**(8): 568-573.

The ability of stem cells to self-renew and generate different lineages during development and organogenesis is a fundamental, tightly controlled, and generally unidirectional process, whereas the 'immortality' of cancer cells could be regarded as pathological self-renewal. The molecular mechanisms that underpin the generation of induced pluripotent stem cells are remarkably similar to those that are deregulated in cancer - so much so that aberrant reprogramming is tumorigenic. The similarities also suggest that mutations in genes implicated in DNA methylation dynamics might represent a hallmark of cancers with a stem cell origin, and they highlight an alternative view of cancer that may be of clinical benefit.

Gomez, D. L., et al. (2016). "Telomerase as a Cancer Target. Development of New Molecules." Curr Top Med Chem **16**(22): 2432-2440.

Telomeres are the terminal part of the chromosome containing a long repetitive and noncodifying sequence that has as function protecting the chromosomes. In normal cells, telomeres lost part of such repetitive sequence in each mitosis, until telomeres reach a critical point, triggering at that time senescence and cell death. However, in most of tumor cells in each cell division a part of the telomere is lost, however the appearance of an enzyme called telomerase synthetize the segment that just has been lost, therefore conferring to tumor cells the immortality hallmark. Telomerase is significantly overexpressed in 80-95% of all malignant tumors, being present at low levels in few normal cells, mostly stem cells. Due to these characteristics, telomerase has become an attractive target for new and more effective anticancer agents. The capability of inhibiting telomerase in tumor cells should lead to telomere shortening, senescence and apoptosis. In this work, we analyze the different strategies for telomerase inhibition, either in development, preclinical or clinical stages taking into account their strong points and their caveats. We covered strategies such as nucleosides analogs, oligonucleotides, small molecule inhibitors, G-quadruplex stabilizers, immunotherapy, gene therapy, molecules that affect the telomere/ telomerase associated proteins, agents from microbial sources, among others, providing a balanced evaluation of the status of the inhibitors of this powerful target together with an analysis of the challenges ahead.

Guo, Y., et al. (2020). "Bisphenol A and polychlorinated biphenyls enhance the cancer stem cell properties of human ovarian cancer cells by activating the WNT signaling pathway." Chemosphere **246**: 125775.

Cancer stem cells (CSCs) are a very small subpopulation that have stem-cell qualities, such as exhibiting self-renewal, immortality, and pluripotency, and the capability to differentiate into different tumor cell subtypes. CSCs contribute to tumor onset, expansion, metastasis, resistance and recurrence. Meanwhile, organic pollutants, including nonpersistent pollutants, such as bisphenol A (BPA), and persistent pollutants, such as polychlorinated biphenyls (PCBs), are toxic chemicals that can be readily ingested via dietary exposure and other exposure routes and can accumulate through the food chain. Many organic pollutants increase the risk of ovarian cancer depending on their estrogenic effects. Nonetheless, most previous studies have focused on the toxic effects of these pollutants on the proliferation, metastasis and development of ovarian cancer cells. However, little research has investigated the adverse effect of these pollutants on ovarian cancer stem cells. The current study found that BPA, PCB126 and PCB153 greatly enhanced the formation of cancer stem-like cell spheres of OVCAR-3 cells (human ovarian cancer cells) under low-dose exposure. In parallel, the CD44(high)CD24(low) cell subpopulation was increased in treated cells relative to untreated cells. Elevated expression of cancer stem cell markers, including ALDH1A1, CD133, SOX2, NANOG and OCT4, was demonstrated in treated cells compared to untreated cells. In summary, these data

demonstrate that the oncogenic effects of pollutants can be evaluated according to changes in caner stem cell properties.

Gupta, S., et al. (2018). "HPV: Molecular pathways and targets." Curr Probl Cancer **42**(2): 161-174.

Infection of high-risk human papillomaviruses (HPVs) is a prerequisite for the development of cervical carcinoma. HPV infections are also implicated in the development of other types of carcinomas. Chronic or persistent infection of HPV is essential but HPV alone is inadequate, additional endogenous or exogenous cues are needed along with HPV to induce cervical carcinogenesis. The strategies that high-risk HPVs have developed in differentiating epithelial cells to reach a DNA-synthesis competent state leading to tumorigenic transformation are basically due to overexpression of the E6 and E7 oncoproteins and the activation of diverse cellular regulatory or signaling pathways that are targeted by them. Moreover, the Wnt/beta-catenin/Notch and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathways are deregulated in various cancers, and have also been implicated in HPV-induced cancers. These are basically related to the "cancer hallmarks," and include sustaining proliferative signals, the evasion of growth suppression and immune destruction, replicative immortality, inflammation, invasion, metastasis and angiogenesis, as well as genome instability, resisting cell death, and deregulation of cellular energetics. These information could eventually aid in identifying or developing new diagnostic, prognostic biomarkers, and may contribute to design more effective targeted therapeutics and treatment strategies. Although surgery, chemotherapy and radiotherapy can cure more than 90% of women with early stage cervical cancer, the recurrent and metastatic disease remains a major cause of cancer mortality. Numerous efforts have been made to design new drugs and develop gene therapies to treat cervical cancer. In recent years, research on treatment strategies has proposed several options, including the role of HPV E5, E6, and E7 oncogenes, which are retained and overexpressed in most of the cervical cancers and whose respective oncoproteins are critical to the induction and maintenance of the malignant phenotype. Other efforts have been focused on antitumor immunotherapy strategies. It is known that during the development of cervical cancer, a cascade of abnormal events is induced, including disruption of cell cycle control, perturbation of antitumor immune response, alteration of gene expression, deregulation of microRNA and cancer stem cell and stemness related markers expression could serve as novel molecular targets for reliable diagnosis and treatment of HPV-positive cancers. However, the search for new proposals for disease control and prevention has brought new findings and approaches in the context of molecular biology indicating innovations and perspectives in the early detection and prevention of the disease. Thus, in this article, we discuss molecular signaling pathways activated by HPV and potential targets or biomarkers for early detection or prevention and the treatment of HPV-associated cancers.

Hafner, S. J., et al. (2017). "Long noncoding RNAs in normal and pathological pluripotency." Semin Cell Dev Biol **65**: 1-10.

The striking similarities between pluripotent and cancer cells, such as immortality and increased stress resistance, have long been acknowledged. Numerous studies searched for and successfully identified common molecular players and pathways, thus providing an entirely new challenge and potential therapeutic angle by targeting cancer cells or a specific stem population of the tumor via pluripotency associated processes. However, these strategies have until now mainly been restricted to proteins. Nonetheless, it has become clear over the past decade that the overwhelming majority of the genome produces noncoding transcripts, many of which have proven both functional and crucial for key cellular processes, including stemness maintenance. Moreover, numerous long noncoding RNAs are deregulated in cancer, but little is known concerning their functions and molecular mechanisms. Consequently, it seems essential to integrate the noncoding transcripts into the picture of the stemness-cancer connection. Whereas a number of studies have addressed the expression of lncRNAs in cancer stem cells, no systematic approach has yet been undertaken to identify lncRNAs implicated in the maintenance of the embryonic stemness state that is hijacked by cancer cells. The aim of this review is to highlight long noncoding RNAs with shared functions in stemness and cancer and to outline the current state of a field in its infancy, the search for long noncoding transcripts in cancer stem cells.

Hannen, R. and J. W. Bartsch (2018). "Essential roles of telomerase reverse transcriptase hTERT in cancer stemness and metastasis." FEBS Lett **592**(12): 2023-2031.

Maintenance of chromosomal telomere length is a hallmark of cancer cells and a prerequisite for stemness. In 85-90% of all human cancers, telomere length maintenance is achieved by reactivation of telomerase, whereas in the remaining 10-15% cancers, alternative lengthening of telomeres (ALT) is observed. Reactivation of telomerase occurs by various mechanisms, one of which is accumulation of point mutations in the promoter region of the gene

encoding the protein subunit hTERT. There are numerous studies linking either hTERT overexpression or the presence of hTERT mutations to an aggressive phenotype of several human cancers. Recent findings demonstrate that hTERT expression is not only associated with replicative immortality, but also with cancer cell motility and stem cell phenotype. However, the mechanisms by which hTERT affects cancer cell migration, invasion, and distant metastasis on the one hand, and stemness and resistance on the other hand, are still poorly understood. Within this review, we aim to provide an overview on the functional involvement of hTERT in these cellular processes, focusing on metastasis formation and maintenance of stemness in different human cancers.

Hiebert, L. S., et al. (2021). "Coloniality, clonality, and modularity in animals: The elephant in the room." J Exp Zool B Mol Dev Evol **336**(3): 198-211.

Nearly half of the animal phyla contain species that propagate asexually via agametic reproduction, often forming colonies of genetically identical modules, that is, ramets, zooids, or polyps. Clonal reproduction, colony formation, and modular organization have important consequences for many aspects of organismal biology. Theories in ecology, evolution, and development are often based on unitary and, mainly, strictly sexually reproducing organisms, and though colonial animals dominate many marine ecosystems and habitats, recognized concepts for the study of clonal species are often lacking. In this review, we present an overview of the study of colonial and clonal animals, from the historic interests in this subject to modern research in a range of topics, including immunology, stem cell biology, aging, biogeography, and ecology. We attempt to portray the fundamental questions lying behind the biology of colonial animals, focusing on how colonial animals challenge several dogmas in biology as well as the remaining puzzles still to be answered, of which there are many.

Hirai, T., et al. (2022). "Evaluation of the reproducibility and positive controls of cellular immortality test for the detection of immortalized cellular impurities in human cell-processed therapeutic products." Regen Ther **21**: 540-546.

INTRODUCTION: Contamination of human cell-processed therapeutic products (hCTPs) with tumorigenic/immortalized cellular impurities is a major concern in the manufacturing and quality control of hCTPs. The cellular immortality test based on cell growth analysis is a method for detecting tumorigenic/immortalized cellular impurities in hCTPs. However, the performance of the cellular immortality test has not yet been well characterized. In this study, we examined the reproducibility of the cellular immortality test in detecting HeLa cells as a model of tumorigenic cellular impurities, as well as the applicability of other models of cellular impurities with different tumorigenicity to the cellular immortality test. METHODS: Using HeLa cells as a model for cellular impurities, we measured the growth rate of human mesenchymal stem cells (hMSCs) supplemented with HeLa cells at concentrations ranging from 0.01 to 0.0001% at each passage in three laboratories and evaluated the reproducibility of the detection of immortalized cellular impurities. In addition, HEK293 cells (another immortalized cell line) and MRC-5 cells (a non-immortalized cell line) were employed as cellular impurity models that exhibit different growth characteristics from HeLa cells, and the ability of the cellular immortality test to detect these different impurities when mixed with hMSCs was examined. RESULTS: In the multisite study, the growth rate of hMSCs supplemented with 1 and 10 HeLa cells (0.0001% and 0.001%) significantly increased and reached a plateau in all three laboratories, whereas those of hMSCs alone eventually decreased. Moreover, when hMSCs were supplemented with 10 and 100 HEK293 and MRC-5 cells (0.001% and 0.01%), the growth rate significantly increased. The growth rate of hMSCs supplemented with HEK293 cells increased with passage and remained high, whereas that of hMSCs supplemented with MRC-5 cells eventually decreased, as in the case of hMSCs alone. CONCLUSIONS: These results indicate that the cellular immortality test is reproducible and can detect immortalized (i.e., potentially tumorigenic) cells such as HEK293 cells with a lower growth rate than HeLa cells by discriminating against normal cells, which could contribute to ensuring the safety and quality of hCTPs.

Hiyama, E. and K. Hiyama (2007). "Telomere and telomerase in stem cells." Br J Cancer **96**(7): 1020-1024.

Telomeres, guanine-rich tandem DNA repeats of the chromosomal end, provide chromosomal stability, and cellular replication causes their loss. In somatic cells, the activity of telomerase, a reverse transcriptase that can elongate telomeric repeats, is usually diminished after birth so that the telomere length is gradually shortened with cell divisions, and triggers cellular senescence. In embryonic stem cells, telomerase is activated and maintains telomere length and cellular immortality; however, the level of telomerase activity is low or absent in the majority of stem cells regardless of their proliferative capacity. Thus, even in stem cells, except for embryonal stem cells and cancer stem cells, telomere shortening occurs during replicative ageing,

possibly at a slower rate than that in normal somatic cells. Recently, the importance of telomere maintenance in human stem cells has been highlighted by studies on dyskeratosis congenital, which is a genetic disorder in the human telomerase component. The regulation of telomere length and telomerase activity is a complex and dynamic process that is tightly linked to cell cycle regulation in human stem cells. Here we review the role of telomeres and telomerase in the function and capacity of the human stem cells.

Hoffman, L. M. and M. K. Carpenter (2005). "Human embryonic stem cell stability." Stem Cell Rev **1**(2): 139-144.

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

Hohaus, S., et al. (1997). "Telomerase activity in human hematopoietic progenitor cells." Haematologica **82**(3): 262-268.

BACKGROUND AND OBJECTIVE: Telomerase is the enzyme that stabilizes and elongates the telomeric ends of chromosomes. It is expressed in germline and malignant cells and absent in most human somatic cells. The selective expression of telomerase has thus been proposed to be a basis for the immortality of germline and malignant cells. Recently, telomerase activity has been observed in human bone marrow (BM) and peripheral blood (PB) samples. The objective of our study was to further characterize the telomerase-expressing population in BM and PB. METHODS: CD34+ cells were isolated from BM and PB, cultured in vitro, and telomerase activity was assessed by the PCR-based TRAP assay. RESULTS: Telomerase activity in human BM and PB could be almost exclusively assigned to the hematopoietic progenitor cell fraction expressing the CD34 antigen. We observed telomerase activity in CD34+ cells from BM and cytokine-mobilized PB. CD34+ cells lacking co-expression of CD33 demonstrated higher levels of telomerase than myeloid committed CD34+/CD33+ cells. In vitro culture of CD34+ cells in the presence of a cocktail of growth factors inducing differentiation resulted in a decrease of telomerase activity. Telomerase activity increased in peripheral blood during cytokine-induced mobilization of hematopoietic progenitor cells. INTERPRETATION AND CONCLUSIONS: Our data demonstrate that at least a portion of the hematopoietic stem/progenitor cell fraction expresses telomerase and downregulates its expression through differentiation.

Holland, A. M. and E. G. Stanley (2009). "Stems cells and the price of immortality." Stem Cell Res **2**(1): 26-28.

Holm, T. M., et al. (2005). "Global loss of imprinting leads to widespread tumorigenesis in adult mice." Cancer Cell **8**(4): 275-285.

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

Irons, R. D. and P. J. Kerzic (2014). "Cytogenetics in benzene-associated myelodysplastic syndromes and acute myeloid leukemia: new insights into a disease continuum." Ann N Y Acad Sci **1310**: 84-88.

Hematopoiesis in health and disease results from complex interactions between primitive hematopoietic stem cells (HSCs) and the extrinsic influences of other cells in the bone marrow (BM) niche. Advances in stem cell biology, molecular genetics, and computational biology reveal that the immortality, self-renewal, and maintenance of blood homeostasis generally attributed to individual HSCs are functions of the cells' behavior in the normal BM environment. Here we discuss how these advances, together with results of outcomes-based clinical epidemiology studies, provide new insight into the importance of epigenetic events in leukemogenesis.

For the chemical benzene (Bz), development of myeloid neoplasms depends predominantly on alterations within the microenvironments in which they arise. The primary persistent disease in Bz myelotoxicity is myelodysplastic syndrome, which precedes cytogenetic injury. Evidence indicates that acute myeloid leukemia arises as a secondary event, subsequent to evolution of the leukemia-initiating cell phenotype within the altered BM microenvironment. Further explorations into the nature of chemical versus de novo disease should consider this mechanism, which is biologically distinct from previous models of clonal cytogenetic injury. Understanding alterations of homeostatic regulation in the BM niche is important for validation of models of leukemogenesis, monitoring at-risk populations, and development of novel treatment and prevention strategies.

Isfort, R. J. and R. A. LeBoeuf (1995). "The Syrian hamster embryo (SHE) cell transformation system: a biologically relevant in vitro model--with carcinogen predicting capabilities--of in vivo multistage neoplastic transformation." Crit Rev Oncog **6**(3-6): 251-260.

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

Iskender, B., et al. (2015). "Myrtucommulone-A treatment decreases pluripotency- and multipotency-associated marker expression in bladder cancer cell line HTB-9." J Nat Med **69**(4): 543-554.

Cancer and stem cells exhibit similar features, including self-renewal, differentiation and immortality. The expression of stem-cell-related genes in cancer cells is demonstrated to be potentially correlated with cancer cell behaviour, affecting both drug response and tumor recurrence. There is an emerging body of evidence that subpopulations of tumors carry a distinct molecular sign and are selectively resistant to chemotherapy. Therefore, it is important to find novel therapeutic agents that could suppress the stem-like features of cancer cells while inhibiting their proliferation. Myrtucommulone-A (MC-A) is an active compound of a nonprenylated acylphloroglucinol isolated from the leaves of myrtle. Here we have investigated the potential of MC-A in inhibiting the expression of self-renewal regulatory factors and cancer stem cell markers in a bladder cancer cell line HTB-9. We used RT-PCR, immunocytochemistry, flow cytometry and western blotting to examine the expression of pluripotency- and multipotency-associated markers with or without treatment with MC-A. Treatment with MC-A not only decreased cancer cell viability and proliferation but also resulted in a decrease in the expression of pluripotency- and multipotency-associated markers such as NANOG, OCT-4, SOX-2, SSEA-4, TRA-1-60, CD90, CD73 and CD44. MC-A treatment was also observed to decrease the sphere-forming ability of HTB-9 cells. In summary, this study provides valuable information on the presence of stem-cell marker expression in HTB-9 cells and our results imply that MC-A could be utilized to target cancer cells with stem-like characteristics.

Ivancich, M., et al. (2017). "Treating Cancer by Targeting Telomeres and Telomerase." Antioxidants (Basel) **6**(1).

Telomerase is expressed in more than 85% of cancer cells. Tumor cells with metastatic potential may have a high telomerase activity, allowing cells to escape from the inhibition of cell proliferation due to shortened telomeres. Human telomerase primarily consists of two main components: hTERT, a catalytic subunit, and hTR, an RNA template whose sequence is complimentary to the telomeric 5'-dTTAGGG-3' repeat. In humans, telomerase activity is typically restricted to renewing tissues, such as germ cells and stem cells, and is generally absent in normal cells.

While hTR is constitutively expressed in most tissue types, hTERT expression levels are low enough that telomere length cannot be maintained, which sets a proliferative lifespan on normal cells. However, in the majority of cancers, telomerase maintains stable telomere length, thereby conferring cell immortality. Levels of hTERT mRNA are directly related to telomerase activity, thereby making it a more suitable therapeutic target than hTR. Recent data suggests that stabilization of telomeric G-quadruplexes may act to indirectly inhibit telomerase action by blocking hTR binding. Telomeric DNA has the propensity to spontaneously form intramolecular G-quadruplexes, four-stranded DNA secondary structures that are stabilized by the stacking of guanine residues in a planar arrangement. The functional roles of telomeric G-quadruplexes are not completely understood, but recent evidence suggests that they can stall the replication fork during DNA synthesis and inhibit telomere replication by preventing telomerase and related proteins from binding to the telomere. Long-term treatment with G-quadruplex stabilizers induces a gradual reduction in the length of the G-rich 3' end of the telomere without a reduction of the total telomere length, suggesting that telomerase activity is inhibited. However, inhibition of telomerase, either directly or indirectly, has shown only moderate success in cancer patients. Another promising approach of targeting the telomere is the use of guanine-rich oligonucleotides (GROs) homologous to the 3' telomere overhang sequence (T-oligos). T-oligos, particularly a specific 11-base oligonucleotide (5'-dGTTAGGGTTAG-3') called T11, have been shown to induce DNA damage responses (DDRs) such as senescence, apoptosis, and cell cycle arrest in numerous cancer cell types with minimal or no cytostatic effects in normal, non-transformed cells. As a result, T-oligos and other GROs are being investigated as prospective anticancer therapeutics. Interestingly, the DDRs induced by T-oligos in cancer cells are similar to the effects seen after progressive telomere degradation in normal cells. The loss of telomeres is an important tumor suppressor mechanism that is commonly absent in transformed malignant cells, and hence, T-oligos have garnered significant interest as a novel strategy to combat cancer. However, little is known about their mechanism of action. In this review, we discuss the current understanding of how T-oligos exert their antiproliferative effects in cancer cells and their role in inhibition of telomerase. We also discuss the current understanding of telomerase in cancer and various therapeutic targets related to the telomeres and telomerase.

Jager, K. and M. Walter (2016). "Therapeutic Targeting of Telomerase." Genes (Basel) **7**(7).

Telomere length and cell function can be preserved by the human reverse transcriptase telomerase (hTERT), which synthesizes the new telomeric DNA from a RNA template, but is normally restricted to cells needing a high proliferative capacity, such as stem cells. Consequently, telomerase-based therapies to elongate short telomeres are developed, some of which have successfully reached the stage I in clinical trials. Telomerase is also permissive for tumorigenesis and 90% of all malignant tumors use telomerase to obtain immortality. Thus, reversal of telomerase upregulation in tumor cells is a potential strategy to treat cancer. Natural and small-molecule telomerase inhibitors, immunotherapeutic approaches, oligonucleotide inhibitors, and telomerase-directed gene therapy are useful treatment strategies. Telomerase is more widely expressed than any other tumor marker. The low expression in normal tissues, together with the longer telomeres in normal stem cells versus cancer cells, provides some degree of specificity with low risk of toxicity. However, long term telomerase inhibition may elicit negative effects in highly-proliferative cells which need telomerase for survival, and it may interfere with telomere-independent physiological functions. Moreover, only a few hTERT molecules are required to overcome senescence in cancer cells, and telomerase inhibition requires proliferating cells over a sufficient number of population doublings to induce tumor suppressive senescence. These limitations may explain the moderate success rates in many clinical studies. Despite extensive studies, only one vaccine and one telomerase antagonist are routinely used in clinical work. For complete eradication of all subpopulations of cancer cells a simultaneous targeting of several mechanisms will likely be needed. Possible technical improvements have been proposed including the development of more specific inhibitors, methods to increase the efficacy of vaccination methods, and personalized approaches. Telomerase activation and cell rejuvenation is successfully used in regenerative medicine for tissue engineering and reconstructive surgery. However, there are also a number of pitfalls in the treatment with telomerase activating procedures for the whole organism and for longer periods of time. Extended cell lifespan may accumulate rare genetic and epigenetic aberrations that can contribute to malignant transformation. Therefore, novel vector systems have been developed for a 'mild' integration of telomerase into the host genome and loss of the vector in rapidly-proliferating cells. It is currently unclear if this technique can also be used in human beings to treat chronic diseases, such as atherosclerosis.

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

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

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

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

Keith, W. N. (2004). "From stem cells to cancer: balancing immortality and neoplasia." Oncogene **23**(29): 5092-5094.

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

Klingler, K., et al. (1988). "Transformation of single myeloid precursor cells by the malignant histiocytosis sarcoma virus (MHSV): generation of growth-factor-independent myeloid colonies and permanent cell lines." J Cell Physiol **135**(1): 32-38.

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

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

Stem cells possess two basic characteristics: they are able to renew themselves and to develop into different cell types. The link between normal stem cells and tumor cells could be examined in three aspects: what are the differences and similarities in the control of self-renewal capacity between stem cells and tumor cells; whether tumor cells arise from stem cells; do tumorous stem cells exist? Since tumor cells also exhibit self-renewal capacity, it seems plausible that their regulation is similar to that of the stem cells. The infinite self-renewal ability (immortalization) is

assured by several, so far only partly known, mechanisms. One of these is telomerase activity, another important regulatory step for survival is the inhibition of apoptosis. Other signal transduction pathways in stem cell regulation may also play certain roles in carcinogenesis: e.g. Notch, Sonic hedgehog (SHH), and Wnt signals. Existence of tumor stem cells was suggested since it is simpler to retain the self-renewal capacity than to reactivate the immortality program in an already differentiated cell. Moreover, stem cells live much longer than the differentiated ones, and so they are exposed for a long period of time to impairments, collecting gene errors leading to the breakdown of the regulation. However, it is still an open question whether all cells in the tumor possess the capacity that produces this tissue or not, that is: are there tumor stem cells or there are not. If tumor stem cells exist, they would be the main target for therapy: only these must be killed since the other tumor cells possess limited proliferative capacity, therefore limited life span. The only problem is that during tumor progression stem-like cells can develop continuously and the identification but mainly the prevention of their formation is still a great challenge.

Kornbluth, S. and R. Fissore (2015). "Vertebrate Reproduction." Cold Spring Harb Perspect Biol **7**(10): a006064.

Vertebrate reproduction requires a myriad of precisely orchestrated events-in particular, the maternal production of oocytes, the paternal production of sperm, successful fertilization, and initiation of early embryonic cell divisions. These processes are governed by a host of signaling pathways. Protein kinase and phosphatase signaling pathways involving Mos, CDK1, RSK, and PP2A regulate meiosis during maturation of the oocyte. Steroid signals-specifically testosterone-regulate spermatogenesis, as does signaling by G-protein-coupled hormone receptors. Finally, calcium signaling is essential for both sperm motility and fertilization. Altogether, this signaling symphony ensures the production of viable offspring, offering a chance of genetic immortality.

Kovalenko, O. A., et al. (2010). "Expression of (NES-)hTERT in cancer cells delays cell cycle progression and increases sensitivity to genotoxic stress." PLoS One **5**(5): e10812.

Telomerase is a reverse transcriptase associated with cellular immortality through telomere maintenance. This enzyme is activated in 90% of human cancers, and inhibitors of telomerase are currently in clinical trials to counteract tumor growth. Many aspects of telomerase biology have been investigated for therapy, particularly inhibition of the enzyme, but little was done regarding its subcellular shuttling. We have recently shown that mutations in the nuclear export signal of hTERT, the catalytic component of telomerase, led to a mutant ((NES-)hTERT) that failed to immortalize cells despite nuclear localization and catalytic activity. Expression of (NES-)hTERT in primary fibroblast resulted in telomere-based premature senescence and mitochondrial dysfunction. Here we show that expression of (NES-)hTERT in LNCaP, SQ20B and HeLa cells rapidly and significantly decreases their proliferation rate and ability to form colonies in soft agar while not interfering with endogenous telomerase activity. The cancer cells showed increased DNA damage at telomeric and extra-telomeric sites, and became sensitive to ionizing radiation and hydrogen peroxide exposures. Our data show that expression of (NES-)hTERT efficiently counteracts cancer cell growth in vitro in at least two different ways, and suggest manipulation with the NES of hTERT or its subcellular shuttling as a new strategy for cancer treatment.

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.

Kroll, J. (2005). "Chaperones and longevity." Biogerontology **6**(5): 357-361.

That evolution of longevity may depend on alterations in the expression of relatively few regulatory genes has been inferred from the rapid increase in lifespan during evolution of the hominid species (Cutler RG (1979) Mech Ageing Dev 9: 337-354). Also the inherent immortality of the embryonic stem cells implies that replicative senescence

(Hayflick L (1997) Biochem Mosc 62: 1180-1190) as possibly aging of species are epigenetic phenomena. Evidence is presented to suggest that the epigenetic changes of the longevity determinants to a significant extend concerns the molecular chaperones. Specific involvement of RNA chaperones in cell immortalization and defective RecQ-DNA chaperones in syndromes of premature aging suggest that DNA/RNA - chaperones probably rank high among the determinants of cellular and species longevity.

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

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

Lackner, D. H., et al. (2012). "Organismal propagation in the absence of a functional telomerase pathway in Caenorhabditis elegans." EMBO J **31**(8): 2024-2033.

To counteract replication-dependent telomere shortening most eukaryotic cells rely on the telomerase pathway, which is crucial for the maintenance of proliferative potential of germ and stem cell populations of multicellular organisms. Likewise, cancer cells usually engage the telomerase pathway for telomere maintenance to gain immortality. However, in approximately 10% of human cancers telomeres are maintained through telomerase-independent alternative lengthening of telomeres (ALT) pathways. Here, we describe the generation and characterization of C. elegans survivors in a strain lacking the catalytic subunit of telomerase and the nematode telomere-binding protein CeOB2. These clonal strains, some of which have been propagated for >180 generations, represent the first example of a multicellular organism with canonical telomeres that can survive without a functional telomerase pathway. The animals display the heterogeneous telomere length characteristic for ALT cells, contain single-stranded C-circles, a transcription profile pointing towards an adaptation to chronic stress and are therefore a unique and valuable tool to decipher the ALT mechanism.

Lapinska, K., et al. (2018). "Cancer Progenitor Cells: The Result of an Epigenetic Event?" Anticancer Res **38**(1): 1-6.

The concept of cancer stem cells was proposed in the late 1990s. Although initially the idea seemed controversial, the existence of cancer stem cells is now well established. However, the process leading to the formation of cancer stem cells is still not clear and thus requires further research. This article discusses epigenetic events that possibly produce cancer progenitor cells from predisposed cells by the influence of their environment. Every somatic cell possesses an epigenetic signature in terms of histone modifications and DNA methylation, which are obtained during lineage-specific differentiation of pluripotent stem cells, which is specific to that particular tissue. We call this signature an epigenetic switch. The epigenetic switch is not fixed. Our epigenome alters with aging. However, depending on the predisposition of the cells of a particular tissue and their microenvironment, the balance of the switch (histone modifications and the DNA methylation) may be tilted to immortality in a few cells, which generates cancer progenitor cells.

Laursen, M. B., et al. (2014). "Human B-cell cancer cell lines as a preclinical model for studies of drug effect in diffuse large B-cell lymphoma and multiple myeloma." Exp Hematol **42**(11): 927-938.

Drug resistance in cancer refers to recurrent or primary refractory disease following drug therapy. At the cellular level, it is a consequence of molecular functions that ultimately enable the cell to resist cell death-one of the classical hallmarks of cancer. Thus, drug resistance is a fundamental aspect of the cancer cell phenotype, in parallel with sustained proliferation, immortality, angiogenesis, invasion, and metastasis. Here we present a preclinical model of human B-cell cancer cell lines used to identify genes involved in specific drug resistance. This process includes a standardized technical setup for specific drug screening, analysis of global gene expression, and the statistical considerations required to develop resistance gene signatures. The state of the art is illustrated by the first-step classical drug screen (including the CD20 antibody rituximab, the DNA intercalating topoisomerase II inhibitor doxorubicin, the mitotic inhibitor vincristine, and the alkylating

agents cyclophosphamide and melphalan) along with the generation of gene lists predicting the chemotherapeutic outcome as validated retrospectively in clinical trial datasets. This B-cell lineage-specific preclinical model will allow us to initiate a range of laboratory studies, with focus on specific gene functions involved in molecular resistance mechanisms.

Lawrenz, B., et al. (2004). "Highly sensitive biosafety model for stem-cell-derived grafts." Cytotherapy **6**(3): 212-222.

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

Lee, M., et al. (2018). "Telomere sequence content can be used to determine ALT activity in tumours." Nucleic Acids Res **46**(10): 4903-4918.

The replicative immortality of human cancer cells is achieved by activation of a telomere maintenance mechanism (TMM). To achieve this, cancer cells utilise either the enzyme telomerase, or the Alternative Lengthening of Telomeres (ALT) pathway. These distinct molecular pathways are incompletely understood with respect to activation and propagation, as well as their associations with clinical outcomes. We have identified significant differences in the telomere repeat composition of tumours that use ALT compared to tumours that do not. We then employed a machine learning approach to stratify tumours according to telomere repeat content with an accuracy of 91.6%. Importantly, this classification approach is applicable across all tumour types. Analysis of pathway mutations that were under-represented in ALT tumours, across 1,075 tumour samples, revealed that the autophagy, cell cycle control of chromosomal replication, and transcriptional regulatory network in embryonic stem cells pathways are involved in the survival of ALT tumours. Overall, our approach demonstrates that telomere sequence content can be used to stratify ALT activity in cancers, and begin to define the molecular pathways involved in ALT activation.

Li, K., et al. (2023). "AKAP12 promotes cancer stem cell-like phenotypes and activates STAT3 in colorectal cancer." Clin Transl Oncol **25**(11): 3263-3276.

BACKGROUND: Cancer stem cells (CSCs) have unique biological characteristics, including tumorigenicity, immortality, and chemoresistance. Colorectal CSCs have been identified and isolated from colorectal cancers by various methods. AKAP12, a scaffolding protein, is considered to act as a potential suppressor in colorectal cancer, but its role in CSCs remains unknown. In this study, we investigated the function of AKAP12 in Colorectal CSCs. METHODS: Herein, Colorectal CSCs were enriched by cell culture with a serum-free medium. CSC-associated characteristics were evaluated by Flow cytometry assay and qPCR. AKAP12 gene expression was regulated by lentiviral transfection assay. The tumorigenicity of AKAP12 in vivo by constructing a tumor xenograft model. The related pathways were explored by qPCR and Western blot. RESULTS: The depletion of AKAP12 reduced colony formation, sphere formation, and expression of stem cell markers in colorectal cancer cells, while its knockdown decreased the volume and weight of tumor xenografts in vivo. AKAP12 expression levels also affected the expression of stemness markers associated with STAT3, potentially via regulating the expression of protein kinase C. CONCLUSION: This study suggests Colorectal CSCs overexpress AKAP12 and maintain stem cell characteristics through the AKAP12/PKC/STAT3 pathway. AKAP12 may be an

important therapeutic target for blocking the development of colorectal cancer in the field of cancer stem cells.

Li Wan Po, A. (2020). "Genomic research delivering on promises: From rejuvenation to vaccines and pharmacogenetics." J Clin Pharm Ther **45**(3): 585-589.

WHAT IS KNOWN AND OBJECTIVE: There has been astounding progress made in the treatment of disease over recent years. This progress is particularly marked in cell therapy and in the personalization of therapy based on genetic insight, an approach known as genomic medicine. Our objective is to comment on the progress made in cell and genomic medicine against an historical backcloth of the search for rejuvenation. COMMENT: In 1741, close to seven decades after Antoine van Leeuwenhoek first saw his microscopic animalcules, Abraham Trembley, a tutor in Leiden, reported on an organism that could regenerate itself. The strange organism was thought to hold the secret of life. If it does, we have yet to prise the secret out. However, the ensuing study of cell programming and induced stem cells has shed considerable light on cellular development and provided new insights on the rejuvenative capacity of organisms. Inventive scientists have provided a deeper understanding of cell replication and, from this, developed new medicines for an increasing range of diseases. Targeted therapies, oligonucleotide therapy, therapeutic monoclonal antibodies and pharmacogenetics are all new therapeutic areas originating from the improved insights. More will surely follow. WHAT IS NEW AND CONCLUSION: Immortality is for the gods, but man's search for its elusive secrets, perhaps as old as man himself, will continue. Huge leaps have been made, and effective medicines have been developed from our improved insights into the mechanism of life. However, only the foolish will predict how far this new knowledge will lead us, and more particularly, at what speed new therapies will follow.

Li, Y., et al. (2021). "ZNF217: the cerberus who fails to guard the gateway to lethal malignancy." Am J Cancer Res **11**(7): 3378-3405.

The aberrant expression of the zinc finger protein 217 (ZNF217) promotes multiple malignant phenotypes, such as replicative immortality, maintenance of proliferation, malignant heterogeneity, metastasis, and cell death resistance, via diverse mechanisms, including transcriptional activation, mRNA N(6)-methyladenosine (m(6)A) regulation, and protein interactions. The induction of these cellular processes by ZNF217 leads to therapeutic resistance and patients' poor outcomes. However, few ZNF217 related clinical applications or trials, have been reported. Moreover, looming observations about ZNF217 roles in m(6)A regulation and cancer immune response triggered significant attention while lacking critical evidence. Thus, in this review, we revisit the literature about ZNF217 and emphasize its importance as a prognostic biomarker for early prevention and as a therapeutic target.

Liggett, L. A. and J. DeGregori (2017). "Changing mutational and adaptive landscapes and the genesis of cancer." Biochim Biophys Acta Rev Cancer **1867**(2): 84-94.

By the time the process of oncogenesis has produced an advanced cancer, tumor cells have undergone extensive evolution. The cellular phenotypes resulting from this evolution have been well studied, and include accelerated growth rates, apoptosis resistance, immortality, invasiveness, and immune evasion. Yet with all of our current knowledge of tumor biology, the details of early oncogenesis have been difficult to observe and understand. Where different oncogenic mutations may work together to enhance the survival of a tumor cell, in isolation they are often pro-apoptotic, pro-differentiative or pro-senescent, and therefore often, somewhat paradoxically, disadvantageous to a cell. It is also becoming clear that somatic mutations, including those in known oncogenic drivers, are common in tissues starting at a young age. These observations raise the question: how do we largely avoid cancer for most of our lives? Here we propose that evolutionary forces can help explain this paradox. As humans and other organisms age or experience external insults such as radiation or smoking, the structure and function of tissues progressively degrade, resulting in altered stem cell niche microenvironments. As tissue integrity declines, it becomes less capable of supporting and maintaining resident stem cells. These stem cells then find themselves in a microenvironment to which they are poorly adapted, providing a competitive advantage to those cells that can restore their functionality and fitness through mutations or epigenetic changes. The resulting oncogenic clonal expansions then increase the odds of further cancer progression. Understanding how the causes of cancer, such as aging or smoking, affect tissue microenvironments to control the impact of mutations on somatic cell fitness can help reconcile the discrepancy between marked mutation accumulation starting early in life and the somatic evolution that leads to cancer at advanced ages or following carcinogenic insults. This article is part of a Special Issue entitled: Evolutionary principles - heterogeneity in cancer?, edited by Dr. Robert A. Gatenby.

Lin, K. W. and J. Yan (2005). "The telomere length

dynamic and methods of its assessment." J Cell Mol Med **9**(4): 977-989.

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

Liu, J. P. and R. Chen (2015). "Stressed SIRT7: facing a crossroad of senescence and immortality." Clin Exp Pharmacol Physiol **42**(6): 567-569.

SIRT7 with coenzyme NAD catalyzes protein de-acetylation. In stress response, SIRT7 regulates protein folding in mitochondria with unknown mechanisms. Decreases in SIRT7 entrain hematopoietic stem cell senescence, but increasing SIRT7 causes elevation of hematopoietic stem cell regenerative function. We discuss the recent findings on SIRT7 and its binding proteins, NRF1 and GABPbeta1, in decision making between the choices of inducing cell aging and immortality.

Liu, N., et al. (2020). "Role of telomerase in the tumour microenvironment." Clin Exp Pharmacol Physiol **47**(3): 357-364.

Telomeres are specialized genomic structures that protect chromosomal ends to maintain genomic stability. Telomeric length is primarily regulated by the telomerase complex, essentially consisting of an RNA template (TERC), an enzymatic subunit (telomerase reverse transcriptase, TERT). In humans, telomerase activity is repressed during embryonic differentiation and is absent in most somatic cells. However, it is upregulated or reactivated in 80%-90% of the primary tumours in humans. The human TERT (hTERT) plays a pivotal role in cellular immortality and tumourigenesis. However, the molecular mechanisms of telomerase functioning in cancer have not been fully understood beyond the telomere maintenance. Several research groups, including ours, have demonstrated that hTERT possesses vital functions independent of its telomere maintenance, including angiogenesis, inflammation, cancer cell stemness, and epithelial-mesenchymal transformation (EMT). All these telomere-independent activities of hTERT may contribute to the regulation of the dynamics and homeostasis of the tumour microenvironment (TME), thereby promoting tumour growth and development. Cancer progression and metastasis largely depend upon the interactions between cancer cells and their microenvironment. In this review, the involvement of TERT in the tumour microenvironment and the underlying implications in cancer therapeutics have been summarized.

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

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

Marczynska, B., et al. (1991). "Phorbol ester promotes growth and transformation of carcinogen-exposed nonhuman primate cells in vitro." Anticancer Res **11**(5): 1711-1717.

Kidney cells established in vitro from a white-lipped marmoset (106) were exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) alone or in combination with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Low (0.1 micrograms/ml, 4 times), intermediate (1 microgram/ml) and high (1 microgram/ml, 4 times) doses of MNNG resulted in 100%, 50% and 2.8% of cell survival, respectively.

High and low doses of MNNG had no effect on cell transformation. Upon exposure of cells to an intermediate dose of MNNG, 106 cells ecquired immortality and evolved into permanent cell line, 106-1M. However, the cells retained normal morphology and anchorage dependence. Chronic applications of TPA (0.1 micrograms/ml, 13 times) promoted 106-1M cells to morphological transformation and anchorage-independent growth but not to tumorigenicity in nude mice (106-1MT cell line). Chromosome analysis revealed only numerical changes in 106 cells and both numerical and structural aberrations in transformed 106-1MT cells. These changes in marmoset cells usually reflected cell culture instability leading to either senescence or to longer survival of cells in vitro. Chronic treatment with TPA did not result in downregulation of protein kinase C (PKC) in transformed 106-1MT cells. Instead, an additional species of PKC appeared in these cells.

Matzuk, M. M. (2004). "Germ-line immortality." Proc Natl Acad Sci U S A **101**(47): 16395-16396.

McCulloch, E. A., et al. (1984). "Hemopoietic stem cells: their roles in human leukemia and certain continuous cell lines." J Cell Physiol Suppl **3**: 13-20.

Hemopoietic stem cells may give rise to progeny like themselves or undergo determination; this event is followed by a series of maturation divisions ending in proliferatively inert but functional cells. In normal hemopoiesis and acute leukemia stem cell renewal is not exact; proliferative capacity is lost gradually. As a consequence, clonal populations cannot be continued indefinitely. Postdeterministic differentiation normally leads to cellular diversity; following transformation this diversity is increased, with the production of blast cells together with one or more myelopoietic lineage. The blasts are heterogeneous both in their proliferative capacity and their phenotypes, as determined using immunologically defined markers. Both self-renewal and determination are considered to be irreversible in vivo. By contrast, in continuous myelopoietic cell lines self-renewal is sufficiently precise to confer immortality on the populations. Furthermore, both determination and renewal may in some instances be reversible. The differences between normal or leukemic hemopoiesis in vivo and continuous lines in culture limits the value of the latter for studies of normal blood formation or the clonal hemopathies.

McLaren, A. (1992). "Embryology. The quest for immortality." Nature **359**(6395): 482-483.

McLaren, A. (2001). "Mammalian germ cells: birth, sex, and immortality." Cell Struct Funct **26**(3): 119-122.

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

Menendez, J. A., et al. (2012). "Metformin is synthetically lethal with glucose withdrawal in cancer cells." Cell Cycle **11**(15): 2782-2792.

Glucose deprivation is a distinctive feature of the tumor microecosystem caused by the imbalance between poor supply and an extraordinarily high consumption rate. The metabolic reprogramming from mitochondrial respiration to aerobic glycolysis in cancer cells (the "Warburg effect") is linked to oncogenic transformation in a manner that frequently implies the inactivation of metabolic checkpoints such as the energy rheostat AMP-activated protein kinase (AMPK). Because the concept of synthetic lethality in oncology can be applied not only to genetic and epigenetic intrinsic differences between normal and cancer cells but also to extrinsic ones such as altered microenvironment, we recently hypothesized that stress-energy mimickers such as the AMPK agonist metformin should produce metabolic synthetic lethality in a glucose-starved cell culture milieu imitating the adverse tumor growth conditions in vivo. Under standard high-glucose conditions, metformin supplementation mostly caused cell cycle arrest without signs of apoptotic cell death. Under glucose withdrawal stress, metformin supplementation circumvented the ability of oncogenes (e.g., HER2) to protect breast cancer cells from glucose-deprivation apoptosis. Significantly, representative cell models of breast cancer heterogeneity underwent massive

apoptosis (by >90% in some cases) when glucose-starved cell cultures were supplemented with metformin. Our current findings may uncover crucial issues regarding the cell-autonomous metformin's anti-cancer actions: (1) The offently claimed clinically irrelevant, non-physiological concentrations needed to observe the metformin's anti-cancer effects in vitro merely underlie the artifactual interference of erroneous glucose-rich experimental conditions that poorly reflect glucose-starved in vivo conditions; (2) the preferential killing of cancer stem cells (CSC) by metformin may simply expose the best-case scenario for its synthetically lethal activity because an increased dependency on Warburg-like aerobic glycolysis (hyperglycolytic phenotype) is critical to sustain CSC stemness and immortality; (3) the microenvironment-mediated contextual synthetic lethality of metformin should be expected to enormously potentiate the anti-cancer effect of anti-angiogenesis agents that promote severe oxygen and glucose deprivation in certain areas of the tumor tissues.

Merle, P. and C. Trepo (2009). "Molecular mechanisms underlying hepatocellular carcinoma." Viruses **1**(3): 852-872.

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

Minh-Thai, T. N., et al. (2021). "A Comprehensive Conceptual and Computational Dynamics Framework for Autonomous Regeneration Systems." Artif Life **27**(2): 80-104.

Many biological organisms regenerate structure and function after damage. Despite the long history of research on molecular mechanisms, many questions remain about algorithms by which cells can cooperate towards the same invariant morphogenetic outcomes. Therefore, conceptual frameworks are needed not only for motivating hypotheses for advancing the understanding of regeneration processes in living organisms, but also for regenerative medicine and synthetic biology. Inspired by planarian regeneration, this study offers a novel generic conceptual framework that hypothesizes mechanisms and algorithms by which cell collectives may internally represent an anatomical target morphology towards which they build after damage. Further, the framework contributes a novel nature-inspired computing method for self-repair in engineering and robotics. Our framework, based on past in vivo and in silico studies on planaria, hypothesizes efficient novel mechanisms and algorithms to achieve complete and accurate regeneration of a simple in silico flatwormlike organism from any damage, much like the body-wide immortality of planaria, with minimal information and algorithmic complexity. This framework that extends our previous circular tissue repair model integrates two levels of organization: tissue and organism. In Level 1, three individual in silico tissues (head, body, and tail-each with a large number of tissue cells and a single stem cell at the centre) repair themselves through efficient local communications. Here, the contribution extends our circular tissue model to other shapes and invests them with tissue-wide immortality through an information field holding the minimum body plan. In Level 2, individual tissues combine to form a simple organism. Specifically, the three stem cells form a network that coordinates organism-wide regeneration with the help of Level 1. Here we contribute novel concepts for collective decision-making by stem cells for stem cell regeneration and large-scale recovery. Both levels (tissue cells and stem cells) represent networks that perform simple neural computations and form a feedback control system. With simple and limited cellular computations, our framework minimises computation and algorithmic complexity to achieve complete recovery. We report results from computer simulations of the framework to demonstrate its robustness in recovering the organism after any injury. This comprehensive hypothetical framework that significantly extends the existing biological regeneration models offers a new way to conceptualise the information-processing aspects of regeneration, which may also help design living and non-living self-

repairing agents.

Mochnacky, F., et al. (2021). ""Neural stem cells puzzle" missing piece: telomerase reverse transcriptase expression patterns during nervous system ontogenesis in rats." J Physiol Pharmacol **72**(6).

For almost three decades, neural stem cells remain still up-to-date and enigmatic topic. The main problem for their studying is the non-existence of an exclusive neural stem cell marker and the heterogeneity of them across the nervous system. As one of the novel markers of neural stem/progenitor cells may serve telomerase reverse transcriptase (TERT), a catalytic subunit of the telomerase enzyme, responsible for retaining the cell immortality. Thus, the aim of our study was to reveal if TERT, as an enzyme for ensuring the immortality of proliferating cells, could be used as a potential marker of neural stem/progenitor cells during the ontogenesis of the rat central nervous system. In this study, we used various markers related to neural stem or progenitor cells character and examined their co-localization with TERT expression. Our experiments were performed on the tissue of the brain and spinal cord during several stages of postnatal development and the neural tube during the 14(th) embryonal day. Cytoplasmatic TERT expression was found in alar plate progenitors and ventral horn neuroblasts of E14 rats. In the postnatal stages of spinal cord ontogenesis, a cytoplasmatic expression in neurons and nuclear expression in astrocytes was defined. In the brain, nuclear TERT expression was found in neural progenitor cells of neurogenic areas. This study provides the first comparative study of TERT expression across the central nervous system ontogenesis. The nuclear presence of TERT may be used as a potential marker of neural stem/progenitor cells, however, further studies are required to confirm these assumptions.

Monk, M., et al. (2001). "Isolation of novel developmental genes from human germ cell, oocyte and embryo cDNA by differential display." Reprod Fertil Dev **13**(1): 51-57.

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

Morris, B. J., et al. (2015). "FOXO3: A Major Gene for Human Longevity--A Mini-Review." Gerontology **61**(6): 515-525.

BACKGROUND: The gene FOXO3, encoding the transcription factor forkhead box O-3 (FoxO3), is one of only two for which genetic polymorphisms have exhibited consistent associations with longevity in diverse human populations. OBJECTIVE: Here, we review the multitude of actions of FoxO3 that are relevant to health, and thus healthy ageing and longevity. METHODS: The study involved a literature search for articles retrieved from PubMed using FoxO3 as keyword. RESULTS: We review the molecular genetics of FOXO3 in longevity, then current knowledge of FoxO3 function relevant to ageing and lifespan. We describe how FoxOs are involved in energy metabolism, oxidative stress, proteostasis, apoptosis, cell cycle regulation, metabolic processes, immunity, inflammation and stem cell maintenance. The single FoxO in Hydra confers immortality to this fresh water polyp, but as more complex organisms evolved, this role has been usurped by the need for FoxO to control a broader range of specialized pathways across a wide spectrum of tissues assisted by the advent of as many as 4 FoxO subtypes in mammals. The major themes of FoxO3 are similar, but not identical, to other FoxOs and include regulation of cellular homeostasis, particularly of stem cells, and of inflammation, which is a common theme of age-related diseases. Other functions concern metabolism, cell cycle arrest, apoptosis, destruction of potentially damaging reactive oxygen species and proteostasis. CONCLUSIONS: The mechanism by which longevity-associated alleles of FOXO3 reduce age-related mortality is currently of great clinical

interest. The prospect of optimizing FoxO3 activity in humans to increase lifespan and reduce age-related diseases represents an exciting avenue of clinical investigation. Research strategies directed at developing therapeutic agents that target FoxO3, its gene and proteins in the pathway(s) FoxO3 regulates should be encouraged and supported.

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

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

Nakamura, H., et al. (2019). "Pulmonary carcinosarcoma characterized by small round cells with neuroendocrine, myogenic, and chondrogenic differentiation: An extremely rare case." Pathol Int **69**(5): 282-287.

Carcinosarcoma is a clonal tumor developed through sarcomatoid changes in a carcinoma via the epithelial-mesenchymal transition (EMT). Here, we present an extremely rare case of pulmonary carcinosarcoma characterized by components suggesting pluripotency, namely neuroendocrine, myogenic, and chondrogenic differentiation, based on immunohistochemical analysis. A 42-year-old Japanese man was admitted to our hospital. Analysis of tumor tissue after right upper lobe lobectomy revealed a transition between carcinomatous and sarcomatous components. Immunohistochemical analysis suggested immortality owing to complete loss of p53 and diffuse expression of p16 in both the carcinomatous and sarcomatous components. There were also scattered cell groups expressing aldehyde dehydrogenase 1 family member A1, SOX2, CD133, and c-kit, suggesting the possible presence of cancer stem cells. Our findings in this case suggested that the EMT may play a key role in mediating the immortality of tumor cells in carcinosarcoma and facilitating the pluripotency of cancer stem cells.

Negishi, Y., et al. (2000). "Multipotency of a bone marrow stromal cell line, TBR31-2, established from ts-SV40 T antigen gene transgenic mice." Biochem Biophys Res Commun **268**(2): 450-455.

Bone marrow is believed to contain multipotential stromal stem cells which can differentiate into osteoblasts, chondrocytes, adipocytes, and myoblasts (Prockop, D. J. Science 276, 71-74, 1997). Therefore, characterization and identification of the stem-like cell within the stromal cells are important to understand bone marrow function in relation to the hematopoietic microenvironment, and repair/regeneration of tissue defects. TBR31-2 cell, a bone marrow stromal cell line established from bone marrow of transgenic mice harboring temperature-sensitive (ts) simian virus (SV) 40T-antigen gene for immortality, is induced toward both adipocytic and osteogenic cells under conditions of the inactivation of T-antigen (Okuyama, R., Yanai, N., Obinata, M. Exp. Cell Res. 218, 424-429, 1995). In this work, using a semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, mRNA expressions of tissue-specific differentiation markers for adipocyte (lipoprotein lipase), osteoblast (type I collagen and osteocalcin), chondrocyte (type II and X collagen), and muscle cell (desmin) were examined during a long-term culture of the cell. In addition, histochemical studies showed the appearance of adipocytic, osteoblastic, chondrocytic, and muscle cells during this long-term culture. Thus, TBR31-2, which has characteristics of an undifferentiated cell, has the potential to express the multipotential cell lineages. These results indicated that a multipotential progenitor cell including potential to differentiate into a muscle cell and which is situated in the mesenchymal cell lineage was first obtained.

Negrini, S., et al. (2020). "Anti-cancer Immunotherapies Targeting Telomerase." Cancers (Basel) **12**(8).

Telomerase is a reverse transcriptase that maintains telomeres length, compensating for the attrition of chromosomal ends that occurs during each replication cycle. Telomerase is expressed in germ cells and stem cells, whereas it is virtually undetectable in adult somatic cells. On the other hand, telomerase is broadly expressed in the majority of human tumors playing a crucial role in the replicative behavior and immortality of cancer cells. Several

studies have demonstrated that telomerase-derived peptides are able to bind to HLA (human leukocyte antigen) class I and class II molecules and effectively activate both CD8(+) and CD4(+) T cells subsets. Due to its broad and selective expression in cancer cells and its significant immunogenicity, telomerase is considered an ideal universal tumor-associated antigen, and consequently, a very attractive target for anti-cancer immunotherapy. To date, different telomerase targeting immunotherapies have been studied in pre-clinical and clinical settings, these approaches include peptide vaccination and cell-based vaccination. The objective of this review paper is to discuss the role of human telomerase in cancer immunotherapy analyzing recent developments and future perspectives in this field.

Nelson, J. O., et al. (2019). "Germline stem cell homeostasis." Curr Top Dev Biol **135**: 203-244.

In many species, germline stem cells (GSCs) function to sustain gametogenesis throughout the life of organismal life span. As the source of gametes, the only cell type that can pass the genetic information to the next generation, GSCs play a fundamental role in maximizing the quantity of gametes that animals produce, while ensuring their highest quality. GSCs are maintained by the signals from their niches, and germ cells that exited the niche undergo differentiation to generate functional gametes. GSC population is sustained by a multitude of mechanisms such as asymmetric stem cell divisions and dedifferentiation of partially differentiated germ cells. In this review, we summarize the mechanisms that maintain GSC homeostasis to ensure life-long production of functional gametes.

Nelson, J. O., et al. (2023). "rDNA magnification is a unique feature of germline stem cells." Proc Natl Acad Sci U S A **120**(47): e2314440120.

Ribosomal DNA (rDNA) encodes ribosomal RNA and exists as tandem repeats of hundreds of copies in the eukaryotic genome to meet the high demand of ribosome biogenesis. Tandemly repeated DNA elements are inherently unstable; thus, mechanisms must exist to maintain rDNA copy number (CN), in particular in the germline that continues through generations. A phenomenon called rDNA magnification was discovered over 50 y ago in Drosophila as a process that recovers the rDNA CN on chromosomes that harbor minimal CN. Our recent studies indicated that rDNA magnification is the mechanism to maintain rDNA CN under physiological conditions to counteract spontaneous CN loss that occurs during aging. Our previous studies that explored the mechanism of rDNA magnification implied that asymmetric division of germline stem cells (GSCs) may be particularly suited to achieve rDNA magnification. However, it remains elusive whether GSCs are the unique cell type that undergoes rDNA magnification or differentiating germ cells are also capable of magnification.

Noormohammadi, A., et al. (2018). "Mechanisms of protein homeostasis (proteostasis) maintain stem cell identity in mammalian pluripotent stem cells." Cell Mol Life Sci **75**(2): 275-290.

Protein homeostasis, or proteostasis, is essential for cell function, development, and organismal viability. The composition of the proteome is adjusted to the specific requirements of a particular cell type and status. Moreover, multiple metabolic and environmental conditions challenge the integrity of the proteome. To maintain the quality of the proteome, the proteostasis network monitors proteins from their synthesis through their degradation. Whereas somatic stem cells lose their ability to maintain proteostasis with age, immortal pluripotent stem cells exhibit a stringent proteostasis network associated with their biological function and intrinsic characteristics. Moreover, growing evidence indicates that enhanced proteostasis mechanisms play a central role in immortality and cell fate decisions of pluripotent stem cells. Here, we will review new insights into the melding fields of proteostasis and pluripotency and their implications for the understanding of organismal development and survival.

Noormohammadi, A., et al. (2016). "Somatic increase of CCT8 mimics proteostasis of human pluripotent stem cells and extends C. elegans lifespan." Nat Commun **7**: 13649.

Human embryonic stem cells can replicate indefinitely while maintaining their undifferentiated state and, therefore, are immortal in culture. This capacity may demand avoidance of any imbalance in protein homeostasis (proteostasis) that would otherwise compromise stem cell identity. Here we show that human pluripotent stem cells exhibit enhanced assembly of the TRiC/CCT complex, a chaperonin that facilitates the folding of 10% of the proteome. We find that ectopic expression of a single subunit (CCT8) is sufficient to increase TRiC/CCT assembly. Moreover, increased TRiC/CCT complex is required to avoid aggregation of mutant Huntingtin protein. We further show that increased expression of CCT8 in somatic tissues extends Caenorhabditis elegans lifespan in a TRiC/CCT-dependent manner. Ectopic expression of CCT8 also ameliorates the age-associated demise of proteostasis and corrects proteostatic deficiencies in worm models of Huntington's disease. Our results suggest proteostasis is a common principle that links organismal longevity

with hESC immortality.

Obradovic, D. (2025). "Five-factor theory of aging and death due to aging." Arch Gerontol Geriatr **129**: 105665.

This new theory of aging explains that aging and death due to aging are due to five factors, and also explains how these factors are interconnected and jointly lead to aging and death of the organism, pointing to many facts that strongly support it. The first factor is the harmful changes that occur in cellular structures. The second factor is the cessation of cell division in adult organisms, which leads to the inability to restore cellular structures. The third factor is the feature that cells do not die due to the accumulation of harmful changes that occur in the cells during the life of the organism. The fourth factor is the inability of stem cells to regenerate tissue by replacing such cells with new ones, because somatic cells do not die and there are no signals that stimulate the proliferation of stem cells and their differentiation into new ones that would replace dead cells. The fifth factor is that all cells die suddenly, due to the cessation of one of the vital functions of the organism, and not gradually during life, due to a decrease in the functionality of cells caused by the introduction of harmful changes in cellular structures, which would allow stem cells to regenerate tissues and keep the body young. Also, to show that this aging theory is valid, the theory gives its view of the evolution of five factors, which according to this theory lead to aging, which gives strong support to this theory.

Ohgushi, M., et al. (2015). "Rho-Signaling-Directed YAP/TAZ Activity Underlies the Long-Term Survival and Expansion of Human Embryonic Stem Cells." Cell Stem Cell **17**(4): 448-461.

Human embryonic stem cells (hESCs) can survive and proliferate for an extended period of time in culture, but unlike that of tumor-derived cells, this form of cellular immortality does not depend on genomic aberrations. In this study, we sought to elucidate the molecular basis of this long-term growth property of hESCs. We found that the survival of hESCs depends on the small GTPase Rho and its activator AKAP-Lbc. We show that AKAP-Lbc/Rho signaling sustains the nuclear function of the transcriptional cofactors YAP and TAZ by modulating actin microfilament organization. By inducing reprogramming and differentiation, we found that dependency on this Rho signaling pathway is associated with the pluripotent state.

Oshimura, M., et al. (1988). "Cytogenetic changes in rat tracheal epithelial cells during early stages of carcinogen-induced neoplastic progression." Cancer Res **48**(3): 702-708.

The cytogenetic changes in enhanced growth (EG) variants of rat tracheal epithelial cells in culture were examined. These variants which are detectable at 35 days after carcinogen exposure are the first phenotypic alteration in the multistep neoplastic process studied in this model system. Karyotypic analysis of N-methyl-N'-nitro-N-nitrosoguanidine-induced EG variants at Day 35 was made possible by the development of an in situ method of cytogenetic analysis on intact colonies containing too few cells for conventional chromosome preparation methods. Of the transformed EG variant colonies in both control and N-methyl-N'-nitro-N-nitrosoguanidine-treated groups, 62-78% had abnormal karyotypes which included numerical and structural changes. There were no specific chromosome changes, although aberrations of chromosomes 3 and 4 were recurrently observed. However, some colonies of even the most morphologically transformed EG variants were composed of only diploid cells. To confirm this finding 10 EG variant colonies were bisected and half of the clone was prepared for chromosome analysis and the other half was subcultured to measure the clonogenicity and karyotypes of the cells. Cells from 3 colonies plated very poorly on 3T3 feeders and therefore no karyotypic analysis of the colony-forming cells was possible; the cells of the 3 parental colonies were diploid.

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.

Pascual-Torner, M., et al. (2022). "Comparative genomics of mortal and immortal cnidarians unveils novel keys behind rejuvenation." Proc Natl Acad Sci U S A **119**(36): e2118763119.

Turritopsis dohrnii is the only metazoan able to rejuvenate repeatedly after its medusae reproduce, hinting at biological immortality and challenging our understanding of aging. We present and compare whole-genome assemblies of T. dohrnii and the nonimmortal Turritopsis rubra using automatic and manual annotations, together with the transcriptome of life cycle reversal (LCR) process of T. dohrnii. We have identified variants and expansions of genes associated with replication, DNA repair, telomere maintenance, redox environment, stem cell population, and intercellular communication. Moreover, we have found silencing of polycomb repressive complex 2 targets and activation of pluripotency targets during LCR, which points to these transcription factors as pluripotency inducers in T. dohrnii. Accordingly, we propose these factors as key elements in the ability of T. dohrnii to undergo rejuvenation.

Pech, M. F., et al. (2015). "High telomerase is a hallmark of undifferentiated spermatogonia and is required for maintenance of male germline stem cells." Genes Dev **29**(23): 2420-2434.

Telomerase inactivation causes loss of the male germline in worms, fish, and mice, indicating a conserved dependence on telomere maintenance in this cell lineage. Here, using telomerase reverse transcriptase (Tert) reporter mice, we found that very high telomerase expression is a hallmark of undifferentiated spermatogonia, the mitotic population where germline stem cells reside. We exploited these high telomerase levels as a basis for purifying undifferentiated spermatogonia using fluorescence-activated cell sorting. Telomerase levels in undifferentiated spermatogonia and embryonic stem cells are comparable and much greater than in somatic progenitor compartments. Within the germline, we uncovered an unanticipated gradient of telomerase activity that also enables isolation of more mature populations. Transcriptomic comparisons of Tert(High) undifferentiated spermatogonia and Tert(Low) differentiated spermatogonia by RNA sequencing reveals marked differences in cell cycle and key molecular features of each compartment. Transplantation studies show that germline stem cell activity is confined to the Tert(High) cKit(-) population. Telomere shortening in telomerase knockout strains causes depletion of undifferentiated spermatogonia and eventual loss of all germ cells after undifferentiated spermatogonia drop below a critical threshold. These data reveal that high telomerase expression is a fundamental characteristic of germline stem cells, thus explaining the broad dependence on telomerase for germline immortality in metazoans.

Peng, W. M., et al. (2002). "Transplanted neuronal precursors migrate and differentiate in the developing mouse brain." Cell Res **12**(3-4): 223-228.

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

Petersen, I. (2021). "Classification and Treatment of Diseases in the Age of Genome Medicine Based on Pathway Pathology." Int J Mol Sci **22**(17).

The focus of pathology as a biomedical discipline is the identification of the pathomechanisms of diseases and the integration of this knowledge into routine diagnosis and classification. Standard tools are macroscopic and microscopic analysis complemented by immunohistochemistry and molecular pathology. So far, classification has been based on the paradigm of cellular pathology established by Rudolf Virchow and others more than 150 years ago, stating that diseases originate from diseased cells. This dogma is meanwhile challenged by the fact that cells can be fully reprogrammed. Many diseases are nowadays

considered to originate from undifferentiated stem cells, induced into a diseased state by genetic or epigenetic alterations. In addition, the completion of the Human Genome Project, with the identification of more than 20.000 genes and a much higher number of gene variants and mutations, led to the concept that diseases are dominated by genetics/epigenetics rather than cells of origin. The axiom of cellular pathology, however, still holds true, as cells are the smallest animate units from which diseases originate. Medical doctors and researchers nowadays have to deal with a tremendous amount of data. The International Classification of Diseases will expand from 14.400 entities/codes in ICD-10 to more than 55.000 in ICD-11. In addition, large datasets generated by "genomics", e.g., whole-genome sequencing, expression profiling or methylome analysis, are meanwhile not only applied in research but also introduced into clinical settings. It constitutes a major task to incorporate all the data into routine medical work. Pathway pathology may help solve this problem. It is based on the realization that diseases are characterized by three essential components: (i) cells of origin/cellular context and (ii) the alteration of cellular as well as (iii) molecular/signal transduction pathways. The concept is illustrated by elaborating on two key cellular pathways, i.e., the cellular senescence of normal cells and the immortality of cancer cells, and by contrasting single cell/single pathway diseases, such as mycoplasma and coughing pneumonia, with complex diseases such as cancer, with multiple cell types as well as multiple affected cellular and signaling pathways. Importantly, the concept of pathway pathology is not just intended to classify disease, but also to conceive new treatment modalities. This article is dedicated to Dr. Leonard Hayflick, who made basic discoveries in pathway pathology not only by identifying cells causing disease (Mycoplasma pneumoniae) and establishing cell strains for treating disease (WI-38 for viral vaccines), but also by first describing cellular senescence and immortality.

Petralia, R. S., et al. (2014). "Aging and longevity in the simplest animals and the quest for immortality." Ageing Res Rev **16**: 66-82.

Here we review the examples of great longevity and potential immortality in the earliest animal types and contrast and compare these to humans and other higher animals. We start by discussing aging in single-celled organisms such as yeast and ciliates, and the idea of the immortal cell clone. Then we describe how these cell clones could become organized into colonies of different cell types that lead to multicellular animal life. We survey aging and longevity in all of the basal metazoan groups including ctenophores (comb jellies), sponges, placozoans, cnidarians (hydras, jellyfish, corals and sea anemones) and myxozoans. Then we move to the simplest bilaterian animals (with a head, three body cell layers, and bilateral symmetry), the two phyla of flatworms. A key determinant of longevity and immortality in most of these simple animals is the large numbers of pluripotent stem cells that underlie the remarkable abilities of these animals to regenerate and rejuvenate themselves. Finally, we discuss briefly the evolution of the higher bilaterians and how longevity was reduced and immortality lost due to attainment of greater body complexity and cell cycle strategies that protect these complex organisms from developing tumors. We also briefly consider how the evolution of multiple aging-related mechanisms/pathways hinders our ability to understand and modify the aging process in higher organisms.

Pfeffer, N. (2008). "What British women say matters to them about donating an aborted fetus to stem cell research: a focus group study." Soc Sci Med **66**(12): 2544-2554.

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

had co-produced a tendency to refuse to donate aborted fetuses.

Pilsworth, J. A., et al. (2018). "TERT promoter mutation in adult granulosa cell tumor of the ovary." Mod Pathol **31**(7): 1107-1115.

The telomerase reverse transcriptase (TERT) gene is highly expressed in stem cells and silenced upon differentiation. Cancer cells can attain immortality by activating TERT to maintain telomere length and telomerase activity, which is a crucial step of tumorigenesis. Two somatic mutations in the TERT promoter (C228T; C250T) have been identified as gain-of-function mutations that promote transcriptional activation of TERT in multiple cancers, such as melanoma and glioblastoma. A recent study investigating TERT promoter mutations in ovarian carcinomas found C228T and C250T mutations in 15.9% of clear cell carcinomas. However, it is unknown whether these mutations are frequent in other ovarian cancer subtypes, in particular, sex cord-stromal tumors including adult granulosa cell tumors. We performed whole-genome sequencing on ten adult granulosa cell tumors with matched normal blood and identified a TERT C228T promoter mutation in 50% of tumors. We found that adult granulosa cell tumors with mutated TERT promoter have increased expression of TERT mRNA and exhibited significantly longer telomeres compared to those with wild-type TERT promoter. Extension cohort analysis using allelic discrimination revealed the TERT C228T mutation in 51 of 229 primary adult granulosa cell tumors (22%), 24 of 58 recurrent adult granulosa cell tumors (41%), and 1 of 22 other sex cord-stromal tumors (5%). There was a significant difference in overall survival between patients with TERT C228T promoter mutation in the primary tumors and those without it ($p = 0.00253$, log-rank test). In seven adult granulosa cell tumors, we found the TERT C228T mutation present in recurrent tumors and absent in the corresponding primary tumor. Our data suggest that TERT C228T promoter mutations may have an important role in progression of adult granulosa cell tumors.

Piper, S. L., et al. (2012). "Inducible immortality in hTERT-human mesenchymal stem cells." J Orthop Res **30**(12): 1879-1885.

Human mesenchymal stem cells (hMSCs) are attractive candidates for tissue engineering and cell-based therapy because of their multipotentiality and availability in adult donors. However, in vitro expansion and differentiation of these cells is limited by replicative senescence. The proliferative capacity of hMSCs can be enhanced by ectopic expression of telomerase, allowing for long-term culture. However, hMSCs with constitutive telomerase expression demonstrate unregulated growth and even tumor formation. To address this problem, we used an inducible Tet-On gene expression system to create hMSCs in which ectopic telomerase expression can be induced selectively by the addition of doxycycline (i-hTERT hMSCs). i-hTERT hMSCs have inducible hTERT expression and telomerase activity, and are able to proliferate significantly longer than wild type hMSCs when hTERT expression is induced. They stop proliferating when hTERT expression is turned off and can be rescued when expression is re-induced. They retain multipotentiality in vitro even at an advanced age. We also used a selective inhibitor of telomere elongation to show that the mechanism driving immortalization of hMSCs by hTERT is dependent upon maintenance of telomere length. Thanks to their extended lifespan, preserved multipotentiality and controlled growth, i-hTERT hMSCs may prove to be a useful tool for the development and testing of novel stem cell therapies.

Postovit, L. M., et al. (2007). "The commonality of plasticity underlying multipotent tumor cells and embryonic stem cells." J Cell Biochem **101**(4): 908-917.

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

Price, J. E., et al. (1986). "Cellular immortality,

clonogenicity, tumorigenicity and the metastatic phenotype." Eur J Cancer Clin Oncol **22**(3): 349-355.

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.

Pyne, N. J. and S. Pyne (2020). "Recent advances in the role of sphingosine 1-phosphate in cancer." FEBS Lett **594**(22): 3583-3601.

Sphingosine 1-phosphate (S1P) is a bioactive lipid that binds to a family of G protein-coupled receptors (S1P(1-5)) and intracellular targets, such as HDAC1/2, that are functional in normal and pathophysiologic cell biology. There is a significant role for sphingosine 1-phosphate in cancer underpinning the so-called hallmarks, such as transformation and replicative immortality. In this review, we survey the most recent developments concerning the role of sphingosine 1-phosphate receptors, sphingosine kinase and S1P lyase in cancer and the prognostic indications of these receptors and enzymes in terms of disease-specific survival and recurrence. We also provide evidence for identification of new therapeutic approaches targeting sphingosine 1-phosphate to prevent neovascularisation, to revert aggressive and drug-resistant cancers to more amenable forms sensitive to chemotherapy, and to induce cytotoxicity in cancer cells. Finally, we briefly describe current advances in the development of isoform-specific inhibitors of sphingosine kinases for potential use in the treatment of various cancers, where these enzymes have a predominant role. This review will therefore highlight sphingosine 1-phosphate signalling as a promising translational target for precision medicine in stratified cancer patients.

Qi, W., et al. (2021). "The secreted endoribonuclease ENDU-2 from the soma protects germline immortality in C. elegans." Nat Commun **12**(1): 1262.

Multicellular organisms coordinate tissue specific responses to environmental information via both cell-autonomous and non-autonomous mechanisms. In addition to secreted ligands, recent reports implicated release of small RNAs in regulating gene expression across tissue boundaries. Here, we show that the conserved poly-U specific endoribonuclease ENDU-2 in C. elegans is secreted from the soma and taken-up by the germline to ensure germline immortality at elevated temperature. ENDU-2 binds to mature mRNAs and negatively regulates mRNA abundance both in the soma and the germline. While ENDU-2 promotes RNA decay in the soma directly via its endoribonuclease activity, ENDU-2 prevents misexpression of soma-specific genes in the germline and preserves germline immortality independent of its RNA-cleavage activity. In summary, our results suggest that the secreted RNase ENDU-2 regulates gene expression across tissue boundaries in response to temperature alterations and contributes to maintenance of stem cell immortality, probably via retaining a stem cell specific program of gene expression.

Qi, W., et al. (2021). "Protection of germline immortality by the soma via a secreted endoribonuclease." Bioessays **43**(12): e2100195.

In sexually reproducing organisms maintenance of germ stem cell immortality is fundamental for transmitting genetic material to future generations. While previous research has mainly considered intrinsic regulatory mechanisms in the germline, our recent study has found a direct contribution of somatic cells in preserving germline immortality via the somatically expressed endoribonuclease ENDU-2 in Caenorhabditis elegans. We have identified ENDU-2 as a secreted protein that can be taken up by the germline. Here, we discuss how ENDU-2 might uncouple its RNA-binding and RNA-

cleavage activities to control gene expression via either an endoribonuclease dependent or an independent way. We also speculate on a possible functional conservation of its mammalian homologs in mediating cell-cell communication as well as its potential significance in understanding human pathogenesis such as cancer development.

Qiu, B., et al. (2012). "Expression and correlation of Bcl-2 with pathological grades in human glioma stem cells." Oncol Rep **28**(1): 155-160.

The anti-apoptotic gene, B-cell lymphoma-2 (Bcl-2), has been reported to be overexpressed in gliomas and is related to tumor prognosis, suggesting a potential therapeutic target. Additionally, recent studies have demonstrated the existence of brain glioma stem cells (BGSCs) which are tumorigenic, self-renewable and dominate the biological behavior of gliomas. Currently BGSCs are committed as a new target of glioma therapies. However, few studies have focused on the expression of Bcl-2 in BGSCs. We performed a series of experiments to culture BGSCs from eight clinical specimens, followed by real-time RT-PCR and immunoassays to compare the expression levels of Bcl-2 in BGSCs and their corresponding primary glioma cells (PGCs). The results showed that Bcl-2 mRNA and protein expression levels are higher in BGSCs compared to their counterparts, and the expression levels are related to glioma malignancies. As an anti-apoptotic gene, Bcl-2 assigns immortality characteristics to cells, which coincide with the pivotal biological feature of BGSCs. The experimental results indicated that BGSCs would evade apoptosis for higher Bcl-2 expression, and may interpret the drug resistance of glioma to cytotoxic drugs and other pro-apoptotic agents. New therapies targeting Bcl-2 must induce apoptosis in BGSCs, thus, resulting in treatment or even eradication of glioma.

Rahman, R., et al. (2009). "Cellular immortality in brain tumours: an integration of the cancer stem cell paradigm." Biochim Biophys Acta **1792**(4): 280-288.

Brain tumours are a diverse group of neoplasms that continue to present a formidable challenge in our attempt to achieve curable intervention. Our conceptual framework of human brain cancer has been redrawn in the current decade. There is a gathering acceptance that brain tumour formation is a phenotypic outcome of dysregulated neurogenesis, with tumours viewed as abnormally differentiated neural tissue. In relation, there is accumulating evidence that brain tumours, similar to leukaemia and many solid tumours, are organized as a developmental hierarchy which is maintained by a small fraction of cells endowed with many shared properties of tissue stem cells. Proof that neurogenesis persists throughout adult life, compliments this concept. Although the cancer cell of origin is unclear, the proliferative zones that harbour stem cells in the embryonic, post-natal and adult brain are attractive candidates within which tumour-initiation may ensue. Dysregulated, unlimited proliferation and an ability to bypass senescence are acquired capabilities of cancerous cells. These abilities in part require the establishment of a telomere maintenance mechanism for counteracting the shortening of chromosomal termini.

Rando, T. A. (2006). "Stem cells, ageing and the quest for immortality." Nature **441**(7097): 1080-1086.

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

Rao, F., et al. (2011). "Medaka tert produces multiple variants with differential expression during differentiation in vitro and in vivo." Int J Biol Sci **7**(4): 426-439.

Embryonic stem (ES) cells have immortality for self-renewal and pluripotency. Differentiated human cells undergo replicative senescence. In human, the telomerase reverse transcriptase (Tert), namely the catalytic subunit of telomerase, exhibits differential expression to regulate telomerase activity governing cellular immortality or senescence, and telomerase activity or tert expression is a routine marker of pluripotent ES cells. Here we have identified the medaka tert gene and determined its expression and telomerase activity in vivo and in vitro. We found that the medaka tert locus produces five variants called terta to terte encoding isoforms TertA to TertE. The longest TertA consists of 1090 amino acid residues and displays a maximum of 34% identity to the human TERT and all the signature motifs of the Tert family. TertB to TertE are novel isoforms and have considerable truncation due to alternative splicing. The terta RNA is ubiquitous in embryos, adult tissues and cell lines, and accompanies ubiquitous telomerase activity in vivo and in vitro as revealed by TRAP assays. The tertb RNA was restricted to the testis, absent in embryos before gastrulation and barely detectable in various cell lines The tertc transcript was

absent in undifferentiated ES cells but became evident upon ES cell differentiation, in vivo it was barely detectable in early embryos and became evident when embryogenesis proceeds. Therefore, ubiquitous terta expression correlates with ubiquitous telomerase activity in medaka, and expression of other tert variants appears to delineate cell differentiation in vitro and in vivo.

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

Many workers have immortalised neural precursor cells by applying a variety of techniques including transfection and retroviral-mediated gene insertion using a variety of oncogenes including c-myc, neu, and the SV40 T antigen. This study made use of a conditionally immortalised hippocampal cell population derived from the H-2Kb-tsA58 transgenic mouse. In this mouse the tsA58 gene is under the control of the H-2Kb major histocompatibility complex class I promoter.

Raz, A. A. and Y. M. Yamashita (2021). "Molding immortality from a plastic germline." Curr Opin Cell Biol **73**: 1-8.

Germ cells are uniquely capable of maintaining cellular immortality, allowing them to give rise to new individuals in generation after generation. Recent studies have identified that the germline state is plastic, with frequent interconversion between germline differentiation states and across the germline/soma border. Therefore, features that grant germline immortality must be inducible, with other cells undergoing some form of rejuvenation to a germline state. In this review, we summarize the breadth of our current interpretations of germline plasticity and the ways in which these fate conversion events can aid our understanding of the underlying hallmarks of germline immortality.

Rha, S. Y., et al. (2000). "Effect of telomere and telomerase interactive agents on human tumor and normal cell lines." Clin Cancer Res **6**(3): 987-993.

Shortening of telomeres along with an up-regulation of telomerase is implicated in the immortality of tumor cells. Targeting either telomeres or telomerase with specific compounds has been proposed as an anticancer strategy. Because telomerase activity and telomeres are found in normal cells, telomere or telomerase targeting agents could induce side effects in normal tissues. We evaluated the effects of telomere and telomerase interactive agents in human tumor and normal cell lines to try to determine the potential side effects those agents might induce in patients. Toxicity of the G-quadruplex interactive porphyrins (TMPyP4, TMPyP2) and azidothymidine (AZT) were tested using a cell-counting technique against normal human cell lines (CRL-2115 and CRL-2120, fibroblasts; NHEK-Ad, adult keratinocytes; CCL-241, small intestinal cells; NCM 460, colonic mucosal epithelial cells) and human tumor cell lines (MDA-MB 231 and Hs 578T, breast cancer; SK-N-FI, neuroblastoma; HeLa, cervix cancer; MIA PaCa-2, pancreatic cancer; HT-29 and HCT-116, colon cancer; DU 145, prostatic cancer cell line). Telomerase activity of these cell lines was measured by a non-PCR-based conventional assay. The effects of TMPgammaP2, TMPyP4, and AZT were also evaluated against normal human bone marrow specimens, using a granulocyte-macrophage colony-forming assay (CFU-GM). AZT showed very low cytotoxic effects against normal and tumor cell lines, with the IC50 values above 200 microM. The IC50 values for TMPyP2 and TMPyP4 in normal human cell lines were in the range of 2.9-48.3 microM and 1.7-15.5 microM, respectively, whereas in tumor cell lines the IC50 values were 11.4-53 microM and 9.0-28.2 microM, respectively.

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.

Robinson, M., et al. (2015). "Optimizing Differentiation Protocols for Producing Dopaminergic Neurons from Human Induced Pluripotent Stem Cells for Tissue Engineering Applications: Supplementary Issue: Stem Cell Biology." Biomark Insights **10 Suppl 1**: 61-70.

Parkinson's disease (PD) is a neurodegenerative disorder that results when the dopaminergic neurons (DNs) present in the substantia nigra necessary for voluntary motor control are depleted, making patients with this disorder ideal candidates for cell replacement therapy. Human induced pluripotent stem cells (hiPSCs), obtained by reprogramming adult cells, possess the properties of pluripotency and immortality while enabling the possibility of patient-specific therapies.

Romaniuk-Drapala, A., et al. (2021). "hTERT Downregulation Attenuates Resistance to DOX, Impairs FAK-Mediated Adhesion, and Leads to Autophagy Induction in Breast Cancer Cells." Cells **10**(4).

Telomerase is known to contribute to telomere maintenance and to provide cancer cell immortality. However, numerous reports are showing that the function of the enzyme goes far beyond chromosome ends. The study aimed to explore how telomerase downregulation in MCF7 and MDA-MB-231 breast cancer cells affects their ability to survive. Consequently, sensitivity to drug resistance, proliferation, and adhesion were assessed. The lentiviral-mediated human telomerase reverse transcriptase (hTERT) downregulation efficiency was performed at gene expression and protein level using qPCR and Western blot, respectively. Telomerase activity was evaluated using the Telomeric Repeat Amplification Protocol (TRAP) assay. The study revealed that hTERT downregulation led to an increased sensitivity of breast cancer cells to doxorubicin which was demonstrated in MTT and clonogenic assays. During a long-term doubling time assessment, a decreased population doubling level was observed. Interestingly, it did not dramatically affect cell cycle distribution. hTERT downregulation was accompanied by an alteration in beta1-integrin- and by focal adhesion kinase (FAK)-driven pathways together with the reduction of target proteins phosphorylation, i.e., paxillin and c-Src. Additionally, autophagy activation was observed in MDA-MB-231 cells manifested by alternations in Atg5, Beclin 1, LC3II/I ratio, and p62. These results provide new evidence supporting the possible therapeutic potential of telomerase downregulation leading to induction of autophagy and cancer cells elimination.

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.

Rothstein, J. D. and E. Y. Snyder (2004). "Reality and immortality--neural stem cells for therapies." Nat Biotechnol **22**(3): 283-285.

Roy, D., et al. (2021). "Is Mycobacterium tuberculosis carcinogenic to humans?" FASEB J **35**(9): e21853.

We highlight the ability of the tuberculosis (TB) causing bacterial pathogen, Mycobacterium tuberculosis (Mtb), to induce key characteristics that are associated with established IARC classified Group 1 and Group 2A carcinogenic agents. There is sufficient evidence from epidemiological case-control, cohort and meta-analysis studies of increased lung cancer (LC) risk in pre-existing/active/old TB cases. Similar to carcinogens and other pathogenic infectious agents, exposure to aerosol-containing Mtb sprays in mice produce malignant transformation of cells that result in squamous cell carcinoma. Convincing, mechanistic data show several characteristics shared between TB and LC which include chronic inflammation, genomic instability and replicative immortality, just to name a few cancer hallmarks. These hallmarks of cancer may serve as precursors to malignant transformation. Together, these findings form the basis of our postulate that Mtb is a complete human pulmonary carcinogen. We also discuss how Mtb may act as both an initiating agent and promoter of tumor growth. Forthcoming experimental studies will not only serve as proof-of-concept but will also

pivot our understanding of how to manage/treat TB cases as well as offer solutions to clinical conundrums of TB lesions masquerading as tumors. Clinical validation of our concept may also help pave the way for next generation personalized medicine for the management of pulmonary TB/cancer particularly for cases that are not responding well to conventional chemotherapy or TB drugs.

Rushing, E. J., et al. (1997). "Expression of telomerase RNA component correlates with the MIB-1 proliferation index in ependymomas." J Neuropathol Exp Neurol **56**(10): 1142-1146.

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

Saini, A., et al. (2013). "'From death, lead me to immortality' - mantra of ageing skeletal muscle." Curr Genomics **14**(4): 256-267.

Skeletal muscle is a post-mitotic tissue maintained by repair and regeneration through a population of stem cell-like satellite cells. Following muscle injury, satellite cell proliferation is mediated by local signals ensuring sufficient progeny for tissue repair. Age-related changes in satellite cells as well as to the local and systemic environment potentially impact on the capacity of satellite cells to generate sufficient progeny in an ageing organism resulting in diminished regeneration. 'Rejuvenation' of satellite cell progeny and regenerative capacity by environmental stimuli effectors suggest that a subset of age-dependent satellite cell changes may be reversible. Epigenetic regulation of satellite stem cells that include DNA methylation and histone modifications which regulate gene expression are potential mechanisms for such reversible changes and have been shown to control organismal longevity. The area of health and ageing that is likely to benefit soonest from advances in the biology of adult stem cells is the emerging field of regenerative medicine. Further studies are needed to elucidate the mechanisms by which epigenetic modifications regulate satellite stem cell function and will require an increased understanding of stem-cell biology, the environment of the aged tissue and the interaction between the two.

Saretzki, G. (2009). "Telomerase, mitochondria and oxidative stress." Exp Gerontol **44**(8): 485-492.

Telomerase plays an important role in cellular proliferation capacity and survival under conditions of stress. A large part of this protective function is due to telomere capping and maintenance. Thus it contributes to cellular immortality in stem cells and cancer. Recently, evidence has accumulated that telomerase can contribute to cell survival and stress resistance in a largely telomere-independent manner. Telomerase has been shown to shuttle dynamically between different cellular locations. Under increased oxidative stress telomerase is excluded from the nucleus and can be found within the mitochondria.

Savage, A. M., et al. (2021). "Germline competent mesoderm: the substrate for vertebrate germline and somatic stem cells?" Biol Open **10**(10).

In vitro production of tissue-specific stem cells [e.g. haematopoietic stem cells (HSCs)] is a key goal of regenerative medicine. However, recent efforts to produce fully functional tissue-specific stem cells have fallen short. One possible cause of shortcomings may be that model organisms used to characterize basic vertebrate embryology (Xenopus, zebrafish, chick) may employ molecular mechanisms for stem cell specification that are not conserved in humans, a prominent example being the specification of primordial germ cells (PGCs). Germ plasm irreversibly specifies PGCs in many models; however, it is not conserved in humans, which produce PGCs from tissue termed germline-competent mesoderm (GLCM). GLCM is not conserved in organisms containing germ plasm, or even in mice, but

understanding its developmental potential could unlock successful production of other stem cell types.

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

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

Sell, S. and H. L. Leffert (2008). "Liver cancer stem cells." J Clin Oncol **26**(17): 2800-2805.

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

Sheldrake, A. R. (2022). "Cellular senescence, rejuvenation and potential immortality." Proc Biol Sci **289**(1970): 20212434.

Ageing, death, and potential immortality lie at the heart of biology, but two seemingly incompatible paradigms coexist in different research communities and have done since the nineteenth century. The universal senescence paradigm sees senescence as inevitable in all cells. Damage accumulates. The potential immortality paradigm sees some cells as potentially immortal, especially unicellular organisms, germ cells and cancerous cells. Recent research with animal cells, yeasts and bacteria show that damaged cell constituents do in fact build up, but can be diluted by growth and cell division, especially by asymmetric cell division. By contrast, mammalian embryonic stem cells and many cancerous and 'immortalized' cell lines divide symmetrically, and yet replicate indefinitely. How do they acquire their potential immortality? I suggest they are rejuvenated by excreting damaged cell constituents in extracellular vesicles. If so, our understanding of cellular senescence, rejuvenation and potential immortality could be brought together in a new synthesis, which I call the cellular rejuvenation hypothesis: damaged cell constituents build up in all cells, but cells can be rejuvenated either by growth and cell division or, in 'immortal' cell lines, by excreting damaged cell constituents. In electronic supplementary material, appendix, I outline nine ways in which this hypothesis could be tested.

Shin, P. K., et al. (2019). "Walnut phenolic extracts reduce telomere length and telomerase activity in a colon cancer stem cell model." Nutr Res Pract **13**(1): 58-63.

BACKGROUND/OBJECTIVES: Telomeres are located at the chromosomal ends and progressively shortened during each cell cycle. Telomerase, which is regulated by hTERT and c-MYC, maintains telomeric DNA sequences. Especially, telomerase is active in cancer and stem cells to maintain telomere length for replicative immortality. Recently we reported that walnut phenolic extract (WPE) can reduce cell viability in a colon cancer stem cell (CSC) model. We, therefore, investigated the effect of WPE on telomere maintenance in the same model. MATERIALS/METHODS: CD133(+)CD44(+) cells from HCT116, a human colon cancer cell line, were sorted by Fluorescence-activated cell sorting (FACS) and treated with WPE at the concentrations of 0, 10, 20, and 40 microg/mL for 6 days. Telomere lengths were assessed by quantitative real-time PCR (qRT-PCR) using telomere specific primers and DNA extracted from the cells, which was further adjusted with single-copy gene and reference DNA (ddC(t)). Telomerase activity was also measured by qRT-PCR after incubating the PCR mixture with cell protein extracts, which was adjusted with reference DNA (dC(t)). Transcriptions of hTERT and c-MYC were determined using conventional RT-PCR. RESULTS:

Telomere length of WPE-treated cells was significantly decreased in a dose-dependent manner (5.16 +/- 0.13 at 0 microg/mL, 4.79 +/- 0.12 at 10 microg/mL, 3.24 +/- 0.08 at 20 microg/mL and 3.99 +/- 0.09 at 40 microg/mL; P = 0.0276). Telomerase activities concurrently decreased with telomere length (1.47 +/- 0.04, 1.09 +/- 0.01, 0.76 +/- 0.08, and 0.88 +/- 0.06; P = 0.0067). There was a positive correlation between telomere length and telomerase activity (r = 0.9090; P < 0.0001). Transcriptions of both hTERT and c-MYC were also significantly decreased in the same manner. CONCLUSION: In the present cell culture model, WPE reduced telomere maintenance, which may provide a mechanistic link to the effect of walnuts on the viability of colon CSCs.

Singh, M. (2016). "Pediatrics in 21(st) Century and Beyond." Indian J Pediatr **83**(12-13): 1420-1425.

Pediatrics is a dynamic discipline and there is awareness and hope for actualizing outstanding achievements in the field of child health in 21(st) century and beyond. Improved lifestyle and quality of children's health is likely to reduce the burden of adult diseases and enhance longevity because seeds of most adult diseases are sown in childhood. Identification and decoding of human genome is expected to revolutionize the practice of pediatrics. The day is not far off when a patient will walk into doctor's chamber with an electronic or digital health history on a CD or palmtop and a decoded genomic constitution. There will be reduced burden of genetic diseases because of selective abortions of "defective" fetuses and replacement of "bad" genes with "good" ones by genetic engineering. Availability of totipotent stem cells and developments in transplant technology are likely to revolutionize the management of a variety of hematologic cancers and life-threatening genetic disorders. The possibility of producing flawless designer babies by advances in assisted reproductive technologies (ARTs) is likely to be mired by several ethical and legal issues.The availability of newer vaccines by recombinant technology for emerging infective and for non-infective lifestyle diseases is likely to improve survival and quality of life. There is going to be a greater focus on the "patient" having the disease rather than "disease" per se by practicing holistic pediatrics by effective utilization of alternative or complementary strategies for health care. Due to advances in technology, pediatrics may get further dehumanized. A true healer cannot simply rely on technology; there must be a spiritual bond between the patient and the physician by exploiting the concept of psycho-neuro-immunology and body-mind interactions. In the years to come, physicians are likely to play "god" but medicine can't achieve immortality because anything born must die in accordance with nature's recycling blueprint. The medical science is likely to improve longevity but our goal should be to improve the quality of life.

So, A. Y., et al. (2014). "Dual mechanisms by which miR-125b represses IRF4 to induce myeloid and B-cell leukemias." Blood **124**(9): 1502-1512.

The oncomir microRNA-125b (miR-125b) is upregulated in a variety of human neoplastic blood disorders and constitutive upregulation of miR-125b in mice can promote myeloid and B-cell leukemia. We found that miR-125b promotes myeloid and B-cell neoplasm by inducing tumorigenesis in hematopoietic progenitor cells. Our study demonstrates that miR-125b induces myeloid leukemia by enhancing myeloid progenitor output from stem cells as well as inducing immortality, self-renewal, and tumorigenesis in myeloid progenitors. Through functional and genetic analyses, we demonstrated that miR-125b induces myeloid and B-cell leukemia by inhibiting interferon regulatory factor 4 (IRF4) but through distinct mechanisms; it induces myeloid leukemia through repressing IRF4 at the messenger RNA (mRNA) level without altering the genomic DNA and induces B-cell leukemia via genetic deletion of the gene encoding IRF4.

Soady, K. and M. J. Smalley (2012). "Slugging their way to immortality: driving mammary epithelial cells into a stem cell-like state." Breast Cancer Res **14**(5): 319.

Delineating the molecular factors that define and maintain the mammary stem cell state is vital for understanding normal development and tumourigenesis. A recent study by Guo and colleagues identifies two master transcriptional regulators of mammary stem cells, Slug and Sox9, ectopic expression of which confers stem cell attributes on differentiated mammary epithelial cells. Slug and Sox9 expression was also shown to determine in vivo metastatic potential of human breast cancer cell lines. Understanding these factors in the context of normal lineage differentiation is an important step toward elucidating the mammary epithelial cell hierarchy and the origins of cancer stem cells.

Stevanovic, M., et al. (2023). "The Role of SOX Transcription Factors in Ageing and Age-Related Diseases." Int J Mol Sci **24**(1).

The quest for eternal youth and immortality is as old as humankind. Ageing is an inevitable physiological process accompanied by many functional declines that are driving factors for age-related diseases. Stem cell exhaustion is one of the major hallmarks of ageing. The SOX transcription factors play well-known roles in self-renewal and

differentiation of both embryonic and adult stem cells. As a consequence of ageing, the repertoire of adult stem cells present in various organs steadily declines, and their dysfunction/death could lead to reduced regenerative potential and development of age-related diseases. Thus, restoring the function of aged stem cells, inducing their regenerative potential, and slowing down the ageing process are critical for improving the health span and, consequently, the lifespan of humans. Reprograming factors, including SOX family members, emerge as crucial players in rejuvenation. This review focuses on the roles of SOX transcription factors in stem cell exhaustion and age-related diseases, including neurodegenerative diseases, visual deterioration, chronic obstructive pulmonary disease, osteoporosis, and age-related cancers. A better understanding of the molecular mechanisms of ageing and the roles of SOX transcription factors in this process could open new avenues for developing novel strategies that will delay ageing and prevent age-related diseases.

Stolzing, A., et al. (2007). "Fusion and regenerative therapies: is immortality really recessive?" Rejuvenation Res **10**(4): 571-586.

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

Sugihara, M., et al. (1999). "Decreased expression of telomerase-associated RNAs in the proliferation of stem cells in comparison with continuous expression in malignant tumors." Int J Oncol **15**(6): 1075-1080.

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

Sugiki, T., et al. (2007). "Hyaline cartilage formation and enchondral ossification modeled with KUM5 and OP9 chondroblasts." J Cell Biochem **100**(5): 1240-1254.

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

microarray data of the expression of cell surface markers and cell-type-specific genes resulted in grouping of KUM5 and OP9 cells into the same subcategory of "chondroblast," that is, a distinct cell type group. We here show that these two cell lines exhibit the unique characteristics of hyaline cartilage formation and enchondral ossification in vitro and in vivo.

Surani, M. A. (2012). "Cellular reprogramming in pursuit of immortality." Cell Stem Cell **11**(6): 748-750.

The discovery that phenotypic diversity among differentiated cells results from epigenetic and not genetic differences, and can be reset to restore pluripotency, promises revolutionary advances in medicine. I discuss how this and related seminal discoveries have brought us to an exciting future.

Takahashi, K., et al. (2003). "Role of ERas in promoting tumour-like properties in mouse embryonic stem cells." Nature **423**(6939): 541-545.

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

Tam, C., et al. (2019). "LncRNAs with miRNAs in regulation of gastric, liver, and colorectal cancers: updates in recent years." Appl Microbiol Biotechnol **103**(12): 4649-4677.

Long noncoding RNA (lncRNA) is a kind of RNAi molecule composed of hundreds to thousands of nucleotides. There are several major types of functional lncRNAs which participate in some important cellular pathways. LncRNA-RNA interaction controls mRNA translation and degradation or serves as a microRNA (miRNA) sponge for silencing. LncRNA-protein interaction regulates protein activity in transcriptional activation and silencing. LncRNA guide, decoy, and scaffold regulate transcription regulators of enhancer or repressor region of the coding genes for alteration of expression. LncRNA plays a role in cellular responses including the following activities: regulation of chromatin structural modification and gene expression for epigenetic and cell function control, promotion of hematopoiesis and maturation of immunity, cell programming in stem cell and somatic cell development, modulation of pathogen infection, switching glycolysis and lipid metabolism, and initiation of autoimmune diseases. LncRNA, together with miRNA, are considered the critical elements in cancer development. It has been demonstrated that tumorigenesis could be driven by homeostatic imbalance of lncRNA/miRNA/cancer regulatory factors resulting in biochemical and physiological alterations inside the cells. Cancer-driven lncRNAs with other cellular RNAs, epigenetic modulators, or protein effectors may change gene expression level and affect the viability, immortality, and motility of the cells that facilitate cancer cell cycle rearrangement, angiogenesis, proliferation, and metastasis. Molecular medicine will be the future trend for development. LncRNA/miRNA could be one of the potential candidates in this category. Continuous studies in lncRNA functional discrepancy between cancer cells and normal cells and regional and rational genetic differences of lncRNA profiles are critical for clinical research which is beneficial for clinical practice.

Tanabe, K., et al. (2014). "Induction of pluripotency by defined factors." Proc Jpn Acad Ser B Phys Biol Sci **90**(3): 83-96.

The "reversion of cell fate from differentiated states back into totipotent or pluripotent states" has been an interest of many scientists for a long time. With the help of knowledge accumulated by those scientists, we succeeded in converting somatic cells to a pluripotent cell lineage by the forced expression of defined factors. These established induced pluripotent stem (iPS) cells have similar features to embryonic stem (ES) cells, including pluripotency and immortality. The iPS cell technology provides unprecedented opportunities for regenerative medicine and drug discovery.

Tewari, A. B., et al. (2023). "Extirpating the cancer stem cell hydra: Differentiation therapy and Hyperthermia therapy for targeting the cancer stem cell hierarchy." Clin Exp Med **23**(7): 3125-3145.

Ever since the discovery of cancer stem cells (CSCs), they have progressively attracted more

attention as a therapeutic target. Like the mythical hydra, this subpopulation of cells seems to contribute to cancer immortality, spawning more cells each time that some components of the cancer cell hierarchy are destroyed. Traditional modalities focusing on cancer treatment have emphasized apoptosis as a route to eliminate the tumor burden. A major problem is that cancer cells are often in varying degrees of dedifferentiation contributing to what is known as the CSCs hierarchy and cells which are known to be resistant to conventional therapy. Differentiation therapy is an experimental therapeutic modality aimed at the conversion of malignant phenotype to a more benign one. Hyperthermia therapy (HT) is a modality exploiting the changes induced in cells by the application of heat produced to aid in cancer therapy. While differentiation therapy has been successfully employed in the treatment of acute myeloid leukemia, it has not been hugely successful for other cancer types. Mounting evidence suggests that hyperthermia therapy may greatly augment the effects of differentiation therapy while simultaneously overcoming many of the hard-to-treat facets of recurrent tumors. This review summarizes the progress made so far in integrating hyperthermia therapy with existing modules of differentiation therapy. The focus is on studies related to the successful application of both hyperthermia and differentiation therapy when used alone or in conjunction for hard-to-treat cancer cell niche with emphasis on combined approaches to target the CSCs hierarchy.

Tomlinson, P. B. and B. A. Huggett (2012). "Cell longevity and sustained primary growth in palm stems." Am J Bot **99**(12): 1891-1902.

Longevity, or organismal life span, is determined largely by the period over which constituent cells can function metabolically. Plants, with modular organization (the ability continually to develop new organs and tissues) differ from animals, with unitary organization (a fixed body plan), and this difference is reflected in their respective life spans, potentially much longer in plants than animals. We draw attention to the observation that palm trees, as a group of monocotyledons without secondary growth comparable to that of lignophytes (plants with secondary growth from a bifacial cambium), retain by means of sustained primary growth living cells in their trunks throughout their organismal life span. Does this make palms the longest-lived trees because they can grow as individuals for several centuries? No conventional lignophyte retains living metabolically active differentiated cell types in its trunk for this length of time, even though the tree as a whole can exist for millennia. Does this contrast also imply that the long-lived cells in a palm trunk have exceptional properties, which allows this seeming immortality? We document the long-life of many tall palm species and their inherent long-lived stem cell properties, comparing such plants to conventional trees. We provide a summary of aspects of cell age and life span in animals and plants. Cell replacement is a feature of animal function, whereas conventional trees rely on active growth centers (meristems) to sustain organismal development. However, the long persistence of living cells in palm trunks is seen not as evidence for unique metabolic processes that sustain longevity, but is a consequence of unique constructional features. This conclusion suggests that the life span of plant cells is not necessarily genetically determined.

Torres-Padilla, M. E. and R. Ciosk (2013). "A germline-centric view of cell fate commitment, reprogramming and immortality." Development **140**(3): 487-491.

To ensure species continuity, the tantalising developmental plasticity of early embryonic cells, also called totipotency, must be transmitted to the offspring. This responsibility rests within the reproductive cell lineage: the germ line. At the recent EMBO/EMBL symposium 'Germline - Immortality through Totipotency', researchers discussed the mechanisms that establish and control totipotency, with an eye towards the mechanisms that may endow germ cells with the ability to propagate totipotency across generations.

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

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

combination of cytology and telomerase activity accounted for 21 of the total 29 cases (72.4%). These results suggest that the measurement of telomerase activity in body cavity effusions may be useful as an adjunctive tool for cytological and clinicopathological diagnosis and that this technique is potentially applicable to remnant cytological materials.

Tousian, H., et al. (2020). "Looking for immortality: Review of phytotherapy for stem cell senescence." Iran J Basic Med Sci **23**(2): 154-166.

In this paper, we discussed natural agents with protective effects against stem cell senescence. Different complications have been observed due to stem cell senescence and the most important of them is "Aging". Senescent cells have not normal function and their secretary inflammatory factors induce chronic inflammation in body which causes different pathologies. Stem cell senescence also has been investigated in different diseases or as drug adverse effects. We searched databases such as Embase, Pubmed and Web of Science with keywords "stem cell", "progenitor cell", "satellite", "senescence" and excluded keywords "cancer", "tumor", "malignancy" and "carcinoma" without time limitation until May 2019. Among them we chose 52 articles that have investigated protective effects of natural agents (extracts or molecules) against cellular senescence in different kind of adult stem cells. Most of these studies were in endothelial progenitor cells, hematopoietic stem cells, mesenchymal stem cells, adipose-derived stem cells and few were about other kinds of stem cells. Most studied agents were resveratrol and ginseng which are also commercially available as supplement. Most protective molecular targets were telomerase and anti-oxidant enzymes to preserve genome integrity and reduce senescence-inducing signals. Due to the safe and long history of herbal usage in clinic, phytotherapy can be used for preventing stem cell senescence and their related complication. Resveratrol and ginseng can be the first choice for this aim due to their protective mechanisms in various kinds of stem cells and their long term clinical usage.

Tsuneishi, R., et al. (2021). "Ammonia-based enrichment and long-term propagation of zone I hepatocyte-like cells." Sci Rep **11**(1): 11381.

Ammonia has a cytotoxic effect and can therefore be used as a selection agent for enrichment of zone I hepatocytes. However, it has not yet been determined whether ammonia-treated hepatocyte-like cells are able to proliferate in vitro. In this study, we employed an ammonia selection strategy to purify hepatocyte-like cells that were differentiated from human embryonic stem cells (ESCs) and from induced pluripotent stem cells (iPSCs). The resistance to cytotoxicity or cell death by ammonia is likely attributable to the metabolism of ammonia in the cells. In addition to ammonia metabolism-related genes, ammonia-selected hepatocytes showed increased expression of the cytochrome P450 genes. Additionally, the ammonia-selected cells achieved immortality or at least an equivalent life span to human pluripotent stem cells without affecting expression of the liver-associated genes. Ammonia treatment in combination with in vitro propagation is useful for obtaining large quantities of hepatocytes.

Turkalo, T. K., et al. (2023). "A non-genetic switch triggers alternative telomere lengthening and cellular immortalization in ATRX deficient cells." Nat Commun **14**(1): 939.

Alternative Lengthening of Telomeres (ALT) is an aberrant DNA recombination pathway which grants replicative immortality to approximately 10% of all cancers. Despite this high prevalence of ALT in cancer, the mechanism and genetics by which cells activate this pathway remain incompletely understood. A major challenge in dissecting the events that initiate ALT is the extremely low frequency of ALT induction in human cell systems. Guided by the genetic lesions that have been associated with ALT from cancer sequencing studies, we genetically engineered primary human pluripotent stem cells to deterministically induce ALT upon differentiation. Using this genetically defined system, we demonstrate that disruption of the p53 and Rb pathways in combination with ATRX loss-of-function is sufficient to induce all hallmarks of ALT and results in functional immortalization in a cell type-specific manner. We further demonstrate that ALT can be induced in the presence of telomerase, is neither dependent on telomere shortening nor crisis, but is rather driven by continuous telomere instability triggered by the induction of differentiation in ATRX-deficient stem cells.

Umezawa, A., et al. (2019). "Amnion-derived cells as a reliable resource for next-generation regenerative medicine." Placenta **84**: 50-56.

The placenta is composed of the amnion, chorionic plate, villous and smooth chorion, decidua basalis, and umbilical cord. The amnion is a readily obtainable source of a large number of cells and cell types, including epithelium, mesenchyme, and endothelium, and is thus an allogeneic resource for regenerative medicine. Endothelial cells are obtained from large arteries and veins in the amniotic membrane as well as the umbilical cord. The amnion-derived cells exhibit transdifferentiation capabilities, including chondrogenesis and cardiomyogenesis, by introduction of transcription factors, in addition to

their original and potential phenotypes. The amnion is also a source for production of induced pluripotent stem cells (AM-iPSCs). The AM-iPSCs exhibit stable phenotypes, such as multipotency and immortality, and a unique gene expression pattern. Through the use of amnion-derived cells, as well as other placenta-derived cells, preclinical proof of concept has been achieved in a mouse model of muscular dystrophy.

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.

Varga, A. C. and J. L. Wrana (2005). "The disparate role of BMP in stem cell biology." Oncogene **24**(37): 5713-5721.

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

Vilchez, D., et al. (2013). "FOXO4 is necessary for neural differentiation of human embryonic stem cells." Aging Cell **12**(3): 518-522.

Proteostasis is critical for maintaining cell function and proteome stability may play an important role in human embryonic stem cell (hESC) immortality. Notably, hESC populations exhibit a high assembly of active proteasomes, a key node of the proteostasis network. FOXO4, an insulin/IGF-1 responsive transcription factor, regulates proteasome activity in hESCs. We find that loss of FOXO4 reduces the potential of hESCs to differentiate into neural lineages. Therefore, FOXO4 crosses evolutionary boundaries and links hESC function to invertebrate longevity modulation.

Vinnitsky, V. (2014). "The development of a malignant tumor is due to a desperate asexual self-cloning process in which cancer stem cells develop the ability to mimic the genetic program of germline cells." Intrinsically Disord Proteins **2**(1): e29997.

To date there is no explanation why the development of almost all types of solid tumors occurs sharing a similar scenario: (1) creation of a cancer stem cell (CSC), (2) CSC multiplication and formation of a multicellular tumor spheroid (TS), (3) vascularization of the TS and its transformation into a vascularized primary tumor, (4) metastatic spreading of CSCs, (5) formation of a metastatic TSs and its transformation into metastatic tumors, and (6) potentially endless repetition of this cycle of events. The above gaps in our knowledge are related to the biology of cancer and specifically to tumorigenesis, which covers the process from the creation of a CSC to the formation of a malignant tumor and the development of metastases. My Oncogerminative Theory of Tumorigenesis considers tumor formation as a dynamic self-organizing process that mimics a self-organizing process of early embryo development. In the initial step in that process, gene mutations combined with epigenetic dysregulation cause somatic

cells to be reprogrammed into CSCs, which are immortal pseudo-germline cells. Mimicking the behavior of fertilized germline cells, the CSC achieves immortality by passing through the stages of its life-cycle and developing into a pseudo-blastula-stage embryo, which manifests in the body as a malignant tumor. In this view, the development of a malignant tumor from a CSC is a phenomenon of developmental biology, which we named a desperate asexual self-cloning event. The theory explains seven core characteristics of malignant tumors: (1) CSC immortality, (2) multistep development of a malignant tumor from a single CSC, (3) heterogeneity of malignant tumor cell populations, (4) metastatic spread of CSCs, (5) invasive growth, (6) malignant progression, and (7) selective immune tolerance toward cancer cells. The Oncogerminative Theory of Tumorigenesis suggests new avenues for discovery of revolutionary therapies to treat, prevent, and eradicate cancer.

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

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

Vogel, G. (2005). "Stem cells. Scientists chase after immortality in a petri dish." Science **309**(5743): 1982-1983.

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

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

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

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

Wang, H. and J. J. Unternaehrer (2019). "Epithelial-mesenchymal Transition and Cancer Stem Cells: At

the Crossroads of Differentiation and Dedifferentiation." Dev Dyn **248**(1): 10-20.

In this review, we explore the connections between epithelial-mesenchymal transition (EMT) and differentiation status. EMTs in development have been described as differentiation events, while in most cases EMTs in cancer have been depicted as dedifferentiation events. We will briefly summarize both embryo development and cancer progression with regard to the involvement of EMT and cell differentiation status. We further present the studies that provide evidence that EMT results in both differentiation and dedifferentiation. Finally, we present our resolution to this dilemma by suggesting that EMT brings about dedifferentiation that enables subsequent differentiation. In normal development, EMT events may cause a partial reversal of differentiation to overcome differentiation barriers. When EMT is aberrantly activated in cancer, cells gain attributes of stem cells that contribute to self-renewal capabilities and are able to differentiate to all cell types represented in the tumor. The resulting cancer stem cells attain hallmarks of cancer, including replicative immortality, resistance to cell death, and invasiveness. Developmental Dynamics 248:10-20, 2019. (c) 2018 Wiley Periodicals, Inc.

Watanabe, H., et al. (2009). "Immortality and the base of multicellular life: Lessons from cnidarian stem cells." Semin Cell Dev Biol **20**(9): 1114-1125.

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

Wazir, U., et al. (2019). "Correlation of TERT and Stem Cell Markers in the Context of Human Breast Cancer." Cancer Genomics Proteomics **16**(2): 121-127.

BACKGROUND: Telomerase reverse transcriptase (TERT) has a well-known role in carcinogenesis due to its functions in inducing cell immortality and preventing senescence. In this study, the relationships between TERT and a panel of known stem cell markers was examined in order to direct future enquiries into the role of 'stem-ness' in human breast cancer. MATERIALS AND METHODS: Breast cancer tissues (n=124) and adjacent normal tissues (n=30) underwent reverse transcription and quantitative polymerase chain reaction. Transcript levels were analyzed for the correlation with that of TERT. RESULTS: A significant direct correlation was found in cancerous tissue between TERT and BMI1 proto-oncogene polycomb ring finger 4 (BMI1; n=88, p<0.001), nestin (NES; n=88, p<0.001), POU domain, class 5, transcription factor 1 (POU5F1; n=88, p<0.001), aldehyde dehydrogenase 1 family member A2 (ALDH1A2; n=87, p=0.0298), cyclin-dependent kinase inhibitor 1A (CDKN1A; n=88, p<0.001), integrin subunit beta 1 (ITGNB1; n=88, p<0.001), integrin subunit alpha 6 (ITGA6; n=88, p<0.001), cluster of differentiation antigen 24 (CD24; n=88, p=0.0114), MET proto-oncogene (MET; n=78, p<0.001) and noggin (NOG; n=88, p<0.001). CONCLUSION: The evidence presented in this article of possible interactions between TERT and a discrete subset of known stem cell markers would significantly contribute to further enquiries regarding clonal dynamics in the context of human breast cancer.

Williams, D. (2015). "Thyroid Growth and Cancer." Eur Thyroid J **4**(3): 164-173.

It is proposed that most papillary thyroid cancers originate in infancy and childhood, based on the early rise in sporadic thyroid carcinoma incidence, the pattern of radiation-induced risk (highest in those exposed as infants), and the high prevalence of sporadic papillary thyroid cancers in children and adolescents (ultrasound screening after the Fukushima accident). The early origin can be linked to the growth pattern of follicular cells, with a high mitotic rate in infancy falling to very low replacement levels in adult life. The cell of origin of thyroid cancers, the differentiated follicular cell, has a limited growth potential. Unlike cancers originating in stem cells, loss of the usually tight link between differentiation and replicative senescence is required for immortalisation. It is suggested that this loss distinguishes larger clinically significant papillary thyroid cancers from micro-papillary thyroid cancers of little clinical

significance. Papillary carcinogenesis can then be divided into 3 stages: (1) initiation, the first mutation in the carcinogenic cascade, for radiation-induced papillary thyroid cancers usually a RET rearrangement, (2) progression, acquisition of the additional mutations needed for low-grade malignancy, and (3) escape, further mutations giving immortality and a higher net growth rate. Most papillary thyroid cancers will not have achieved full immortality by adulthood, and remain as so-called micro-carcinomas with a very low growth rate.

Yamashita, Y. M. (2023). "Asymmetric Stem Cell Division and Germline Immortality." Annu Rev Genet **57**: 181-199.

Germ cells are the only cell type that is capable of transmitting genetic information to the next generation, which has enabled the continuation of multicellular life for the last 1.5 billion years. Surprisingly little is known about the mechanisms supporting the germline's remarkable ability to continue in this eternal cycle, termed germline immortality. Even unicellular organisms age at a cellular level, demonstrating that cellular aging is inevitable. Extensive studies in yeast have established the framework of how asymmetric cell division and gametogenesis may contribute to the resetting of cellular age. This review examines the mechanisms of germline immortality-how germline cells reset the aging of cells-drawing a parallel between yeast and multicellular organisms.

Yang, T. and K. Rycaj (2015). "Targeted therapy against cancer stem cells." Oncol Lett **10**(1): 27-33.

Research into cancer stem cells (CSCs), which have the ability to self-renew and give rise to more mature (differentiated) cancer cells, and which may be the cells responsible for the overall organization of a tumor, has progressed rapidly and concomitantly with recent advances in studies of normal tissue stem cells. CSCs have been reported in a wide spectrum of human tumors. Like normal tissue stem cells, CSCs similarly exhibit significant phenotypic and functional heterogeneity. The ability of CSCs to self-renew results in the immortality of malignant cells at the population level, whereas the ability of CSCs to differentiate, either fully or partially, generates the cellular hierarchy and heterogeneity commonly observed in solid tumors. CSCs also appear to have maximized their pro-survival mechanisms leading to their relative resistance to anti-cancer therapies and subsequent relapse. Studies in animal models of human cancers have also provided insight into the heterogeneity and characteristics of CSCs, helping to establish a platform for the development of novel targeted therapies against specific CSCs. In the present study, we briefly review the most recent progress in dissecting CSC heterogeneity and targeting CSCs in various human tumor systems. We also highlight a few examples of CSC-targeted drug development and clinical trials, with the ultimate aim of developing more effective therapeutic regimens that are capable of preventing tumor recurrence and metastasis.

Yoshioka, K., et al. (2015). "Development of cancer-initiating cells and immortalized cells with genomic instability." World J Stem Cells **7**(2): 483-489.

Cancers that develop after middle age usually exhibit genomic instability and multiple mutations. This is in direct contrast to pediatric tumors that usually develop as a result of specific chromosomal translocations and epigenetic aberrations. The development of genomic instability is associated with mutations that contribute to cellular immortalization and transformation. Cancer occurs when cancer-initiating cells (CICs), also called cancer stem cells, develop as a result of these mutations. In this paper, we explore how CICs develop as a result of genomic instability, including looking at which cancer suppression mechanisms are abrogated.

Yui, J., et al. (1998). "Telomerase activity in candidate stem cells from fetal liver and adult bone marrow." Blood **91**(9): 3255-3262.

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

levels of telomerase activity. Given the replication-dependent loss of telomeric DNA in hematopoietic cells, these observations suggest that the observed telomerase activity in candidate stem cells is either expressed in a minor subset of stem cells or, more likely, is not sufficient to prevent telomere shortening.

Zeng, X. (2007). "Human embryonic stem cells: mechanisms to escape replicative senescence?" Stem Cell Rev **3**(4): 270-279.

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

Zhang, D. Y., et al. (2021). "Mechanisms of cancer stem cell senescence: Current understanding and future perspectives." Clin Exp Pharmacol Physiol **48**(9): 1185-1202.

Cancer stem cells (CSCs) are a small population of heterogeneous tumor cells with the capacity of self-renewal and aberrant differentiation for immortality and divergent lineages of cancer cells. In contrast to bulky tumor cells, CSCs remain less differentiated and resistant to therapy even when targeted with tissue-specific antigenic markers. This makes CSCs responsible for not only tumor initiation, development, but also tumor recurrence. Emerging evidence suggests that CSCs can undergo cell senescence, a non-proliferative state of cells in response to stress. While cell senescence attenuates tumor cell proliferation, it is commonly regarded as a tumor suppressive mechanism. However, mounting research indicates that CSC senescence also provides these cells with the capacity to evade cytotoxic effects from cancer therapy, exacerbating cancer relapse and metastasis. Recent studies demonstrate that senescence drives reprogramming of cancer cell toward stemness and promotes CSC generation. In this review, we highlight the origin, heterogeneity and senescence regulatory mechanisms of CSCs, the complex relationship between CSC senescence and tumor therapy, and the recent beneficial effects of senotherapy on eliminating senescent tumor cells.

Zheng, L., et al. (2018). "Regulation of c-MYC transcriptional activity by transforming growth factor-beta 1-stimulated clone 22." Cancer Sci **109**(2): 395-402.

c-MYC stimulates cell proliferation through the suppression of cyclin-dependent kinase (CDK) inhibitors including P15 (CDKN2B) and P21 (CDKN1A). It also activates E-box-mediated transcription of various target genes including telomerase reverse transcriptase (TERT) that is involved in cellular immortality and tumorigenesis. Transforming growth factor-beta 1 (TGF-beta1)-stimulated clone 22 (TSC-22/TSC22D1) encodes a highly conserved leucine zipper protein that is induced by various stimuli, including TGF-beta. TSC-22 inhibits cell growth in mammalian cells and in Xenopus embryos. However, underlying mechanisms of growth inhibition by TSC-22 remain unclear. Here, we show that TSC-22 physically interacts with c-MYC to inhibit the recruitment of c-MYC on the P15 (CDKN2B) and P21 (CDKN1A) promoters, effectively inhibiting c-MYC-mediated suppression of P15 (CDKN2B) and also P21 (CDKN1A) promoter activities. In contrast, TSC-22 enhances c-MYC-mediated activation of the TERT promoter. Additionally, the expression of TSC-22 in embryonic stem cells inhibits cell growth without affecting its pluripotency-related gene expression. These results indicate that TSC-22 differentially regulates c-MYC-mediated transcriptional activity to regulate cell proliferation.

Zhou, Y., et al. (2013). "Stem cells' exodus: a journey to immortality." Dev Cell **24**(2): 113-114.

Stem cell niches provide a regulatory microenvironment that retains stem cells and promotes self-renewal. Recently in Developmental Cell, Rinkevich et al. (2013) showed that cell islands (CIs) of Botryllus schlosseri, a colonial chordate, provide niches for maintaining cycling stem cells that migrate from degenerated CIs to newly formed buds.

Zhu, S., et al. (2022). "Mutation or not, what directly establishes a neoplastic state, namely cellular immortality and autonomy, still remains unknown and should be prioritized in our research." J Cancer **13**(9): 2810-2843.

Although the concept that cancer is caused by mutations has been widely accepted, there still are ample data deprecating it. For example, embryonic cells displaced in non-embryonic environments may

develop to cancer, whereas cancer cells placed in embryonic environments may be reverted to phenotypic normal. Although many intracellular or extracellular aberrations are known to be able to initiate a lengthy tumorigenesis, the molecular or cellular alterations that directly establish a neoplastic state, namely cellular immortality and autonomy, still remain unknown. Hereditary traits are encoded not only by gene sequences but also by karyotype and DNA or chromosomal structures that may be altered via non-mutational mechanisms, such as post-translational modifications of nuclear proteins, to initiate tumorigenesis. However, the immortal and autonomous nature of neoplasms makes them "new" organisms, meaning that neoplasms should have mutations to distinguish themselves from their host patients in the genome. Neoplasms are malignant if they bear epigenetic or genetic alterations in mutator genes, i.e. the genes whose alterations accelerate other genes to mutate, whereas neoplasms are benign if their epigenetic or genetic aberrations occur only in non-mutator genes. Future mechanistic research should be focused on identifying the alterations that directly establish cellular immortality and autonomy. Benign tumors may have many fewer alterations and thus be much better models than cancers for such research.

Zimmermann, S., et al. (2003). "Lack of telomerase activity in human mesenchymal stem cells." Leukemia **17**(6): 1146-1149.

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

11/25/2024

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