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Stem Cell

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Stem Cell and Telomere Research Literatures

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

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 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2360127/).

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

Adams, P. D., et al. (2015). "Aging-Induced Stem Cell Mutations as Drivers for Disease and Cancer." Cell Stem Cell **16**(6): 601-612.

Aging is characterized by a decrease in genome integrity, impaired organ maintenance, and an increased risk of cancer, which coincide with clonal dominance of expanded mutant stem and progenitor cell populations in aging tissues, such as the intestinal epithelium, the hematopoietic system, and the male germline. Here we discuss possible explanations for age-associated increases in the initiation and/or progression of mutant stem/progenitor clones and highlight the roles of stem cell quiescence, replication-associated DNA damage, telomere shortening, epigenetic alterations, and metabolic challenges as determinants of stem cell mutations and clonal dominance in aging.

Ahmed, R., et al. (2016). "Human Stem Cell-like Memory T Cells Are Maintained in a State of Dynamic Flux." Cell Rep **17**(11): 2811-2818.

Adaptive immunity requires the generation of memory T cells from naive precursors selected in the thymus. The key intermediaries in this process are stem cell-like memory T (TSCM) cells, multipotent progenitors that can both self-renew and replenish more differentiated subsets of memory T cells. In theory, antigen specificity within the TSCM pool may be imprinted statically as a function of largely dormant cells and/or retained dynamically by more transitory

subpopulations. To explore the origins of immunological memory, we measured the turnover of TSCM cells in vivo using stable isotope labeling with heavy water. The data indicate that TSCM cells in both young and elderly subjects are maintained by ongoing proliferation. In line with this finding, TSCM cells displayed limited telomere length erosion coupled with high expression levels of active telomerase and Ki67. Collectively, these observations show that TSCM cells exist in a state of perpetual flux throughout the human lifespan.

Akhtari, M., et al. (2013). "Therapy-related myeloid neoplasms after autologous hematopoietic stem cell transplantation in lymphoma patients." Cancer Biol Ther **14**(12): 1077-1088.

Lymphoma patients treated with autologous transplantation (ASCT) live an increasingly long life with the recent advancement in therapeutic modalities. This has resulted in an increase in the incidence of therapy-related myeloid neoplasms (t-MN), which is one of the leading causes of non-relapse mortality. Several observational studies have linked the development of t-MN after ASCT with the intensity and frequency of chemotherapy, particularly alkylating agents, use of total body irradiation (TBI), and peripheral blood progenitor cells. In addition, role of genetic factors is increasingly being identified. It is postulated that the use of chemotherapy prior to ASCT results in DNA damage of progenitor cells, mitochondrial dysfunction, and altered gene expression related to DNA repair, metabolism as well as hematopoietic regulation. Cytogenetic studies have shown the presence of abnormalities in the peripheral blood progenitor cells prior to ASCT. It is, therefore, likely that the reinfusion of peripheral blood progenitor cells, proliferative stress on infused progenitor cells during hematopoietic regeneration and associated telomere shortening ultimately result in clonal hematopoiesis and blastic transformation. Cytopenias, myelodysplasia, or cytogenetic abnormalities are common and can be transient after ASCT; therefore, only when present together, they do confirm the diagnosis of t-MN. Attempts to reduce the occurrence of t-MN should be directed toward minimizing the exposure to the identified risk factors. Although the median survival is few months to less than a year, studies have shown the promising role of allogeneic transplantation in select young t-MN patients without high-risk cytogenetics. In this review we will explain the recent findings in the field of t-MN in lymphoma patients that have implications for identifying the molecular and genetic mechanisms of leukemogenesis and discuss potential strategies to reduce the risk of t-MN in this patient population.

Akiyama, M., et al. (2000). "Shortening of telomeres in recipients of both autologous and allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **25**(4): 441-447.

Telomere length of peripheral blood mononuclear cells (PBMCs) from 23 autologous HSCT patients ranging from 4 to 61 years old, and 46 allogeneic HSCT recipients from 6 to 52 years old were studied to confirm whether excessive shortening of telomeres is associated with HSCT. After autologous HSCT, telomere length of PBMCs ranged from 6.8 to 12.0 kb. The comparison between transplanted PBMCs and PBMCs after autologous HSCT showed shortening by up to 1.9 kb (mean +/- s.d.: 0.64 +/- 0.50 kb). There was a difference between autologous HSCT patients and normal volunteers in the slopes of regression lines. After allogeneic HSCT, telomere length of PBMCs ranged from 6.8 to 12.0 kb. Telomeres of recipients were up to 2.1 kb (0.60 +/- 0.468 kb) shorter than those of donors. The slope of regression lines for allogeneic HSCT patients and normal volunteers were parallel. Although all patients were transplanted with more than 2.0 x 10(8) cells/kg, telomere length did not correlate with the number of transplanted cells. There was no significant correlation between telomere length and recovery of hematological parameters. However, three patients with an average telomere length of 6.8 kb after HSCT took a longer period to reach the normal hematological state. Taken together, these data suggest that most HSCTs are performed within the biological safety range of telomeres, while the patients who have telomeres shorter than 7.0 kb after HSCT should be observed carefully for long-term hematopoiesis and the occurrence of hematopoietic disorders.

Allsopp, R. (2013). "Short telomeres flirt with stem cell commitment." Cell Stem Cell **12**(4): 383-384.

High expression of telomerase in embryonic stem cells (ESCs) is important for their maintenance, but whether telomere length affects lineage commitment is unknown. In this issue of Cell stem cell, Pucci et al. (2013) reveal that ESCs with short telomeres exhibit unstable differentiation by inducing altered DNA methylation.

Alraies, A., et al. (2017). "Variation in human dental pulp stem cell ageing profiles reflect contrasting proliferative and regenerative capabilities." BMC Cell Biol **18**(1): 12.

BACKGROUND: Dental pulp stem cells (DPSCs) are increasingly being recognized as a viable cell source for regenerative medicine. Although significant variations in their ex vivo expansion are well-established, DPSC proliferative heterogeneity remains poorly understood, despite such characteristics influencing their regenerative and therapeutic potential. This study assessed clonal human DPSC regenerative potential and the impact of cellular senescence on these responses, to better understand DPSC functional behaviour. RESULTS: All DPSCs were negative for

hTERT. Whilst one DPSC population reached >80 PDs before senescence, other populations only achieved <40 PDs, correlating with DPSCs with high proliferative capacities possessing longer telomeres (18.9 kb) than less proliferative populations (5-13 kb). High proliferative capacity DPSCs exhibited prolonged stem cell marker expression, but lacked CD271. Early-onset senescence, stem cell marker loss and positive CD271 expression in DPSCs with low proliferative capacities were associated with impaired osteogenic and chondrogenic differentiation, favouring adipogenesis. DPSCs with high proliferative capacities only demonstrated impaired differentiation following prolonged expansion (>60 PDs). CONCLUSIONS: This study has identified that proliferative and regenerative heterogeneity is related to contrasting telomere lengths and CD271 expression between DPSC populations. These characteristics may ultimately be used to selectively screen and isolate high proliferative capacity/multi-potent DPSCs for regenerative medicine exploitation.

Amit, M., et al. (2000). "Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture." Dev Biol **227**(2): 271-278.

Embryonic stem (ES) cell lines derived from human blastocysts have the developmental potential to form derivatives of all three embryonic germ layers even after prolonged culture. Here we describe the clonal derivation of two human ES cell lines, H9.1 and H9.2. At the time of the clonal derivation of the H9.1 and H9.2 ES cell lines, the parental ES cell line, H9, had already been continuously cultured for 6 months. After an additional 8 months of culture, H9.1 and H9.2 ES cell lines continued to: (1) actively proliferate, (2) express high levels of telomerase, and (3) retain normal karyotypes. Telomere lengths, while somewhat variable, were maintained between 8 and 12 kb in high-passage H9.1 and H9.2 cells. High-passage H9.1 and H9.2 cells both formed teratomas in SCID-beige mice that included differentiated derivatives of all three embryonic germ layers. These results demonstrate the pluripotency of single human ES cells, the maintenance of pluripotency during an extended period of culture, and the long-term self-renewing properties of cultured human ES cells. The remarkable developmental potential, proliferative capacity, and karyotypic stability of human ES cells distinguish them from adult cells.

Armstrong, L., et al. (2004). "A role for nucleoprotein Zap3 in the reduction of telomerase activity during embryonic stem cell differentiation." Mech Dev **121**(12): 1509-1522.

Telomerase, the enzyme which maintains the ends of linear chromosomes in eukaryotic cells is found in murine embryonic stem cells; however, its activity is downregulated during in vitro differentiation. Previous work has indicated that this is due to the transcriptional downregulation of murine reverse transcriptase unit (mTert) of telomerase. To investigate the factors that cause the transcriptional repression of mTert we defined a 300 bp region which is essential for its transcription and performed site directed mutagenesis and electrophoretic mobility shift assays. This analysis indicated that Sp1, Sp3 and c-Myc bind to the GC-boxes and E-boxes, respectively, within the promoter and help activate the transcription of mTert gene. We also identified a novel binding sequence, found repeated within the mTert core region, which when mutated caused increased mTert expression. Yeast one hybrid screening combined with electrophoretic mobility shift assays indicated that the nuclear protein Zap3 binds to this site and its overexpression leads to the downregulation of mTert during differentiation. This suggests that regulation of mTert transcription is a complex process which depends on a quantitative balance between transcription factors that cause activation or repression of this gene. Overexpression of Zap3 in murine embryonic stem cells results in reduction in telomerase activity and telomere length as well as reduced proliferative capacity and limited ability to contribute to the development of haematopoietic cells upon differentiation.

Azevedo, R. I., et al. (2013). "Long-term immune reconstitution of naive and memory t cell pools after haploidentical hematopoietic stem cell transplantation." Biol Blood Marrow Transplant **19**(5): 703-712.

Haploidentical hematopoietic stem cell transplantation (HSCT) constitutes an important alternative for patients lacking a human leukocyte antigen (HLA)-matched donor. Although the use of haploidentical donors is increasingly common, the long-term impact of generating a donor-derived immune system in the context of an HLA-mismatched thymic environment remains poorly characterized. We performed an in-depth assessment of immune reconstitution in a group of haploidentical HSCT recipients 4 to 6 years posttransplantation, in parallel with the respective parental donors and age-matched healthy control subjects. Our data show that the proportion of naive and memory subsets in the recipients, both within CD8(+) and CD4(+) T cells, more closely resembled that observed in age-matched control subjects than in the donors. HSCT recipients displayed relatively high signal-joint T cell-receptor excision circle levels and a high frequency of the recent thymic emigrant-enriched CD31(+) subset within naive CD4(+) and naive regulatory T cells. Moreover, CD8(+), CD4(+), and regulatory T cells from HSCT recipients displayed a diverse T cell repertoire. These results support a key role for thymic output in T cell reconstitution. Nevertheless, HSCT recipients had

significantly shorter telomeres within a naive-enriched CD4(+) T cell population than age-matched control subjects, despite the similar telomere length observed within the most differentiated CD8(+) and CD4(+) T cell subsets. Overall, our data suggest that long-term immune reconstitution was successfully achieved after haploidentical HSCT, a process that appears to have largely relied on de novo T cell production.

Babor, F., et al. (2019). "Presence of centromeric but absence of telomeric group B KIR haplotypes in stem cell donors improve leukaemia control after HSCT for childhood ALL." Bone Marrow Transplant **54**(11): 1847-1858.

Although allogeneic hematopoietic stem-cell transplantation (HSCT) provides high cure rates for children with high-risk acute lymphoblastic leukaemia (ALL), relapses remain the main cause of treatment failure. Whereas donor killer cell immunoglobulin-like receptor (KIR) genotype was shown to impact on relapse incidence in adult myeloid leukaemia similar studies in paediatric ALL are largely missing. Effect of donor KIR genotype on transplant outcome was evaluated in 317 children receiving a first myeloablative HSCT from an HLA-matched unrelated donor or sibling within the prospective ALL-SCT-BFM-2003 trial. Analysis of donor KIR gene polymorphism revealed that centromeric presence and telomeric absence of KIR B haplotypes was associated with reduced relapse risk. A centromeric/telomeric KIR score (ct-KIR score) integrating these observations correlated with relapse risk (hazard ratio (HR) 0.58; P = 0.002) while it had no impact on graft-versus-host disease or non-relapse mortality. In multivariable analyses ct-KIR score was associated with reduced relapse risk (HR 0.58; P = 0.003) and a trend towards improved event-free survival (HR 0.76; P = 0.059). This effect proved independent of MRD level prior to HSCT. Our data suggest that in children with ALL undergoing HSCT after myeloablative conditioning, donor selection based on KIR genotyping holds promise to improve clinical outcome by decreasing relapse risk and prolonged event-free survival.

Baerlocher, G. M., et al. (2009). "Cellular senescence of white blood cells in very long-term survivors after allogeneic hematopoietic stem cell transplantation: the role of chronic graft-versus-host disease and female donor sex." Blood **114**(1): 219-222.

In this single-center, cross-sectional study, we evaluated 44 very long-term survivors with a median follow-up of 17.5 years (range, 11-26 years) after hematopoietic stem cell transplantation. We assessed the telomere length difference in human leukocyte antigen-identical donor and recipient sibling pairs and searched for its relationship with clinical factors. The telomere length (in kb, mean +/- SD) was significantly shorter in all recipient blood cells compared with their donors' blood cells (P < .01): granulocytes (6.5 +/- 0.9 vs 7.1 +/- 0.9), naive/memory T cells (5.7 +/- 1.2 vs 6.6 +/- 1.2; 5.2 +/- 1.0 vs 5.7 +/- 0.9), B cells (7.1 +/- 1.1 vs 7.8 +/- 1.1), and natural killer/natural killer T cells (4.8 +/- 1.0 vs 5.6 +/- 1.3). Chronic graft-versus-host disease (P < .04) and a female donor (P < .04) were associated with a greater difference in telomere length between donor and recipient. Critically short telomeres have been described in degenerative diseases and secondary malignancies. If this hypothesis can be confirmed, identification of recipients at risk for cellular senescence could become part of monitoring long-term survivors after hematopoietic stem cell transplantation.

Barbaro, P. and A. Vedi (2016). "Survival after Hematopoietic Stem Cell Transplant in Patients with Dyskeratosis Congenita: Systematic Review of the Literature." Biol Blood Marrow Transplant **22**(7): 1152-1158.

Dyskeratosis congenita (DC) is a multisystem disorder, with a disruption in telomere biology leading to very short telomeres underpinning its pathophysiology. Bone marrow failure is a key feature in DC and is the leading cause of mortality. Hematopoietic stem cell transplantation (HSCT) is the only curative option for bone marrow failure in DC; however, small case reports and series have suggested a poor outcome after HSCT. We undertook a systematic review of all reported patients with DC who underwent HSCT to better characterize outcome and to identify factors associated with improved survival. The outcome of 109 patients found in the literature was poor, with 5- and 10-year survival estimates of only 57% and 23%, respectively. Patients transplanted after 2000 had improved early survival, with 5-year survival estimates of 70%; however, longer term survival was similar (28%). Pulmonary disease, infection, and graft failure were the leading causes of death. Prognosis after development of pulmonary disease post-HSCT was poor, with only 4 of 15 patients surviving at last follow-up. Multivariate analysis identified age >20 years at HSCT, HSCT before 2000, and alternate donor source to be poor prognostic markers. Reduced-intensity conditioning was not significantly found to be associated with improved survival. This review shows the poor outcome after HSCT in patients with DC and highlights the need for future collaborative clinical trials and extended follow-up of this rare patient population to define whether changes in therapy will lead to improved survival.

Beatty, G. L., et al. (2009). "Functional unresponsiveness and replicative senescence of myeloid leukemia antigen-specific CD8+ T cells after allogeneic stem cell transplantation." Clin Cancer Res **15**(15): 4944-4953.

PURPOSE: The therapeutic effect of allogeneic hematopoietic stem cell transplantation (HSCT) for patients with myeloid malignancies has been attributed in part to a graft-versus-leukemia effect that is dependent on donor T lymphocytes. CD8(+) T-cell responses to MHC class I-restricted tumor epitopes, not just allogeneic antigens, may help mediate antileukemia effects after HSCT, but the specificity and function of such cells are not completely understood. EXPERIMENTAL DESIGN: We examined the diversity, phenotype, and functional potential of leukemia-associated antigen-specific CD8(+) T cells in patients with myeloid leukemia following allogeneic HSCT. Screening for antigen-specific T cells was accomplished with a peptide/MHC tetramer library. RESULTS: Patients with acute myelogenous leukemia or chronic myelogenous leukemia in remission following HSCT exhibited significant numbers of peripheral blood CD8(+) T cells that recognized varying combinations of epitopes derived from leukemia-associated antigens. However, these cells failed to proliferate, release cytokines, or degranulate in response to antigen-specific stimuli. As early as 2 months after HSCT, CD8(+) T cells from patients were predominantly CD28(-) CD57(+) and had relatively short telomeres, consistent with cellular senescence. CONCLUSIONS: Circulating leukemia-specific CD8(+) T cells are prominent in myeloid leukemia patients after HSCT, but such cells are largely functionally unresponsive, most likely due to replicative senescence. These findings carry important implications for the understanding of the graft-versus-leukemia effect and for the rational design of immunotherapeutic strategies for patients with myeloid leukemias.

Beck, S., et al. (2011). "Telomerase activity-independent function of TERT allows glioma cells to attain cancer stem cell characteristics by inducing EGFR expression." Mol Cells **31**(1): 9-15.

Telomerase reverse transcriptase (TERT), the catalytic subunit of the enzyme telomerase, is robustly expressed in cancer cells. TERT enables cells to avoid chromosome shortening during repeated replication by maintaining telomere length. However, several lines of evidence indicate that many cancer cells exhibit shorter telomere length than normal tissues, implying an additional function of TERT in tumor formation and progression. Here, we report a telomerase activity-independent function of TERT that induces cancer stemness in glioma cells. Overexpression of TERT712, a telomerase activity-deficient form of TERT, in U87MG cells promoted cell self-renewal in vitro, and induced EGFR expression and formation of gliomas exhibiting cellular heterogeneity in vivo. In patients with glioblastoma multiforme, TERT expression showed a high correlation with EGFR expression, which is closely linked to the stemness gene signature. Induction of differentiation and TERT-knockdown in glioma stem cells led to a marked reduction in EGFR expression, cancer stemness, and anticancer drug resistance. Together, our findings indicate that TERT plays a crucial role in tumor progression by promoting cancer stemness through expression of EGFR.

Beerman, I., et al. (2013). "Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging." Cell Stem Cell **12**(4): 413-425.

The functional potential of hematopoietic stem cells (HSCs) declines during aging, and in doing so, significantly contributes to hematopoietic pathophysiology in the elderly. To explore the relationship between age-associated HSC decline and the epigenome, we examined global DNA methylation of HSCs during ontogeny in combination with functional analysis. Although the DNA methylome is generally stable during aging, site-specific alterations of DNA methylation occur at genomic regions associated with hematopoietic lineage potential and selectively target genes expressed in downstream progenitor and effector cells. We found that age-associated HSC decline, replicative limits, and DNA methylation are largely dependent on the proliferative history of HSCs, yet appear to be telomere-length independent. Physiological aging and experimentally enforced proliferation of HSCs both led to DNA hypermethylation of genes regulated by Polycomb Repressive Complex 2. Our results provide evidence that epigenomic alterations of the DNA methylation landscape contribute to the functional decline of HSCs during aging.

Beier, F., et al. (2011). "Identification of CD133(-)/telomerase(low) progenitor cells in glioblastoma-derived cancer stem cell lines." Cell Mol Neurobiol **31**(3): 337-343.

Glioblastoma multiforme (GBM) is paradigmatic for the investigation of cancer stem cells (CSC) in solid tumors. The CSC hypothesis implies that tumors are maintained by a rare subpopulation of CSC that gives rise to rapidly proliferating progenitor cells. Although the presence of progenitor cells is crucial for the CSC hypothesis, progenitor cells derived from GBM CSC are yet uncharacterized. We analyzed human CD133(+) CSC lines that were directly derived from CD133(+) primary astrocytic GBM. In these CSC lines, CD133(+)/telomerase(high) CSC give rise to non-tumorigenic, CD133(-)/telomerase(low) progenitor cells. The proliferation of the progenitor cell population results in significant telomere shortening as compared to the CD133(+) compartment comprising CSC. The average difference in telomere length as determined by a modified multi-color flow fluorescent in situ hybridization was 320 bp corresponding to 4-8 cell

divisions. Taken together, we demonstrate that CD133(+) primary astrocytic GBM comprise proliferating, CD133(-)/telomerase(low) progenitor cell population characterized by low telomerase activity and shortened telomeres as compared to CSC.

Bereshchenko, O., et al. (2012). "Pontin is essential for murine hematopoietic stem cell survival." Haematologica **97**(9): 1291-1294.

Pontin is a highly conserved DNA helicase/ATPase which is a component of several macromolecular complexes with functions that include DNA repair, telomere maintenance and tumor suppression. While Pontin is known to be essential in yeast, fruit flies and frogs, its physiological role in mammalian organisms remains to be determined. We here find that Pontin is highly expressed in embryonic stem cells and hematopoietic tissues. Through germline inactivation of Ruvbl1, the gene encoding Pontin, we found it to be essential for early embryogenesis, as Ruvbl1 null embryos could not be recovered beyond the blastocyst stage where proliferation of the pluripotent inner cell mass was impaired. Conditional ablation of Ruvbl1 in hematopoietic tissues led to bone marrow failure. Competitive repopulation experiments showed that this included the loss of hematopoietic stem cells through apopotosis. Pontin is, therefore, essential for the function of both embryonic pluripotent cells and adult hematopoietic stem cells.

Bertolo, A., et al. (2015). "Canine Mesenchymal Stem Cell Potential and the Importance of Dog Breed: Implication for Cell-Based Therapies." Cell Transplant **24**(10): 1969-1980.

The study of canine bone marrow-derived mesenchymal stem cells (MSCs) has a prominent position in veterinary cell-based applications. Yet the plethora of breeds, their different life spans, and interbreed variations provide unclearness on what can be achieved specifically by such therapies. In this study, we compared a set of morphological, physiological, and genetic markers of MSCs derived from large dog breeds, namely, Border collie, German shepherd, Labrador, Malinois, Golden retriever, and Hovawart. We compared colony-forming units (CFUs) assay, population doubling time (PDT), senescence-associated beta-galactosidase (SA-beta-gal) activity, telomere length, and gene expression of MSCs, as well as the ability of cells to differentiate to osteogenic, adipogenic, and chondrogenic phenotypes. The influence of the culture media alpha-MEM, low-glucose DMEM, and high-glucose DMEM, used in cell isolation and expansion, was investigated in the presence and absence of basic fibroblast growth factor (bFGF). Initial cell yield was not affected by culturing medium, but MSCs expanded best in alpha-MEM supplemented with bFGF. After isolation, the number of MSCs was similar among breeds--as shown by equivalent CFUs--except in the Hovawart samples, which had fivefold less CFU. Telomere lengths were similar among breeds. MSCs divided actively only for 4 weeks in culture (PDT = approximately 50 h/division), except Border collie cells divided for a longer time than cells from other groups. The percentage of senescent cells increased linearly in all breeds with time, with a faster rate in German shepherd, Labrador, and Golden retriever. Border collie cells underwent efficient osteogenic differentiation, Hovawart cells performed the best in chondrogenic differentiation, and Labrador cells in both, while German shepherd cells had the lower differentiation potential. MSCs from all breeds preserved the same adipogenic differentiation potential. In conclusion, despite variations, isolated MSCs can be expanded and differentiated in vitro, and all breeds are eligible for MSC-based therapies.

Blagoev, K. B. (2011). "Organ aging and susceptibility to cancer may be related to the geometry of the stem cell niche." Proc Natl Acad Sci U S A **108**(48): 19216-19221.

Telomere loss at each cell replication limits the proliferative capacity of normal cells, including adult stem cells. Entering replicative senescence protects dividing cells from neoplastic transformation, but also contributes to aging of the tissue. Recent experiments have shown that intestinal mouse stem cells divide symmetrically, at random make decisions to remain stem cells or to differentiate, and gradually lose telomeric DNA. A cell's decision whether to differentiate or to remain a stem cell depends on the local cellular and chemical environment and thus tissue architecture is expected to play role in cell proliferation dynamics. To take into account the structure of the stem cell niche in determining its proliferative potential and susceptibility to cancer, a theoretical model is introduced and the niche proliferative potential is quantified for different architectures. The niche proliferative potential is quantitatively related to the proliferative potential of the individual stem cells for different structural classes of the stem cell niche. Stem cells at the periphery of a niche are under pressure to divide and to differentiate, as well as to maintain the stem cell niche boundary, and thus the geometry of the stem cell niche is expected to play a role in determining the stem cell division sequence and differentiation. Smaller surface-to-volume ratio is associated with higher susceptibility to cancer, higher tissue renewal capacity, and decreased aging rate. Several testable experimental predictions are discussed, as well the presence of stochastic effects.

Boettcher, S., et al. (2020). "Clonal hematopoiesis in donors and long-term survivors of related allogeneic hematopoietic stem cell transplantation." Blood **135**(18): 1548-1559.

Clonal hematopoiesis (CH) is associated with age and an increased risk of myeloid malignancies, cardiovascular risk, and all-cause mortality. We tested for CH in a setting where hematopoietic stem cells (HSCs) of the same individual are exposed to different degrees of proliferative stress and environments, ie, in long-term survivors of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and their respective related donors (n = 42 donor-recipient pairs). With a median follow-up time since allo-HSCT of 16 years (range, 10-32 years), we found a total of 35 mutations in 23 out of 84 (27.4%) study participants. Ten out of 42 donors (23.8%) and 13 out of 42 recipients (31%) had CH. CH was associated with older donor and recipient age. We identified 5 cases of donor-engrafted CH, with 1 case progressing into myelodysplastic syndrome in both donor and recipient. Four out of 5 cases showed increased clone size in recipients compared with donors. We further characterized the hematopoietic system in individuals with CH as follows: (1) CH was consistently present in myeloid cells but varied in penetrance in B and T cells; (2) colony-forming units (CFUs) revealed clonal evolution or multiple independent clones in individuals with multiple CH mutations; and (3) telomere shortening determined in granulocytes suggested approximately 20 years of added proliferative history of HSCs in recipients compared with their donors, with telomere length in CH vs non-CH CFUs showing varying patterns. This study provides insight into the long-term behavior of the same human HSCs and respective CH development under different proliferative conditions.

Bojovic, B., et al. (2013). "Stem cell expansion during carcinogenesis in stem cell-depleted conditional telomeric repeat factor 2 null mutant mice." Oncogene **32**(43): 5156-5166.

To examine the role of telomeric repeat-binding factor 2 (TRF2) in epithelial tumorigenesis, we characterized conditional loss of TRF2 expression in the basal layer of mouse epidermis. These mice exhibit some characteristics of dyskeratosis congenita, a human stem cell depletion syndrome caused by telomere dysfunction. The epidermis in conditional TRF2 null mice exhibited DNA damage response and apoptosis, which correlated with stem cell depletion. The stem cell population in conditional TRF2 null epidermis exhibited shorter telomeres than those in control mice. Squamous cell carcinomas induced in conditional TRF2 null mice developed with increased latency and slower growth due to reduced numbers of proliferating cells as the result of increased apoptosis. TRF2 null epidermal stem cells were found in both primary and metastatic tumors. Despite the low-grade phenotype of the conditional TRF2 null primary tumors, the number of metastatic lesions was similar to control cancers. Basal cells from TRF2 null tumors demonstrated extreme telomere shortening and dramatically increased numbers of telomeric signals by fluorescence in situ hybridization due to increased genomic instability and aneuploidy in these cancers. DNA damage response signals were detected at telomeres in TRF2 null tumor cells from these mice. The increased genomic instability in these tumors correlated with eightfold expansion of the transformed stem cell population compared with that in control cancers. We concluded that genomic instability resulting from loss of TRF2 expression provides biological advantages to the cancer stem cell population.

Bonab, M. M., et al. (2006). "Aging of mesenchymal stem cell in vitro." BMC Cell Biol **7**: 14.

BACKGROUND: A hot new topic in medical treatment is the use of mesenchymal stem cells (MSC) in therapy. The low frequency of this subpopulation of stem cells in bone marrow (BM) necessitates their in vitro expansion prior to clinical use. We evaluated the effect of long term culture on the senescence of these cells. RESULTS: The mean long term culture was 118 days and the mean passage number was 9. The average number of PD decreased from 7.7 to 1.2 in the 10th passage. The mean telomere length decreased from 9.19 Kbp to 8.7 kbp in the 9th passage. Differentiation potential dropped from the 6th passage on. The culture's morphological abnormalities were typical of the Hayflick model of cellular aging. CONCLUSION: We believe that MSC enter senescence almost undetectably from the moment of in vitro culturing. Simultaneously these cells are losing their stem cell characteristics. Therefore, it is much better to consider them for cell and gene therapy early on.

Brosh, R. M., Jr., et al. (2017). "Fanconi Anemia: A DNA repair disorder characterized by accelerated decline of the hematopoietic stem cell compartment and other features of aging." Ageing Res Rev **33**: 67-75.

Fanconi Anemia (FA) is a rare autosomal genetic disorder characterized by progressive bone marrow failure (BMF), endocrine dysfunction, cancer, and other clinical features commonly associated with normal aging. The anemia stems directly from an accelerated decline of the hematopoietic stem cell compartment. Although FA is a complex heterogeneous disease linked to mutations in 19 currently identified genes, there has been much progress in understanding the molecular pathology involved. FA is broadly considered a DNA repair disorder and the FA gene products, together with other DNA repair factors, have been implicated in interstrand cross-link (ICL) repair. However, in addition to the defective DNA damage response, altered epigenetic regulation, and telomere defects, FA is also marked by elevated levels of inflammatory mediators in

circulation, a hallmark of faster decline in not only other hereditary aging disorders but also normal aging. In this review, we offer a perspective of FA as a monogenic accelerated aging disorder, citing the latest evidence for its multi-factorial deficiencies underlying its unique clinical and cellular features.

Brown, R. L. (2003). "Stem cell exhaustion and atherosclerosis." J Anti Aging Med **6**(3): 279; discussion 280.

Casale, A. M., et al. (2019). "Heterochromatin protein 1 (HP1) is intrinsically required for post-transcriptional regulation of Drosophila Germline Stem Cell (GSC) maintenance." Sci Rep **9**(1): 4372.

A very important open question in stem cells regulation is how the fine balance between GSCs self-renewal and differentiation is orchestrated at the molecular level. In the past several years much progress has been made in understanding the molecular mechanisms underlying intrinsic and extrinsic controls of GSC regulation but the complex gene regulatory networks that regulate stem cell behavior are only partially understood. HP1 is a dynamic epigenetic determinant mainly involved in heterochromatin formation, epigenetic gene silencing and telomere maintenance. Furthermore, recent studies have revealed the importance of HP1 in DNA repair, sister chromatid cohesion and, surprisingly, in positive regulation of gene expression. Here, we show that HP1 plays a crucial role in the control of GSC homeostasis in Drosophila. Our findings demonstrate that HP1 is required intrinsically to promote GSC self-renewal and progeny differentiation by directly stabilizing the transcripts of key genes involved in GSCs maintenance.

Cesselli, D., et al. (2011). "Effects of age and heart failure on human cardiac stem cell function." Am J Pathol **179**(1): 349-366.

Currently, it is unknown whether defects in stem cell growth and differentiation contribute to myocardial aging and chronic heart failure (CHF), and whether a compartment of functional human cardiac stem cells (hCSCs) persists in the decompensated heart. To determine whether aging and CHF are critical determinants of the loss in growth reserve of the heart, the properties of hCSCs were evaluated in 18 control and 23 explanted hearts. Age and CHF showed a progressive decrease in functionally competent hCSCs. Chronological age was a major predictor of five biomarkers of hCSC senescence: telomeric shortening, attenuated telomerase activity, telomere dysfunction-induced foci, and p21(Cip1) and p16(INK4a) expression. CHF had similar consequences for hCSCs, suggesting that defects in the balance between cardiomyocyte mass and the pool of nonsenescent hCSCs may condition the evolution of the decompensated myopathy. A correlation was found previously between telomere length in circulating bone marrow cells and cardiovascular diseases, but that analysis was restricted to average telomere length in a cell population, neglecting the fact that telomere attrition does not occur uniformly in all cells. The present study provides the first demonstration that dysfunctional telomeres in hCSCs are biomarkers of aging and heart failure. The biomarkers of cellular senescence identified here can be used to define the birth date of hCSCs and to sort young cells with potential therapeutic efficacy.

Chang, W. F., et al. (2019). "Compromised Chondrocyte Differentiation Capacity in TERC Knockout Mouse Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer." Int J Mol Sci **20**(5).

Mammalian telomere lengths are primarily regulated by telomerase, consisting of a reverse transcriptase protein (TERT) and an RNA subunit (TERC). We previously reported the generation of mouse Terc(+/-) and Terc(-/-) embryonic stem cells (ntESCs) by somatic cell nuclear transfer. In the present work, we investigated the germ layer development competence of Terc(-/-), Terc(+/-) and wild-type (Terc(+/+)) ntESCs. The telomere lengths are longest in wild-type but shortest in Terc(-/-) ntESCs, and correlate reversely with the population doubling time. Interestingly, while in vitro embryoid body (EB) differentiation assay reveals EB size difference among ntESCs of different genotypes, the more stringent in vivo teratoma assay demonstrates that Terc(-/-) ntESCs are severely defective in differentiating into the mesodermal lineage cartilage. Consistently, in a directed in vitro chondrocyte differentiation assay, the Terc(-/-) cells failed in forming Collagen II expressing cells. These findings underscore the significance in maintaining proper telomere lengths in stem cells and their derivatives for regenerative medicine.

Chapman, E. J., et al. (2008). "Genes involved in differentiation, stem cell renewal, and tumorigenesis are modulated in telomerase-immortalized human urothelial cells." Mol Cancer Res **6**(7): 1154-1168.

The expression of hTERT, the catalytic subunit of telomerase, immortalizes normal human urothelial cells (NHUC). Expression of a modified hTERT, without the ability to act in telomere maintenance, did not immortalize NHUC, confirming that effects at telomeres are required for urothelial immortalization. Previous studies indicate that inhibition of telomerase has an immediate effect on urothelial carcinoma (UC) cell line viability, before sufficient divisions to account for telomere attrition, implicating non-telomere effects of telomerase in UC. We analyzed the effects of telomerase on gene expression in isogenic mortal and hTERT-transduced NHUC. hTERT expression led to consistent alterations in the expression of genes predicted to be of

phenotypic significance in tumorigenesis. A subset of expression changes were detected soon after transduction with hTERT and persisted with continued culture. These genes (NME5, PSCA, TSPYL5, LY75, IGFBP2, IGF2, CEACAM6, XG, NOX5, KAL1, and HPGD) include eight previously identified as polycomb group targets. TERT-NHUC showed overexpression of the polycomb repressor complex (PRC1 and PRC4) components, BMI1 and SIRT1, and down-regulation of multiple PRC targets and genes associated with differentiation. TERT-NHUC at 100 population doublings, but not soon after transduction, showed increased saturation density and an attenuated differentiation response, indicating that these are not acute effects of telomerase expression. Some of the changes in gene expression identified may contribute to tumorigenesis. Expression of NME5 and NDN was down-regulated in UC cell lines and tumors. Our data supports the concept of both telomere-based and non-telomere effects of telomerase and provides further rationale for the use of telomerase inhibitors in UC.

Chen, J. (2011). "Hematopoietic stem cell development, aging and functional failure." Int J Hematol **94**(1): 3-10.

Hematopoietic stem cells (HSCs) are found in yolk sac, fetal liver, umbilical cord blood, placenta, and amniotic fluid during mammalian embryonic development. In adults, HSCs reside in marrow cavity of long bones where they self-renew and differentiate to replenish short-lived mature blood cells. HSCs exist in very low frequencies within specific "niches" where they interact with the surrounding environment through molecular associations. Overall HSC function can last much longer than a normal lifetime, but HSCs do show functional senescence with characteristic features of decreased self-renewal, reduced clonal stability, reduced homing and engraftment, and biased lineage commitment. The progressive shortening of telomeres with increasing age, especially under conditions with specific mutations in the telomerase gene complex, could predispose patients to HSC dysfunction and bone marrow failure diseases. Continuous investigation into HSC biology should facilitate the utilization of HSCs as a therapeutic modality and helps to prevent HSC malfunction.

Chen, R., et al. (2015). "Telomerase Deficiency Causes Alveolar Stem Cell Senescence-associated Low-grade Inflammation in Lungs." J Biol Chem **290**(52): 30813-30829.

Mutations of human telomerase RNA component (TERC) and telomerase reverse transcriptase (TERT) are associated with a subset of lung aging diseases, but the mechanisms by which TERC and TERT participate in lung diseases remain unclear. In this report, we show that knock-out (KO) of the mouse gene Terc or Tert causes pulmonary alveolar stem cell replicative senescence, epithelial impairment, formation of alveolar sacs, and characteristic inflammatory phenotype. Deficiency in TERC or TERT causes a remarkable elevation in various proinflammatory cytokines, including IL-1, IL-6, CXCL15 (human IL-8 homolog), IL-10, TNF-alpha, and monocyte chemotactic protein 1 (chemokine ligand 2 (CCL2)); decrease in TGF-beta1 and TGFbetaRI receptor in the lungs; and spillover of IL-6 and CXCL15 into the bronchoalveolar lavage fluids. In addition to increased gene expressions of alpha-smooth muscle actin and collagen 1alpha1, suggesting myofibroblast differentiation, TERC deficiency also leads to marked cellular infiltrations of a mononuclear cell population positive for the leukocyte common antigen CD45, low-affinity Fc receptor CD16/CD32, and pattern recognition receptor CD11b in the lungs. Our data demonstrate for the first time that telomerase deficiency triggers alveolar stem cell replicative senescence-associated low-grade inflammation, thereby driving pulmonary premature aging, alveolar sac formation, and fibrotic lesion.

Chilosi, M., et al. (2010). "Epithelial stem cell exhaustion in the pathogenesis of idiopathic pulmonary fibrosis." Sarcoidosis Vasc Diffuse Lung Dis **27**(1): 7-18.

New paradigms have been recently proposed in the pathogenesis of idiopathic pulmonary fibrosis (IPF), evidencing that in IPF the cumulative action of an accelerated parenchymal senescence determined by either telomere dysfunction or genetic defects, together with the concurrent noxious activity of tobacco smoking, are able to severely compromise the regenerative potential of parenchymal epithelial stem cells, triggering a cascade of molecular signals and events (scarring, bronchiolar proliferation, abnormal remodelling) eventually leading to severe and irreversible functional impairment. New pathogenic schemes focus on the complex molecular mechanisms driving in a vicious circle the different signalling pathways (e.g. Wnt/ -catenin, TGF-beta, caveolin-1, etc.) potentially involved in epithelial-mesenchymal transition and irreversible lung remodelling.

Choudhury, A. R., et al. (2007). "Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation." Nat Genet **39**(1): 99-105.

Telomere shortening limits the proliferative lifespan of human cells by activation of DNA damage pathways, including upregulation of the cell cycle inhibitor p21 (encoded by Cdkn1a, also known as Cip1 and Waf1)) (refs. 1-5). Telomere shortening in response to mutation of the gene encoding telomerase is associated with impaired organ maintenance and shortened lifespan in humans and in mice. The in vivo function of p21 in the context of telomere dysfunction

is unknown. Here we show that deletion of p21 prolongs the lifespan of telomerase-deficient mice with dysfunctional telomeres. p21 deletion improved hematolymphopoiesis and the maintenance of intestinal epithelia without rescuing telomere function. Moreover, deletion of p21 rescued proliferation of intestinal progenitor cells and improved the repopulation capacity and self-renewal of hematopoietic stem cells from mice with dysfunctional telomeres. In these mice, apoptotic responses remained intact, and p21 deletion did not accelerate chromosomal instability or cancer formation. This study provides experimental evidence that telomere dysfunction induces p21-dependent checkpoints in vivo that can limit longevity at the organismal level.

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

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

Dabelsteen, S., et al. (2009). "Epithelial cells derived from human embryonic stem cells display p16INK4A senescence, hypermotility, and differentiation properties shared by many P63+ somatic cell types." Stem Cells **27**(6): 1388-1399.

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

Dalle, J. H. and R. Peffault de Latour (2016). "Allogeneic hematopoietic stem cell transplantation for inherited bone marrow failure syndromes." Int J Hematol **103**(4): 373-379.

Inherited bone marrow failure (IBMF) syndromes are a heterogeneous group of rare hematological disorders characterized by the impairment of hematopoiesis, which harbor specific

clinical presentations and pathogenic mechanisms. Some of these syndromes may progress through clonal evolution, myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Most prominent are failures of DNA repair such as Fanconi Anemia and much rarer failure of ribosomal apparatus, e.g., Diamond Blackfan Anemia or of telomere elongation such as dyskeratosis congenita. In these congenital disorders, hematopoietic stem cell transplantation (HSCT) is often a consideration. However, HSCT will not correct the underlying disease and possible co-existing extra-medullary (multi)-organ defects, but will improve BMF. Indications as well as transplantation characteristics are most of the time controversial in this setting because of the rarity of reported cases. The present paper proposes a short overview of current practices.

De Angelis, A., et al. (2010). "Anthracycline cardiomyopathy is mediated by depletion of the cardiac stem cell pool and is rescued by restoration of progenitor cell function." Circulation **121**(2): 276-292.

BACKGROUND: Anthracyclines are the most effective drugs available in the treatment of neoplastic diseases; however, they have profound consequences on the structure and function of the heart, which over time cause a cardiomyopathy that leads to congestive heart failure. METHODS AND RESULTS: Administration of doxorubicin in rats led to a dilated myopathy, heart failure, and death. To test whether the effects of doxorubicin on cardiac anatomy and function were mediated by alterations in cardiac progenitor cells (CPCs), these cells were exposed to the anthracycline, which increased the formation of reactive oxygen species and caused increases in DNA damage, expression of p53, telomere attrition, and apoptosis. Additionally, doxorubicin resulted in cell-cycle arrest at the G2/M transition, which led to a significant decrease in CPC growth. Doxorubicin elicited multiple molecular adaptations; the massive apoptotic death that occurred in CPCs in the presence of anthracycline imposed on the surviving CPC pool the activation of several pathways aimed at preservation of the primitive state, cell division, lineage differentiation, and repair of damaged DNA. To establish whether delivery of syngeneic progenitor cells opposed the progression of doxorubicin cardiotoxicity, enhanced green fluorescent protein-labeled CPCs were injected in the failing myocardium; this treatment promoted regeneration of cardiomyocytes and vascular structures, which improved ventricular performance and rate of animal survival. CONCLUSIONS: Our results raise the possibility that autologous CPCs can be obtained before antineoplastic drugs are given to cancer patients and subsequently administered to individuals who are particularly sensitive to the cardiotoxicity of these agents for prevention or management of heart failure.

Deezagi, A. (2014). "Differential sensitivity of telomerase from human hematopoietic stem cells and leukemic cell lines to mild hyperthermia." Cell Biochem Biophys **69**(3): 681-691.

We have investigated the effects of hyperthermia (HT) on cell proliferation and telomerase activity of human hematopoietic stem cells (HSCs) and compared with human leukemic cell lines (TF-1, K562 and HL-60). The cells were exposed to HT at 42 and 43 degrees C up to 120 min. The cells were incubated at 37 degrees C for 96 h. Then the cells were collected and assayed for cell proliferation, viability, telomerase activity, and terminal restriction fragment (TRF) lengths. The enzyme activity from HSCs was decreased up to 68.6 at 42 and 85.1 % at 43 degrees C for 120 min. This inhibition in leukemic cells was up to 28.9 and 53.6 % in TF-1; 53 and 63.9 % in K562; 45.2 and 61.1 % in HL-60 cells. The treated cells showed TRF lengths about 5.3 kb for control HL-60 cells, 5.0 kb for HL-60 cells treated at 42 and 4.5 kb at 43 degrees C for 120 min. In HSCs, the TRF length was about 4.5 kb for untreated cells and 4.0-4.5 kb for treated cells at 42 and 43 degrees C for 120 min. The time response curves indicated that, inhibition of the enzyme activity in leukemic cells was dependent to the time of exposure to HT. But in HSCs, the inhibition was reached to steady state at 15 min exposure to 43 degrees C heat stress. TRF length was constant at treated two types of cells, which implies that in cells subjected to mild HT no telomere shortening was observed.

Deschenes-Simard, X., et al. (2019). "Circumventing senescence is associated with stem cell properties and metformin sensitivity." Aging Cell **18**(2): e12889.

Most cancers arise in old individuals, which also accumulate senescent cells. Cellular senescence can be experimentally induced by expression of oncogenes or telomere shortening during serial passage in culture. In vivo, precursor lesions of several cancer types accumulate senescent cells, which are thought to represent a barrier to malignant progression and a response to the aberrant activation of growth signaling pathways by oncogenes (oncogene toxicity). Here, we sought to define gene expression changes associated with cells that bypass senescence induced by oncogenic RAS. In the context of pancreatic ductal adenocarcinoma (PDAC), oncogenic KRAS induces benign pancreatic intraepithelial neoplasias (PanINs), which exhibit features of oncogene-induced senescence. We found that the bypass of senescence in PanINs leads to malignant PDAC cells characterized by gene signatures of epithelial-mesenchymal transition, stem cells, and mitochondria. Stem cell properties were similarly acquired in PanIN cells treated with LPS, and in primary fibroblasts and mammary epithelial cells that bypassed Ras-induced senescence after reduction of ERK signaling.

Intriguingly, maintenance of cells that circumvented senescence and acquired stem cell properties was blocked by metformin, an inhibitor of complex I of the electron transport chain or depletion of STAT3, a protein required for mitochondrial functions and stemness. Thus, our studies link bypass of senescence in premalignant lesions to loss of differentiation, acquisition of stemness features, and increased reliance on mitochondrial functions.

Diao, D., et al. (2018). "Telomeric epigenetic response mediated by Gadd45a regulates stem cell aging and lifespan." EMBO Rep **19**(10).

Progressive attrition of telomeres triggers DNA damage response (DDR) and limits the regenerative capacity of adult stem cells during mammalian aging. Intriguingly, telomere integrity is not only determined by telomere length but also by the epigenetic status of telomeric/sub-telomeric regions. However, the functional interplay between DDR induced by telomere shortening and epigenetic modifications in aging remains unclear. Here, we show that deletion of Gadd45a improves the maintenance and function of intestinal stem cells (ISCs) and prolongs lifespan of telomerase-deficient mice (G3Terc (-/-)). Mechanistically, Gadd45a facilitates the generation of a permissive chromatin state for DDR signaling by inducing base excision repair-dependent demethylation of CpG islands specifically at sub-telomeric regions of short telomeres. Deletion of Gadd45a promotes chromatin compaction in sub-telomeric regions and attenuates DDR initiation at short telomeres of G3Terc (-/-) ISCs. Treatment with a small molecule inhibitor of base excision repair reduces DDR and improves the maintenance and function of G3Terc (-/-) ISCs. Taken together, our study proposes a therapeutic approach to enhance stem cell function and prolong lifespan by targeting epigenetic modifiers.

Elmahadi, S., et al. (2016). "Allogeneic hematopoietic stem cell transplantation for dyskeratosis congenita." Curr Opin Hematol **23**(6): 501-507.

PURPOSE OF REVIEW: Dyskeratosis congenita is an inherited bone marrow failure syndrome caused by defects in telomere maintenance. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for bone marrow failure because of dyskeratosis congenita. The present review summarizes the literature with respect to the diagnosis and treatment of patients with dyskeratosis congenita who received HSCT, and discusses the recent progress in the management of dyskeratosis congenita. RECENT FINDINGS: The recent systematic review of the literature showed poor long-term outcome, with 10-year survival estimates of only 23% in 109 patients with dyskeratosis congenita who received HSCT. Multivariate analysis identified age greater than 20 years at HSCT, HSCT before 2000, and alternative donor source to be poor prognostic markers. HSCT for dyskeratosis congenita is characterized by a marked decline in long-term survival because of late deaths from pulmonary complications. However, a prospective study using danazol showed promising results in gain in telomere length and hematologic responses. SUMMARY: A recent prospective study may support the recommendation that HSCT is not indicated for patients with dyskeratosis congenita; instead, they should receive androgen, particularly danazol, as a first-line therapy. Another option may be routine use of androgen after HSCT for the prophylaxis of pulmonary fibrosis.

Estrada, J. C., et al. (2013). "Human mesenchymal stem cell-replicative senescence and oxidative stress are closely linked to aneuploidy." Cell Death Dis **4**: e691.

In most clinical trials, human mesenchymal stem cells (hMSCs) are expanded in vitro before implantation. The genetic stability of human stem cells is critical for their clinical use. However, the relationship between stem-cell expansion and genetic stability is poorly understood. Here, we demonstrate that within the normal expansion period, hMSC cultures show a high percentage of aneuploid cells that progressively increases until senescence. Despite this accumulation, we show that in a heterogeneous culture the senescence-prone hMSC subpopulation has a lower proliferation potential and a higher incidence of aneuploidy than the non-senescent subpopulation. We further show that senescence is linked to a novel transcriptional signature that includes a set of genes implicated in ploidy control. Overexpression of the telomerase catalytic subunit (human telomerase reverse transcriptase, hTERT) inhibited senescence, markedly reducing the levels of aneuploidy and preventing the dysregulation of ploidy-controlling genes. hMSC-replicative senescence was accompanied by an increase in oxygen consumption rate (OCR) and oxidative stress, but in long-term cultures that overexpress hTERT, these parameters were maintained at basal levels, comparable to unmodified hMSCs at initial passages. We therefore propose that hTERT contributes to genetic stability through its classical telomere maintenance function and also by reducing the levels of oxidative stress, possibly, by controlling mitochondrial physiology. Finally, we propose that aneuploidy is a relevant factor in the induction of senescence and should be assessed in hMSCs before their clinical use.

Farahzadi, R., et al. (2020). "Mesenchymal Stem Cells Could Be Considered as a Candidate for Further Studies in Cell-Based Therapy of Alzheimer's Disease via Targeting the Signaling Pathways." ACS Chem Neurosci **11**(10): 1424-1435.

Mesenchymal stem cells (MSCs) are of particular interest because of their potential in regenerative medicine. Stem cell-based therapies cast a new hope for neurodegenerative disease treatment as a regeneration strategy, including treatment for Alzheimer's disease (AD). A multitude of cytokines and factors secreted from MSCs are known to give such multifunctional properties, but associated mechanisms of these factors have yet to be entirely understood. To better understand the in vitro effect of MSCs on a neurodegenerative disorder, we treated primary cortical and hippocampal neural cells with amyloid beta (Abeta) as an in vitro cell line model for AD. For this purpose, bone marrow-derived MSCs (BMSCs) were cocultured with Abeta-treated neural cells, collected at day 3, and subjected to absolute telomere length measurement and telomerase activity assay. Next, the gene and protein expression levels of mTOR, p-mTOR, AMPK, p-AMPK, GSK-3beta, p-GSK-3beta, Wnt3, and beta-catenin were investigated. Also, after 3 days of coculture treatment, the supernatant was collected from both groups for cytokine measurement. It was found that telomere length as a biomarker in neurodegenerative disorder as well as telomerase activity had significantly increased in the experimental group, and the presence of IL-6, IL-10, and TGF-beta was obviously significant in the cocultured media. Also, BMSCs significantly changed the gene and protein expression of mTOR, AMPK, GSK-3beta, and Wnt3/beta-catenin signaling pathways components. It was concluded that the mentioned effects of MSCs on neural cells as an in vitro cell line model for AD as a therapeutic agent can be related to the signaling network.

Farooqi, A., et al. (2019). "Identification of patient-derived glioblastoma stem cell (GSC) lines with the alternative lengthening of telomeres phenotype." Acta Neuropathol Commun **7**(1): 76.

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.

Fehrer, C. and G. Lepperdinger (2005). "Mesenchymal stem cell aging." Exp Gerontol **40**(12): 926-930.

Stem cells are located throughout the adult body of higher organisms, supporting a continuous renewal and repair of tissues. Unique abilities of stem cells are self-renewal and multipotential differentiation. It is, therefore, of critical importance for an organism to maintain and control quantity and quality of stem cells within a given pool. Otherwise, when something goes awry within a stem cell, it is likely to have far-reaching effects. Mesenchymal stem cells (MSC) derived from various sources such as bone marrow or fat have been expanded in culture and differentiated in vitro into several lineages such as adipocytes, osteocytes or chondrocytes. In particular, aged human MSC show a decline in differentiation potential as well as in proliferation rate. The latter most likely reflects the fact that aged MSC suffer from eroded telomeres. Besides the individual age of the cell, stem and progenitor cell functions are influenced by the cellular environment, i.e. the niche and the architecture of the tissue, they reside in. This contribution reviews current knowledge about MSC aging (in vitro or in vivo), and respective difficulties for tissue engineering and stem cell therapy.

Fierabracci, A., et al. (2008). "Identification of an adult stem/progenitor cell-like population in the human thyroid." J Endocrinol **198**(3): 471-487.

There is evidence that tissue-specific stem cells reside in certain adult tissues. Their specific properties remain elusive, because they are rare and heterogeneous in parent tissues; furthermore, technical difficulties have been encountered in the identification and characterization of their progeny. The aim of this

study was to isolate stem/progenitor cells from the human thyroid. We devised a method based on the enzymatic digestion of fresh surgical thyroid specimens, followed by culture of cells in the presence of epidermal growth factor and basic fibroblast growth factor. We also used markers that identify and characterize these cells. Spheroids with self-replicative potential were obtained from all thyroid specimens. The isolated population contained a subset of CD34+ CD45- cells and it was able, in differentiation conditions, to generate follicles with thyroid hormonal production. In support of the plasticity concept, we obtained evidence that, when most freshly isolated spheroids were co-cultured with a neuroblastoma cell line, they produced progeny expressing the neuronal marker beta-tubulin III. Spheroids were also able to undergo adipogenic differentiation when cultured in adipogenic medium. We conclude that a predominant functional type of stem/progenitor cell exists within the thyroid, with an intrinsic ability to generate thyroidal cells and the potential to produce non-thyroidal cells.

Flores, I., et al. (2006). "Telomerase regulation and stem cell behaviour." Curr Opin Cell Biol **18**(3): 254-260.

Telomerase expression is restricted to a few cell types of the adult organism, most notably germ cells and stem/progenitor cells. Telomerase activity in germ cells is sufficient to prevent telomere shortening with age. Stem cells, however, do not have sufficient telomerase to prevent telomere shortening associated with continuous tissue renewal with increasing age. Indeed, telomerase levels in the adult organism are thought to be rate-limiting for longevity. This is supported by rare human syndromes caused by mutations in telomerase components, which are characterized by premature loss of tissue renewal and premature death. More recently, the role of telomerase and telomere length in stem cells is starting to be elucidated.

Flores, I. and M. A. Blasco (2009). "A p53-dependent response limits epidermal stem cell functionality and organismal size in mice with short telomeres." PLoS One **4**(3): e4934.

Telomere maintenance is essential to ensure proper size and function of organs with a high turnover. In particular, a dwarf phenotype as well as phenotypes associated to premature loss of tissue regeneration, including the skin (hair loss, hair graying, decreased wound healing), are found in mice deficient for telomerase, the enzyme responsible for maintaining telomere length. Coincidental with the appearance of these phenotypes, p53 is found activated in several tissues from these mice, where is thought to trigger cellular senescence and/or apoptotic responses. Here, we show that p53 abrogation rescues both the small size phenotype and restitutes the functionality of epidermal stem cells (ESC) of telomerase-deficient mice with dysfunctional telomeres. In particular, p53 ablation restores hair growth, skin renewal and wound healing responses upon mitogenic induction, as well as rescues ESCmobilization defects in vivo and defective ESC clonogenic activity in vitro. This recovery of ESC functions is accompanied by a downregulation of senescence markers and an increased proliferation in the skin and kidney of telomerase-deficient mice with critically short telomeres without changes in apoptosis rates. Together, these findings indicate the existence of a p53-dependent senescence response acting on stem/progenitor cells with dysfunctional telomeres that is actively limiting their contribution to tissue regeneration, thereby impinging on tissue fitness.

Flores, I. and M. A. Blasco (2010). "The role of telomeres and telomerase in stem cell aging." FEBS Lett **584**(17): 3826-3830.

Stem cells regenerate our bodies. In a similar manner to match ignition, stem cell "ignition" has to be precisely tuned to avoid uncontrolled proliferation as may occur in tumors or, inversely, the lack of proliferation as happens in degenerative disorders. During the last years it has become evident that telomeres and telomerase are main components of the stem cell "ignition" mechanism, providing a way to restrain cancer and delay aging.

Flores, I., et al. (2008). "The longest telomeres: a general signature of adult stem cell compartments." Genes Dev **22**(5): 654-667.

Identification of adult stem cells and their location (niches) is of great relevance for regenerative medicine. However, stem cell niches are still poorly defined in most adult tissues. Here, we show that the longest telomeres are a general feature of adult stem cell compartments. Using confocal telomere quantitative fluorescence in situ hybridization (telomapping), we find gradients of telomere length within tissues, with the longest telomeres mapping to the known stem cell compartments. In mouse hair follicles, we show that cells with the longest telomeres map to the known stem cell compartments, colocalize with stem cell markers, and behave as stem cells upon treatment with mitogenic stimuli. Using K15-EGFP reporter mice, which mark hair follicle stem cells, we show that GFP-positive cells have the longest telomeres. The stem cell compartments in small intestine, testis, cornea, and brain of the mouse are also enriched in cells with the longest telomeres. This constitutes the description of a novel general property of adult stem cell compartments. Finally, we make the novel finding that telomeres shorten with age in different mouse stem cell compartments, which parallels a decline in stem cell functionality, suggesting that telomere loss may contribute to stem cell dysfunction with age.

Fong, C. Y., et al. (2010). "Derivation efficiency, cell proliferation, freeze-thaw survival, stem-cell properties and differentiation of human Wharton's jelly stem cells." Reprod Biomed Online **21**(3): 391-401.

Human mesenchymal stem cells (MSC) are non-controversial multipotent stem cells. Their presence in umbilical cord blood (UCB) has been debated in some studies and others report low counts per cord blood unit and poor proliferation rates. On the other hand, Wharton's jelly of human umbilical cords appears to be a rich source of human MSC. This study derived 13 human Wharton's jelly stem cell (WJSC) lines from 13 human umbilical cords (100%) and recovered 4.7 +/- 0.2 x 10(6) live WJSC/cm of cord before culture. Complex culture medium produced greater proliferation rates of the WJSC in culture compared with simple medium. The mean population doubling times were 24.47 +/- 0.33 to 26.25 +/- 0.50 h in complex medium. The stem-cell markers of the WJSC were retained for at least 10 passages in both media. After programmed machine freezing, the thaw-survival rates of WJSC were 85-90% and they could be differentiated into neurons. Given the high derivation efficiency, availability of large numbers of fresh live cells, high expansion capabilities, prolonged maintenance of stem-cell properties and differentiation potential, it is proposed that human WJSC may be frozen at the same time as UCB in cord blood banks for regenerative medicine purposes.

Fukada, S., et al. (2014). "Adult stem cell and mesenchymal progenitor theories of aging." Front Cell Dev Biol **2**: 10.

Advances in medical science and technology allow people live longer lives, which results in age-related problems. Humans cannot avoid the various aged-related alterations of aging; in other words, humans cannot remain young at molecular and cellular levels. In 1956, Harman proposed the "free radical theory of aging" to explain the molecular mechanisms of aging. Telomere length, and accumulation of DNA or mitochondrial damage are also considered to be mechanisms of aging. On the other hand, stem cells are essential for maintaining tissue homeostasis by replacing parenchymal cells; therefore, the stem cell theory of aging is also used to explain the progress of aging. Importantly, the stem cell theory of aging is likely related to other theories. In addition, recent studies have started to reveal the essential roles of tissue-resident mesenchymal progenitors/stem cells/stromal cells in maintaining tissue homeostasis, and some evidence of their fundamental roles in the progression of aging has been presented. In this review, we discuss how stem cell and other theories connect to explain the progress of aging. In addition, we consider the mesenchymal progenitor theory of aging to describing the process of aging.

Fukunaga, A., et al. (2007). "Altered homeostasis of CD4(+) memory T cells in allogeneic hematopoietic stem cell transplant recipients: chronic graft-versus-host disease enhances T cell differentiation and exhausts central memory T cell pool." Biol Blood Marrow Transplant **13**(10): 1176-1184.

An increased risk of late infection is a serious complication after allogeneic hematopoietic stem cell transplantation (AHSCT), especially for recipients with defective CD4(+) T cell recovery. Although chronic graft-versus-host disease (cGVHD) negatively influences CD4(+) T cell reconstitution, the mechanisms leading to this defect are not well understood. We found that the proportion of CD27(-) CD4(+) T cells was remarkably increased in ASHCT recipients with cGVHD or with repetitive infectious episodes. Isolated CD27(-) CD4(+) T cells from ASHCT recipients had significantly shortened telomere length, displayed enhanced vulnerability to activation-induced cell death, and showed extremely reduced clonal diversity, when compared with CD27(-) CD4(+) T cells from healthy donors. Also, CD27(+) CD4(+) T cells from AHSCT recipients easily lost their expression of CD27 in response to antigen stimulation regardless of cGVHD status. Taken together, these data indicate that homeostasis of memory CD4(+) T cells from AHSCT recipients is altered, and that they easily transit into CD27(-) effector memory T cells. Increased in vivo T cell stimulation observed in recipients with cGVHD further promotes the transition to effector memory cells, a change that decreases the central memory CD4(+) T cell pool and consequently weakens the recipient's defense against persistently infecting pathogens.

Ganuza, M., et al. (2012). "Genetic inactivation of Cdk7 leads to cell cycle arrest and induces premature aging due to adult stem cell exhaustion." EMBO J **31**(11): 2498-2510.

Cyclin-dependent kinase (Cdk)7, the catalytic subunit of the Cdk-activating kinase (CAK) complex has been implicated in the control of cell cycle progression and of RNA polymerase II (RNA pol II)-mediated transcription. Genetic inactivation of the Cdk7 locus revealed that whereas Cdk7 is completely dispensable for global transcription, is essential for the cell cycle via phosphorylation of Cdk1 and Cdk2. In vivo, Cdk7 is also indispensable for cell proliferation except during the initial stages of embryonic development. Interestingly, widespread elimination of Cdk7 in adult tissues with low proliferative indexes had no phenotypic consequences. However, ablation of conditional Cdk7 alleles in tissues with elevated cellular turnover led to the efficient repopulation of these tissues with Cdk7-expressing cells most likely derived from adult stem cells that may have escaped the inactivation of their targeted Cdk7 alleles. This

process, a physiological attempt to maintain tissue homeostasis, led to the attrition of adult stem cell pools and to the appearance of age-related phenotypes, including telomere shortening and early death.

Garcia-Lavandeira, M., et al. (2009). "A GRFa2/Prop1/stem (GPS) cell niche in the pituitary." PLoS One **4**(3): e4815.

BACKGROUND: The adult endocrine pituitary is known to host several hormone-producing cells regulating major physiological processes during life. Some candidates to progenitor/stem cells have been proposed. However, not much is known about pituitary cell renewal throughout life and its homeostatic regulation during specific physiological changes, such as puberty or pregnancy, or in pathological conditions such as tumor development. PRINCIPAL FINDINGS: We have identified in rodents and humans a niche of non-endocrine cells characterized by the expression of GFRa2, a Ret co-receptor for Neurturin. These cells also express b-Catenin and E-cadherin in an oriented manner suggesting a planar polarity organization for the niche. In addition, cells in the niche uniquely express the pituitary-specific transcription factor Prop1, as well as known progenitor/stem markers such as Sox2, Sox9 and Oct4. Half of these GPS (GFRa2/Prop1/Stem) cells express S-100 whereas surrounding elongated cells in contact with GPS cells express Vimentin. GFRa2+-cells form non-endocrine spheroids in culture. These spheroids can be differentiated to hormone-producing cells or neurons outlining the neuroectoderm potential of these progenitors. In vivo, GPSs cells display slow proliferation after birth, retain BrdU label and show long telomeres in its nuclei, indicating progenitor/stem cell properties in vivo. SIGNIFICANCE: Our results suggest the presence in the adult pituitary of a specific niche of cells characterized by the expression of GFRa2, the pituitary-specific protein Prop1 and stem cell markers. These GPS cells are able to produce different hormone-producing and neuron-like cells and they may therefore contribute to postnatal pituitary homeostasis. Indeed, the relative abundance of GPS numbers is altered in Cdk4-deficient mice, a model of hypopituitarism induced by the lack of this cyclin-dependent kinase. Thus, GPS cells may display functional relevance in the physiological expansion of the pituitary gland throughout life as well as protection from pituitary disease.

Geiger, H. and K. L. Rudolph (2009). "Aging in the lympho-hematopoietic stem cell compartment." Trends Immunol **30**(7): 360-365.

Cells of the immune system are progeny of a single primitive cell type, the hematopoietic stem cell (HSC). Aging in most strains of mice is associated with a reduction in HSC frequency and a reduction in HSC function. Aged HSCs demonstrate reduced differentiation toward the lymphoid lineage, and this might be a relevant factor influencing immunosenescence. The molecular mechanisms of HSC aging need to be determined in more detail, but current studies have identified, among others, a role for telomere dysfunction in inducing cell intrinsic checkpoints and environmental alterations, which both skews and reduces stem cell differentiation and function. Reverting or ameliorating aging of HSCs might be a crucial step to restoring immuno-competence in the elderly.

Geng, S., et al. (2015). "Study on the Dynamic Biological Characteristics of Sca-1(+) Hematopoietic Stem and Progenitor Cell Senescence." Stem Cells Int **2015**: 954120.

The researches in the dynamic changes of the progress of HSCs aging are very limited and necessary. In this study, male C57BL/6 mice were divided into 5 groups by age. We found that the superoxide damage of HSPCs started to increase from the middle age (6 months old), with notably reduced antioxidation ability. In accordance with that, the senescence of HSPCs also started from the middle age, since the self-renewal and differentiation ability remarkably decreased, and senescence-associated markers SA-beta-GAL increased in the 6-month-old and the older groups. Interestingly, the telomere length and telomerase activity increased to a certain degree in the 6-month-old group. It suggested an intrinsic spontaneous ability of HSPCs against aging. It may provide a theoretical and experimental foundation for better understanding the senescence progress of HSPCs. And the dynamic biological characteristics of HSPCs senescence may also contribute to the clinical optimal time for antiaging drug intervention.

Giraddi, R. R., et al. (2015). "Stem and progenitor cell division kinetics during postnatal mouse mammary gland development." Nat Commun **6**: 8487.

The cycling properties of mammary stem and progenitor cells is not well understood. To determine the division properties of these cells, we administered synthetic nucleosides for varying periods of time to mice at different stages of postnatal development and monitored the rate of uptake of these nucleosides in the different mammary cell compartments. Here we show that most cell division in the adult virgin gland is restricted to the oestrogen receptor-expressing luminal cell lineage. Our data also demonstrate that the oestrogen receptor-expressing, milk and basal cell subpopulations have telomere lengths and cell division kinetics that are not compatible with these cells being hierarchically organized; instead, our data indicate that in the adult homeostatic gland, each cell type is largely maintained by its own restricted progenitors. We also observe that transplantable stem cells are largely

quiescent during oestrus, but are cycling during dioestrus when progesterone levels are high.
Gonzalez-Estevez, C. and I. Flores (2020). "Fasting for stem cell rejuvenation." Aging (Albany NY) **12**(5): 4048-4049.
Greenwood, M. J. and P. M. Lansdorp (2003). "Telomeres, telomerase, and hematopoietic stem cell biology." Arch Med Res **34**(6): 489-495.

Telomeres are composed of the tandem DNA repeats and associated proteins that cap the end of linear chromosomes. They provide stability to the chromosome and protect against DNA loss associated with cellular replication. Telomeres are maintained by the reverse transcriptase, telomerase. The regulation of telomere length and telomerase activity is a complex and dynamic process that is tightly linked to cell cycle regulation. Hematopoietic stem cells have an impressive but finite proliferative potential and demonstrate telomeric shortening during replicative aging despite expression of low levels of telomerase. Recently, the important role of telomeres in human illness has been highlighted by studies of the rare genetic disorder dyskeratosis congenita. Here we review the role of telomeres and telomerase in the function and regulation of the hematopoietic stem cell compartment and their importance in hematologic disease.

Gu, B. W., et al. (2011). "Accelerated hematopoietic stem cell aging in a mouse model of dyskeratosis congenita responds to antioxidant treatment." Aging Cell **10**(2): 338-348.

Mutations in DKC1, encoding telomerase associated protein dyskerin, cause X-linked dyskeratosis congenita (DC), a bone marrow (BM) failure, and cancer susceptibility syndrome. Decreased accumulation of telomerase RNA resulting in excessive telomere shortening and premature cellular senescence is thought to be the primary cause of disease in X-linked DC. Affected tissues are those that require constant renewal by stem cell activity. We previously showed that in Dkc1(Delta15) mice, which contain a mutation that is a copy of a human mutation causing DC, mutant cells have a telomerase-dependent proliferative defect and increased accumulation of DNA damage in the first generation before the telomeres are short. We now demonstrate the presence of the growth defect in Dkc1(Delta15) mouse embryonic fibroblasts in vitro and show that accumulation of DNA damage and levels of reactive oxygen species increase with increasing population doublings. Treatment with the antioxidant, N-acetyl cysteine (NAC), partially rescued the growth disadvantage of mutant cells in vitro and in vivo. Competitive BM repopulation experiments showed that the Dkc1(Delta15) mutation is associated with a functional stem cell defect that becomes more severe with increasing age, consistent with accelerated senescence, a hallmark of DC hematopoiesis. This stem cell phenotype was partially corrected by NAC treatment. These results suggest that a pathogenic Dkc1 mutation accelerates stem cell aging, that increased oxidative stress might play a role in the pathogenesis of X-linked DC, and that some manifestations of DC may be prevented or delayed by antioxidant treatment.

Hao, H., et al. (2013). "Culturing on Wharton's jelly extract delays mesenchymal stem cell senescence through p53 and p16INK4a/pRb pathways." PLoS One **8**(3): e58314.

Mesenchymal stem cells (MSCs) hold great therapeutic potential. However, MSCs undergo replication senescence during the in vitro expansion process. Wharton's jelly from the human umbilical cord harbors a large number of MSCs. In this study, we hypothesized that Wharton's jelly would be beneficial for in vitro expansion of MSCs. Wharton's jelly extract (WJEs), which is mainly composed of extracellular matrix and cytokines, was prepared as coating substrate. Human MSCs were isolated and cultured on WJE-coated plates. Although the proliferation capacity of cells was not augmented by WJE in early phase culture, adynamic growth in late-phase culture was clearly reduced, suggesting that the replicative senescence of MSCs was efficiently slowed by WJE. This was confirmed by beta-galactosidase staining and telomere length measurements of MSCs in late-phase culture. In addition, the decreased differentiation ability of MSCs after long-term culture was largely ameliorated by WJE. Reactive oxygen species (ROS), p53, and p16INK4a/pRb expression increased with passaging. Analysis at the molecular level revealed that WJE-based culture efficiently suppressed the enhancement of intracellular ROS, p53, and p16INK4a/pRb in MSCs. These data demonstrated that WJE provided an ideal microenvironment for MSCs culture expansion in vitro preserved MSC properties by delaying MSCs senescence, and allowed large numbers of MSCs to be obtained for basic research and clinical therapies.

Havenith, S. H., et al. (2012). "Analysis of stem-cell-like properties of human CD161++IL-18Ralpha+ memory CD8+ T cells." Int Immunol **24**(10): 625-636.

CD161(++)IL-18Ralpha(+)CD8(+) human T cells have recently been identified as a new subset of memory cells but their exact role remains unclear. CD161(++)IL-18Ralpha(+)CD8(+), mucosal-associated invariant T cells express a semi-invariant TCR Valpha7.2-Jalpha33, which recognizes the MHC-related protein 1. On the basis of properties including the expression of the ABC-B1 transporter, cKit expression and survival after chemotherapy, CD161(++)IL-18Ralpha(+)CD8(+) T cells have been designated as 'stem' cells. Here we analyse location and

functional properties of CD161(++)IL-18Ralpha(+) CD8(+) T cells and question whether they have other traits that would mark them as genuine 'stem' cells. CD161(++)IL-18Ralpha(+)CD8(+) T cells were found in peripheral blood, spleen and bone marrow but interestingly hardly at all in lymph nodes (LNs), which may possibly be explained by the finding that these cells express a specific set of chemokine receptors that allows migration to inflamed tissue rather than to LNs. In addition to TCR ligation and co-stimulation, CD161(++)IL-18Ralpha(+) CD8(+) T cells require cytokines for proliferation. The CD161(++)IL-18Ralpha(+) CD8(+) pool contains cells reactive towards peptides, derived from both persisting and cleared viruses. Although CD161(++)IL-18Ralpha(+) CD8(+) T cells express the ABC-B1 transporter, they have shorter telomeres and less telomerase activity and do not express aldehyde dehydrogenase. Finally, CD161(++)IL-18Ralpha(+) CD8(+) T cells show similarities to terminally differentiated T cells, expressing IFNgamma, KLRG1 and the transcription factor Blimp-1. In conclusion, CD161(++)IL-18Ralpha(+) CD8(+) T cells lack many features of typical 'stem' cells, but appear rather to be a subset of effector-type cells.

Hidema, S., et al. (2016). "Transgenic expression of Telomerase reverse transcriptase (Tert) improves cell proliferation of primary cells and enhances reprogramming efficiency into the induced pluripotent stem cell." Biosci Biotechnol Biochem **80**(10): 1925-1933.

The enzymatic activity of telomerase is important for the extension of the telomere repeat sequence and overcoming cellular senescence. We generated a conditional transgenic mouse line, carrying the telomerase reverse transcriptase (Tert) expression cassette, controlled by the Cre-loxP-mediated recombination. In our study, Cre recombinase expression efficiently activated Tert expression, resulting in its increased enzymatic activity, which extended the period of cellular proliferation until the keratinocytes entered senescence. This suggests that transgenic Tert expression is effective in enhancing primary cell proliferation. Notably, Tert expression increased colony formation of induced pluripotent stem (iPS) cells after the introduction of four reprogramming factors, Oct-4, klf4, SOX-2, and c-Myc into the transgenic fibroblasts. To the best of our knowledge, this is the first study to show that the transgenic Tert expression enhances reprogramming efficiency of iPS cells, which indicates a critical role for Tert in the reprogramming process.

Hirao, A. (2006). "[Regulation of hematopoietic stem cell self-renewal by aging-related genes]." Rinsho Ketsueki **47**(5): 379-382.

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.

Holyoake, T. L., et al. (2002). "Elucidating critical mechanisms of deregulated stem cell turnover in the chronic phase of chronic myeloid leukemia." Leukemia **16**(4): 549-558.

Chronic myeloid leukemia (CML) has been studied intensively for many years; yet its treatment remains problematic and its biology remains elusive. In chronic phase, the leukemic clone appears to be maintained by a small number of BCR-ABL-positive hematopoietic stem cells that differentiate normally and amplify slowly. In contrast, as these cells enter the intermediate stages of lineage restriction, their progeny are selectively expanded and generate an enlarged pool of neoplastic progenitors. Recent analyses of purified subsets of primitive CML cells have provided a coherent explanation for this dichotomous behavior of BCR-ABL-positive stem and progenitor cells based on the discovery of an unusual autocrine IL-3/G-CSF mechanism activated in them. This only partially counteracts in vivosignals that maintain normal stem cells in a quiescent state but, when active in CML stem cells, promotes their differentiation in favor of their self-renewal. In more differentiated CML progenitors, the same mechanism has a more potent mitogenic effect which is then extinguished when the cells enter the terminal stages of differentiation. Thus, further expansion of the clone is limited until inevitably additional mutations are acquired that further distort or override the regulatory mechanisms still operative in the chronic phase.

Hosokawa, K., et al. (2017). "Functional analysis of Protection of Telomeres 1a (Pot1a) in regulation of hematopoietic stem cell aging." Rinsho Ketsueki **58**(8): 942-949.

Repeated cell divisions induce DNA damage accumulation, which impairs stem cell function during

aging. However, the general molecular mechanisms by which this occurs remain unclear. Herein, we show that the expression of protection of telomeres 1a (Pot1a), a component of shelterin, is crucial for prevention of telomeric DNA damage response (DDR) and maintenance of hematopoietic stem cell (HSC) activity during aging. We observed that HSCs express high levels of Pot1a during development, and this expression declines with aging. Knockdown of Pot1a induced an age-related phenotype, characterized by increased telomeric DDR and reduced long-term reconstitution activity. In contrast, treatment with exogenous Pot1a protein prevented telomeric DDR, which decreased stem cell activity and partially rejuvenated HSC activity. These results highlight a general, reversible mechanism by which aging compromises mammalian stem cell activity, with widespread implications for regenerative medicine.

Huang, L., et al. (2011). "Stem cell-like properties of human umbilical cord lining epithelial cells and the potential for epidermal reconstitution." Cytotherapy **13**(2): 145-155.

BACKGROUND AIMS: Stem cells are particularly attractive for many cell-based therapeutic interventions because of their ability to self-renew and their capacity to differentiate into site-specific differentiating cells. Restoration of the integrity of epithelial continuity is an essential aspect of wound repair and tissue regeneration. We are currently looking at the potential of human umbilical cord lining cells as a source of epithelial stem cells with appropriate differentiation capacity for potential epidermal reconstitution. METHODS: We isolated human umbilical cord lining epithelial cells (CLEC) and characterized their phenotype from the perspective of proliferative potential, telomere length, expression of epidermal differentiation markers, as well as stem cell-specific markers, and clonogenicity. Their potential for epidermal reconstitution was investigated in an organotypic culture model. RESULTS: The results demonstrated that CLEC present a long telomere length and have a relatively high proliferative potential and passaging ability in culture. CLEC display some of the stem cell-specific markers for epithelial as well as pluripotent stem cells, including CK19, p63, OCT-4, SSEA-4, TRA-1-60, SOX2 and Nanog. CLEC are capable of generating a fully stratified epithelium in organotypic culture. CONCLUSIONS: The potential of CLEC to be used in clinical applications for specialized epithelial reconstruction is still unexplored. The demonstration that CLEC have stem cell-like properties and are capable of generating fully stratified epithelium provides support for their potential clinical application in epidermal reconstitution.

Imamura, M. (2000). "[Hematopoietic stem cell transplantation and aging]." Nihon Ronen Igakkai Zasshi **37**(9): 709-711.

Infante, A., et al. (2014). "Prelamin A accumulation and stress conditions induce impaired Oct-1 activity and autophagy in prematurely aged human mesenchymal stem cell." Aging (Albany NY) **6**(4): 264-280.

Aging, a time-dependent functional decline of biological processes, is the primary risk factor in developing diseases such as cancer, cardiovascular or degenerative diseases. There is a real need to understand the human aging process in order to increase the length of disease-free life, also known as "health span". Accumulation of progerin and prelamin A are the hallmark of a group of premature aging diseases but have also been found during normal cellular aging strongly suggesting similar mechanisms between healthy aging and LMNA-linked progeroid syndromes. How this toxic accumulation contributes to aging (physiological or pathological) remains unclear. Since affected tissues in age-associated disorders and in pathological aging are mainly of mesenchymal origin we propose a model of human aging based on mesenchymal stem cells (hMSCs) which accumulate prelamin A. We demonstrate that prelamin A-accumulating hMSCs have a premature aging phenotype which affects their functional competence in vivo. The combination of prelamin A accumulation and stress conditions enhance the aging phenotype by dysregulating the activity of the octamer binding protein Oct-1This experimental model has been fundamental to identify a new role for Oct-1 in hMSCs aging.

Isoda, T., et al. (2013). "Irreversible leukoencephalopathy after reduced-intensity stem cell transplantation in a dyskeratosis congenita patient with TINF2 mutation." J Pediatr Hematol Oncol **35**(4): e178-182.

Hematopoietic stem cell transplantation (HSCT) for dyskeratosis congenita (DC) is challenging due to severe treatment-related adverse effects. Development of pulmonary fibrosis or veno-occlusive disease is well described in DC. However, neurological complication after HSCT has not been reported. A 9-year-old Japanese male with DC harboring the TINF2 mutation received reduced-intensity HSCT. Unfortunately, patient developed posterior reversible encephalopathy syndrome-like symptoms plausibly result by combination of thrombotic microangiopathy, graft-versus-host disease, and persistent hypertension and has been persisted mental retardation. Therefore, to decrease risk in DC cases after HSCT, strict control of hypertension, graft-versus-host disease, and thrombotic microangiopathy is required.

Jorgensen, H. G. and T. L. Holyoake (2001). "A comparison of normal and leukemic stem cell biology in Chronic Myeloid Leukemia." Hematol Oncol **19**(3): 89-106.

Chronic Myeloid Leukemia (CML), a myeloproliferative disease of stem cell origin, is characterized by the presence of the Philadelphia (Ph) chromosome and the bcr-abl oncogene. The BCR-ABL fusion gene product, thought to be causative in CML, has multiple effects on diverse cell functions such as growth, differentiation and turnover as well as adhesion and apoptosis. Persistent Ph-negative progenitors co-exist with leukemic cells, both in the marrow and blood of patients, in the early chronic phase of the disease. Despite accumulating knowledge of hemopoiesis and the disease process, CML remains incurable with conventional chemotherapy. Nonetheless, with the efficacy of the ABL tyrosine kinase inhibitor STI-571 (signal transduction inhibitor 571) as a novel therapy in CML recently being realized in clinical trials, it is therefore timely to review our current understanding of the cell biology of this fascinating disease.

Jose, S. S., et al. (2018). "The Telomerase Complex Directly Controls Hematopoietic Stem Cell Differentiation and Senescence in an Induced Pluripotent Stem Cell Model of Telomeropathy." Front Genet **9**: 345.

Telomeropathies are rare disorders associated with impaired telomere length control mechanisms that frequently result from genetic mutations in the telomerase complex. Dyskeratosis congenita is a congenital progressive telomeropathy in which mutation in the telomerase RNA component (TERC) impairs telomere maintenance leading to accelerated cellular senescence and clinical outcomes resembling premature aging. The most severe clinical feature is perturbed hematopoiesis and bone-marrow failure, but the underlying mechanisms are not fully understood. Here, we developed a model of telomerase function imbalance using shRNA to knockdown TERC expression in human induced pluripotent stem cells (iPSCs). We then promoted in vitro hematopoiesis in these cells to analyze the effects of TERC impairment. Reduced TERC expression impaired hematopoietic stem-cell (HSC) differentiation and increased the expression of cellular senescence markers and production of reactive oxygen species. Interestingly, telomere length was unaffected in shTERC knockdown iPSCs, leading to conclusion that the phenotype is controlled by non-telomeric functions of telomerase. We then assessed the effects of TERC-depletion in THP-1 myeloid cells and again observed reduced hematopoietic and myelopoietic differentiative potential. However, these cells exhibited impaired telomerase activity as verified by accelerated telomere shortening. shTERC-depleted iPSC-derived and THP-1-derived myeloid precursors had lower phagocytic capacity and increased ROS production, indicative of senescence. These findings were confirmed using a BIBR1532 TERT inhibitor, suggesting that these phenotypes are dependent on telomerase function but not directly linked to telomere length. These data provide a better understanding of the molecular processes driving the clinical signs of telomeropathies and identify novel roles of the telomerase complex other than regulating telomere length.

Joseph, I., et al. (2010). "The telomerase inhibitor imetelstat depletes cancer stem cells in breast and pancreatic cancer cell lines." Cancer Res **70**(22): 9494-9504.

Cancer stem cells (CSC) are rare drug-resistant cancer cell subsets proposed to be responsible for the maintenance and recurrence of cancer and metastasis. Telomerase is constitutively active in both bulk tumor cell and CSC populations but has only limited expression in normal tissues. Thus, inhibition of telomerase has been shown to be a viable approach in controlling cancer growth in nonclinical studies and is currently in phase II clinical trials. In this study, we investigated the effects of imetelstat (GRN163L), a potent telomerase inhibitor, on both the bulk cancer cells and putative CSCs. When breast and pancreatic cancer cell lines were treated with imetelstat in vitro, telomerase activity in the bulk tumor cells and CSC subpopulations were inhibited. Additionally, imetelstat treatment reduced the CSC fractions present in the breast and pancreatic cell lines. In vitro treatment with imetelstat, but not control oligonucleotides, also reduced the proliferation and self-renewal potential of MCF7 mammospheres and resulted in cell death after <4 weeks of treatment. In vitro treatment of PANC1 cells showed reduced tumor engraftment in nude mice, concomitant with a reduction in the CSC levels. Differences between telomerase activity expression levels or telomere length of CSCs and bulk tumor cells in these cell lines did not correlate with the increased sensitivity of CSCs to imetelstat, suggesting a mechanism of action independent of telomere shortening for the effects of imetelstat on the CSC subpopulations. Our results suggest that imetelstat-mediated depletion of CSCs may offer an alternative mechanism by which telomerase inhibition may be exploited for cancer therapy.

Ju, Z., et al. (2007). "A dual role of p21 in stem cell aging." Ann N Y Acad Sci **1100**: 333-344.

A decline in adult stem cell function occurs during aging, likely contributing to the decline in organ homeostasis and regeneration with age. An emerging field in aging research is to analyze molecular pathways limiting adult stem cell function in response to macromolecular damage accumulation during aging. Current data suggest that the p21 cell cycle inhibitor

has a dual role in stem cell aging: On one hand, p21 protects adult stem cells from acute genotoxic stress by preventing inappropriate cycling of acutely damaged stem cells. On the other hand, p21 activation impairs stem cell function and survival of aging telomere dysfunctional mice indicating that p21 checkpoint function is disadvantageous in the context of chronic and persistent damage, which accumulates during aging. This article focuses on these dual roles of p21 in aging stem cells.

Kalkman, H. O. (2009). "Altered growth factor signaling pathways as the basis of aberrant stem cell maturation in schizophrenia." Pharmacol Ther **121**(1): 115-122.

In recent years evidence has accumulated that the activity of the signaling cascades of Neuregulin-1, Wnt, TGF-beta, BDNF-p75 and DISC1 is different between control subjects and patients with schizophrenia. These pathways are involved in embryonic and adult neurogenesis and neuronal maturation. A review of the clinical data indicates that in schizophrenia the Wnt pathway is most likely hypoactive, whereas the Nrg1-ErbB4, the TGF-beta- and the BDNF-p75-pathways are hyperactive. Haplo-insuffiency of the DISC1 gene is currently the best established schizophrenia risk factor. Preclinical experiments indicate that suppression of DISC1 signaling leads to accelerated dendrite development in neuronal stem cells, accelerated migration and aberrant integration into the neuronal network. Other preclinical experiments show that increasing NRG1-, BDNF- and TGF-beta signaling and decreasing Wnt signaling, also promotes adult neuronal differentiation and migration. Thus deviations in these pathways detected in schizophrenia could contribute to premature neuronal differentiation, accelerated migration and inappropriate insertion into the neuronal network. Initial clinical findings are confirmatory: neuronal stem cells isolated from nasal biopsies from schizophrenia patients display signs of accelerated development, whilst increased erosion of telomeres and bone age provide further support for accelerated cell maturation in schizophrenia.

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

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

Kamminga, L. M. and G. de Haan (2006). "Cellular memory and hematopoietic stem cell aging." Stem Cells **24**(5): 1143-1149.

Hematopoietic stem cells (HSCs) balance self-renewal and differentiation in order to sustain lifelong blood production and simultaneously maintain the HSC pool. However, there is clear evidence that HSCs are subject to quantitative and qualitative exhaustion. In this review, we briefly discuss several known aspects of the stem cell aging process, including DNA damage, telomere shortening, and oxidative stress. Besides these known players, there is increasing evidence that higher order chromatin structure, largely defined by the histone code and affecting transcriptional activity, is important. A model is suggested which describes how epigenetic regulation of gene transcription by modulation of the chromatin structure in stem cells can account for regulation of the aging program.

Kaneko, S. (2016). "In Vitro Generation of Antigen-Specific T Cells from Induced Pluripotent Stem Cells of Antigen-Specific T Cell Origin." Methods Mol Biol **1393**: 67-73.

Induced pluripotent stem (iPS) cells derived from T lymphocyte (T-iPS cells) preserve the T cell receptor (TCR) alpha and beta gene rearrangements identical to the original T cell clone. Re-differentiated CD8 single positive alphabeta T cells from the T-iPS cells exhibited antigen-specific cytotoxicity, improved proliferative response, and elongation of telomere

indicating rejuvenation of antigen specific T cell immunity in vitro. To regenerate antigen specific cytotoxic T lymphocytes (CTL), first, we have optimized a method for reprogramming-resistant CD8 T cell clones into T-iPS cells by using sendaiviral vectors. Second, we have optimized stepwise differentiation methods for inducing hematopoietic progenitor cells, T cell progenitors, and functionally matured CD8 single positive CTL. These protocols provide useful in vitro tools and models both for research of antigen-specific T cell immunotherapy and for research of normal and pathological thymopoiesis.

Kawano, Y., et al. (2011). "Low telomerase activity in CD4+ regulatory T cells in patients with severe chronic GVHD after hematopoietic stem cell transplantation." Blood **118**(18): 5021-5030.

CD4(+)CD25(+)Foxp3(+) regulatory T cells (Treg) play an important role in the control of chronic graft-versus-host disease (cGVHD). In this study, we examined telomere length and telomerase activity of Treg and conventional CD4(+) T cells (Tcon) in 61 patients who survived more than 2 years after allogeneic hematopoietic stem cell transplantation. Cell proliferation and expression of Bcl-2 were also measured in each subset. Treg telomere length was shorter and Treg telomerase activity was increased compared with Tcon ($P < .0001$). After transplantation, Treg were also more highly proliferative than Tcon ($P < .0001$). Treg number, telomerase activity, and expression of Bcl-2 were each inversely associated with severity of cGVHD. These data indicate that activation of telomerase is not sufficient to prevent telomere shortening in highly proliferative Treg. However, telomerase activation is associated with increased Bcl-2 expression and higher Treg numbers in patients with no or mild cGVHD. In contrast, patients with moderate or severe cGVHD have fewer Treg with lower levels of telomerase activity and Bcl-2 expression. These results suggest that failure to activate Treg telomerase may restrict proliferative capacity and increase apoptotic susceptibility, resulting in the loss of peripheral tolerance and the development of cGVHD.

Khattar, E., et al. (2019). "Rap1 regulates hematopoietic stem cell survival and affects oncogenesis and response to chemotherapy." Nat Commun **10**(1): 5349.

Increased levels and non-telomeric roles have been reported for shelterin proteins, including RAP1 in cancers. Herein using Rap1 null mice, we provide the genetic evidence that mammalian Rap1 plays a major role in hematopoietic stem cell survival, oncogenesis and response to chemotherapy. Strikingly, this function of RAP1 is independent of its association with the telomere or with its known partner TRF2. We show that RAP1 interacts with many members of the DNA damage response (DDR) pathway. RAP1 depleted cells show reduced interaction between XRCC4/DNA Ligase IV and DNA-PK, and are impaired in DNA Ligase IV recruitment to damaged chromatin for efficient repair. Consistent with its role in DNA damage repair, RAP1 loss decreases double-strand break repair via NHEJ in vivo, and consequently reduces B cell class switch recombination. Finally, we discover that RAP1 levels are predictive of the success of chemotherapy in breast and colon cancer.

Kim, P. J., et al. (2015). "Direct evaluation of myocardial viability and stem cell engraftment demonstrates salvage of the injured myocardium." Circ Res **116**(7): e40-50.

RATIONALE: The mechanism of functional restoration by stem cell therapy remains poorly understood. Novel manganese-enhanced MRI and bioluminescence reporter gene imaging were applied to follow myocardial viability and cell engraftment, respectively. Human-placenta-derived amniotic mesenchymal stem cells (AMCs) demonstrate unique immunoregulatory and precardiac properties. In this study, the restorative effects of 3 AMC-derived subpopulations were examined in a murine myocardial injury model: (1) unselected AMCs, (2) ckit(+)AMCs, and (3) AMC-derived induced pluripotent stem cells (MiPSCs). OBJECTIVE: To determine the differential restorative effects of the AMC-derived subpopulations in the murine myocardial injury model using multimodality imaging. METHODS AND RESULTS: SCID (severe combined immunodeficiency) mice underwent left anterior descending artery ligation and were divided into 4 treatment arms: (1) normal saline control (n=14), (2) unselected AMCs (n=10), (3) ckit(+)AMCs (n=13), and (4) MiPSCs (n=11). Cardiac MRI assessed myocardial viability and left ventricular function, whereas bioluminescence imaging assessed stem cell engraftment during a 4-week period. Immunohistological labeling and reverse transcriptase polymerase chain reaction of the explanted myocardium were performed. The unselected AMC and ckit(+)AMC-treated mice demonstrated transient left ventricular functional improvement. However, the MiPSCs exhibited a significantly greater increase in left ventricular function compared with all the other groups during the entire 4-week period. Left ventricular functional improvement correlated with increased myocardial viability and sustained stem cell engraftment. The MiPSC-treated animals lacked any evidence of de novo cardiac differentiation. CONCLUSION: The functional restoration seen in MiPSCs was characterized by increased myocardial viability and sustained engraftment without de novo cardiac differentiation, indicating salvage of the injured myocardium.

Kim, Y. Y., et al. (2013). "Anti-aging effects of vitamin C on human pluripotent stem cell-derived cardiomyocytes." Age (Dordr) **35**(5): 1545-1557.

Human pluripotent stem cells (hPSCs) have arisen as a source of cells for biomedical research due to their developmental potential. Stem cells possess the promise of providing clinicians with novel treatments for disease as well as allowing researchers to generate human-specific cellular metabolism models. Aging is a natural process of living organisms, yet aging in human heart cells is difficult to study due to the ethical considerations regarding human experimentation as well as a current lack of alternative experimental models. hPSC-derived cardiomyocytes (CMs) bear a resemblance to human cardiac cells and thus hPSC-derived CMs are considered to be a viable alternative model to study human heart cell aging. In this study, we used hPSC-derived CMs as an in vitro aging model. We generated cardiomyocytes from hPSCs and demonstrated the process of aging in both human embryonic stem cell (hESC)- and induced pluripotent stem cell (hiPSC)-derived CMs. Aging in hESC-derived CMs correlated with reduced membrane potential in mitochondria, the accumulation of lipofuscin, a slower beating pattern, and the downregulation of human telomerase RNA (hTR) and cell cycle regulating genes. Interestingly, the expression of hTR in hiPSC-derived CMs was not significantly downregulated, unlike in hESC-derived CMs. In order to delay aging, vitamin C was added to the cultured CMs. When cells were treated with 100 muM of vitamin C for 48 h, anti-aging effects, specifically on the expression of telomere-related genes and their functionality in aging cells, were observed. Taken together, these results suggest that hPSC-derived CMs can be used as a unique human cardiomyocyte aging model in vitro and that vitamin C shows anti-aging effects in this model.

Koziel, J. E. and B. S. Herbert (2015). "The telomerase inhibitor imetelstat alone, and in combination with trastuzumab, decreases the cancer stem cell population and self-renewal of HER2+ breast cancer cells." Breast Cancer Res Treat **149**(3): 607-618.

Cancer stem cells (CSCs) are thought to be responsible for tumor progression, metastasis, and recurrence. HER2 overexpression is associated with increased CSCs, which may explain the aggressive phenotype and increased likelihood of recurrence for HER2(+) breast cancers. Telomerase is reactivated in tumor cells, including CSCs, but has limited activity in normal tissues, providing potential for telomerase inhibition in anti-cancer therapy. The purpose of this study was to investigate the effects of a telomerase antagonistic oligonucleotide, imetelstat (GRN163L), on CSC and non-CSC populations of HER2(+) breast cancer cell lines. The effects of imetelstat on CSC populations of HER2(+) breast cancer cells were measured by ALDH activity and CD44/24 expression by flow cytometry as well as mammosphere assays for functionality. Combination studies in vitro and in vivo were utilized to test for synergism between imetelstat and trastuzumab. Imetelstat inhibited telomerase activity in both subpopulations. Moreover, imetelstat alone and in combination with trastuzumab reduced the CSC fraction and inhibited CSC functional ability, as shown by decreased mammosphere counts and invasive potential. Tumor growth rate was slower in combination-treated mice compared to either drug alone. Additionally, there was a trend toward decreased CSC marker expression in imetelstat-treated xenograft cells compared to vehicle control. Furthermore, the observed decrease in CSC marker expression occurred prior to and after telomere shortening, suggesting that imetelstat acts on the CSC subpopulation in telomere length-dependent and -independent mechanisms. Our study suggests addition of imetelstat to trastuzumab may enhance the effects of HER2 inhibition therapy, especially in the CSC population.

Krylova, T. A., et al. (2005). "[Continuous human embryonic stem cell lines]." Tsitologiia **47**(2): 121-129.

A new continuous human embryonic stem cell line (HESC-5) derived from a blastocyst is described. The cultured cell passed over 200 population doublings, which exceeds the Hayflick's limit sufficiently. The cells maintained a stable proliferative activity, high activity of alkaline phosphatase, and expression of transcription factor Oct-4 and of surface antigens SSEA-3, SSEA-4 and TRA-1-60 known to be characteristic of embryonic stem cells of the human origin. Immunofluorescent detection of antigens, characteristic of ectoderm, endoderm and mesoderm in the new cell line HESC-5, and in the previously described other four stem cell lines confirms the ability of these cells to retain their pluripotency under in vitro condition. In addition, in all the cell lines, a high telomerase activity was revealed, which controls a stable telomere length and, hence, an unlimited ESC proliferation. Unlike other cell lines, HESC-5 was found, under specific conditions, to spontaneously differentiate into hematopoietic cells. A morphological similarity was shown between ESC colonies cultivated both on a feeder layer and in the non-feeder system.

Ksiazek, K. (2009). "A comprehensive review on mesenchymal stem cell growth and senescence." Rejuvenation Res **12**(2): 105-116.

In recent years mesenchymal stem cells (MSCs) have generated a great deal of excitement as an attractive alternative to embryonic stem cells (ESCs) in cell-based regenerative medicine. In contrast to cells of embryonic origin, however, the clinical application of MSCs is heavily restricted by their finite ability of self-

renewal, in which they resemble the rest of the somatic cells. Yet the mechanisms controlling MSC proliferation and senescence remain unclear. This review summarizes recent advances in our understanding of the factors affecting MSC expansion in vitro and discusses the pattern of their senescence with particular emphasis on the role of telomere shortening, activation of effectory pathways, and oxidative stress. The issues associated with MSC growth and senescence will be shown in the context of other somatic cells, and all of the parallels and disparities will be delineated precisely.

Kuniakova, M., et al. (2015). "Somatic stem cell aging and malignant transformation--impact on therapeutic application." Cell Mol Biol Lett **20**(5): 743-756.

Somatic stem cells possess unique properties of self-renewal and plasticity which make them promising candidates for use in tissue engineering and regenerative medicine, in addition to serving as efficient delivery vehicles in site-specific therapy. In the case of therapeutic application, it is essential to isolate and culture stem cells in vitro, to obtain them in sufficient quantities. Although long-term cultivation provides an adequate number of cells, it has been shown that this approach is associated with increased risk of transformation of cultured cells, which presents a significant biological hazard. This article reviews information about biological features and cellular events which occur during long-term cultivation of somatic stem cells, with respect to their safe utilization in potential clinical practice.

Lafferty-Whyte, K., et al. (2009). "A gene expression signature classifying telomerase and ALT immortalization reveals an hTERT regulatory network and suggests a mesenchymal stem cell origin for ALT." Oncogene **28**(43): 3765-3774.

Telomere length is maintained by two known mechanisms, the activation of telomerase or alternative lengthening of telomeres (ALT). The molecular mechanisms regulating the ALT phenotype are poorly understood and it is unknown how the decision of which pathway to activate is made at the cellular level. We have shown earlier that active repression of telomerase gene expression by chromatin remodelling of the promoters is one mechanism of regulation; however, other genes and signalling networks are likely to be required to regulate telomerase and maintain the ALT phenotype. Using gene expression profiling, we have uncovered a signature of 1305 genes to distinguish telomerase-positive and ALT cell lines. By combining this with the gene expression profiles of liposarcoma tissue samples, we refined this signature to 297 genes. A network analysis of known interactions between genes within this signature revealed a regulatory signalling network consistent with a model of human telomerase reverse transcriptase (hTERT) repression in ALT cell lines and liposarcomas. This network expands on our existing knowledge of hTERT regulation and provides a platform to understand differential regulation of hTERT in different tumour types and normal tissues. We also show evidence to suggest a novel mesenchymal stem cell origin for ALT immortalization in cell lines and mesenchymal tissues.

Lagunas, A. M., et al. (2019). "Paracrine Interaction of Cancer Stem Cell Populations Is Regulated by the Senescence-Associated Secretory Phenotype (SASP)." Mol Cancer Res **17**(7): 1480-1492.

Dyskeratosis congenita is a telomere DNA damage syndrome characterized by defective telomere maintenance, bone marrow failure, and increased head and neck cancer risk. The Pot1b(-/-);Terc(+/-) mouse exhibits some features of dyskeratosis congenita, but head and neck cancer was not reported in this model. To model the head and neck cancer phenotype, we created unique Pot1b- and p53-null-mutant models which allow genetic lineage tracing of two distinct stem cell populations. Loss of Pot1b expression depleted stem cells via ATR/Chk1/p53 signaling. Tumorigenesis was inhibited in Pot1b(-/-);p53(+/+) mice due to cellular senescence. Pot1b(-/-);p53(-/-) tumors also exhibited senescence, but proliferated and metastasized with expansion of Lgr6(+) stem cells indicative of senescence-associated secretory phenotype. Selective depletion of the small K15(+) stem cell fraction resulted in reduction of Lgr6(+) cells and inhibition of tumorigenesis via senescence. Gene expression studies revealed that K15(+) cancer stem cells regulate Lgr6(+) cancer stem cell expansion via chemokine signaling. Genetic ablation of the chemokine receptor Cxcr2 inhibited cancer stem cell expansion and tumorigenesis via senescence. The effects of chemokines were primarily mediated by PI3K signaling, which is a therapeutic target in head and neck cancer. IMPLICATIONS: Paracrine interactions of cancer stem cell populations impact therapeutic options and patient outcomes.

Lansdorp, P. M. (1998). "Stem cell biology for the transfusionist." Vox Sang **74 Suppl 2**: 91-94.

The limited life-span of most blood cells requires continuous production of cells which in adults may exceed 1012 cells/day. This impressive production of cells (approximately 4.1015 cells over a life time) is achieved by the proliferation and differentiation of committed progenitor cells which themselves are derived from a population of pluripotent stem cells with self-renewal potential. In adults, the large majority of stem cells are found in the bone marrow among cells with a CD34 + CD38- phenotype. Interestingly, small but significant numbers of such cells can be found in the circulation. The frequency of circulating CD34 + CD38- cells can be dramatically increased by treatment with certain compounds including cytokines. Such

"mobilized" peripheral blood stem cells have become an important alternative to bone marrow in stem cell transplantation procedures primarily because engraftment is more rapid. The latter is almost certainly related to the increased numbers of primitive CD34 + CD38- cells capable of engrafting the bone marrow in blood versus bone marrow stem cell grafts [1]. Paradoxically, the large majority of "candidate" stem cells in adult bone marrow are quiescent cells. One possibility is that stem cells, like other somatic cells, have only a limited replicative potential (< 100 divisions). This hypothesis is supported by two key observations and the consideration that, in theory, 52 divisions can yield 4.1015 cells. First, it was shown that "candidate" stem cells purified from fetal and adult tissue display marked functional differences in turn-over time and the ability to produce cells with stem cell properties [2]. Secondly, these functional differences were found to correlate with a measurable loss of telomere repeats [3], despite the presence of low but readily detectable levels of telomerase in all purified cell fractions [4,5]. In order to address questions about the role of telomeres in normal and malignant hematopoiesis, we developed quantitative fluorescence in situ hybridization [6]. With this technique the length of telomere repeats at individual chromosome ends can be reliably estimated using optical density measurements from digital images of metaphase chromosomes after fluorescence in situ hybridization with directly labeled (CCCTAA)3--Peptide Nucleic Acid Probe [6,7]. Furthermore, we recently showed that this method can be adapted to measure the total telomere repeat content of cells by flow cytometry [8]. Here some issues in studies of hematopoietic stem cells are discussed in relation to rapidly accumulating information about telomere biology.

Lansdorp, P. M., et al. (1994). "Age-related decline in proliferative potential of purified stem cell candidates." Blood Cells **20**(2-3): 376-380; discussion 380-371.

Recent studies in our laboratory have shown striking differences in the functional properties of candidate hematopoietic stem cells purified from fetal, neonatal, and adult human tissues. These differences include the ability to produce CD34+ cells, the turnover rate, and the fraction of cells that respond to a mixture of cytokines. All these parameters decrease with the age of the cell donor, and some of these observations are summarized here. Extensive qualitative changes in hematopoietic cells from various stages of development should be taken into account in the design of novel therapeutic strategies.

Lee, G. H., et al. (2020). "Immunosenescent characteristics of T cells in young patients following haploidentical haematopoietic stem cell transplantation from parental donors." Clin Transl Immunology **9**(4): e1124.

Objectives: Paediatric and adolescent patients in need of allogeneic haematopoietic stem cell transplantation (HSCT) generally receive stem cells from older, unrelated or parental donors when a sibling donor is not available. Despite encouraging clinical outcomes, it has been suggested that immune reconstitution accompanied by increased replicative stress and a large difference between donor and recipient age may worsen immunosenescence in paediatric recipients. Methods: In this study, paired samples were collected at the same time from donors and recipients of haploidentical haematopoietic stem cell transplantation (HaploSCT). We then conducted flow cytometry-based phenotypic and functional analyses and telomere length (TL) measurements of 21 paired T-cell sets from parental donors and children who received T-cell-replete HaploSCT with post-transplant cyclophosphamide (PTCy). Results: Senescent T cells, CD28(-) or CD57(+) cells, were significantly expanded in patients. Further, not only CD4(+)CD28(-) T cells, but also CD4(+)CD28(+) T cells showed reduced cytokine production capacity and impaired polyfunctionality compared with parental donors, whereas their TCR-mediated proliferation capacity was comparable. Of note, the TL in patient T cells was preserved, or even slightly longer, in senescent T cells compared with donor cells. Regression analysis showed that senescent features of CD4(+) and CD8(+) T cells in patients were influenced by donor age and the frequency of CD28(-) cells, respectively. Conclusion: Our data suggest that in paediatric HaploSCT, premature immunosenescent changes occur in T cells from parental donors, and therefore, long-term immune monitoring should be conducted.

Lepperdinger, G. (2011). "Inflammation and mesenchymal stem cell aging." Curr Opin Immunol **23**(4): 518-524.

In adults, mesenchymal stromal cells contain tissue-specific multipotent stem cells, MSC, which can be found throughout the body. With advancing age, tight controls of regulatory networks, which guide MSC biology, gradually deteriorate. Aberrations within the MSC microenvironment such as chronic inflammation eventually lead to adverse manifestations, such as the accumulation of fat deposits in bone and muscles, impaired healing and fibrosis after severe injury, or altered hematopoiesis and autoimmunity. MSC can also specifically interact with a large variety of immune cells, and in doing so, they secrete cytoprotective and immunoregulatory molecules, which together with intercellular contacts mediate immune modulatory processes. This review comprehends the current knowledge regarding molecular mechanisms and cellular interactions that occur in stem cell niches, which are jointly shared

between MSC and hematopoietic stem and progenitor cells, as well as those intracellular interdependences taking place between mesenchymal and a wide variety of hematopoietic progeny in particular T lymphocytes, which eventually perturb tissue homeostasis and immunology at advanced age.

Li, P., et al. (2015). "A tight control of Rif1 by Oct4 and Smad3 is critical for mouse embryonic stem cell stability." Cell Death Dis **6**: e1588.

Prolonged culture of embryonic stem cells (ESCs) leads them to adopt embryonal carcinoma cell features, creating enormous dangers for their further application. The mechanism involved in ESC stability has not, however, been extensively studied. We previously reported that SMAD family member 3 (Smad3) has an important role in maintaining mouse ESC stability, as depletion of Smad3 results in cancer cell-like properties in ESCs and Smad3-/- ESCs are prone to grow large, malignant teratomas. To understand how Smad3 contributes to ESC stability, we performed microarray analysis to compare the transcriptome of wild-type and Smad3-/- ESCs. We found that Rif1 (RAP1-associated protein 1), a factor important for genomic stability, is significantly upregulated in Smad3-/- ESCs. The expression level of Rif1 needs to be tightly controlled in ESCs, as a low level of Rif1 is associated with ESC differentiation, but a high level of Rif1 is linked to ESC transformation. In ESCs, Oct4 activates Rif1, whereas Smad3 represses its expression. Oct4 recruits Smad3 to bind to Rif1 promoter, but Smad3 joining facilitates the loading of a polycomb complex that generates a repressive epigenetic modification on Rif1 promoter, and thus maintains the expression of Rif1 at a proper level in ESCs. Interestingly, Rif1 short hairpin RNA (shRNA)-transduced Smad3-/- ESCs showed less malignant properties than the control shRNA-transduced Smad3-/- ESCs, suggesting a critical role of Rif1 in maintaining the stability of ESCs during proliferation.

Li, T., et al. (2015). "Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay." EMBO J **34**(12): 1630-1647.

Nonsense-mediated mRNA decay (NMD) is a post-transcriptional mechanism that targets aberrant transcripts and regulates the cellular RNA reservoir. Genetic modulation in vertebrates suggests that NMD is critical for cellular and tissue homeostasis, although the underlying mechanism remains elusive. Here, we generate knockout mice lacking Smg6/Est1, a key nuclease in NMD and a telomerase cofactor. While the complete loss of Smg6 causes mouse lethality at the blastocyst stage, inducible deletion of Smg6 is compatible with embryonic stem cell (ESC) proliferation despite the absence of telomere maintenance and functional NMD. Differentiation of Smg6-deficient ESCs is blocked due to sustained expression of pluripotency genes, normally repressed by NMD, and forced down-regulation of one such target, c-Myc, relieves the differentiation block. Smg6-null embryonic fibroblasts are viable as well, but are refractory to cellular reprograming into induced pluripotent stem cells (iPSCs). Finally, depletion of all major NMD factors compromises ESC differentiation, thus identifying NMD as a licensing factor for the switch of cell identity in the process of stem cell differentiation and somatic cell reprograming.

Li, T., et al. (2016). "SET1A Cooperates With CUDR to Promote Liver Cancer Growth and Hepatocyte-like Stem Cell Malignant Transformation Epigenetically." Mol Ther **24**(2): 261-275.

Long noncoding RNA CUDR plays an important role during tumorigenesis. Herein, we demonstrate that SET1A cooperates with CUDR to accelerate hepatocarcinogenesis and promote malignant transformation of hepatocyte-like stem cells. Mechanistically, CUDR enhances the phosphorylation of RB1, C-myc expression, and the interplay between the SET1A and pRB1. Notably, CUDR acts as a sponge cushion that shows a link between SET1A and pRB1, producing a activated pRB1-SET1A complex. On the other hand, the pRB1-SET1A complex may carry methyls(me) to occupy the position of H3K4, resulting in specific tri-methylation of forth lysine of histone H3 (H3K4me3). Thereby, the H3K4me3 loads on the TRF2 promoter region which causes the TRF2 overexpression. Ultimately, the excessive TRF2 binds to telomere repeat DNA, prolonging the telomere length. These findings provide the first demonstration that SET1A cooperates with CUDR to play a positive potential role during hepatocarcinogenesis and hepatocyte-like stem cells' malignant transformation epigenetically.

Li, Y., et al. (2016). "Vitamin C alleviates aging defects in a stem cell model for Werner syndrome." Protein Cell **7**(7): 478-488.

Werner syndrome (WS) is a premature aging disorder that mainly affects tissues derived from mesoderm. We have recently developed a novel human WS model using WRN-deficient human mesenchymal stem cells (MSCs). This model recapitulates many phenotypic features of WS. Based on a screen of a number of chemicals, here we found that Vitamin C exerts most efficient rescue for many features in premature aging as shown in WRN-deficient MSCs, including cell growth arrest, increased reactive oxygen species levels, telomere attrition, excessive secretion of inflammatory factors, as well as disorganization of nuclear lamina and heterochromatin. Moreover, Vitamin C restores in vivo viability of MSCs in a mouse model. RNA sequencing analysis indicates that Vitamin C alters the expression of a series of genes involved in chromatin condensation, cell cycle

regulation, DNA replication, and DNA damage repair pathways in WRN-deficient MSCs. Our results identify Vitamin C as a rejuvenating factor for WS MSCs, which holds the potential of being applied as a novel type of treatment of WS.

Ligeiro, D., et al. (2016). "KIR genotypic diversity in Portuguese and analysis of KIR gene allocation after allogeneic hematopoietic stem cell transplantation." HLA **87**(5): 375-380.

The diversity of killer-cell immunoglobulin-like receptors (KIR) genes was evaluated in Portuguese and the observed genotypic profiles were found related to the ones reported in European populations. The KIR repertoire after hematopoietic stem cell transplantation is determined by these gene frequencies and the KIR group B motifs are the less common. We estimated donor-KIR/recipient-ligand interactions in transplants with related donors and unrelated donors found in a local registry or from abroad. A large fraction of transplants had all three ligands of inhibitory receptors, and therefore, in theory were not prone to natural killer cell (NK) mediated alloreactivity. Furthermore, the distribution of KIR alloreactive interactions was found independent of the donor-recipient genetic proximity, probably because of different gene segregation and comparable KIR frequencies in the donor pools.

Ling, L., et al. (2020). "Enhancing the Efficacy of Stem Cell Therapy with Glycosaminoglycans." Stem Cell Reports **14**(1): 105-121.

Human mesenchymal stem cell (hMSC) therapy offers significant potential for osteochondral regeneration. Such applications require their ex vivo expansion in media frequently supplemented with fibroblast growth factor 2 (FGF2). Particular heparan sulfate (HS) fractions stabilize FGF2-FGF receptor complexes. We show that an FGF2-binding HS variant (HS8) accelerates the expansion of freshly isolated bone marrow hMSCs without compromising their naivety. Importantly, the repair of osteochondral defects in both rats and pigs is improved after treatment with HS8-supplemented hMSCs (MSC(HS8)), when assessed histologically, biomechanically, or by MRI. Thus, supplementing hMSC culture media with an HS variant that targets endogenously produced FGF2 allows the elimination of exogenous growth factors that may adversely affect their therapeutic potency.

Liu, D., et al. (2018). "Integrated Genome-Wide Analysis of Gene Expression and DNA Copy Number Variations Highlights Stem Cell-Related Pathways in Small Cell Esophageal Carcinoma." Stem Cells Int **2018**: 3481783.

Purpose/Objectives. Primary small cell esophageal carcinoma (SCEC) represents a rare and aggressive malignancy without any prospective clinical trial or established treatment strategy at present. Although previous studies have indicated similarities between SCEC and small cell lung cancer (SCLC) in terms of their clinical manifestations, pathology, and morphology, very little genetic information is available on this highly malignant tumor. At present, patients with SCEC are staged and treated according to the guidelines established for SCLC. However, early recurrence and distant metastasis are common, and long-time survivors are rare. Current options available for patients with relapsed SCEC are fairly unsatisfactory, and their prognosis is generally poor. Novel therapeutic approaches against SCEC are therefore urgently needed and require a deeper understanding of the underlying genetic mechanisms. The current investigation aims to characterize the gene expression profile and copy number variations (CNVs) in SCEC to clarify molecular markers and pathways that may possess clinical significance. Materials/Methods. De novo expression array was carried out on three matched sets of primary SCEC and adjacent normal tissue samples procured from the institutional tissue bank, utilizing the Affymetrix HG U133 Plus 2.0 Array. After individual tissue normalization, the statistical software GeneSpring GX 12.5 was used to determine differentially expressed genes (DEGs) in the tumors relative to their paired normal tissues. Gene enrichments in addition to functional annotation and gene interaction networks were performed using DAVID 6.8 and STRING 10.0, respectively. A gene alteration was determined to be recurrent if it was observed in at least 2 samples. Chromosomes X and Y were not included in calculations as gender differences are a known source of analysis bias. The DEGs of at least one SCEC sample could be mapped to the CNV regions (fold change (FC) >/= 2 and false discovery rate (FDR) < 0.01) after gene expression profiling by RefSeq Transcript ID. These overlapped genes were subjected to the functional annotation using DAVID 6.8. In order to elucidate the effect of CNV on mRNA expression, we integrated the genome-wide copy number data and gene expression in 3 paired samples. CNV-associated gene expression aberration (CNV-FC) was calculated for the recurrent DEGs using previously published integrated microarray data as reference. Pearson's correlation coefficient was employed to determine if there was a statistical correlation between the gene expression log2 ratios and their copy numbers using the SPSS 19.0 software. Genes that possessed CNV-FC >/= 2 and r >/= 0.6 ($p < 0.05$) were determined to be genes potentially associated with cancer. Results. High-quality DNA and total RNA were first extracted from both SCEC and normal tissues. Microarray data showed significant upregulation in WNT gene sets and downregulation in the PTEN and notch gene sets in SCEC. Functional annotation showed that genes associated with DNA replication, mitosis, cell cycle,

DNA repair, telomere maintenance, RB, and p53 pathways were significantly altered in SCEC compared to corresponding noncancerous tissues (Benjamini p < 0.05). Thirteen recurrent CNVs were found in all SCEC samples by array CGH. Chromosomal regions with gain were located in 14q11.2, and regions with loss were located in 4q22.3-23.3, 3q25.31-q29, 5p15.31-15.2, 8q21.11-24.3, and 9p23-13.1 in all samples. In two samples, the 14q11.2-32.33 region was amplified, whereas 3p26.3-25.3, 4p16.3-11, 4q11-22.3, 4q23-25, 8p23.3, and 16p13.3 were deleted. We further identified 306 genes that consistently differed in copy number and expression (194 upregulated and 112 downregulated) between the SCEC and noncancerous tissues in all three samples. These genes were significantly enriched with those involved in cell cycle, mitosis, DNA repair, P53 pathway, and RB pathway, according to their functional annotation. These 306 DEGs also included network genes of the above pathways such as NUF2, CCNE2, NFIB, ETV5, KLF5, ATAD2, NDC80, and ZWINT. In addition, 39 individual DEGs demonstrated a minimum 2-fold copy number-associated expression change (median: 5.35, 95% CI: 4.53-16.98) and Pearson's correlation coefficient >/= 0.6 (p < 0.05), of which PTP4A3 showed the highest correlation (CNV-FC = 21362.13; Pearson's correlation coefficient = 0.9983; p = 0.037). Two distinct groups of genes belonging to each SCEC and nonmalignant tissues were observed upon unsupervised two-way (genes and samples) hierarchical clustering. Conclusions. The current investigation is the first to produce data regarding the genomic signature of SCEC at the transcription level and in relation to CNVs. Our preliminary data indicate possible key roles of WNT and notch signaling in SCEC and overexpressed PTP4A3 as a potential therapeutic target. Further validation of our findings is warranted.

Liu, Z., et al. (2013). "Telomerase reverse transcriptase promotes epithelial-mesenchymal transition and stem cell-like traits in cancer cells." Oncogene **32**(36): 4203-4213.

Telomerase activation through induction of telomerase reverse transcriptase (hTERT) contributes to malignant transformation by stabilizing telomeres. Clinical studies demonstrate that higher hTERT expression is associated with cancer progression and poor outcomes, but the underlying mechanism is unclear. Because epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs) are key factors in cancer metastasis and relapse, and hTERT has been shown to exhibit multiple biological activities independently of its telomere-lengthening function, we address a potential role of hTERT in EMT and CSCs using gastric cancer (GC) as a model. hTERT overexpression promotes, whereas its inhibition suppresses, EMT and stemness of GC cells, respectively. Transforming growth factor (TGF)-beta1 and beta-catenin-mediated EMT was abolished by small interfering RNA depletion of hTERT expression. hTERT interacts with beta-catenin, enhances its nuclear localization and transcriptional activity, and occupies the beta-catenin target vimentin promoter. All these hTERT effects were independent of its telomere-lengthening function or telomerase activity. hTERT and EMT marker expression correlates positively in GC samples. Mouse experiments demonstrate the in vivo stimulation of hTERT on cancer cell colonization. Collectively, hTERT stimulates EMT and induces stemness of cancer cells, thereby promoting cancer metastasis and recurrence. Thus, targeting hTERT may prevent cancer progression by inhibiting EMT and CSCs.

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

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

Maimon, I., et al. (2014). "Without children is required for Stat-mediated zfh1 transcription and for germline stem cell differentiation." Development **141**(13): 2602-2610.

Tissue homeostasis is maintained by balancing stem cell self-renewal and differentiation. How surrounding cells support this process has not been entirely resolved. Here we show that the chromatin and telomere-binding factor Without children (Woc) is required for maintaining the association of escort cells (ECs) with germ cells in adult ovaries. This tight association is essential for germline stem cell (GSC) differentiation into cysts. Woc is also required in larval ovaries for the association of intermingled cells (ICs) with primordial

germ cells. Reduction in the levels of two other proteins, Stat92E and its target Zfh1, produce phenotypes similar to woc in both larval and adult ovaries, suggesting a molecular connection between these three proteins. Antibody staining and RT-qPCR demonstrate that Zfh1 levels are increased in somatic cells that contact germ cells, and that Woc is required for a Stat92E-mediated upregulation of zfh1 transcription. Our results further demonstrate that overexpression of Zfh1 in ECs can rescue GSC differentiation in woc-deficient ovaries. Thus, Zfh1 is a major Woc target in ECs. Stat signalling in niche cells has been previously shown to maintain GSCs non-autonomously. We now show that Stat92E also promotes GSC differentiation. Our results highlight the Woc-Stat-Zfh1 module as promoting somatic encapsulation of germ cells throughout their development. Each somatic cell type can then provide the germline with the support it requires at that particular stage. Stat is thus a permissive factor, which explains its apparently opposite roles in GSC maintenance and differentiation.

Marion, R. M., et al. (2009). "Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells." Cell Stem Cell **4**(2): 141-154.

Telomere shortening is associated with organismal aging. iPS cells have been recently derived from old patients; however, it is not known whether telomere chromatin acquires the same characteristics as in ES cells. We show here that telomeres are elongated in iPS cells compared to the parental differentiated cells both when using four (Oct3/4, Sox2, Klf4, cMyc) or three (Oct3/4, Sox2, Klf4) reprogramming factors and both from young and aged individuals. We demonstrate genetically that, during reprogramming, telomere elongation is usually mediated by telomerase and that iPS telomeres acquire the epigenetic marks of ES cells, including a low density of trimethylated histones H3K9 and H4K20 and increased abundance of telomere transcripts. Finally, reprogramming efficiency of cells derived from increasing generations of telomerase-deficient mice shows a dramatic decrease in iPS cell efficiency, a defect that is restored by telomerase reintroduction. Together, these results highlight the importance of telomere biology for iPS cell generation and functionality.

Mason, S., et al. (2014). "Standardization and safety of alveolar bone-derived stem cell isolation." J Dent Res **93**(1): 55-61.

Cell therapies utilizing mesenchymal stem cells (MSCs) could overcome limitations of traditional treatments for reconstructing craniofacial tissues. This large-scale study explored a standardized methodology for the isolation and clinical-scale expansion of alveolar bone marrow-derived MSCs (aBMSCs). We harvested 103 alveolar bone marrow samples from 45 patients using 1 of 3 standardized methodologies. Following aBMSC isolation, cells were characterized through cell-surface marker expression and lineage-specific differentiation. Long-term cultures (> 50 population doublings [PDs]) were evaluated for transformational changes through senescence, gene expression, and karyotyping. Finally, aBMSC bone-forming potential was determined in vivo. More than 0.5 cc of bone marrow was needed to predictably isolate aBMSCs, and, regardless of methodology for harvest, cell-surface marker expression of CD73, CD90, CD105, and Stro-1 was similar for aBMSCs, being 89.8%, 98.8%, 93.8%, and 3.2%, respectively; all cells were negative for CD11b, CD19, and CD45. aBMSCs exhibited multipotency, and karyotypes were normal up to 30 PDs, with significant cell senescence beginning following 35 PDs. Additionally, aBMSCs induced ectopic bone formation following subcutaneous transplantation into mice. These findings demonstrate a predictable approach for the isolation and safe clinical-scale expansion of aBMSCs, and thus, their clinical use could be considered for craniofacial regenerative therapies.

Mazzini, L., et al. (2008). "Stem cell treatment in Amyotrophic Lateral Sclerosis." J Neurol Sci **265**(1-2): 78-83.

Amyotrophic Lateral Sclerosis is a progressive fatal neurodegenerative disease that targets motor neurons. Its origin is unknown but a main role of reactive astrogliosis and microglia activation in the pathogenesis has been recently demonstrated. Surrounding neurons with healthy adjoining cells completely stops motor neuron death in some cases. Hence stem cell transplantation might represent a promising therapeutic strategy. In this study MSCs were isolated from bone marrow of 9 patients with definite ALS. Growth kinetics, immunophenotype, telomere length and karyotype were evaluated during in vitro expansion. No significant differences between donors or patients were observed. The patients received intraspinal injections of autologous MSCs at the thoracic level and monitored for 4 years. No significant acute or late side effects were evidenced. No modification of the spinal cord volume or other signs of abnormal cell proliferation were observed. Four patients show a significant slowing down of the linear decline of the forced vital capacity and of the ALS-FRS score. Our results seem to demonstrate that MSCs represent a good chance for stem cell cell-based therapy in ALS and that intraspinal injection of MSCs is safe also in the long term. A new phase 1 study is carried out to verify these data in a larger number of patients.

Mei, Y., et al. (2018). "RIF1 promotes tumor growth and cancer stem cell-like traits in NSCLC by protein

phosphatase 1-mediated activation of Wnt/beta-catenin signaling." Cell Death Dis **9**(10): 942.

Wnt/beta-catenin signaling is essential for proliferation and maintenance of cancer stem cell-like traits of various cancer cells. In non-small-cell lung carcinoma (NSCLC), the mechanisms underlying the hyperactivation of Wnt signaling remain unclear, as mutations in APC and beta-catenin genes are rare in NSCLC. RIF1 has been shown upregulated in breast and cervical cancer, this study intends to find out the potential effects of the expression and biological functions of RIF1 in NSCLC. Here we revealed that RIF1 was highly expressed in NCSLC at both mRNA and protein levels. RIF1 expression was significantly associated with clinical stage ($P < 0.05$) and prognosis ($P < 0.001$) of NSCLC patients. RIF1 knockdown inhibited NSCLC cell growth in vitro and in vivo, whereas overexpression of RIF1 in NSCLC cell lines promoted cell growth, cell cycle progression and cancer stem cell (CSC)-like properties via promoting PP1-AXIN interaction and thereby activating Wnt/beta-catenin signaling. Inhibition of PP1 in RIF1-overexpressed cells counteracted the effects of RIF1 on cell growth and CSC-like phenotype, as well as the Wnt/beta-catenin signaling. RIF1 expression was positively correlated with beta-catenin at the protein level in 32 NSCLC tissues. RIF1 expression closely related to MYC ($r = 0.28$, $P < 0.001$) and CCND1 ($r = 0.14$, $P < 0.01$) expression at the mRNA level in cohorts of The Cancer Genome Atlas (TCGA). These results indicated that RIF1 had an oncogenic role as a novel positive regulator of Wnt/beta-catenin signaling by directing PP1 to dephosphorylate AXIN; this novel mechanism may present a new therapeutic target for NSCLC.

Mimeault, M. and S. K. Batra (2010). "Recent advances on skin-resident stem/progenitor cell functions in skin regeneration, aging and cancers and novel anti-aging and cancer therapies." J Cell Mol Med **14**(1-2): 116-134.

Recent advances in skin-resident adult stem/progenitor cell research have revealed that these immature and regenerative cells with a high longevity provide critical functions in maintaining skin homeostasis and repair after severe injuries along the lifespan of individuals. The establishment of the functional properties of distinct adult stem/progenitor cells found in skin epidermis and hair follicles and extrinsic signals from their niches, which are deregulated during their aging and malignant transformation, has significantly improved our understanding on the etiopathogenesis of diverse human skin disorders and cancers. Particularly, enhanced ultraviolet radiation exposure, inflammation and oxidative stress and telomere attrition during chronological aging may induce severe DNA damages and genomic instability in the skin-resident stem/progenitor cells and their progenies. These molecular events may result in the alterations in key signalling components controlling their self-renewal and/or regenerative capacities as well as the activation of tumour suppressor gene products that trigger their growth arrest and senescence or apoptotic death. The progressive decline in the regenerative functions and/or number of skin-resident adult stem/progenitor cells may cause diverse skin diseases with advancing age. Moreover, the photoaging, telomerase re-activation and occurrence of different oncogenic events in skin-resident adult stem/progenitor cells may also culminate in their malignant transformation into cancer stem/progenitor cells and skin cancer initiation and progression. Therefore, the anti-inflammatory and anti-oxidant treatments and stem cell-replacement and gene therapies as well as the molecular targeting of their malignant counterpart, skin cancer-initiating cells offer great promise to treat diverse skin disorders and cancers.

Minamino, T. and I. Komuro (2008). "Vascular aging: insights from studies on cellular senescence, stem cell aging, and progeroid syndromes." Nat Clin Pract Cardiovasc Med **5**(10): 637-648.

Epidemiological studies have shown that age is the chief risk factor for atherosclerotic cardiovascular diseases, but the molecular mechanisms that underlie the increase in risk conferred by aging remain unclear. Evidence suggests that the cardiovascular repair system is impaired with advancing age, thereby inducing age-associated cardiovascular dysfunction. Such impairment could be attributable to senescence of cardiovascular tissues at the cellular level as a result of telomere shortening, DNA damage, and genomic instability. In fact, the replicative ability of cardiovascular cells, particularly stem cells and/or progenitor cells, has been shown to decline with age. Recently, considerable progress has been made in understanding the pathogenesis of human progeroid syndromes that feature cardiovascular aging. Most of the genes responsible have a role in DNA metabolism, and mutated forms of these genes result in alterations of the response to DNA damage and in decreased cell proliferation, which might be common features of a phenotype of aging. Here we review the cardiovascular research on cellular senescence, stem cell aging, and progeroid syndromes and discuss the potential role of cellular senescence in the mechanisms underlying both normal aging and premature aging syndromes.

Miyazaki, T., et al. (2012). "Telomestatin impairs glioma stem cell survival and growth through the disruption of telomeric G-quadruplex and inhibition of the proto-oncogene, c-Myb." Clin Cancer Res **18**(5): 1268-1280.

PURPOSE: Glioma stem cells (GSC) are a critical therapeutic target of glioblastoma multiforme (GBM). EXPERIMENTAL DESIGN: The effects of a G-quadruplex ligand, telomestatin, were evaluated using patient-derived GSCs, non-stem tumor cells (non-GSC), and normal fetal neural precursors in vitro and in vivo. The molecular targets of telomestatin were determined by immunofluorescence in situ hybridization (iFISH) and cDNA microarray. The data were then validated by in vitro and in vivo functional assays, as well as by immunohistochemistry against 90 clinical samples. RESULTS: Telomestatin impaired the maintenance of GSC stem cell state by inducing apoptosis in vitro and in vivo. The migration potential of GSCs was also impaired by telomestatin treatment. In contrast, both normal neural precursors and non-GSCs were relatively resistant to telomestatin. Treatment of GSC-derived mouse intracranial tumors reduced tumor sizes in vivo without a noticeable cell death in normal brains. iFISH revealed both telomeric and non-telomeric DNA damage by telomestatin in GSCs but not in non-GSCs. cDNA microarray identified a proto-oncogene, c-Myb, as a novel molecular target of telomestatin in GSCs, and pharmacodynamic analysis in telomestatin-treated tumor-bearing mouse brains showed a reduction of c-Myb in tumors in vivo. Knockdown of c-Myb phenocopied telomestatin-treated GSCs both in vitro and in vivo, and restoring c-Myb by overexpression partially rescued the phenotype. Finally, c-Myb expression was markedly elevated in surgical specimens of GBMs compared with normal tissues. CONCLUSIONS: These data indicate that telomestatin potently eradicates GSCs through telomere disruption and c-Myb inhibition, and this study suggests a novel GSC-directed therapeutic strategy for GBMs.

Moore, M. A. (1997). "Stem cell proliferation: ex vivo and in vivo observations." Stem Cells **15 Suppl 1**: 239-248; discussion 248-251.

The contribution of stem cells to the early phases of hematopoietic engraftment is controversial, as is the issue of the number of stem cells required for successful long-term engraftment in man. An extensive body of data exists in the murine system and the degree to which this can be extrapolated to man is discussed. Other variables involving stem cells include their efficiency of homing to the bone marrow, their previous proliferative history influencing telomere length and their ability to upregulate telomerase. The extent to which stem cell numbers increase in vivo upon transplantation, or ex vivo upon cytokine stimulation, is discussed and the question as to the ability of nonstochastic extrinsic influences to alter stem cell self-renewal probability is raised.

Morcos, Y. A. T., et al. (2019). "A Novel Tissue and Stem Cell Specific TERF1 Splice Variant Is Downregulated in Tumour Cells." Int J Mol Sci **21**(1).

In this study, we describe the identification of a novel splice variant of TERF1/PIN2, one of the main components of the telomeric shelterin complex. This new splice variant is identical to TERF1, apart from a 30 amino acid internal insertion near to the C-terminus of TERF1. Based on genome comparison analyses and RNA expression data, we show that this splice variant is conserved among hominidae but absent from all other species. RNA expression and histological analyses show specific expression in human spermatogonial and hematopoietic stem cells (HSCs), while all other analyzed tissues lack the expression of this TERF1-isoform, hence the name TERF1-tsi (TERF1-tissue-specific-isoform). In addition, we could not detect any expression in primary human cells and established cancer cell lines. Immunohistochemistry results involving two new rabbit polyclonal antibodies, generated against TERF1-tsi specific peptides, indicate nuclear localization of TERF1-tsi in a subset of spermatogonial stem cells. In line with this observation, immunofluorescence analyzes in various cell lines consistently revealed that ectopic TERF1-tsi localizes to the cell nucleus, mainly but not exclusively at telomeres. In a first attempt to evaluate the impact of TERF1-tsi in the testis, we have tested its expression in normal testis samples versus matched tumor samples from the same patients. Both RT-PCR and IHC show a specific downregulation of TERF1-tsi in tumor samples while the expression of TERF1 and PIN2 remains unchanged.

Moschidou, D., et al. (2013). "Human mid-trimester amniotic fluid stem cells cultured under embryonic stem cell conditions with valproic acid acquire pluripotent characteristics." Stem Cells Dev **22**(3): 444-458.

Human mid-trimester amniotic fluid stem cells (AFSC) have promising applications in regenerative medicine, being broadly multipotent with an intermediate phenotype between embryonic (ES) and mesenchymal stem cells (MSC). Despite this propluripotent phenotype, AFSC are usually cultured in adherence in a serum-based expansion medium, and how expansion in conditions sustaining pluripotency might affect their phenotype remains unknown. We recently showed that early AFSC from first trimester amniotic fluid, which endogenously express Sox2 and Klf4, can be reprogrammed to pluripotency without viral vectors using the histone deacetylase inhibitor valproic acid (VPA). Here, we show that mid-trimester AFSC cultured under MSC conditions contained a subset of cells endogenously expressing telomerase, CD24, OCT4, C-MYC, and SSEA4, but low/null levels of SOX2, NANOG, KLF4, SSEA3, TRA-1-60, and

TRA-1-81, with cells unable to form embryoid bodies (EBs) or teratomas. In contrast, AFSC cultured under human ESC conditions were smaller in size, grew faster, formed colonies, upregulated OCT4 and C-MYC, and expressed KLF4 and SOX2, but not NANOG, SSEA3, TRA-1-60, and TRA-1-81. Supplementation with VPA for 5 days further upregulated OCT4, KLF4, and SOX2, and induced expression of NANOG, SSEA3, TRA-1-60, and TRA-1-81, with cells now able to form EBs and teratomas. We conclude that human mid-trimester AFSC, which may be isolated autologously during pregnancy without ethics restriction, can acquire pluripotent characteristics without the use of ectopic factors. Our data suggest that this medium-dependant approach to pluripotent mid-trimester AFSC reflects true reprogramming and not the selection of prepluripotent cells.

Movahednia, M. M., et al. (2015). "Differential effects of the extracellular microenvironment on human embryonic stem cell differentiation into keratinocytes and their subsequent replicative life span." Tissue Eng Part A **21**(7-8): 1432-1443.

Culture microenvironment plays a critical role in the propagation and differentiation of human embryonic stem cells (hESCs) and their differentiated progenies. Although high efficiency of hESC differentiation to keratinocytes (hESC-Kert) has been achieved, little is known regarding the effects of early culture microenvironment and pertinent extracellular matrix (ECM) interactions during epidermal commitment on subsequent proliferative capacity of hESC-Kert. The aim of this study is to evaluate the effects of the different ECM microenvironments during hESC differentiation on subsequent replicative life span of hESC-Kert. In doing so, H1-hESCs were differentiated to keratinocytes (H1-Kert) in two differentiation systems. The first system employed autologous fibroblast feeder support, in which keratinocytes (H1-Kert(ACC)) were derived by coculture of hESCs with hESC-derived fibroblasts (H1-ebFs). The second system employed a novel decellularized matrix from H1-ebFs to create a dermoepidermal junction-like (DEJ) matrix. H1-Kert(AFF) were derived by differentiation of hESCs on the feeder-free system employing the DEJ matrix. Our study indicated that the feeder-free system with the use of DEJ matrix was more efficient in differentiation of hESCs toward epidermal progenitors. However, the feeder-free system was not sufficient to support the subsequent replicative capacity of differentiated keratinocytes. Of note, H1-Kert(AFF) showed limited replicative capacity with reduced telomere length and early cellular senescence. We further showed that the lack of cell-cell interactions during epidermal commitment led to heightened production of TGF-beta1 by hESC-Kert during extended culture, which in turn was responsible for resulting in the limited replicative life span with cellular senescence of hESC-Kert derived under the feeder-free culture system. This study highlights for the first time the importance of the culture microenvironment and cell-ECM interactions during differentiation of hESCs on subsequent replicative life span and cellular senescence of the differentiated keratinocytes, with implications for use of these cells for applications in tissue engineering and regenerative medicine.

Niedernhofer, L. J. (2008). "DNA repair is crucial for maintaining hematopoietic stem cell function." DNA Repair (Amst) **7**(3): 523-529.

Richard Cornall and collaborators recently developed a mouse model of Ligase IV syndrome with growth retardation and immunodeficiency due to a defect in nonhomologous end-joining (NHEJ) of DNA double-strand breaks. They demonstrated age-dependent loss of hematopoietic stem cell function in these mice. Simultaneously, Irving Weissman and colleagues demonstrated a similar phenomenon in Ku80(-/-) mice defective in NHEJ and telomere maintenance, Xpd(TTD) mice defective in nucleotide excision repair, and late generation mTr(-/-) missing telomerase activity. These studies strongly support the hypothesis that genomic stress causes aging by limiting the ability of stem cells to indefinitely maintain tissue homeostasis.

Norddahl, G. L., et al. (2012). "Reduced repression of cytokine signaling ameliorates age-induced decline in hematopoietic stem cell function." Aging Cell **11**(6): 1128-1131.

Aging causes profound effects on the hematopoietic stem cell (HSC) pool, including an altered output of mature progeny and enhanced self-propagation of repopulating-defective HSCs. An important outstanding question is whether HSCs can be protected from aging. The signal adaptor protein LNK negatively regulates hematopoiesis at several cellular stages. It has remained unclear how the enhanced sensitivity to cytokine signaling caused by LNK deficiency affects hematopoiesis upon aging. Our findings demonstrate that aged LNK-/- HSCs displayed a robust overall reconstitution potential and gave rise to a hematopoietic system with a balanced lineage distribution. Although aged LNK-/- HSCs displayed a distinct molecular profile in which reduced proliferation was central, little or no difference in the proliferation of aged LNK-/- HSCs was observed after transplantation when compared to aged WT HSCs. This coincided with equal telomere maintenance in WT and LNK-/- HSCs. Collectively, our studies suggest that enhanced cytokine signaling can counteract functional age-related HSC decline.

Notara, M., et al. (2013). "The impact of age on the physical and cellular properties of the human limbal stem cell niche." Age (Dordr) **35**(2): 289-300.

The limbal niche in the corneoscleral junction of the eye, habitat of the limbal epithelial stem cells (LESC), facilitates corneal epithelial regeneration by providing physical support and chemical signalling. Anatomical structures within the limbus, namely, limbal epithelial crypts and focal stromal projections, are believed to function as a putative niche for LESCs. In this study, the impact of age on the topography of this niche was investigated. Also, the relationship between niche topography and limbal epithelial cell phenotype was assessed. Ex vivo imaging of the limbus in cadaveric tissue of donors aged from infancy to 90 years was carried out using electron and confocal microscopy. The data suggested that the area occupied by the crypts was sharply reduced after the age of 60 years. The niche microstructures also became smoother with donor age. The phenotypic assessment of cultured limbal epithelial cells harvested from donors of different ages showed that the levels of putative stem cell markers as well as telomerase activity and telomere length remained unchanged, regardless of niche topography. However, the colony forming efficiency of the cultures was significantly reduced with age ($p < 0.05$). This is the first comprehensive study of the effect of age on the structural and phenotypic characteristics of the human limbal niche. The results have a significant biological value as they suggest a correlation of limbal architecture with decline of re-epithelialisation rate in older patients. Overall, the data also suggest that LESCs harvested from younger donors may be more suitable for cultured LESC therapy production.

O'Connell, N., et al. (2014). "Successful treatment with rituximab and mycophenolate mofetil of refractory autoimmune hemolytic anemia post-hematopoietic stem cell transplant for dyskeratosis congenita due to TINF2 mutation." Pediatr Transplant **18**(1): E22-24.

AIHA following allogeneic HSCT is appearing more frequently in the literature. It occurs as a result of donor cell-derived antibodies targeting donor red cell antigens. Little guidance exists on the management of such patients, particularly in the pediatric setting. First-line conventional treatment is corticosteroids and/or immunoglobulin therapy with monoclonal antibody therapy reserved for treatment failure. We report our experience of a child refractory to immunoglobulin and steroid therapy who required several infusions of rituximab and immunomodulatory therapy to obtain a clinically significant response.

Pandey, S., et al. (2020). "Sphingosine kinases protect murine embryonic stem cells from sphingosine-induced cell cycle arrest." Stem Cells **38**(5): 613-623.

Sphingosine-1-phosphate (S1P) is a bioactive lipid molecule regulating organogenesis, angiogenesis, cell proliferation, and apoptosis. S1P is generated by sphingosine kinases (SPHK1 and SPHK2) through the phosphorylation of ceramide-derived sphingosine. Phenotypes caused by manipulating S1P metabolic enzymes and receptors suggested several possible functions for S1P in embryonic stem cells (ESCs), yet the mechanisms by which S1P and related sphingolipids act in ESCs are controversial. We designed a rigorous test to evaluate the requirement of S1P in murine ESCs by knocking out both Sphk1 and Sphk2 to create cells incapable of generating S1P. To accomplish this, we created lines mutant for Sphk2 and conditionally mutant (floxed) for Sphk1, allowing evaluation of ESCs that transition to double-null state. The Sphk1/2-null ESCs lack S1P and accumulate the precursor sphingosine. The double-mutant cells fail to grow due to a marked cell cycle arrest at G2/M. Mutant cells activate expression of telomere elongation factor genes Zscan4, Tcstv1, and Tcstv3 and display longer telomeric repeats. Adding exogenous S1P to the medium had no impact, but the cell cycle arrest is partially alleviated by the expression of a ceramide synthase 2, which converts excess sphingosine into ceramide. The results indicate that sphingosine kinase activity is essential in mouse ESCs for limiting the accumulation of sphingosine that otherwise drives cell cycle arrest.

Pang, L. Y. and D. J. Argyle (2009). "Using naturally occurring tumours in dogs and cats to study telomerase and cancer stem cell biology." Biochim Biophys Acta **1792**(4): 380-391.

The recently described cancer stem cell theory opens up many new challenges and opportunities to identify targets for therapeutic intervention. However, the majority of cancer related therapeutic studies rely upon rodent models of human cancer that rarely translate into clinical success in human patients. Naturally occurring cancers in dogs, cats and humans share biological features, including molecular targets, telomerase biology and tumour genetics. Studying cancer stem cell biology and telomere/telomerase dynamics in the cancer bearing pet population may offer the opportunity to develop a greater understanding of cancer biology in the natural setting and evaluate the development of novel therapies targeted at these systems.

Pazianos, G., et al. (2003). "The elements of stem cell self-renewal: a genetic perspective." Biotechniques **35**(6): 1240-1247.

Every day, the body produces billions of new blood cells. Each of these is derived from a rare cell in the bone marrow called the hematopoietic stem cell (HSC). Because most mature blood cells have a limited lifespan, the ability of HSCs to self-renew and

replenish the mature cell compartment is critical to sustaining life. While great progress has been made in isolating HSCs and defining their functional and phenotypic characteristics, the molecular mechanisms that regulate their self-renewal remain a mystery. Over the last few years, alterations in HSC frequency and self-renewal capacity in transgenic and knock-out mice have led to the identification of novel mediators of HSC homeostasis in vivo. These genetically modified mice have revealed that maintenance of survival, proliferation, quiescence, and normal telomere length all contribute to the self-renewal of HSCs. They also highlight the need to test in context of the normal microenvironment the role of signaling molecules such as Notch and Wnt, which have emerged recently as important regulators of HSC self-renewal. The emerging picture these data provide of the regulation of self-renewal in HSCs has provided a better understanding of the basic biology of stem cells and holds promise for designing strategies to improve bone marrow transplantation.

Pelicci, P. G. (2004). "Do tumor-suppressive mechanisms contribute to organism aging by inducing stem cell senescence?" J Clin Invest **113**(1): 4-7.

Stem/progenitor cells ensure tissue and organism homeostasis and might represent a frequent target of transformation. Although these cells are potentially immortal, their life span is restrained by signaling pathways (p19-p53; p16-Rb) that are activated by DNA damage (telomere dysfunction, environmental stresses) and lead to senescence or apoptosis. Execution of these checkpoint programs might lead to stem cell depletion and organism aging, while their inactivation contributes to tumor formation.

Pereboom, T. C., et al. (2011). "A zebrafish model of dyskeratosis congenita reveals hematopoietic stem cell formation failure resulting from ribosomal protein-mediated p53 stabilization." Blood **118**(20): 5458-5465.

Dyskeratosis congenita (DC) is a bone marrow failure disorder characterized by shortened telomeres, defective stem cell maintenance, and highly heterogeneous phenotypes affecting predominantly tissues that require high rates of turnover. Here we present a mutant zebrafish line with decreased expression of nop10, one of the known H/ACA RNP complex genes with mutations linked to DC. We demonstrate that this nop10 loss results in 18S rRNA processing defects and collapse of the small ribosomal subunit, coupled to stabilization of the p53 tumor suppressor protein through small ribosomal proteins binding to Mdm2. These mutants also display a hematopoietic stem cell deficiency that is reversible on loss of p53 function. However, we detect no changes in telomere length in nop10 mutants. Our data support a model of DC whereupon in early development mutations involved in the H/ACA complex contribute to bone marrow failure through p53 deregulation and loss of initial stem cell numbers while their role in telomere maintenance does not contribute to DC until later in life.

Phermthai, T., et al. (2016). "Successful derivation of xeno-free mesenchymal stem cell lines from endometrium of infertile women." Reprod Biol **16**(4): 261-268.

Transplantation of mesenchymal stem cells (MSC) can effectively repair endometrial deficiencies, including infertile patients with a problem of inadequate endometrium thickness. Although, MSC derived from different organ sources have a similarity of MSC specific characteristics, endometrial stem cells (EMSC) are temporally regulated throughout the menstrual cycle in a micro-environmental niche found only in endometrial tissue. Given the micro-environment niche, developing treatments for endometrial disorders with EMSC should be a top priority. To provide EMSC that afford safety for therapeutic usage, we have established a completely xeno-free EMSC line derivation protocol using human allogenic umbilical cord serum instead of animal derived reagents, and proved that it is feasible to generate xeno-free EMSC lines from infertile patient donors using these conditions. Our results demonstrate the successful derivation of xeno-free EMSC lines from 10 out of 10 infertile patients. The resultant xeno-free EMSC lines showed typical MSC morphology, phenotypic markers, differentiation capacity, telomere length and normal karyotypes. They showed superior proliferation capability, but lower expression of proto-oncogenes, to the lines generated under standard (animal derived reagents) culture. Biosafety of xeno-free EMSC lines also displayed in retention of immunosuppressive ability, epigenetic stability by imprinted genes expression, proto-oncogenes expression and no mutation of specific codon on p53 tumor suppressor gene. Taken together, these data indicate that our cells may be safe for clinical use. In conclusion, we have succeeded in establishing completely xeno-free EMSC lines and demonstrate for the first time that autogenic and xeno-free EMSC lines can be generated from infertile women.

Piccin, D. and C. M. Morshead (2010). "Potential and pitfalls of stem cell therapy in old age." Dis Model Mech **3**(7-8): 421-425.

Our increasing understanding of resident stem cell populations in various tissues of the adult body provides promise for the development of cell-based therapies to treat trauma and disease. With the sharp rise in the aging population, the need for effective regenerative medicine strategies for the aged is more important then ever. Yet, the vast majority of research fuelling our understanding of the mechanisms that

control stem cell behaviour, and their role in tissue regeneration, is conducted in young animals. Evidence collected in the last several years indicates that, although stem cells remain active into old age, changes in the stem cells and their microenvironments inhibit their regenerative potential. An understanding of both the cell-intrinsic stem cell changes, as well as concomitant changes to the stem cell niche and the systemic environment, are crucial for the development of regenerative medicine strategies that might be successful in aged patients.

Pirmoradi, S., et al. (2018). "Curcumin Affects Adipose Tissue-Derived Mesenchymal Stem Cell Aging Through TERT Gene Expression." Drug Res (Stuttg) **68**(4): 213-221.

Aging and losing cell survival is one of the main problems in cell therapy. Aging of Mesenchymal Stem Cells (MSCs) is associated with a rise in intracellular reactive oxygen species, decrease in telomerase reverse transcriptase (TERT) expression and finally eroded telomere ends. Given that the production of free radicals in the aging process is effective, the use of antioxidants can help in scavenging free radicals and prevent the aging of cells. The aim of this study is to evaluate the effects of curcumin on proliferation, aging and TERT expression of rat adipose tissue-derived stem cells (rADSC). rADSCs were isolated from inguinal rat adipose tissue and their viabilities were assessed by MTT after exposure to different concentrations of curcumin. Flow-cytometry was performed for investigating the cell surface markers. Adipogenic and osteogenic differentiation were carried out to evaluate the pluripotency of rADSCs. Telomerase expression and percentage of senescent cells were evaluated using real-time PCR and senescence-associated beta-galactosidase activity, respectively. The results demonstrated significant proliferation of rADSCs after 48 h treatment with 1 and 5 microM curcumin. Additionally, these concentrations could significantly reduce the population doubling time and aging of rADSCs at different passages. The findings of SA-ss-gal staining showed that curcumin significantly decreased the number of senescent cells in the 5 and 7 cell passages. Moreover, expression levels of TERT increased in the presence of 1 and 5 microM curcumin than control group ($P<0.001$). As a conclusion, curcumin may be a good candidate to improve lifespan of rADSCs through promoting TERT gene expression.

Porter, D. L. and C. H. June (2005). "T-cell reconstitution and expansion after hematopoietic stem cell transplantation: 'T' it up!" Bone Marrow Transplant **35**(10): 935-942.

Adoptive immunotherapy is the isolation and infusion of antigen-specific or nonspecific lymphocytes. Adoptive therapy with T cells may have a role in replacing, repairing, or enhancing immune function damaged by cytotoxic therapies, and rapid lymphocyte recovery may improve outcome after autologous and allogeneic stem cell transplantation (SCT). Recently, a plethora of information on the basic mechanisms of T-cell biology and regulation of cellular immune responses has emerged, permitting the development of new forms of adoptive cell therapy. Efficient ex vivo culture method for T-cell subsets affords the possibility of adoptive transfer of T cells engineered with enhanced capacity for central memory, effector cytotoxicity, Th1, Th2, veto cell, and T regulatory functions. Studies show that homeostatic T-cell proliferation is important for effective adoptive immunotherapy and pretreatment with chemotherapy may enhance the effects of infused T cells. Replicative senescence, in part due to telomere erosion, likely limits successful adoptive immunotherapy, though it may be possible to maintain T-cell pools by enforced expression of telomerase. Clinical trials now demonstrate that it is possible to enhance immune reconstitution after SCT with cytokines or infusions of ex vivo costimulated expanded T cells. These data all support the premise that adoptive therapy can accelerate reconstitution of cellular immunity with enhanced antitumor effects following SCT.

Pu, H., et al. (2015). "CUDR promotes liver cancer stem cell growth through upregulating TERT and C-Myc." Oncotarget **6**(38): 40775-40798.

Cancer up-regulated drug resistant (CUDR) is a novel non-coding RNA gene. Herein, we demonstrate excessive CUDR cooperates with excessive CyclinD1 or PTEN depletion to accelerate liver cancer stem cells growth and liver stem cell malignant transformation in vitro and in vivo. Mechanistically, we reveal the decrease of PTEN in cells may lead to increase binding capacity of CUDR to CyclinD1. Therefore, CUDR-CyclinD1 complex loads onto the long noncoding RNA H19 promoter region that may lead to reduce the DNA methylation on H19 promoter region and then to enhance the H19 expression. Strikingly, the overexpression of H19 increases the binding of TERT to TERC and reduces the interplay between TERT with TERRA, thus enhancing the cell telomerase activity and extending the telomere length. On the other hand, insulator CTCF recruits the CUDR-CyclinD1 complx to form the composite CUDR-CyclinD1-insulator CTCF complex which occupancied on the C-myc gene promoter region, increasing the outcome of oncogene C-myc. Ultimately, excessive TERT and C-myc lead to liver cancer stem cell and hepatocyte-like stem cell malignant proliferation. To understand the novel functions of long noncoding RNA CUDR will help in the development of new liver cancer therapeutic and diagnostic approaches.

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. A strategy based upon the synthesis of telomeric repeat sequences by the ribonucleoprotein telomerase, is prevalent in approximately 90% of human tumours studied, including the majority of brain tumours. This review will provide a developmental perspective with respect to normal (neurogenesis) and aberrant (tumourigenesis) cellular turnover, differentiation and function. Within this context our current knowledge of brain tumour telomere/telomerase biology will be discussed with respect to both its developmental and therapeutic relevance to the hierarchical model of brain tumourigenesis presented by the cancer stem cell paradigm.

Rinaldi, S., et al. (2014). "Stem cell senescence. Effects of REAC technology on telomerase-independent and telomerase-dependent pathways." Sci Rep **4**: 6373.

Decline in the gene expression of senescence repressor Bmi1, and telomerase, together with telomere shortening, underlay senescence of stem cells cultured for multiple passages. Here, we investigated whether the impairment of senescence preventing mechanisms can be efficiently counteracted by exposure of human adipose-derived stem cells to radio electric asymmetrically conveyed fields by an innovative technology, named Radio Electric Asymmetric Conveyer (REAC). Due to REAC exposure, the number of stem cells positively stained for senescence associated beta-galactosidase was significantly reduced along multiple culturing passages. After a 90-day culture, REAC-treated cells exhibited significantly higher transcription of Bmi1 and enhanced expression of other stem cell pluripotency genes and related proteins, compared to unexposed cells. Transcription of the catalytic telomerase subunit (TERT) was also increased in REAC-treated cells at all passages. Moreover, while telomere shortening occurred at early passages in both REAC-treated and untreated cells, a significant rescue of telomere length could be observed at late passages only in REAC-exposed cells. Thus, REAC-asymmetrically conveyed radio electric fields acted on a gene and protein expression program of both telomerase-independent and telomerase-dependent patterning to optimize stem cell ability to cope with senescence progression.

Rodriguez, R. M., et al. (2013). "Role of sirtuins in stem cell differentiation." Genes Cancer **4**(3-4): 105-111.

Sirtuins play an essential role in the cellular response to environmental stress, promoting DNA repair, telomere stability, cell cycle arrest, cellular senescence, and apoptosis. Much attention has been given to the role of sirtuins in aging and cancer development; however, less is known about their role in stem cell regulation. This review focuses in this topic and discusses the possible implications in adult stem cell aging.

Rodriguez-Brenes, I. A. and D. Wodarz (2016). "Telomeres open a window on stem cell division." Elife **5**: e12481.

Measuring the length distribution of telomeres can reveal information about biological processes that are otherwise difficult to analyze experimentally.

Roy, S., et al. (2019). "Combined treatment with cisplatin and the tankyrase inhibitor XAV-939 increases cytotoxicity, abrogates cancer-stem-like cell phenotype and increases chemosensitivity of head-and-neck squamous-cell carcinoma cells." Mutat Res Genet Toxicol Environ Mutagen **846**: 503084.

Cancer stem-like cells (CSCs) were reported to be linked with tumorigenesis, metastasis and resistant to chemo and radiotherapy in head and neck squamous cell carcinoma (HNSCC). In this study we investigated the role of CSCs in chemoresistance and abrogation of CSC mediated chemoresistance by combinatorial treatment with cisplatin and small molecule tankyrase inhibitor XAV-939. Two cisplatin-resistant HNSCC cells were generated by stepwise dose incremental strategy. We evaluated the chemoresistance, sphere forming capacity, extent of DNA damage and repair capacity in parental and cisplatin-resistant HNSCC cells. Furthermore, the abrogation of CSC mediated chemoresistance was evaluated in HNSCC cells with XAV-939 alone and in combination with cisplatin. It was observed that

cisplatin-resistant HNSCC cell lines exhibited increase in chemoresistance, CSC phenotype and increased DNA repair capacity. We observed that combination of cisplatin and XAV-939 acts synergistically to abrogate chemoresistance by increasing DNA damage. Molecular docking study also revealed similar binding region that could contribute towards synergy predictions between cisplatin and XAV939. In conclusion, this study elucidated that combination of cisplatin and XAV-939 exerted cytotoxic and genotoxic effect to abrogate CSC mediated chemoresistance in HNSCC in synergistic manner.

Sacco, A., et al. (2010). "Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice." Cell **143**(7): 1059-1071.

In Duchenne muscular dystrophy (DMD), dystrophin mutation leads to progressive lethal skeletal muscle degeneration. For unknown reasons, dystrophin deficiency does not recapitulate DMD in mice (mdx), which have mild skeletal muscle defects and potent regenerative capacity. We postulated that human DMD progression is a consequence of loss of functional muscle stem cells (MuSC), and the mild mouse mdx phenotype results from greater MuSC reserve fueled by longer telomeres. We report that mdx mice lacking the RNA component of telomerase (mdx/mTR) have shortened telomeres in muscle cells and severe muscular dystrophy that progressively worsens with age. Muscle wasting severity parallels a decline in MuSC regenerative capacity and is ameliorated histologically by transplantation of wild-type MuSC. These data show that DMD progression results, in part, from a cell-autonomous failure of MuSC to maintain the damage-repair cycle initiated by dystrophin deficiency. The essential role of MuSC function has therapeutic implications for DMD.

Sachs, P. C., et al. (2012). "Defining essential stem cell characteristics in adipose-derived stromal cells extracted from distinct anatomical sites." Cell Tissue Res **349**(2): 505-515.

The discovery of adipose-derived stromal cells (ASCs) has created many opportunities for the development of patient-specific cell-based replacement therapies. We have isolated multiple cell strains of ASCs from various anatomical sites (abdomen, arms/legs, breast, buttocks), indicating widespread distribution of ASCs throughout the body. Unfortunately, there exists a general lack of agreement in the literature as to their "stem cell" characteristics. We find that telomerase activity and expression of its catalytic subunit in ASCs are both below the levels of detection, independent of age and culturing conditions. ASCs also undergo telomere attrition and eventually senesce, while maintaining a stable karyotype without the development of spontaneous tumor-associated abnormalities. Using a set of cell surface markers that have been promoted to identify ASCs, we find that they failed to distinguish ASCs from normal fibroblasts, as both are positive for CD29, CD73 and CD105 and negative for CD14, CD31 and CD45. All of the ASC isolates are multipotent, capable of differentiating into osteocytes, chondrocytes and adipocytes, while fibroblasts show no differentiation potential. Our ASC strains also show elevated expression of genes associated with pluripotent cells, Oct-4, SOX2 and NANOG, when compared to fibroblasts and bone marrow-derived mesenchymal stem cells (BM-MSCs), although the levels were lower than induced pluripotent stem cells (iPS). Together, our data suggest that, while the cell surface profile of ASCs does not distinguish them from normal fibroblasts, their differentiation capacity and the expression of genes closely linked to pluripotency clearly define ASCs as multipotent stem cells, regardless of tissue isolation location.

Saeed, H. and M. Iqtedar (2013). "Stem cell function and maintenance - ends that matter: role of telomeres and telomerase." J Biosci **38**(3): 641-649.

Stem cell research holds a promise to treat and prevent age-related degenerative changes in humans. Literature is replete with studies showing that stem cell function declines with aging, especially in highly proliferative tissues/ organs. Among others, telomerase and telomere damage is one of the intrinsic physical instigators that drive agerelated degenerative changes. In this review we provide brief overview of telomerase-deficient aging affects in diverse stem cells populations. Furthermore, potential disease phenotypes associated with telomerase dysregulation in a specific stem cell population is also discussed in this review. Additionally, the role of telomerase in stem cell driven cancer is also briefly touched upon.

Sanada, F., et al. (2014). "c-Kit-positive cardiac stem cells nested in hypoxic niches are activated by stem cell factor reversing the aging myopathy." Circ Res **114**(1): 41-55.

RATIONALE: Hypoxia favors stem cell quiescence, whereas normoxia is required for stem cell activation, but whether cardiac stem cell (CSC) function is regulated by the hypoxic/normoxic state of the cell is currently unknown. OBJECTIVE: A balance between hypoxic and normoxic CSCs may be present in the young heart, although this homeostatic control may be disrupted with aging. Defects in tissue oxygenation occur in the old myocardium, and this phenomenon may expand the pool of hypoxic CSCs, which are no longer involved in myocyte renewal. METHODS AND RESULTS: Here, we show that the senescent heart is characterized by an increased number of quiescent CSCs with intact telomeres that cannot re-enter the cell cycle and form a differentiated progeny. Conversely, myocyte replacement is controlled only by frequently dividing CSCs with

shortened telomeres; these CSCs generate a myocyte population that is chronologically young but phenotypically old. Telomere dysfunction dictates their actual age and mechanical behavior. However, the residual subset of quiescent young CSCs can be stimulated in situ by stem cell factor reversing the aging myopathy. CONCLUSIONS: Our findings support the notion that strategies targeting CSC activation and growth interfere with the manifestations of myocardial aging in an animal model. Although caution has to be exercised in the translation of animal studies to human beings, our data strongly suggest that a pool of functionally competent CSCs persists in the senescent heart and that this stem cell compartment can promote myocyte regeneration effectively, partly correcting the aging myopathy.

Sastry, P. S. (2004). "Metabolic rate determines haematopoietic stem cell self-renewal." Med Hypotheses **63**(3): 476-480.

The number of haematopoietic stem cells (HSCs) per animal is conserved across species. This means the HSCs need to maintain hematopoiesis over a longer period in larger animals. This would result in the requirement of stem cell self-renewal. At present the three existing models are the stochastic model, instructive model and the third more recently proposed is the chiaro-scuro model. It is a well known allometric law that metabolic rate scales to the three quarter power. Larger animals have a lower metabolic rate, compared to smaller animals. Here it is being hypothesized that metabolic rate determines haematopoietic stem cell self-renewal. At lower metabolic rate the stem cells commit for self-renewal, where as at higher metabolic rate they become committed to different lineages. The present hypothesis can explain the salient features of the different models. Recent findings regarding stem cell self-renewal suggest an important role for Wnt proteins and their receptors known as frizzleds, which are an important component of cell signaling pathway. The role of cGMP in the Wnts action provides further justification for the present hypothesis as cGMP is intricately linked to metabolic rate. One can also explain the telomere homeostasis by the present hypothesis. One prediction of the present hypothesis is with reference to the limit of cell divisions known as Hayflick limit, here it is being suggested that this is the result of metabolic rate in laboratory conditions and there can be higher number of cell divisions in vivo if the metabolic rate is lower.

Schneider, R. P., et al. (2013). "TRF1 is a stem cell marker and is essential for the generation of induced pluripotent stem cells." Nat Commun **4**: 1946.

TRF1 is a component of the shelterin complex that protects chromosome ends. TRF1 deficiency leads to early embryonic lethality and to severe organ atrophy when deleted in adult tissues. Here we generate a reporter mouse carrying a knock-in eGFP-TRF1 fusion allele to study the role of TRF1 in stem cell biology and tissue homeostasis. We find that eGFP-TRF1 expression in mice is maximal in known adult stem cell compartments and show that TRF1 ensures their functionality. eGFP-TRF1 is highly expressed in induced pluripotent stem cells, uncoupled from the telomere elongation associated with reprogramming. Selection of eGFP-TRF1-high induced pluripotent stem cells correlates with higher pluripotency as indicated by their ability to form teratomas and chimeras. We further show that TRF1 is necessary for both induction and maintenance of pluripotency, and that TRF1 is a direct transcriptional target of Oct3/4.

Schultz, M. B. and D. A. Sinclair (2016). "When stem cells grow old: phenotypes and mechanisms of stem cell aging." Development **143**(1): 3-14.

All multicellular organisms undergo a decline in tissue and organ function as they age. An attractive theory is that a loss in stem cell number and/or activity over time causes this decline. In accordance with this theory, aging phenotypes have been described for stem cells of multiple tissues, including those of the hematopoietic system, intestine, muscle, brain, skin and germline. Here, we discuss recent advances in our understanding of why adult stem cells age and how this aging impacts diseases and lifespan. With this increased understanding, it is feasible to design and test interventions that delay stem cell aging and improve both health and lifespan.

Selleri, S., et al. (2011). "In vivo T-cell dynamics during immune reconstitution after hematopoietic stem cell gene therapy in adenosine deaminase severe combined immune deficiency." J Allergy Clin Immunol **127**(6): 1368-1375 e1368.

BACKGROUND: Gene therapy (GT) with hematopoietic stem cells is a promising treatment for inherited immunodeficiencies. OBJECTIVES: Limited information is available on the relative contribution of de novo thymopoiesis and peripheral expansion to T-cell reconstitution after GT as well as on the potential effects of gene transfer on hematopoietic stem cells and lymphocyte replicative lifespan. We studied these issues in patients affected by adenosine deaminase severe combined immune deficiency after low-intensity conditioning and reinfusion of retrovirally transduced autologous CD34(+) cells. METHODS: Immunophenotype, proliferative status, telomere length, and T-cell receptor excision circles were investigated at early and late time points (up to 9 years) after GT treatment. Control groups consisted of pediatric healthy donors and patients undergoing allogeneic bone marrow transplantation (BMT). RESULTS: We observed no telomere shortening in the bone marrow compartment and in granulocytes,

whereas peripheral blood naive T cells from both GT and BMT patients showed a significant reduction in telomere length compared with healthy controls. This was in agreement with the presence of a high fraction of actively cycling naive and memory T cells and lower T-cell receptor excision circles. CONCLUSION: These data indicate that T-cell homeostatic expansion contributes substantially to immune reconstitution, like BMT, and is not associated with senescence in the stem cell compartment.

Shea, L. D. (2006). "Back to the science of stem cell research - CHI's 2nd Annual Meeting." IDrugs **9**(10): 699-701.

Shen, C., et al. (2018). "The Pinx1 Gene Downregulates Telomerase and Inhibits Proliferation of CD133+ Cancer Stem Cells Isolated from a Nasopharyngeal Carcinoma Cell Line by Regulating Trfs and Mad1/C-Myc/p53 Pathways." Cell Physiol Biochem **49**(1): 282-294.

BACKGROUND/AIMS: Cancer stem cells (CSCs) are important factors for the continuous growth, recurrence, and metastasis of malignant tumors. They are responsible for the ineffectiveness of traditional radiotherapy and chemotherapy toward malignant tumors. Currently, stem cells or side-population cells have been isolated from many cancer cell lines and malignant tumor tissues, including nasopharyngeal carcinoma. Exploring the biological characteristics of CSCs for CSC-targeted therapy has gained importance. CSCs possess higher telomerase activity; thus, the use of the gene encoding telomerase inhibitor PinX1 gene to target telomerase in CSCs and inhibit proliferation, invasion, and metastasis of CSCs has become an important means for the treatment of malignant tumors. PinX1 may regulate complex pathways, including TRF1, Mad1/c-Myc, and p53. METHODS: In this study, nasopharyngeal CD133+ CSCs were sorted using CD133 immunomagnetic beads by flow cytometry The successful isolation of CD133+ CSCs was confirmed by examining their surface markers, namely CD44, NaNOG, and SOX2 as well as their ability to undergo in vivo tumorigenesis and in vitro sphere formation, proliferation, migration, and invasion. In addition, CD133+ CSCs were transfected with the constructed PinX1 overexpression plasmid or siRNA and the resulting effects on their proliferation, migration, invasion, and apoptosis were detected using cell counting kit-8 (CCK-8), transwell assay, and scratch test, respectively. Furthermore, their effects on mRNA and protein levels of TRF1, TRF2, Mad1, c-Myc, and p53 were examined using quantitative real-time PCR and western blot, respectively. RESULTS: The overexpression of PinX1 in CD133+ CSCs significantly decreased hTERT ($P < 0.001$), inhibited proliferation, migration, and invasion, induced apoptosis, and significantly decreased c-Myc mRNA levels ($P < 0.001$), while it increased TRF1, Mad1, and p53 mRNA levels (all $P < 0.001$). On the other hand, PinX1 silencing in CD133+ CSCs significantly decreased TRF1, Mad1, and p53 mRNA levels (all $P < 0.01$), while it increased hTERT and c-Myc mRNA levels (all $P < 0.05$).

Shepherd, B. E., et al. (2007). "Hematopoietic stem-cell behavior in nonhuman primates." Blood **110**(6): 1806-1813.

Little is known about the behavior of hematopoietic stem cells (HSCs) in primates because direct observations and competitive-repopulation assays are not feasible. Therefore, we used 2 different and independent experimental strategies, the tracking of transgene expression after retroviral-mediated gene transfer (N = 11 baboons; N = 7 rhesus macaques) and quantitation of the average telomere length of granulocytes (N = 132 baboons; N = 14 macaques), together with stochastic methods, to study HSC kinetics in vivo. The average replication rate for baboon HSCs is once per 36 weeks according to gene-marking analyses and once per 23 weeks according to telomere-shortening analyses. Comparable results were derived from the macaque data. These rates are substantially slower than the average replication rates previously reported for HSCs in mice (once per 2.5 weeks) and cats (once per 8.3 weeks). Because baboons and macaques live for 25 to 45 years, much longer than mice (approximately 2 years) and cats (12-18 years), we can compute that HSCs undergo a relatively constant number (approximately 80-200) of lifetime replications. Thus, our data suggest that the self-renewal capacity of mammalian stem cells in vivo is defined and evolutionarily conserved.

Siegl-Cachedenier, I., et al. (2007). "Telomerase reverses epidermal hair follicle stem cell defects and loss of long-term survival associated with critically short telomeres." J Cell Biol **179**(2): 277-290.

Organ homeostasis and organismal survival are related to the ability of stem cells to sustain tissue regeneration. As a consequence of accelerated telomerase shortening, telomerase-deficient mice show defective tissue regeneration and premature death. This suggests a direct impact of telomere length and telomerase activity on stem cell biology. We recently found that short telomeres impair the ability of epidermal stem cells to mobilize out of the hair follicle (HF) niche, resulting in impaired skin and hair growth and in the suppression of epidermal stem cell proliferative capacity in vitro. Here, we demonstrate that telomerase reintroduction in mice with critically short telomeres is sufficient to correct epidermal HF stem cell defects. Additionally, telomerase reintroduction into these mice results in a normal life span by preventing degenerative pathologies in the absence of increased tumorigenesis.

Spyrou, J., et al. (2019). "Metabolomic and Transcriptional Analyses Reveal Atmospheric Oxygen During Human Induced Pluripotent Stem Cell Generation Impairs Metabolic Reprogramming." Stem Cells **37**(8): 1042-1056.

The transition to pluripotency invokes profound metabolic restructuring; however, reprogramming is accompanied by the retention of somatic cell metabolic and epigenetic memory. Modulation of metabolism during reprogramming has been shown to improve reprogramming efficiency, yet it is not known how metabolite availability during reprogramming affects the physiology of resultant induced pluripotent stem cells (iPSCs). Metabolic analyses of iPSCs generated under either physiological (5%; P-iPSC) or atmospheric (20%; A-iPSC) oxygen conditions revealed that they retained aspects of somatic cell metabolic memory and failed to regulate carbohydrate metabolism with A-iPSC acquiring different metabolic characteristics. A-iPSC exhibited a higher mitochondrial membrane potential and were unable to modulate oxidative metabolism in response to oxygen challenge, contrasting with P-iPSC. RNA-seq analysis highlighted that A-iPSC displayed transcriptomic instability and a reduction in telomere length. Consequently, inappropriate modulation of metabolism by atmospheric oxygen during reprogramming significantly impacts the resultant A-iPSC metabolic and transcriptional landscape. Furthermore, retention of partial somatic metabolic memory in P-iPSC derived under physiological oxygen suggests that metabolic reprogramming remains incomplete. As the metabolome is a regulator of the epigenome, these observed perturbations of iPSC metabolism will plausibly have downstream effects on cellular function and physiology, both during and following differentiation, and highlight the need to optimize nutrient availability during the reprogramming process. Stem Cells 2019;37:1042-1056.

Straathof, K. C., et al. (2009). "Haemopoietic stem-cell transplantation with antibody-based minimal-intensity conditioning: a phase 1/2 study." Lancet **374**(9693): 912-920.

BACKGROUND: Stem-cell transplantation can cure primary immunodeficiencies. However, in patients with pre-existing organ toxicity, patients younger than 1 year, and those with DNA or telomere repair disorders, chemotherapy-based conditioning is poorly tolerated and results in major morbidity and mortality. We tested a novel antibody-based minimal-intensity conditioning (MIC) regimen to assess whether this approach allowed curative donor stem-cell engraftment without non-haemopoietic toxicity. METHODS: 16 high-risk patients underwent stem-cell transplantation for primary immunodeficiencies with an MIC regimen consisting of two rat anti-CD45 monoclonal antibodies YTH 24.5 and YTH 54.12 for myelosuppression, and alemtuzumab (anti-CD52) and fludarabine, and low dose cyclophosphamide for immunosuppression. Donors were matched siblings (n=5), and matched (9) and mismatched (2) unrelated donors. FINDINGS: Antibody-based conditioning was well tolerated, with only two cases of grade 3 and no grade 4 toxicity. Rates of clinically significant acute (n=6, 36%) and chronic graft-versus-host disease (GVHD) (n=5, 31%) were acceptable. 15 of 16 patients (94%) engrafted, of whom 11 (69%) achieved full or high-level mixed chimerism in both lymphoid and myeloid lineages, and three achieved engraftment in the T-lymphoid lineage only. One patient needed retransplantation. At a median of 40 months post-transplant, 13 of 16 patients (81%) in this high-risk cohort were alive and cured from their underlying disease. INTERPRETATION: Monoclonal antibody-based conditioning seems well tolerated and can achieve curative engraftment even in patients with severe organ toxicity or DNA repair defects, or both. This novel approach represents a shift from the paradigm that intensive chemotherapy or radiotherapy, or both, is needed for donor stem-cell engraftment. This antibody-based conditioning regimen may reduce toxicity and late effects and enable SCT in virtually any primary immunodeficiency patient with a matched donor. FUNDING: None.

Suzuki, S., et al. (2017). "Impairment of fetal hematopoietic stem cell function in the absence of Fancd2." Exp Hematol **48**: 79-86.

Fanconi anemia (FA) results from mutations in the genes necessary for DNA damage repair and often leads to progressive bone marrow failure. Although the exhaustion of the bone marrow leads to cytopenias in FA patients as they age, evidence from human FA and mouse model fetal livers suggests that hematopoietic defects originate in utero, which may lead to deficient seeding of the bone marrow. To address this possibility, we examined the consequences of loss of Fancd2, a central component of the FA pathway. Examination of embryonic day 14.5 (E14.5) Fancd2 knockout (KO) fetal livers showed a decrease in total cellularity and specific declines in long-term and short-term hematopoietic stem cell (LT-HSC and ST-HSC, respectively) numbers. Fancd2 KO fetal liver cells display similar functional defects to Fancd2 adult bone marrow cells, including reduced colony-forming units, increased mitomycin C sensitivity, increased LT-HSC apoptosis, and heavily impaired competitive repopulation, implying that these defects are intrinsic to the fetal liver and are not dependent on the accumulation of DNA damage during aging. Telomere shortening, an aging-related mechanism proposed to contribute to HSC apoptosis and bone marrow failure

in FA, was not observed in Fancd2 KO fetal livers. In summary, loss of Fancd2 yields significant defects to fetal liver hematopoiesis, particularly the HSC population, which mimics key phenotypes from adult Fancd2 KO bone marrow independently of aging-accrued DNA damage.

Szychlinska, M. A., et al. (2017). "Mesenchymal Stem Cell-Based Cartilage Regeneration Approach and Cell Senescence: Can We Manipulate Cell Aging and Function?" Tissue Eng Part B Rev **23**(6): 529-539.

Aging is the most prominent risk factor triggering several degenerative diseases, such as osteoarthritis (OA). Due to its poor self-healing capacity, once injured cartilage needs to be reestablished. This process might be approached through resorting to cell-based therapies and/or tissue engineering. Human mesenchymal stem cells (MSCs) represent a promising approach due to their chondrogenic differentiation potential. Presently, in vitro chondrogenic differentiation of MSCs is limited by two main reasons as follows: aging of MSCs, which determines the loss of cell proliferative and differentiation capacity and MSC-derived chondrocyte hypertrophic differentiation, which limits the use of these cells in cartilage tissue regeneration approach. The effect of aging on MSCs is fundamental for stem cell-based therapy development, especially in older subjects.

Tan, K. K., et al. (2014). "Characterization of fetal keratinocytes, showing enhanced stem cell-like properties: a potential source of cells for skin reconstruction." Stem Cell Reports **3**(2): 324-338.

Epidermal stem cells have been in clinical application as a source of culture-generated grafts. Although applications for such cells are increasing due to aging populations and the greater incidence of diabetes, current keratinocyte grafting technology is limited by immunological barriers and the time needed for culture amplification. We studied the feasibility of using human fetal skin cells for allogeneic transplantation and showed that fetal keratinocytes have faster expansion times, longer telomeres, lower immunogenicity indicators, and greater clonogenicity with more stem cell indicators than adult keratinocytes. The fetal cells did not induce proliferation of T cells in coculture and were able to suppress the proliferation of stimulated T cells. Nevertheless, fetal keratinocytes could stratify normally in vitro. Experimental transplantation of fetal keratinocytes in vivo seeded on an engineered plasma scaffold yielded a well-stratified epidermal architecture and showed stable skin regeneration. These results support the possibility of using fetal skin cells for cell-based therapeutic grafting.

Tapia, P. C. (2006). "RhoA, Rho kinase, JAK2, and STAT3 may be the intracellular determinants of longevity implicated in the progeric influence of obesity: Insulin, IGF-1, and leptin may all conspire to promote stem cell exhaustion." Med Hypotheses **66**(3): 570-576.

The aging process in higher mammals is increasingly being shown to feature a potentially substantial contribution from the longitudinal deterioration of normative stem cell dynamics seen with the passage of time. The precise mechanistic sequence producing this phenomenon is not entirely understood, but recent evidence has strongly implicated intracellular downstream effectors of endocrinologic pathways thought to be engaged by the obese state, specifically the insulin, IGF-1, and leptin signaling pathways. Among the intracellular effectors of these signals, a uniquely potent influence on stem cell dynamics may be attributable to Rho/ROCK, JAK kinase activity and STAT3 activity. In particular, it has already been shown that specific tyrosine kinase activities, such as that seen with Rho kinase, are presently thought to be associated with adverse health outcomes in numerous clinical contexts. Furthermore, the Rho GTPase is thought to be contributing to end-stage renal disease. However, in addition to its contribution to organ system dysfunction, the Rho/ROCK pathway has recently been shown to be activated by insulin and IGF-1, providing a tantalizing connection to nutrition and aging science. The JAK-STAT pathway, in contrast, has long been associated with pro-inflammatory cytokines, but has recently been implicated in leptin signaling as well. Importantly, JAK-STAT signaling has, similarly to Rho/ROCK signaling, been implicated as capable of accelerating stem cell proliferation. The implications of these recent determinations, in light of the recent finding of telomere attrition in humans associated with obesity, are that the intracellular determinants of aging may already be known, and the known common influence of these signaling elements on longitudinal stem cell dynamics is a pronounced induction of proliferation, an elevation that has been linked to the pathologic evolution of longitudinal organ-level dysfunction and the organismal-level physiologic decline seen with the inexorable passage of time. Besides the obvious utility for the management for human age-related dysfunction that investigation of pharmacologic inhibitors of these proteins would provide, interventions such as caloric restriction and possibly intermittent fasting may beneficially influence stem cell proliferation dynamics and reduce intracellular correlates of mitogenic drive.

Thornley, I. and M. H. Freedman (2002). "Telomeres, X-inactivation ratios, and hematopoietic stem cell transplantation in humans: a review." Stem Cells **20**(3): 198-204.

The marrow repopulating hematopoietic stem cells (HSCs) in an auto- or allograft represent a small fraction of the normal complement of HSCs, yet are

required to reconstitute hematopoiesis and sustain it for the lifetime of the recipient. Such a burden imposes a "replicative stress" upon hematopoietic stem/progenitor cells. The finding of accelerated telomere shortening in hematopoietic stem cell transplant (HSCT) recipients raised the specter of accelerated hematopoietic aging. Here, we review the HSCT telomere literature and other studies of surrogate markers of HSC behavior conducted in human HSCT recipients. We present a paradigm for posttransplant hematopoietic reconstitution and speculate on the fate of HSCs in the human transplant setting.

Torella, D., et al. (2004). "Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression." Circ Res **94**(4): 514-524.

To determine whether cellular aging leads to a cardiomyopathy and heart failure, markers of cellular senescence, cell death, telomerase activity, telomere integrity, and cell regeneration were measured in myocytes of aging wild-type mice (WT). These parameters were similarly studied in insulin-like growth factor-1 (IGF-1) transgenic mice (TG) because IGF-1 promotes cell growth and survival and may delay cellular aging. Importantly, the consequences of aging on cardiac stem cell (CSC) growth and senescence were evaluated. Gene products implicated in growth arrest and senescence, such as p27Kip1, p53, p16INK4a, and p19ARF, were detected in myocytes of young WT mice, and their expression increased with age. IGF-1 attenuated the levels of these proteins at all ages. Telomerase activity decreased in aging WT myocytes but increased in TG, paralleling the changes in Akt phosphorylation. Reduction in nuclear phospho-Akt and telomerase resulted in telomere shortening and uncapping in WT myocytes. Senescence and death of CSCs increased with age in WT impairing the growth and turnover of cells in the heart. DNA damage and myocyte death exceeded cell formation in old WT, leading to a decreased number of myocytes and heart failure. This did not occur in TG in which CSC-mediated myocyte regeneration compensated for the extent of cell death preventing ventricular dysfunction. IGF-1 enhanced nuclear phospho-Akt and telomerase delaying cellular aging and death. The differential response of TG mice to chronological age may result from preservation of functional CSCs undergoing myocyte commitment. In conclusion, senescence of CSCs and myocytes conditions the development of an aging myopathy.

Torlen, J., et al. (2019). "Effect of Graft-versus-Host Disease Prophylaxis Regimens on T and B Cell Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **25**(6): 1260-1268.

Lymphocyte reconstitution is pivotal for successful long-term outcome after allogeneic hematopoietic stem cell transplantation (HSCT), and conditioning regimen and post-transplantation immunosuppression are risk factors for prolonged immunodeficiency. Nevertheless, the effects of different immunosuppressive protocols on lymphocyte output and replicative capacity have not been investigated. Here we assessed T cell receptor excision circles (TREC), kappa-deleting recombination excision circles (KREC), and T cell telomere length (TL) as proxy markers for immune reconstitution in patients in a prospective randomized trial comparing graft-versus-host disease (GVHD) prophylaxis after transplantation (cyclosporine/methotrexate versus tacrolimus/sirolimus; n=200). Results showed that medians of TREC, KREC, and TL were not significantly different between the prophylaxis groups at any assessment time point during follow-up (24 months), but the kinetics of TREC, KREC, and TL were significantly influenced by other transplantation-related factors. Older recipient age, the use of antithymocyte globulin before graft infusion, and use of peripheral blood stem cell grafts were associated with lower TREC levels, whereas acute GVHD transiently affected KREC levels. Patients with lymphocyte excision circle levels above the median at </=6 months post-transplantation had reduced transplantation-related mortality and superior 5-year overall survival ($P < .05$). We noticed significant T cell telomere shortening in the patient population as a whole during follow-up. Our results suggest that lymphocyte reconstitution after transplantation is not altered by different immunosuppressive protocols. This study has been registered at ClinicalTrials.gov (identifier: NCT00993343).

Tsai, C. C., et al. (2011). "Hypoxia inhibits senescence and maintains mesenchymal stem cell properties through down-regulation of E2A-p21 by HIF-TWIST." Blood **117**(2): 459-469.

Although low-density culture provides an efficient method for rapid expansion of human mesenchymal stem cells (MSCs), MSCs enriched by this method undergo senescence and lose their stem cell properties, which could be preserved by combining low-density and hypoxic culture. The mechanism was mediated through direct down-regulation of E2A-p21 by the hypoxia-inducible factor-1alpha (HIF-1alpha)-TWIST axis. Expansion under normoxia induced E2A and p21 expression, which were abrogated by overexpression of TWIST, whereas siRNA against TWIST up-regulated E2A and p21 in hypoxic cells. Furthermore, siRNA against p21 in normoxic cells enhanced proliferation and increased differentiation potential, whereas overexpression of p21 in hypoxic cells induced a decrease in proliferation and a loss of differentiation capacity. More importantly, MSCs expanded under hypoxic conditions by up to 100

population doublings, exhibited telomerase activity with maintained telomere length, normal karyotyping, and intact genetic integrity, and did not form tumors. These results support low-density hypoxic culture as a method for efficiently expanding MSCs without losing stem cell properties or increasing tumorigenicity.

Ullah, M., et al. (2020). "Emerging role of stem cell-derived extracellular microRNAs in age-associated human diseases and in different therapies of longevity." Ageing Res Rev **57**: 100979.

Organismal aging involves the progressive decline in organ function and increased susceptibility to age-associated diseases. This has been associated with the aging of stem cell populations within the body that decreases the capacity of stem cells to self-renew, differentiate, and regenerate damaged tissues and organs. This review aims to explore how aging is associated with the dysregulation of stem cell-derived extracellular vesicles (SCEVs) and their corresponding miRNA cargo (SCEV-miRNAs), which are short non-coding RNAs involved in post-transcriptional regulation of target genes. Recent evidence has suggested that in aging stem cells, SCEV-miRNAs may play a vital role regulating various processes that contribute to aging: cellular senescence, stem cell exhaustion, telomere length, and circadian rhythm. Hence, further clarifying the age-dependent molecular mechanisms through which SCEV-miRNAs exert their downstream effects may inform a greater understanding of the biology of aging, elucidate their role in stem cell function, and identify important targets for future regenerative therapies. Additionally, current studies evaluating therapeutic role of SCEVs and SCEV-miRNAs in treating several age-associated diseases are also discussed.

Umrath, F., et al. (2020). "iPSC-Derived MSCs Versus Originating Jaw Periosteal Cells: Comparison of Resulting Phenotype and Stem Cell Potential." Int J Mol Sci **21**(2).

Induced pluripotent stem cell-derived mesenchymal stem cell-like cells (iMSCs) are considered to be a promising source of progenitor cells for approaches in the field of bone regeneration. In a previous study, we described the generation of footprint-free induced pluripotent stem cells (iPSCs) from human jaw periosteal cells (JPCs) by transfection of a self-replicating RNA (srRNA) and subsequent differentiation into functional osteogenic progenitor cells. In order to facilitate the prospective transfer into clinical practice, xeno-free reprogramming and differentiation methods were established. In this study, we compared the properties and stem cell potential of the iMSCs produced from JPC-derived iPSCs with the parental primary JPCs they were generated from. Our results demonstrated, on the one hand, a comparable differentiation potential of iMSCs and JPCs. Additionally, iMSCs showed significantly longer telomere lengths compared to JPCs indicating rejuvenation of the cells during reprogramming. On the other hand, proliferation, mitochondrial activity, and senescence-associated beta-galactosidase (SA-beta-gal) activity indicated early senescence of iMSCs. These data demonstrate the requirement of further optimization strategies to improve mesenchymal development of JPC-derived iPSCs in order to take advantage of the best features of reprogrammed and rejuvenated cells.

Uziel, O., et al. (2020). "Premature ageing following allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **55**(7): 1438-1446.

Survivors of hematopoietic cell transplantation (HCT) have been shown to exhibit both clinical and biological features of accelerated ageing. Most studies used frailty measures, comorbidities for clinical assessment and several biological assessment of premature ageing. However, these tests are less suitable for age determination of individual patients. Recently, DNA methylation has emerged as a novel test to measure cellular age. In the present study, we assessed ageing in a cohort of 26 survivors of allogeneic HCT by frailty tests comprising the handgrip and 6 min walk tests and by biological tests including DNA methylation, telomere length and expression of p16INK(4A) and serum levels of IL-6. DNA methylation was evaluated both in blood and buccal epithelial cells. Physiological reserve was markedly reduced in transplant survivors, reflected by 6 min walk test. Increased IL-6 serum levels and p16(ink4A) correlated with accelerated ageing. Overall, the measured age of donor blood cells was significantly higher than these blood cells residing in their respective donors, as reflected by DNA methylation and by buccal epithelium methylation status. These clinical and biological observations suggest that allogeneic HCT is associated with accelerated ageing.

Vicidomini, R., et al. (2017). "Drosophila dyskerin is required for somatic stem cell homeostasis." Sci Rep **7**(1): 347.

Drosophila represents an excellent model to dissect the roles played by the evolutionary conserved family of eukaryotic dyskerins. These multifunctional proteins are involved in the formation of H/ACA snoRNP and telomerase complexes, both involved in essential cellular tasks. Since fly telomere integrity is guaranteed by a different mechanism, we used this organism to investigate the specific role played by dyskerin in somatic stem cell maintenance. To this aim, we focussed on Drosophila midgut, a hierarchically organized and well characterized model for stemness analysis. Surprisingly, the ubiquitous loss of the protein uniquely affects the formation of the larval stem cell

niches, without altering other midgut cell types. The number of adult midgut precursor stem cells is dramatically reduced, and this effect is not caused by premature differentiation and is cell-autonomous. Moreover, a few dispersed precursors found in the depleted midguts can maintain stem identity and the ability to divide asymmetrically, nor show cell-growth defects or undergo apoptosis. Instead, their loss is mainly specifically dependent on defective amplification. These studies establish a strict link between dyskerin and somatic stem cell maintenance in a telomerase-lacking organism, indicating that loss of stemness can be regarded as a conserved, telomerase-independent effect of dyskerin dysfunction.

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

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

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

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

Wang, H., et al. (2016). "Ultrasensitive detection of telomerase activity in a single cell using stem-loop primer-mediated exponential amplification (SPEA) with near zero nonspecific signal." Chem Sci **7**(8): 4945-4950.

Telomerase is a crucial biomarker for cancers. Its reliable and sensitive detection, particularly in a single cell, has great significance for the early diagnosis of cancers, studies on tumor progression and anticancer therapy, which remain a challenge, due to nonspecific amplification. Herein, we developed a novel stem-loop primer-mediated exponential amplification (SPEA) strategy, which can specifically and efficiently amplify the telomerase-elongated telomere repeat unit with near zero nonspecific signal. The SPEA-based assay can accurately detect telomerase activity in the crude lysate of a single cell and is suited for detecting the cellular heterogeneity arising from cell-to-cell variations.

Wang, J., et al. (2011). "Immunoaging induced by hematopoietic stem cell aging." Curr Opin Immunol **23**(4): 532-536.

Hematopoietic stem cells (HSCs) generate all known hematopoietic lineages and self-renew, but the function of HSCs declines during aging, which is characterized as impairments of lymphopoiesis and enhanced myelopoiesis. These aging-associated changes correlate with an increasing risk of myelo-proliferative diseases and impairments in immune function. Recent studies showed that only a subpopulation of HSCs contains a high capacity to undergo lymphoid differentiation but this subpopulation is lost during aging, which contributes to age-related impairments in lymphopoiesis. On the basis of the observations that DNA damage and telomere dysfunction can impair stem cell function, it is possible that accumulation of DNA damage results in the loss of subpopulations of HSCs that are required for the maintenance of lymphopoiesis and immune functions during aging.

Wang, J., et al. (2012). "A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage." Cell **148**(5): 1001-1014.

Checkpoints that limit stem cell self-renewal in response to DNA damage can contribute to cancer protection but may also promote tissue aging. Molecular components that control stem cell responses to DNA damage remain to be delineated. Using in vivo RNAi screens, we identified basic leucine zipper transcription factor, ATF-like (BATF) as a major component limiting self-renewal of hematopoietic stem cells (HSCs) in response to telomere dysfunction and gamma-irradiation. DNA damage induces BATF in a G-CSF/STAT3-dependent manner resulting in lymphoid differentiation of HSCs. BATF deletion improves HSC self-renewal and function in response to gamma-irradiation or telomere shortening but results in accumulation of DNA damage in HSCs. Analysis of bone marrow from patients with myelodysplastic syndrome supports the conclusion that DNA damage-dependent induction of BATF is conserved in human HSCs. Together, these results provide experimental evidence that a BATF-dependent differentiation checkpoint limits self-renewal of HSCs in response to DNA damage.

Wang, L., et al. (2014). "The role of telomeres and telomerase in hematologic malignancies and hematopoietic stem cell transplantation." J Hematol Oncol **7**: 61.

Telomeres are specific nucleoprotein structures at the ends of eukaryotic chromosomes. Telomeres and telomere-associated proteins maintain genome stability by protecting the ends of chromosomes from fusion and degradation. In normal somatic cells, the length of the telomeres gradually becomes shortened with cell division. In tumor cells, the shortening of telomeres length is accelerated under the increased proliferation pressure. However, it will be maintained at an extremely short length as the result of activation of telomerase. Significantly shortened telomeres, activation of telomerase, and altered expression of telomere-associated proteins are common features of various hematologic malignancies and are related with progression or chemotherapy resistance in these diseases. In patients who have received hematopoietic stem cell transplantation (HSCT), the telomere length and the telomerase activity of the engrafted donor cells have a significant influence on HSCT outcomes. Transplantation-related factors should be taken into consideration because of their impacts on telomere homeostasis. As activation of telomerase is widespread in tumor cells, it has been employed as a target point in the treatment of neoplastic hematologic disorders. In this review, the characteristics and roles of telomeres and telomerase both in hematologic malignancies and in HSCT will be summarized. The current status of telomerase-targeted therapies utilized in the treatment of hematologic malignancies will also be reviewed.

Wu, Y., et al. (2018). "Whole-Transcriptome Analysis of CD133+CD144+ Cancer Stem Cells Derived from Human Laryngeal Squamous Cell Carcinoma Cells." Cell Physiol Biochem **47**(4): 1696-1710.

BACKGROUND/AIMS: CD133+CD44+ cancer stem cells previously isolated from laryngeal squamous cell carcinoma (LSCC) cell lines showed strong malignancy and tumorigenicity. However, the molecular mechanism underlying the enhanced malignancy remained unclear. METHODS: Cell proliferation assay, spheroid-formation experiment, RNA sequencing (RNA-seq), miRNA-seq, bioinformatic analysis, quantitative real-time PCR, migration assay, invasion assay, and luciferase reporter assay were used to identify differentially expressed mRNAs, lncRNAs, circRNAs and miRNAs, construct transcription regulatory network, and investigate functional roles and mechanism of circRNA in CD133+CD44+ laryngeal cancer stem cells. RESULTS: Differentially expressed genes in TDP cells were mainly enriched in the biological processes of cell differentiation, regulation of autophagy, negative regulation of cell death, regulation of cell growth, response to hypoxia, telomere maintenance, cellular response to gamma radiation, and regulation of apoptotic signaling, which are closely related to the malignant features of tumor cells. We constructed the regulatory network of differentially expressed circRNAs, miRNAs and mRNAs. qPCR findings for the expression of key genes in the network were consistent with the sequencing data. Moreover, our data revealed that circRNA hg19_circ_0005033 promotes proliferation, migration, invasion, and chemotherapy resistance of laryngeal cancer stem cells. CONCLUSIONS: This study provides potential biomarkers and targets for LSCC diagnosis and therapy, and provide important evidences for the heterogeneity of LSCC cells at the transcription level.

Xu, X., et al. (2018). "Telomeric noncoding RNA promotes mouse embryonic stem cell self-renewal through inhibition of TCF3 activity." Am J Physiol Cell Physiol **314**(6): C712-C720.

Although long noncoding RNAs (lncRNAs) are emerging as new modulators in the fate decision of pluripotent stem cells, the functions of specific lncRNAs remain unclear. Here, we found that telomeric RNA (TERRA or TelRNA), one type of lncRNAs, is highly expressed in mouse embryonic stem cells (mESCs) but declines significantly upon differentiation. TERRA is induced by the Wnt/beta-catenin signaling pathway and can reproduce its self-renewal-promoting effect when overexpressed. Further studies revealed that T cell factor 3 (TCF3) is a potential downstream target of TERRA and mediates the effect of TERRA in mESC maintenance. TERRA inhibits TCF3 transcription, while enforced TCF3

expression abrogates the undifferentiated state of mESCs supported by TERRA. Accordingly, the transcripts of the pluripotency genes Esrrb, Tfcp2l1, and Klf2, repressed by TCF3 in mESCs, are increased in TERRA-overexpressing cells. Our study therefore highlights the important role of TERRA in mESC maintenance and also uncovers a mechanism by which TERRA promotes self-renewal. These data will expand our understanding of the pluripotent regulatory network of ESCs.

Yadav, R. K., et al. (2014). "A high-resolution gene expression map of the Arabidopsis shoot meristem stem cell niche." Development **141**(13): 2735-2744.

The shoot apical meristem (SAM) acts as a reservoir for stem cells. The central zone (CZ) harbors stem cells. The stem cell progenitors differentiate in the adjacent peripheral zone and in the rib meristem located just beneath the CZ. The SAM is further divided into distinct clonal layers: the L1 epidermal, L2 sub-epidermal and L3 layers. Collectively, SAMs are complex structures that consist of cells of different clonal origins that are organized into functional domains. By employing fluorescence-activated cell sorting, we have generated gene expression profiles of ten cell populations that belong to different clonal layers as well as domains along the central and peripheral axis. Our work reveals that cells in distinct clonal layers exhibit greater diversity in gene expression and greater transcriptional complexity than clonally related cell types in the central and peripheral axis. Assessment of molecular functions and biological processes reveals that epidermal cells express genes involved in pathogen defense: the L2 layer cells express genes involved in DNA repair pathways and telomere maintenance, and the L3 layers express transcripts involved in ion balance and salt tolerance besides photosynthesis. Strikingly, the stem cell-enriched transcriptome comprises very few hormone-responsive transcripts. In addition to providing insights into the expression profiles of hundreds of transcripts, the data presented here will act as a resource for reverse genetic analysis and will be useful in deciphering molecular pathways involved in cell type specification and their functions.

Yakushev, E. Y., et al. (2013). "Multifunctionality of PIWI proteins in control of germline stem cell fate." Biochemistry (Mosc) **78**(6): 585-591.

PIWI proteins interacting with specific type of small RNAs (piRNAs) repress transposable elements in animals. Besides, they have been shown to participate in various cellular processes: in the regulation of heterochromatin formation including telomere structures, in the control of translation and the cell cycle, and in DNA rearrangements. PIWI proteins were first identified by their roles in the self-renewal of germline stem cells. PIWI protein functions are not limited to gonadogenesis, but the role in determining the fate of stem cells is their specific feature conserved throughout the evolution of animals. Molecular mechanisms underlying these processes are far from being understood. This review focuses on the role of PIWI proteins in the control of maintenance and proliferation of germinal stem cells and its relation to the known function of PIWI in transposon repression.

Ye, F., et al. (2013). "Protective properties of radio-chemoresistant glioblastoma stem cell clones are associated with metabolic adaptation to reduced glucose dependence." PLoS One **8**(11): e80397.

Glioblastoma stem cells (GSC) are a significant cell model for explaining brain tumor recurrence. However, mechanisms underlying their radiochemoresistance remain obscure. Here we show that most clonogenic cells in GSC cultures are sensitive to radiation treatment (RT) with or without temozolomide (TMZ). Only a few single cells survive treatment and regain their self-repopulating capacity. Cells re-populated from treatment-resistant GSC clones contain more clonogenic cells compared to those grown from treatment-sensitive GSC clones, and repeated treatment cycles rapidly enriched clonogenic survival. When compared to sensitive clones, resistant clones exhibited slower tumor development in animals. Upregulated genes identified in resistant clones via comparative expression microarray analysis characterized cells under metabolic stress, including blocked glucose uptake, impaired insulin/Akt signaling, enhanced lipid catabolism and oxidative stress, and suppressed growth and inflammation. Moreover, many upregulated genes highlighted maintenance and repair activities, including detoxifying lipid peroxidation products, activating lysosomal autophagy/ubiquitin-proteasome pathways, and enhancing telomere maintenance and DNA repair, closely resembling the anti-aging effects of caloric/glucose restriction (CR/GR), a nutritional intervention that is known to increase lifespan and stress resistance in model organisms. Although treatment-introduced genetic mutations were detected in resistant clones, all resistant and sensitive clones were subclassified to either proneural (PN) or mesenchymal (MES) glioblastoma subtype based on their expression profiles. Functional assays demonstrated the association of treatment resistance with energy stress, including reduced glucose uptake, fatty acid oxidation (FAO)-dependent ATP maintenance, elevated reactive oxygen species (ROS) production and autophagic activity, and increased AMPK activity and NAD(+) levels accompanied by upregulated mRNA levels of SIRT1/PGC-1alpha axis and DNA repair genes. These data support the view that treatment resistance may arise from quiescent GSC exhibiting a GR-like phenotype, and suggest that

targeting stress response pathways of resistant GSC may provide a novel strategy in combination with standard treatment for glioblastoma.

Yoon, S. M., et al. (2011). "Epithelial cell adhesion molecule (EpCAM) marks hepatocytes newly derived from stem/progenitor cells in humans." Hepatology **53**(3): 964-973.

UNLABELLED: Epithelial cell adhesion molecule (EpCAM) is a surface marker on human hepatic stem/progenitor cells that is reported as absent on mature hepatocytes. However, it has also been noted that in cirrhotic livers of diverse causes, many hepatocytes have EpCAM surface expression; this may represent aberrant EpCAM expression in injured hepatocytes or, as we now hypothesize, persistence of EpCAM in hepatocytes that have recently derived from hepatobiliary progenitors. To evaluate this concept, we investigated patterns of EpCAM expression in hepatobiliary cell compartments of liver biopsy specimens from patients with all stages of chronic hepatitis B and C, studying proliferation, senescence and telomere lengths. We found that EpCAM(+) hepatocytes were rare in early stages of disease, became increasingly prominent in later stages in parallel with the emergence of ductular reactions, and were consistently arrayed around the periphery of cords of keratin 19(+) hepatobiliary cells of the ductular reaction, with which they shared EpCAM expression. Proliferating cell nuclear antigen (proliferation marker) and p21 (senescence marker) were both higher in hepatocytes in cirrhosis than in normal livers, but ductular reaction hepatobiliary cells had the highest proliferation rate, in keeping with being stem/progenitor cell-derived transit amplifying cells. Telomere lengths in EpCAM(+) hepatocytes in cirrhosis were higher than EpCAM(-) hepatocytes ($P < 0.046$), and relatively shorter than those in the corresponding ductular reaction hepatobiliary cells ($P = 0.057$). CONCLUSION: These morphologic, topographic, immunophenotypic, and molecular data support the concept that EpCAM(+) hepatocytes in chronic viral hepatitis are recent progeny of the hepatobiliary stem/progenitor cell compartment through intermediates of the transit amplifying, ductular reaction hepatobiliary cells.

Yu, Q., et al. (2015). "DNA-damage-induced type I interferon promotes senescence and inhibits stem cell function." Cell Rep **11**(5): 785-797.

Expression of type I interferons (IFNs) can be induced by DNA-damaging agents, but the mechanisms and significance of this regulation are not completely understood. We found that the transcription factor IRF3, activated in an ATM-IKKalpha/beta-dependent manner, stimulates cell-autonomous IFN-beta expression in response to double-stranded DNA breaks. Cells and tissues with accumulating DNA damage produce endogenous IFN-beta and stimulate IFN signaling in vitro and in vivo. In turn, IFN acts to amplify DNA-damage responses, activate the p53 pathway, promote senescence, and inhibit stem cell function in response to telomere shortening. Inactivation of the IFN pathway abrogates the development of diverse progeric phenotypes and extends the lifespan of Terc knockout mice. These data identify DNA-damage-response-induced IFN signaling as a critical mechanism that links accumulating DNA damage with senescence and premature aging.

Yuge, L., et al. (2006). "Microgravity potentiates stem cell proliferation while sustaining the capability of differentiation." Stem Cells Dev **15**(6): 921-929.

A three-dimensional (3D) clinostat is a device for generating multidirectional G force, resulting in an environment with an average of 10(3) G. Here we report that human mesenchymal stem cells (hMSCs) cultured in a 3D-clinostat (group CL) showed marked proliferation (13-fold in a week) compared with cells cultured under normal conditions of 1 G (group C) (4-fold in a week). Flow cytometry revealed a 6-fold increase in the number of hMSCs double-positive for CD44/CD29 or CD90/CD29 in group CL after 7 days in culture, compared with group C. Telomere length remained the same in cells from both groups during culturing. Group C cells showed increasing expression levels of type II collagen and aggrecan over the culture period, whereas group CL cells showed a decrease to undetectable levels. Pellets of hMSCs from each group were explanted into cartilagedefective mice. The transplants from group CL formed hyaline cartilage after 7 days, whereas the transplants from group C formed only noncartilage tissue containing a small number of cells. These results show that hMSCs cultured in a 3D-clinostat possess the strong proliferative characteristic of stem cells and retain their ability to differentiate into hyaline cartilage after transplantation. On the contrary, cells cultured in a 1-G environment do not maintain these features. Simulated microgravity may thus provide an environment to successfully expand stem cell populations in vitro without culture supplements that can adversely affect stem cell-derived transplantations. This method has significant potential for regenerative medicine and developmental biology.

Zeng, X. and M. S. Rao (2007). "Human embryonic stem cells: long term stability, absence of senescence and a potential cell source for neural replacement." Neuroscience **145**(4): 1348-1358.

Unlike normal somatic cells, human embryonic stem cells (hESCs) can proliferate indefinitely in culture in an undifferentiated state where they do not appear to undergo senescence and yet remain nontransformed. Cells maintain their pluripotency both in vivo and in vitro, exhibit high

telomerase activity, and maintain telomere length after prolonged in vitro culture. Thus, hESCs may provide an unlimited cell source for replacement in a number of aging-related neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease as well as other neurological disorders including spinal cord injuries. The ability of hESCs to bypass senescence is lost as hESCs differentiate into fully differentiated somatic cells. Evidence has been accumulated that differences in telomere length, telomerase activity, cell cycle signaling, DNA repair ability, as well as the lack of genomic, mitochondrial and epigenetic changes, may contribute to the lack of senescence in hESC. In this manuscript, we will review recent advances in characterizing hESCs and monitoring changes in these aspects in prolonged cultures. We will focus on the potential roles of several cellular pathways including the telomerase, p53 and the Rb pathways in escaping senescence in hESCs. We will also discuss the genomic and epigenetic changes in long-term hESC culture and their potential roles in bypassing senescence.

Zhang, D., et al. (2020). "Autophagy inhibits the mesenchymal stem cell aging induced by D-galactose through ROS/JNK/p38 signalling." Clin Exp Pharmacol Physiol **47**(3): 466-477.

Autophagy and cellular senescence are two critical responses of mammalian cells to stress and may have a direct relationship given that they respond to the same set of stimuli, including oxidative stress, DNA damage, and telomere shortening. Mesenchymal stem cells (MSCs) have emerged as reliable cell sources for stem cell transplantation and are currently being tested in numerous clinical trials. However, the effects of autophagy on MSC senescence and corresponding mechanisms have not been fully evaluated. Several studies demonstrated that autophagy level increases in aging MSCs and the downregulation of autophagy can delay MSC senescence, which is inconsistent with most studies that showed autophagy could play a protective role in stem cell senescence. To further study the relationship between autophagy and MSC senescence and explore the effects and mechanisms of premodulated autophagy on MSC senescence, we induced the up- or down-regulation of autophagy by using rapamycin (Rapa) or 3-methyladenine, respectively, before MSC senescence induced by D-galactose (D-gal). Results showed that pretreatment with Rapa for 24 hours remarkably alleviated MSC aging induced by D-gal and inhibited ROS generation. p-Jun N-terminal kinases (JNK) and p-38 expression were also clearly decreased in the Rapa group. Moreover, the protective effect of Rapa on MSC senescence can be abolished by enhancing the level of ROS, and p38 inhibitor can reverse the promoting effect of H2 O2 on MSC senescence. In summary, the present study indicates that autophagy plays a protective role in MSC senescence induced by D-gal, and ROS/JNK/p38 signalling plays an important mediating role in autophagy-delaying MSC senescence.

Zhao, Z., et al. (2005). "Establishment and properties of fetal dermis-derived mesenchymal stem cell lines: plasticity in vitro and hematopoietic protection in vivo." Bone Marrow Transplant **36**(4): 355-365.

Human mesenchymal stem cells (hMSCs) are excellent candidates for ex vivo gene transfer and cell therapy in various systems. However, hMSCs are mortal somatic cells, and thus invariably enter an irreversible growth arrest after a finite number of cell divisions in culture. It has been proposed that this is due to telomere shortening. In this study, pGRN145 plasmid containing human telomerase reverse transcriptase (hTRT) was introduced into fetal dermis-derived hMSCs. Single-cell clones positive for telomerase activity and hTRT mRNA were selected and expanded. Single-cell-derived hTRT(+) cells could be expanded rapidly in vitro and passaged up to 70 doublings without showing senescence. FACScan flow cytometer showed that hTRT(+) cells were positive for CD29, CD44, CD105, and CD166, while CD31, CD45, CD34, vWF, and HLA-DR were negative. Under suitable conditions, hTRT(+) cells have the ability of multiple lineage differentiation, including bone, fat, and nerve. Furthermore, transplantation of hTRT(+) cells could protect NOD/SCID mice from lethal irradiation. Thus, these cells may be an ideal cell source for promoting hematopoietic recovery after radiotherapy.

Zheng, Q., et al. (2018). "Inflammatory factor receptor Toll-like receptor 4 controls telomeres through heterochromatin protein 1 isoforms in liver cancer stem cell." J Cell Mol Med **22**(6): 3246-3258.

Toll-like receptor 4 (TLR4) which acts as a receptor for lipopolysaccharide (LPS) has been reported to be involved in carcinogenesis. However, the regulatory mechanism of it has not been elucidated. Herein, we demonstrate that TLR4 promotes the malignant growth of liver cancer stem cells. Mechanistically, TLR4 promotes the expression of histone-lysine N-methyltransferase (SUV39 h2) and increases the formation of trimethyl histone H3 lysine 9-heterochromatin protein 1-telomere repeat binding factor 2 (H3K9me3-HP1-TRF2) complex at the telomeric locus under mediation by long non coding RNA urothelial cancer-associated 1 (CUDR). At the telomeric locus, this complex promotes binding of POT1, pPOT1, Exo1, pExo1, SNM1B and pSNM1B but prevents binding of CST/AAF to telomere, thus controlling telomere and maintaining telomere length. Furthermore, TLR4 enhances interaction between HP1alpha and DNA methyltransferase (DNMT3b), which limits RNA polymerase II deposition on the telomeric repeat-containing RNA (TERRA) promoter

region and its elongation, thus inhibiting transcription of TERRA. Ultimately, TLR4 enhances the telomerase activity by reducing the interplay between telomerase reverse transcriptase catalytic subunit (TERT) and TERRA. More importantly, our results reveal that tri-complexes of HP1 isoforms (alpha, beta and gamma) are required for the oncogenic action of TLR4. This study elucidates a novel protection mechanism of TLR4 in liver cancer stem cells and suggests that TLR4 can be used as a novel therapeutic target for liver cancer.

Zhou, Y., et al. (2011). "Establishment of an aging model of Sca-1+ hematopoietic stem cell and studies on its relative biological mechanisms." In Vitro Cell Dev Biol Anim **47**(2): 149-156.

The objective of this study is to establish the aging model of murine hematopoietic stem cell (HSC) ex vitro and investigate its relative biological mechanism, aimed to build the foundation for searching the methods to delaying HSC aging. Sca-1(+)HSC were isolated and purified from murine bone marrow mono-nucleated cell by magnetic-activated cell sorting. The purity of separated cells was analyzed by flow cytometry (FCM) and the expression of Sca-1 was detected by immunofluorescence. Sca-1(+)HSC induced aging by tert-butylhydroperoxide (t-BHP, final concentration of 100 mumol/L) for 6 h to establish the murine HSC aging model in vitro. Biological characteristics of aging HSC were evaluated by mixed hematopoietic progenitor cell culture in vitro, cell cycle assay and senescence-associated beta-galactosidase (SA-beta-gal) cytochemical staining. Telomere length and telomerase activity were detected by southern blotting and telomere repeat amplification protocol-polymerase chain reaction (TRAP-PCR) augmentation. The expressions of p16(INK4a), P19(Arf), P53, P21(Cip1/Waf1) mRNA were detected by reverse transcription (RT)-PCR. The purity of separated Sca-1(+) HSC was 87.2% and the survival of Sca-1(+) HSC was 96~99%. After 6 h cocultured with 100 mumol/L t-BHP, the ability of aging Sca-1(+) HSC to form mixed hematopoietic progenitor colony, self-renewal and multi-differentiation were decreased significantly. The number of aging Sca-1(+) HSC entered G1 phase of the cell cycle and the percentage of positive cells expressed SA-beta-gal increased significantly. The telomere length shortened and the telomerase activity decreased. The expression of p16(INK4a), p19(Arf), p53, P21(Cip1/Waf1) mRNA increased. The t-BHP can induce Sca-1(+) HSC senescence in vitro. The signal transduction pathways of p16(INK4a)-retinoblastoma and P19(Arf)-Mdm2-P53-P21(Cip1/Waf1) may play key roles in the Sca-1(+) HSC senescence induced by t-BHP.

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