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Stem Cell

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Nanog and Stem Cell Research Literatures

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

Introduction

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

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

Arufe, M. C., et al. (2010). "Chondrogenic potential of subpopulations of cells expressing mesenchymal stem cell markers derived from human synovial membranes." J Cell Biochem **111**(4): 834-845.

In this study we analyzed the chondrogenic potential of subpopulations of mesenchymal stem cells (MSCs) derived from human synovial membranes enriched for CD73, CD106, and CD271 markers. Subpopulations of human synovial membrane MSCs enriched for CD73, CD106, and CD271 markers were isolated using a cytometry sorter and characterized by flow cytometry for MSC markers. The expression of Sox9, Nanog, and Runx2 genes by these cells was measured by reverse transcriptase-polymerase chain reaction. The chondrogenesis of each subpopulation was assessed by culturing the cells in a defined medium to produce spontaneous spheroid formation and differentiation towards chondrocyte-like cells. The examination of the spheroids by histological and immunohistochemical analyses for collagen type II (COL2), aggrecan, collagen type I (COL1), metalloprotease 13 (MMP13), and collagen type X (COLX) levels were performed to assess their chondrogenesis capacity. The adipogenesis and osteogenesis potential of each subpopulation was determined using commercial media; the resulting cells were stained with oil red O or red alizarin to test the degree of differentiation. The subpopulations had different profiles of cells positive for the MSC markers CD44, CD69, CD73, CD90, and CD105 and showed different expression levels of the genes Sox9, Nanog, and Runx2 involved in chondrogenesis, undifferentiation, and osteoblastogenesis, respectively. Immunohistochemical analysis demonstrated that COL1, COL2, COLX, MMP13, and aggrecan were expressed in the spheroids as soon as 14 days of culture. The CD271(+) subpopulation expressed the highest levels of COL2 staining compared to the other subpopulations. CD105 and Runx2 were shown by immunohistochemistry and genetic analysis to have significantly higher expression CD271(+) subpopulation than the other subpopulations. Spheroids formed from CD271-enriched and CD73-enriched MSCs from normal human synovial membranes mimic the native cartilage extracellular matrix more closely than CD106(+) MSCs and are

possible candidates for use in cartilage tissue engineering. Both cell types have potential for promoting the differentiation of MSCs into chondrocytes, presenting new possibilities for achieving intrinsic cartilage repair.

Asumda, F. Z. and P. B. Chase (2011). "Age-related changes in rat bone-marrow mesenchymal stem cell plasticity." BMC Cell Biol **12**: 44.

BACKGROUND: The efficacy of adult stem cells is known to be compromised as a function of age. This therefore raises questions about the effectiveness of autologous cell therapy in elderly patients. RESULTS: We demonstrated that the expression profile of stemness markers was altered in BM-MSCs derived from old rats. BM-MSCs from young rats (4 months) expressed Oct-4, Sox-2 and NANOG, but we failed to detect Sox-2 and NANOG in BM-MSCs from older animals (15 months). Chondrogenic, osteogenic and adipogenic potential is compromised in old BM-MSCs. Stimulation with a cocktail mixture of bone morphogenetic protein (BMP-2), fibroblast growth factor (FGF-2) and insulin-like growth factor (IGF-1) induced cardiomyogenesis in young BM-MSCs but not old BM-MSCs. Significant differences in the expression of gap junction protein connexin-43 were observed between young and old BM-MSCs. Young and old BM-MSCs fused with neonatal ventricular cardiomyocytes in co-culture and expressed key cardiac transcription factors and structural proteins. Cells from old animals expressed significantly lower levels of VEGF, IGF, EGF, and G-CSF. Significantly higher levels of DNA double strand break marker gamma-H2AX and diminished levels of telomerase activity were observed in old BM-MSCs. CONCLUSION: The results suggest age related differences in the differentiation capacity of BM-MSCs. These changes may affect the efficacy of BM-MSCs for use in stem cell therapy.

Balbous, A., et al. (2014). "A mesenchymal glioma stem cell profile is related to clinical outcome." Oncogenesis **3**: e91.

Recent studies have demonstrated a relationship between the expression of stem cell-associated genes and relapses in glioblastoma (GBM), suggesting a key role for tumor stem cells in this process. Although there is increasing interest in this field, glioma stem cells (GSCs) are still poorly characterized, their 'stemness' state and factors maintaining these properties remain largely unknown. We performed an expression profiling analysis of pluripotency in gliomaspheres derived from 11 patients. Comparative analysis between GSCs and H1 and H9 human embryonic stem cells as well as H9-derived neural stem cells indicates major variations in gene expression of pluripotency factors Nanog and OCT4, but a stable pattern for SOX2 suggesting its important function in maintaining pluripotency in GSCs. Our results also showed that all GSC lines have the capacity to commit to neural differentiation and express mesenchymal or endothelial differentiation markers. In addition, hierarchical clustering analysis revealed two groups of GSCs reflecting their heterogeneity and identified COL1A1 and IFITM1 as the most discriminating genes. Similar patterns have been observed in tumors from which gliomaspheres have been established. To determine whether this heterogeneity could be clinically relevant, the expression of both genes was further analyzed in an independent cohort of 30 patients with GBM and revealed strong correlation with overall survival. In vitro silencing of COL1A1 and IFTM1 confirmed the effect of these mesenchymal-associated genes on cell invasion and gliomasphere initiation. Our results indicate that COL1A1 and IFITM1 genes could be considered for use in stratifying patients with GBM into subgroups for risk of recurrence at diagnosis, as well as for prognostic and therapeutic evolution.

Ball, S. G., et al. (2012). "Inhibition of platelet-derived growth factor receptor signaling regulates Oct4 and Nanog expression, cell shape, and mesenchymal stem cell potency." Stem Cells **30**(3): 548-560.

Defining the signaling mechanisms that regulate the fate of adult stem cells is an essential step toward their use in regenerative medicine. Platelet-derived growth factor receptor (PDGFR) signaling plays a crucial role in specifying mesenchymal stem cell (MSC) commitment to mesenchymal lineages. Based on the hypothesis that selective inhibition of signaling pathways involved in differentiation may increase stem cell potency, we examined the role of PDGFR signaling in controlling the fate of human MSCs. Using a small molecular PDGFR inhibitor that induced MSCs toward a more rounded shape, expression of Oct4 and Nanog were markedly upregulated. In these PDGFR inhibitor-treated MSCs, Oct4 and Nanog expression and cell shape were regulated by janus kinase (JAK), MAPK kinase (MEK), and epidermal growth factor receptor (EGFR) signaling. Under defined differentiation conditions, these PDGFR-inhibited MSCs expressed definitive endodermal, ectodermal, and mesodermal markers. We also confirmed that depletion of individual PDGF receptors upregulated expression of Oct4A and Nanog. This study identifies PDGFR signaling as a key regulator of Oct4 and Nanog expression and of MSC potency. Thus, inhibiting these specific receptor tyrosine kinases, which play essential roles in tissue formation,

offers a novel approach to unlock the therapeutic capacity of MSCs.

Boozarpour, S., et al. (2016). "Glial cell derived neurotrophic factor induces spermatogonial stem cell marker genes in chicken mesenchymal stem cells." Tissue Cell **48**(3): 235-241.

Mesenchymal stem cells (MSCs) are known with the potential of multi-lineage differentiation. Advances in differentiation technology have also resulted in the conversion of MSCs to other kinds of stem cells. MSCs are considered as a suitable source of cells for biotechnology purposes because they are abundant, easily accessible and well characterized cells. Nowadays small molecules are introduced as novel and efficient factors to differentiate stem cells. In this work, we examined the potential of glial cell derived neurotrophic factor (GDNF) for differentiating chicken MSCs toward spermatogonial stem cells. MSCs were isolated and characterized from chicken and cultured under treatment with all-trans retinoic acid (RA) or glial cell derived neurotrophic factor. Expression analysis of specific genes after 7days of RA treatment, as examined by RT-PCR, proved positive for some germ cell markers such as CVH, STRA8, PLZF and some genes involved in spermatogonial stem cell maintenance like BCL6b and c-KIT. On the other hand, GDNF could additionally induce expression of POU5F1, and NANOG as well as other genes which were induced after RA treatment. These data illustrated that GDNF is relatively more effective in diverting chicken MSCs towards Spermatogonial stem cell -like cells in chickens and suggests GDNF as a new agent to obtain transgenic poultry, nevertheless, exploitability of these cells should be verified by more experiments.

Chen, C., et al. (2011). "Evidence for epithelial-mesenchymal transition in cancer stem cells of head and neck squamous cell carcinoma." PLoS One **6**(1): e16466.

Initiation, growth, recurrence, and metastasis of head and neck squamous cell carcinomas (HNSCC) have been related to the behavior of cancer stem cells (CSC) that can be identified by their aldehyde-dehydrogenase-isoform-1 (ALDH1) activity. We quantified and enriched ALDH1(+) cells within HNSCC cell lines and subsequently characterized their phenotypical and functional properties like invasion capacity and epithelial-mesenchymal transition (EMT). Spheroid culture enriched CSC from five HNSCC cell lines by up to 5-fold. In spheroid-derived cells (SDC) and the parental monolayer-derived cell line ALDH1, CD44, CD24, E-Cadherin, alpha-SMA, and Vimentin expression was compared by flow-cytometry and immunofluorescence together with proliferation and cell cycle analysis. Invasion activity was evaluated by Matrigel assay and expression of stemness-related transcription factors (TF) Nanog, Oct3/4, Sox2 and EMT-related genes Snail1 and 2, and Twist by real-time PCR. All cell lines formed spheroids that could self-renew and be serially re-passaged. ALDH1 expression was significantly higher in SDC. ALDH1(+) cells showed increased colony-formation. The proportion of cells with a putative CSC marker constellation of CD44(+)/CD24(-) was highly variable (0.5% to 96%) in monolayer and spheroid cultures and overlapped in 0%-33% with the CD44(+)/CD24(-)/ALDH1(+) cell subset. SDC had significantly higher invading activity. mRNA of the stemness-related genes Sox2, Nanog, and Oct3/4 was significantly increased in SDC of all cell lines. Twist was significantly increased in two while Snail2 showed a significant increase in one and a significant decrease in SDC of two cell lines. SDC had a higher G0 phase proportion, showed high-level expression of alpha-SMA and Vimentin, but significantly decreased E-Cadherin expression. HNSCC-lines harbor potential CSC, characterized by ALDH1 and stemness marker TF expression as well as properties like invasiveness, quiescence, and EMT. CSC can be enriched by anchorage-independent culture techniques, which may be important for the investigation of their contribution to therapy resistance, tumor recurrence and metastasis.

Chen, L., et al. (2014). "Mouse induced pluripotent stem cell microenvironment generates epithelial-mesenchymal transition in mouse Lewis lung cancer cells." Am J Cancer Res **4**(1): 80-88.

Induced pluripotent stem (iPS) cells may be a powerful tool in regenerative medicine, but their potential tumorigenicity is a significant challenge for the clinical use of iPS cells. Previously, we succeeded in converting miPS cells into cancer stem cells (CSCs) under the conditions of tumor microenvironment. Both stem cells and tumor cells are profoundly influenced by bi-directional communication with their respective microenvironment, which dictates cell fate determination and behavior. The microenvironment derived from iPS cells has not been well studied. In this paper, we have investigated the effects of secreted factors from Nanog-mouse iPS (miPS) cells on mouse Lewis lung cancer (LLC) cells that are found in the conditioned media. The results demonstrated that miPS cells secrete factors that can convert the epithelia phenotype of LLC cells to a mesenchymal phenotype, and that can promote tumorigenisity, migration and invasion. Furthermore, LLC cells that have been exposed to miPS conditioned medium became resistant to apoptosis. These various biological effects suggest that the miPS microenvironment contain factors that can promote an epithelial-mesenchymal

transition (EMT) through an active Snail-MMP axis or by suppressing differentiation in LLC cells.

Chiou, S. H., et al. (2010). "Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation." Cancer Res **70**(24): 10433-10444.

Epithelial-mesenchymal transition (EMT), a critical process of cancer invasion and metastasis, is associated with stemness property of cancer cells. Though Oct4 and Nanog are homebox transcription factors essential to the self-renewal of stem cells and are expressed in several cancers, the role of Oct4/Nanog signaling in tumorigenesis is still elusive. Here microarray and quantitative real-time PCR analysis showed a parallel, elevated expression of Oct4 and Nanog in lung adenocarcinoma (LAC). Ectopic expressions of Oct4 and Nanog in LACs increased the percentage of CD133-expressing subpopulation and sphere formation, enhanced drug resistance, and promoted EMT. Ectopic expressions of Oct4 and Nanog activated Slug and enhanced the tumor-initiating capability of LAC. Furthermore, double knockdown of Oct4 and Nanog suppressed the expression of Slug, reversed the EMT process, blocked the tumorigenic and metastatic ability, and greatly improved the mean survival time of transplanted immunocompromised mice. The immunohistochemical analysis demonstrated that expressions of Oct4, Nanog, and Slug were present in high-grade LAC, and triple positivity of Oct4/Nanog/Slug indicated a worse prognostic value of LAC patients. Our results support the notion that the Oct4/Nanog signaling controls epithelial-mesenchymal transdifferentiation, regulates tumor-initiating ability, and promotes metastasis of LAC.

Choi, H. Y., et al. (2018). "Generation of a human induced pluripotent stem cell line, KSCBi003-A, from human adipose tissue-derived mesenchymal stem cells using a chromosomal integration-free system." Stem Cell Res **31**: 1-4.

We generated a human induced pluripotent stem cell (hiPSC) line, KSCBi003-A, from adipose tissue-derived mesenchymal stem cells (Ad-MSCs) using a Sendai virus-based gene delivery system. We confirmed that the KSCBi003-A has a normal karyotype and short tandem repeat (STR)-based identities that match the parent cells. We also confirmed that the cell line expresses pluripotent stem cell markers such as Nanog, OCT4, SSEA-4, TRA-1-60, and TRA-1-81. We also analyzed that the KSCBi003-A has an ability to differentiate three germ layers (ectoderm, mesoderm, endoderm). This cell line is registered and available at the National Stem Cell Bank, Korea National Institute of Health.

Dang, H., et al. (2011). "Snail1 induces epithelial-to-mesenchymal transition and tumor initiating stem cell characteristics." BMC Cancer **11**: 396.

BACKGROUND: Tumor initiating stem-like cells (TISCs) are a subset of neoplastic cells that possess distinct survival mechanisms and self-renewal characteristics crucial for tumor maintenance and propagation. The induction of epithelial-mesenchymal-transition (EMT) by TGFbeta has been recently linked to the acquisition of TISC characteristics in breast cancer. In HCC, a TISC and EMT phenotype correlates with a worse prognosis. In this work, our aim is to elucidate the underlying mechanism by which cells acquire tumor initiating characteristics after EMT. METHODS: Gene and protein expression assays and Nanog-promoter luciferase reporter were utilized in epithelial and mesenchymal phenotype liver cancer cell lines. EMT was analyzed with migration/invasion assays. TISC characteristics were analyzed with tumor-sphere self-renewal and chemotherapy resistance assays. In vivo tumor assay was performed to investigate the role of Snail1 in tumor initiation. CONCLUSION: TGFbeta induced EMT in epithelial cells through the up-regulation of Snail1 in Smad-dependent signaling. Mesenchymal liver cancer post-EMT demonstrates TISC characteristics such as tumor-sphere formation but are not resistant to cytotoxic therapy. The inhibition of Snail1 in mesenchymal cells results in decreased Nanog promoter luciferase activity and loss of self-renewal characteristics in vitro. These changes confirm the direct role of Snail1 in some TISC traits. In vivo, the down-regulation of Snail1 reduced tumor growth but was not sufficient to eliminate tumor initiation. In summary, TGFbeta induces EMT and TISC characteristics through Snail1 and Nanog up-regulation. In mesenchymal cells post-EMT, Snail1 directly regulates Nanog expression, and loss of Snail1 regulates tumor growth without affecting tumor initiation.

de Andrade, N. P., et al. (2017). "Cancer stem cell, cytokeratins and epithelial to mesenchymal transition markers expression in oral squamous cell carcinoma derived from ortothopic xenoimplantation of CD44(high) cells." Pathol Res Pract **213**(3): 235-244.

Oral squamous cell carcinoma (OSCC) is the most prevalent neoplasia of oral cavity worldwide and prognosis remains unchanged in decades. Recently, different authors reported that head and neck squamous cell carcinomas have a subpopulation of

tumor initiating cells that apparently correspond to cancer stem cells (CSC) and are also responsible for tumor growth and metastasis. The purpose of the present study was to investigate the microscopic and phenotypic characteristics of OSCC tumors induced after orthotopic xenoimplantation of SCC9(WT) cell line and CSC-enriched subpopulation isolated from SCC9 cell line based on high expression of the putative CSC marker CD44. Different numbers of FACS-sorted SCC9 CD44(high) and CD44(low) cells as well as SCC9(WT) (wild type) were transplanted into the tongue of BALB/C nude (NOD/SCID) mice to evaluate their tumorigenic potential. Sixty days post-induction, tumors were morphologically characterized and immunostained for CSC markers (CD44, Nanog and Bmi-1), epithelial-mesenchymal transition (Snail, Slug) and epithelial differentiating cell markers (cytokeratins 4, 13, 15, 17 and 19), as well as E-cadherin and beta-catenin. The data presented here shows that SCC9 CD44(high) cells have higher ability to form tumors than SCC9 CD44(low) cells, even when significantly lower numbers of SCC9 CD44(high) cells were transplanted. Immunoassessment of tumors derived from SCC9 CD44(high) cells revealed high expression of cytokeratin CK19, beta-catenin, E-cadherin and CD44, and negative or low expression of CK17, CK4, CK15, CK13, Nanog, Bmi-1, Snail and Slug. While tumors derived from SCC9(WT) showed high expression of CK17, CK19, CD44, Nanog, Bmi-1, Snail and Slug, and negative or low expression of CK4, CK15, CK13, beta-catenin and E-cadherin. Thus, SCC9 CD44(high) cells were highly tumorigenic, capable of originating heterogeneous tumors and these tumors have a immunohistochemical profile different from those formed by the wild type cell line.

Ding, Z. and H. Huang (2015). "Mesenchymal stem cells in rabbit meniscus and bone marrow exhibit a similar feature but a heterogeneous multi-differentiation potential: superiority of meniscus as a cell source for meniscus repair." BMC Musculoskelet Disord **16**: 65.

BACKGROUND: The restoration of damaged meniscus has always been a challenge due to its limited healing capacity. Recently, bone marrow-derived mesenchymal stem cells (BMSCs) provide a promising alternative to repair meniscal defects. However, BMSCs are not ideal chondroprogenitor cells for meniscus repair because they have a high propensity for cartilage hypertrophy and bone formation. Our hypothesis is that mesenchymal stem cells (MSCs) reside in meniscus maintain specific traits distinct from others which may be more conducive to meniscus regeneration. METHODS: MSCs were isolated from bone marrow and menisci of the rabbits. The similarities and differences between BMSCs and MMSCs were investigated in vitro by a cell culture model, ex vivo by a rabbit meniscus defect model and in vivo by a nude rat implantation model using histochemistry, immunocytochemistry, qRT-PCR and western blotting. RESULTS: Our data showed that two types of MSCs have universal stem cell characteristics including clonogenicity, multi-potency and self-renewal capacity. They both express stem cell markers including SSEA-4, Nanog, nucleostemin, strol-1, CD44 and CD90. However, MMSCs differed from BMSCs. MMSC colonies were much smaller and grew more slowly than BMSC colonies. Moreover, fewer MMSCs expressed CD34 than BMSCs. Finally, MMSCs always appeared a pronounced tendency to chondrogenic differentiation while BMSCs exhibited significantly greater osteogenic potential, whatever in vitro and in vivo. CONCLUSIONS: This study shows the similarities and differences between MMSCs and BMSCs for the first time. MMSCs are a promising source of mesenchymal stem cells in repairing meniscus defect.

Divya, M. S., et al. (2012). "Umbilical cord blood-derived mesenchymal stem cells consist of a unique population of progenitors co-expressing mesenchymal stem cell and neuronal markers capable of instantaneous neuronal differentiation." Stem Cell Res Ther **3**(6): 57.

INTRODUCTION: Umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) are self-renewing multipotent progenitors with the potential to differentiate into multiple lineages of mesoderm, in addition to generating ectodermal and endodermal lineages by crossing the germline barrier. In the present study we have investigated the ability of UCB-MSCs to generate neurons, since we were able to observe varying degrees of neuronal differentiation from a few batches of UCB-MSCs with very simple neuronal induction protocols whereas other batches required extensive exposure to combination of growth factors in a stepwise protocol. Our hypothesis was therefore that the human UCB-MSCs would contain multiple types of progenitors with varying neurogenic potential and that the ratio of the progenitors with high and low neurogenic potentials varies in different batches of UCB. METHODS: In total we collected 45 UCB samples, nine of which generated MSCs that were further expanded and characterized using immunofluorescence, fluorescence-activated cell sorting and RT-PCR analysis. The neuronal differentiation potential of the UCB-MSCs was analyzed with exposure to combination of growth factors. RESULTS: We could identify two different populations of progenitors within the UCB-MSCs. One population represented progenitors with innate

neurogenic potential that initially express pluripotent stem cell markers such as Oct4, Nanog, Sox2, ABCG2 and neuro-ectodermal marker nestin and are capable of expanding and differentiating into neurons with exposure to simple neuronal induction conditions. The remaining population of cells, typically expressing MSC markers, requires extensive exposure to a combination of growth factors to transdifferentiate into neurons. Interesting to note was that both of these cell populations were positive for CD29 and CD105, indicating their MSC lineage, but showed prominent difference in their neurogenic potential. CONCLUSION: Our results suggest that the expanded UCB-derived MSCs harbor a small unique population of cells that express pluripotent stem cell markers along with MSC markers and possess an inherent neurogenic potential. These pluripotent progenitors later generate cells expressing neural progenitor markers and are responsible for the instantaneous neuronal differentiation; the ratio of these pluripotent marker expressing cells in a batch determines the innate neurogenic potential.

Foja, S., et al. (2013). "Hypoxia supports reprogramming of mesenchymal stromal cells via induction of embryonic stem cell-specific microRNA-302 cluster and pluripotency-associated genes." Cell Reprogram **15**(1): 68-79.

Pluripotency is characterized by specific transcription factors such as OCT4, NANOG, and SOX2, but also by pluripotency-associated microRNAs (miRs). Somatic cells can be reprogrammed by forced expression of these factors leading to induced pluripotent stem cells (iPSCs) with characteristics similar to embryonic stem cells (ESCs). However, current reprogramming strategies are commonly based on viral delivery of the pluripotency-associated factors, which affects the integrity of the genome and impedes the use of such cells in any clinical application. In an effort to establish nonviral, nonintegrating reprogramming strategies, we examined the influence of hypoxia on the expression of pluripotency-associated factors and the ESC-specific miR-302 cluster in primary and immortalized mesenchymal stromal cells (MSCs). The combination of hypoxia and fibroblast growth factor 2 (FGF2) treatments led to the induction of OCT4 and NANOG in an immortalized cell line L87 and primary MSCs, accompanied with increased doubling rates and decreased senescence. Most importantly, the endogenous ECS-specific cluster miR-302 was induced upon hypoxic culture and FGF2 supplementation. Hypoxia also improved reprogramming of MSCs via episomal expression of pluripotency factors. Thus, our data illustrate that hypoxia in combination with FGF2 supplementation efficiently facilitates reprogramming of MSCs.

Galat, V., et al. (2016). "Transgene Reactivation in Induced Pluripotent Stem Cell Derivatives and Reversion to Pluripotency of Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells." Stem Cells Dev **25**(14): 1060-1072.

Induced pluripotent stem cells (iPSCs) have enormous potential in regenerative medicine and disease modeling. It is now felt that clinical trials should be performed with iPSCs derived with nonintegrative constructs. Numerous studies, however, including those describing disease models, are still being published using cells derived from iPSCs generated with integrative constructs. Our experimental work presents the first evidence of spontaneous transgene reactivation in vitro in several cellular types. Our results show that the transgenes were predominantly silent in parent iPSCs, but in mesenchymal and endothelial iPSC derivatives, the transgenes experienced random upregulation of Nanog and c-Myc. Additionally, we provide evidence of spontaneous secondary reprogramming and reversion to pluripotency in mesenchymal stem cells derived from iPSCs. These findings strongly suggest that the studies, which use cellular products derived from iPSCs generated with retro- or lentiviruses, should be evaluated with consideration of the possibility of transgene reactivation. The in vitro model described here provides insight into the earliest events of culture transformation and suggests the hypothesis that reversion to pluripotency may be responsible for the development of tumors in cell replacement experiments. The main goal of this work, however, is to communicate the possibility of transgene reactivation in retro- or lenti-iPSC derivatives and the associated loss of cellular fidelity in vitro, which may impact the outcomes of disease modeling and related experimentation.

Gao, Y., et al. (2014). "All-trans retinoic acid promotes nerve cell differentiation of yolk sac-derived mesenchymal stem cells." Appl Biochem Biotechnol **174**(2): 682-692.

Fetal membranes are abundant; the yolk sac is a source of cell lineages that do not express MHCs and are mainly free from immunological incompatibles when transferred to a recipient. Although data are available especially for hematopoietic stem cells in human and murine; whereas other cell types and species are dramatically unnoticed. Here, we studied the nature and differentiation potential of yolk sac-derived mesenchymal stem cells from a chicken embryo. In this study, we observed the gene expression of pluripotent markers in yolk sac

mesenchymal stem cells (YS-MSCs) and the capacity of YS-MSCs to differentiate into neural-like cells using quantitative RT-PCR, immunocytochemistry, and western blotting. YS-MSCs have a spindle shape and revealed the expression of the MSC-related proteins beta-integrin, CD44, CD71, and CD73, but not CD34. YS-MSCs express pluripotent markers such as octamer-binding transcription factor 4 (Oct4) and Nanog at the protein and mRNA levels. QRT-PCR analyses revealed that YS-MSCs expressed nestin. Immunocytochemical and western blotting data showed that the cells expressed Nestin and microtubule-associated protein 2 (Map-2) for neurons, respectively, after induction of neural differentiation. These findings demonstrate the plasticity of YS-MSCs and their potential for use in cellular replacement therapy for neural diseases.

Ge, X., et al. (2012). "Human amniotic mesenchymal stem cell-derived induced pluripotent stem cells may generate a universal source of cardiac cells." Stem Cells Dev **21**(15): 2798-2808.

Human amniotic mesenchymal stem cells (hAMSCs) demonstrated partially pluripotent characteristics with a strong expression of Oct4 and Nanog genes and immunomodulatory properties characterized by the absence of HLA-DR and the presence of HLA-G and CD59. The hAMSCs were reprogrammed into induced pluripotent stem cells (iPSCs) that generate a promising source of universal cardiac cells. The hAMSC-derived iPSCs (MiPSCs) successfully underwent robust cardiac differentiation to generate cardiomyocytes. This study investigated 3 key properties of the hAMSCs and MiPSCs: (1) the reprogramming efficiency of the partially pluripotent hAMSCs to generate MiPSCs; (2) immunomodulatory properties of the hAMSCs and MiPSCs; and (3) the cardiac differentiation potential of the MiPSCs. The characteristic iPSC colony formation was observed within 10 days after the transduction of the hAMSCs with a single integration polycistronic vector containing 4 Yamanaka factors. Immunohistology and reverse transcription-polymerase chain reaction assays revealed that the MiPSCs expressed stem cell surface markers and pluripotency-specific genes. Furthermore, the hAMSCs and MiPSCs demonstrated immunomodulatory properties enabling successful engraftment in the SVJ mice. Finally, the cardiac differentiation of MiPSCs exhibited robust spontaneous contractility, characteristic calcium transience across the membrane, a high expression of cardiac genes and mature cardiac phenotypes, and a contractile force comparable to cardiomyocytes. Our results demonstrated that the hAMSCs are reprogrammed with a high efficiency into MiPSCs, which possess pluripotent, immunomodulatory, and precardiac properties. The MiPSC-derived cardiac cells express a c-kit cell surface marker, which may be employed to purify the cardiac cell population and enable allogeneic cardiac stem cell therapy.

Ghaem Maghami, R., et al. (2018). "Differentiation of mesenchymal stem cells to germ-like cells under induction of Sertoli cell-conditioned medium and retinoic acid." Andrologia **50**(3).

The aim of this research was to find a way to differentiate germ cells from umbilical cord Wharton's jelly mesenchymal stem cells (MSCs) to support in vitro spermatogenesis. A small piece of Wharton's jelly was cultured in high-glucose Dulbecco's modified Eagle's medium in present of 10% foetal calf serum. After the fourth passage, the cells were isolated and cultured in Sertoli cell-conditioned medium under induction of two different doses of retinoic acid (10(-5), 10(-6) m). The differentiation of MSC to germ-like cells was evaluated by expression of Oct4, Nanog, Plzf, Stra8 and Prm1 genes during different days of culture through qPCR. The results showed that there were downregulation of Oct4 and Nanog and upregulation of pre-meiotic germ cell marker (stra8) and haploid cell marker (Prm1) when MSCs are differentiated over time. The expression of Bax gene (an apoptotic marker) was significantly observed in high dosage of retinoic acid (RA). As a result, RA has positive effects on proliferation and differentiation of MSCs, but its effects are related to dosage. The success of this method can introduce umbilical cord MSC as a source of germ cells for treatment of infertility in future.

Guo, D., et al. (2012). "Cancer stem-like side population cells in the human nasopharyngeal carcinoma cell line cne-2 possess epithelial mesenchymal transition properties in association with metastasis." Oncol Rep **28**(1): 241-247.

It has been recently reported that side population (SP) cells in nasopharyngeal carcinoma (NPC) cell lines display characteristics of cancer stem-like cells. However, the biological behavior and the significance of these cells for NPC progression remain unclear. In this study, we isolated SP cells from the NPC cell line CNE-2 by flow cytometry and investigated their biological characteristics. We discovered that SP cells had stronger colony forming abilities compared to the non-side population (NSP) cells, and observed that some SP cells looked more like the shape of mesenchymal cells when cultured in the common polyHEMA-coated flask. When checked by quantitative real-time PCR, the SP cells expressed higher levels of stemness-related genes Oct4, Sox2 and Nanog, and mesenchymal cell-related genes N-cadherin, vimentin and Snail, while they expressed lower levels of the epithelial cell-related gene, E-

cadherin. Western blot and immunofluorescence staining methods further verified that SP cells expressed higher vimentin and expressed lower E-cadherin levels. Finally, Transwell invasion assay results indicated that the SP cells had higher invasive potential compared to NSP cells. Collectively, our data reveal that SP cells in the CNE-2 cell line not only possess the properties of cancer stem cells, but also have more mesenchymal cell characteristics which are associated with epithelial mesenchymal transition (EMT) and cancer cell invasion and metastasis. These findings are helpful for developing novel targets for effective clinical treatment of NPC.

Han, J., et al. (2012). "Nanog reverses the effects of organismal aging on mesenchymal stem cell proliferation and myogenic differentiation potential." Stem Cells **30**(12): 2746-2759.

Although the therapeutic potential of mesenchymal stem cells (MSCs) is widely accepted, loss of cell function due to donor aging or culture senescence are major limiting factors hampering their clinical application. Our laboratory recently showed that MSCs originating from older donors suffer from limited proliferative capacity and significantly reduced myogenic differentiation potential. This is a major concern, as the patients most likely to suffer from cardiovascular disease are elderly. Here we tested the hypothesis that a single pluripotency-associated transcription factor, namely Nanog, may reverse the proliferation and differentiation potential of bone marrow-derived MSC (BM-MSC) from adult donors. Microarray analysis showed that adult (a)BM-MSC expressing Nanog clustered close to Nanog-expressing neonatal cells. Nanog markedly upregulated genes involved in cell cycle, DNA replication, and DNA damage repair and enhanced the proliferation rate and clonogenic capacity of aBM-MSC. Notably, Nanog reversed the myogenic differentiation potential and restored the contractile function of aBM-MSC to a similar level as that of neonatal (n)BM-MSC. The effect of Nanog on contractility was mediated--at least in part--through activation of the TGF-beta pathway by diffusible factors secreted in the conditioned medium of Nanog-expressing BM-MSC. Overall, our results suggest that Nanog may be used to overcome the effects of organismal aging on aBM-MSC, thereby increasing the potential of MSC from aged donors for cellular therapy and tissue regeneration.

Izumiya, M., et al. (2012). "Chemoresistance is associated with cancer stem cell-like properties and epithelial-to-mesenchymal transition in pancreatic cancer cells." Anticancer Res **32**(9): 3847-3853.

BACKGROUND: The aim of this study was to evaluate whether apoptosis-resistant cancer cells have cancer stem cell (CSC)-like properties. MATERIALS AND METHODS: Panc-1 pancreatic cancer cells were incubated in the presence of 5-fluorouracil (5-FU) for 24 h, and further incubated without 5-FU for 28 days. To assess the capacity of self-renewal, surviving cells were planted for sphere-forming assay. Epithelial-to-mesenchymal transition (EMT) was induced with TGF-beta, then mRNA expression was evaluated by real-time PCR for E-cadherin, SNAIL, and vimentin. The E-Cadherin protein levels were also examined by immunoblot analysis. The Local invasion ability was analyzed by Matrigel invasion assay. RESULTS: The frequency of cells that were capable of initiating spheres was higher in 5-FU-pre treated cells, which also overexpressed stem cell marker genes, OCT4 and NANOG. Matrigel invasion activity of apoptosis-resistant Panc-1 cells was greater than that of control Panc-1 cells. CONCLUSION: Apoptosis-resistant cancer cells have CSC-like properties, i.e., able to initiate sphere formation, express stem cell genes, and respond to EMT stimulation.

Jaramillo-Ferrada, P. A., et al. (2012). "Differential mesengenic potential and expression of stem cell-fate modulators in mesenchymal stromal cells from human-term placenta and bone marrow." J Cell Physiol **227**(9): 3234-3242.

Placenta has attracted increasing attention over the past decade as a stem cell source for regenerative medicine. In particular, the amniochorionic membrane has been shown to harbor populations of mesenchymal stromal cells (MSCs). In this study, we have characterized ex vivo expanded MSCs from the human amniotic (hAMSCs) and chorionic (hCMSCs) membranes of human full-term placentas and adult bone marrow (hBMSCs). Our results show that hAMSCs, hCMSCs, and hBMSCs express typical mesenchymal (CD73, CD90, CD105, CD44, CD146, CD166) and pluripotent (Oct-4, Sox2, Nanog, Lin28, and Klf4) markers but not hematopoietic markers (CD45, CD34). Ex vivo expanded hAMSCs were found to be of fetal origin, while hCMSCs cultures contained only maternal cells. Cell proliferation was significantly higher in hCMSCs, compared to hAMSCs and hBMSCs. Integrin profiling revealed marked differences in the expression of alpha subunits between the three cell sources. Cadherin receptors were consistently expressed on a subset of progenitors (ranging from 1% to 60%), while N-CAM (CD56) was only expressed in hAMSCs and hCMSCs but not in hBMSCs. When induced to differentiate, hAMSCs and hCMSCs displayed strong chondrogenic and osteogenic differentiation potential but very limited capacity for adipogenic conversion. In contrast, hBMSCs showed strong differentiation potential along the three lineages. These results illustrate how MSCs

from different ontological sources display differential expression of cell-fate mediators and mesodermal differentiation capacity.

Kang, E. J., et al. (2010). "In vitro and in vivo osteogenesis of porcine skin-derived mesenchymal stem cell-like cells with a demineralized bone and fibrin glue scaffold." Tissue Eng Part A **16**(3): 815-827.

In vitro and in vivo osteogenesis of skin-derived mesenchymal stem cell-like cells (SDMSCs) with a demineralized bone (DMB) and fibrin glue scaffold were compared. SDMSCs isolated from the ears of adult miniature pigs were evaluated for the expression of transcriptional factors (Oct-4, Sox-2, and Nanog) and MSC marker proteins (CD29, CD44, CD90, and vimentin). The isolated SDMSCs were cocultured in vitro with a mixed DMB and fibrin glue scaffold in a nonosteogenic medium for 1, 2, and 4 weeks. Osteonectin, osteocalcin, and Runx2 were expressed during the culture period and reached maximum at 2 weeks after in vitro coculture. von Kossa-positive bone minerals were also noted in the cocultured medium at 4 weeks. Autogenous porcine SDMSCs (1 x 10(7)) labeled with a tracking dye, PKH26, were grafted into the maxillary sinus with a DMB and fibrin glue scaffold. In the contralateral side, only a scaffold was grafted without SDMSCs (control). In vivo osteogenesis was evaluated from two animals euthanized at 2 and 4 weeks after grafting. In vivo PKH26 staining was detected in all the specimens at both time points. Trabecular bone formation and osteocalcin expression were more pronounced around the grafted materials in the SDMSC-grafted group compared with the control group. New bone generation was initiated from the periphery to the center of the grafted material. The number of proliferating cells increased over time and reached a peak at 4 weeks in both in vivo and in vitro specimens. These findings suggest that autogenous SDMSC grafting with a DMB and fibrin glue scaffold can serve as a predictable alternative to bone grafting in the maxillary sinus floor.

Kermani, A. J., et al. (2008). "Characterization and genetic manipulation of human umbilical cord vein mesenchymal stem cells: potential application in cell-based gene therapy." Rejuvenation Res **11**(2): 379-386.

Stem cells are defined by two main characteristics: self-renewal capacity and commitment to multi-lineage differentiation. The cells have a great therapeutic potential in repopulating damaged tissues as well as being genetically manipulated and used in cell-based gene therapy. Umbilical cord vein is a readily available and inexpensive source of stem cells that are capable of generating various cell types. Despite the recent isolation of human umbilical cord vein mesenchymal stem cells (UVMSC), the self-renewal capacity and the potential clinical application of the cells are not well known. In the present study, we have successfully isolated and cultured human UVMSCs. Our data further revealed that the isolated cells express the self-renewal genes Oct-4, Nanog, ZFX, Bmi-1, and Nucleostemin; but not Zic-3, Hoxb-4, TCL-1, Tbx-3 and Esrrb. In addition, our immunocytochemistry results revealed the expression of SSEA-4, but not SSEA-3, TRA-1-60, and TRA-1-81 embryonic stem cell surface markers in the cells. Also, we were able to transfect the cells with a reporter, enhanced green fluorescent protein (EGFP), and a therapeutic human brain-derived neurotrophic factor (hBDNF) gene by means of electroporation and obtained a stable cell line, which could constantly express both transgenes. The latter data provide further evidence on the usefulness of umbilical cord vein mesenchymal stem cells as a readily available source of stem cells, which could be genetically manipulated and used in cell-based gene therapy applications.

Kim, D. S., et al. (2017). "Cell culture density affects the stemness gene expression of adipose tissue-derived mesenchymal stem cells." Biomed Rep **6**(3): 300-306.

The results of clinical trials using mesenchymal stem cells (MSCs) are controversial due to the heterogeneity of human MSCs and differences in culture conditions. In this regard, it is important to identify gene expression patterns according to culture conditions, and to determine how the cells are expanded and when they should be clinically used. In the current study, stemness gene expression was investigated in adipose tissue-derived MSCs (AT-MSCs) harvested following culture at different densities. AT-MSCs were plated at a density of 200 or 5,000 cells/cm (2). After 7 days of culture, stemness gene expression was examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. The proliferation rate of AT-MSCs harvested at a low density (~50% confluent) was higher than that of AT-MSCs harvested at a high density (~90% confluent). Although there were differences in the expression levels of stemness gene, such as octamer-binding transcription factor 4, nanog homeobox (Nanog), SRY-box 2, Kruppel like factor 4, v-myc avian myelocytomatosis viral oncogene homolog (c-Myc), and lin-28 homolog A, in the AT-MSCs obtained from different donors, RT-qPCR analysis demonstrated differential gene expression patterns according to the cell culture density. Expression levels of stemness genes, particularly Nanog and c-Myc, were upregulated in AT-MSCs

harvested at a low density (~50% confluent) in comparison to AT-MSCs from the same donor harvested at a high density (~90% confluent). These results imply that culture conditions, such as the cell density at harvesting, modulate the stemness gene expression and proliferation of MSCs.

Kim, P. H., et al. (2015). "Stanniocalcin 2 enhances mesenchymal stem cell survival by suppressing oxidative stress." BMB Rep **48**(12): 702-707.

To overcome the disadvantages of stem cell-based cell therapy like low cell survival at the disease site, we used stanniocalcin 2 (STC2), a family of secreted glycoprotein hormones that function to inhibit apoptosis and oxidative damage and to induce proliferation. STC2 gene was transfected into two kinds of stem cells to prolong cell survival and protect the cells from the damage by oxidative stress. The stem cells expressing STC2 exhibited increased cell viability and improved cell survival as well as elevated expression of the pluripotency and self-renewal markers (Oct4 and Nanog) under sub-lethal oxidative conditions. Up-regulation of CDK2 and CDK4 and down-regulation of cell cycle inhibitors p16 and p21 were observed after the delivery of STC2. Furthermore, STC2 transduction activated pAKT and pERK 1/2 signal pathways. Taken together, the STC2 can be used to enhance cell survival and maintain long-term stemness in therapeutic use of stem cells.

Kong, D., et al. (2010). "Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells." PLoS One **5**(8): e12445.

BACKGROUND: Current management of patients diagnosed with prostate cancer (PCa) is very effective; however, tumor recurrence with Castrate Resistant Prostate Cancer (CRPC) and subsequent metastasis lead to poor survival outcome, suggesting that there is a dire need for novel mechanistic understanding of tumor recurrence, which would be critical for designing novel therapies. The recurrence and the metastasis of PCa are tightly linked with the biology of prostate cancer stem cells or cancer-initiating cells that is reminiscent of the acquisition of Epithelial to Mesenchymal Transition (EMT) phenotype. Increasing evidence suggests that EMT-type cells share many biological characteristics with cancer stem-like cells. METHODOLOGY/PRINCIPAL FINDINGS: In this study, we found that PCa cells with EMT phenotype displayed stem-like cell features characterized by increased expression of Sox2, Nanog, Oct4, Lin28B and/or Notch1, consistent with enhanced clonogenic and sphere (prostasphere)-forming ability and tumorigenecity in mice, which was associated with decreased expression of miR-200 and/or let-7 family. Reversal of EMT by re-expression of miR-200 inhibited prostasphere-forming ability of EMT-type cells and reduced the expression of Notch1 and Lin28B. Down-regulation of Lin28B increased let-7 expression, which was consistent with repressed self-renewal capability. CONCLUSIONS/SIGNIFICANCE: These results suggest that miR-200 played a pivotal role in linking the characteristics of cancer stem-like cells with EMT-like cell signatures in PCa. Selective elimination of cancer stem-like cells by reversing the EMT phenotype to Mesenchymal-Epithelial Transition (MET) phenotype using novel agents would be useful for the prevention of tumor recurrence especially by eliminating those cells that are the "Root Cause" of tumor development and recurrence.

Kong, L., et al. (2016). "Overexpression of SDF-1 activates the NF-kappaB pathway to induce epithelial to mesenchymal transition and cancer stem cell-like phenotypes of breast cancer cells." Int J Oncol **48**(3): 1085-1094.

The formation of EMT and EMT-induced CSC-like phenotype is crucial for the metastasis of tumor cells. The stromal cell-derived factor-1 (SDF-1) is upregulated in various human carcinomas, which is closely associated with proliferation, migration, invasion and prognosis of malignancies. However, limited attention has been directed towards the effect of SDF-1 on epithelial to mesenchymal transition (EMT) or cancer stem cell (CSC)-like phenotype formation in breast cancer cells and the related mechanism. In the present study, we screened MCF-7 cells with low SDF-1 expression level for the purpose of evaluating whether SDF-1 is involved in EMT and CSC-like phenotype formation in MCF-7 cells. The pEGFP-N1-SDF-1 plasmid was transfected into MCF-7 cells, and the stably overexpressed SDF-1 in MCF-7 cells was confirmed by real-time PCR and western blot analysis. Colony formation assay, MTT, wound healing assay and Transwell invasion assay demonstrated that overexpression of SDF-1 significantly boosted the proliferation, migration and invasion of MCF-7 cells compared with parental ($P<0.05$). Flow cytometry analysis revealed a notable increase of CD44+/CD24- subpopulation in SDF-1 overexpressing MCF-7 cells ($P<0.001$), accompanied by the apparently elevated ALDH activity and the upregulation of the stem cell markers OCT-4, Nanog, and SOX2 compared with parental ($P<0.01$). Besides, western blot analysis and immunofluorescence assay observed the significant decreased expression of E-cadherin and enhanced expression of slug, fibronectin and vimentin in SDF-1 overexpressed MCF-7 cells in comparison with parental ($P<0.01$). Further study

found that overexpression of SDF-1 induced the activation of NF-kappaB pathway in MCF-7 cells. Conversely, suppressing or silencing p65 expression by antagonist or RNA interference could remarkably increase the expression of E-cadherin in SDF-1 overexpressed MCF-7 cells (P<0.001). Overall, the above results indicated that overexpression of SDF-1 enhanced EMT by activating the NF-kappaB pathway of MCF-7 cells and further induced the formation of CSC-like phenotypes, ultimately promoting the proliferation and metastasis of MCF-7 cells. Therefore, SDF-1 may further be assessed as a potential target for gene therapy of breast cancer.

Latifi, A., et al. (2011). "Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile." J Cell Biochem **112**(10): 2850-2864.

Epithelial mesenchymal transition (EMT) and cancer stem cells (CSC) have been associated with resistance to chemotherapy. Eighty percent of ovarian cancer patients initially respond to platinum-based combination therapy but most return with recurrence and ultimate demise. To better understand such chemoresistance we have assessed the potential role of EMT in tumor cells collected from advanced-stage ovarian cancer patients and the ovarian cancer cell line OVCA 433 in response to cisplatin in vitro. We demonstrate that cisplatin-induced transition from epithelial to mesenchymal morphology in residual cancer cells correlated with reduced E-cadherin, and increased N-cadherin and vimentin expression. The mRNA expression of Snail, Slug, Twist, and MMP-2 were significantly enhanced in response to cisplatin and correlated with increased migration. This coincided with increased cell surface expression of CSC-like markers such as CD44, alpha2 integrin subunit, CD117, CD133, EpCAM, and the expression of stem cell factors Nanog and Oct-4. EMT and CSC-like changes in response to cisplatin correlated with enhanced activation of extracellular signal-regulated kinase (ERK)1/2. The selective MEK inhibitor U0126 inhibited ERK2 activation and partially suppressed cisplatin-induced EMT and CSC markers. In vivo xenotransplantation of cisplatin-treated OVCA 433 cells in zebrafish embryos demonstrated significantly enhanced migration of cells compared to control untreated cells. U0126 inhibited cisplatin-induced migration of cells in vivo, suggesting that ERK2 signaling is critical to cisplatin-induced EMT and CSC phenotypes, and that targeting ERK2 in the presence of cisplatin may reduce the burden of residual tumor, the ultimate cause of recurrence in ovarian cancer patients.

Lee, E. J., et al. (2012). "New culture system for human embryonic stem cells: autologous mesenchymal stem cell feeder without exogenous fibroblast growth factor 2." Differentiation **83**(1): 92-100.

Human embryonic stem (hES) cells have been successfully maintained using human-cell feeder systems or feeder-free systems. However, despite advances in culture techniques, hES cells require supplementation with fibroblast growth factor 2 (FGF-2), an exogenous stemness factor, which is needed to sustain the authentic undifferentiated status. We developed a new culture system for hES cells; this system does not require supplementation with FGF-2 to obtain hES cells that are suitable for tissue engineering and regenerative medicine. This culture system employed mesenchymal stem cells derived from hES cells (hESC-MSCs) as autologous human feeder cells in the absence of FGF-2. The hES cell line SNUhES3 cultured in this new autologous feeder culture system maintained the typical morphology of hES cells and expression of pluripotency-related proteins, SSEA-4, TRA-1-60, OCT4, and alkaline phosphatase, without development of abnormal karyotypes after more than 30 passages. RNA expression of the pluripotency-related genes OCT4 and NANOG was similar to the expression in SNUhES3 cells maintained on xenofeeder STO cells. To identify the mechanism that enables the cells to be maintained without exogenous FGF-2, we checked the secretion of FGF-2 from the mitomycin-C treated autofeeder hESC-MSCs versus xenofeeder STO cells, and confirmed that hESC-MSCs secreted FGF-2 whereas STO cells did not. The level of FGF-2 in the media from the autofeeder system without exogenous FGF-2 was comparable to that from the xenofeeder system with addition of FGF-2. In conclusion, our new culture system for hES cells, which employs a feeder layer of autologous hESC-MSCs, supplies sufficient amounts of secreted FGF-2 to eliminate the requirement for exogenous FGF-2.

Lee, M. W., et al. (2013). "Human bone marrow-derived mesenchymal stem cell gene expression patterns vary with culture conditions." Blood Res **48**(2): 107-114.

BACKGROUND: Because of the heterogeneity of human mesenchymal stem cells (MSCs), methods for cell expansion in culture and the effects on gene expression are critical factors that need to be standardized for preparing MSCs. We investigated gene expression patterns of MSCs with different seeding densities and culture times. METHODS: Bone marrow-derived MSCs were plated at densities from 200 cells/cm (2) to 5,000 cells/cm (2), and the gene expression patterns were evaluated over time using a

reverse-transcription polymerase chain reaction assay. RESULTS: The mRNA levels of factors that play a critical role in cell migration and tissue regeneration, such as podocalyxin-like protein (PODXL), alpha4-integrin, alpha6-integrin, and leukemia inhibitory factor (LIF), were higher in MSCs plated at 200 cells/cm (2) than in MSCs plated at 5,000 cells/cm (2). The mRNA levels of these factors gradually increased for 10 days and then decreased by day 15 in culture. MSCs seeded at 200 cells/cm (2) that were cultured for 10 days expressed high levels of Oct-4 and Nanog. Indoleamine 2,3-dioxygenase, cyclooxygenase-1, and hepatocyte growth factor expression were upregulated in the presence of the proinflammatory cytokine interferon-gamma in these cells. CONCLUSION: We found differences in the gene expression patterns of MSCs under different culture conditions. MSCs from 10-day cultures seeded at a low density were efficiently expanded, expressed PODXL, alpha6-integrin, alpha4-integrin, and LIF, and maintained properties like stemness and immunomodulation. Therefore, ex vivo expansion of MSCs maintained for an adequate culture time after plating at low cell density can provide an effective regenerative medicinal strategy for cell therapies using MSCs.

Leyva-Leyva, M., et al. (2013). "Characterization of mesenchymal stem cell subpopulations from human amniotic membrane with dissimilar osteoblastic potential." Stem Cells Dev **22**(8): 1275-1287.

Human fetal mesenchymal stem cells can be isolated from the amniotic membrane (AM-hMSCs) by enzymatic digestion. The biological properties of this cell population have been characterized; however, few studies have focused on the presence of stem cell subpopulations and their differentiation potential. The aim of the present study was to isolate homogeneous AM-hMSC subpopulations based on the coexpression of surface markers. In addition, we aimed to characterize stem cell subpopulations through the detection of typical stem cell markers and its differentiation potential. In this study, fluorescence-activated cell sorting (FACS) was used to positively select for the surface markers CD44, CD73, and CD105. Two subpopulations were isolated: CD44+ / CD73+ / CD105+ (CD105+), and CD44+ / CD73+ / CD105- (CD105-). To characterize the cell subpopulations, the expression of pluripotency-associated markers was analyzed by reverse transcriptase-polymerase chain reaction and immunofluorescence. Our results showed positive expression of SOX2, SOX3, PAX6, OCT3/4, and NANOG in the CD105+ and CD105(-) cell subpopulations. In contrast, we did not detect expression of SSEA4 or FOXD3 in either subpopulation. Immunophenotypes, such as mesenchymal and hematopoietic markers, were studied by FACS analyses. Our data revealed the expression of the CD49a, CD49d, CD29, integrin alpha9beta1, CD44, CD73, and CD105 antigens in both subpopulations. In contrast, CD90, CD45, CD34, CD14, and HLA-DR expression was not detected. The ability of both subpopulations to differentiate into osteoblasts, adipocytes, and chondrocytes was evidenced using Alizarin red, Oil-Red, and Alcian blue staining, respectively. Furthermore, neuronal differentiation was demonstrated by the expression of GFAP and NEURO-D. Interestingly, we observed a dissimilar osteoblastic differentiation potential between the subpopulations. CD105- cells showed stronger expression of secreted protein acidic and rich in cysteine (SPARC) and osteonectin, which was associated with more effective calcium deposition, than CD105+ cells. In conclusion, we described a systematic method for the isolation of hMSCs that was highly reproducible and generated homogeneous cultures for osteoblast differentiation with an efficient capacity for mineralization.

Li, W. J., et al. (2016). "Hexavalent chromium induces expression of mesenchymal and stem cell markers in renal epithelial cells." Mol Carcinog **55**(2): 182-192.

Cr (VI) causes severe kidney damage. The patient's renal function could gradually recover by spontaneous kidney regeneration. The molecular effect of Cr (VI) on recovery of kidney cells, however, has not been clearly elucidated. Here we show that Cr (VI) induces expression of mesenchymal and stem cell markers, cell markers, such as paxillin, vimentin, alpha-SMA, nanog, and CD133 of HK-2 cells. Moreover, Cr (VI) activates epithelial-to-mesenchymal transition (EMT). By revealing that levels of dihydrodiol dehydrogenase were promptly reduced following Cr (VI) challenge, our data suggested that DDH could be involved in a Cr (VI)-related oxidation to generate massive reactive oxygen species and H2 O2, and to create intracellular hypoxia, which then increased levels of SUMO-1 activating enzyme subunit 2, and sumoylation of eukaryotic elongation factor-2, to mediate the subsequent molecular and cellular responses, e.g., expression of mesenchymal and stem cell markers. Pretreatment with vitamin C reduced Cr (VI)-related cellular effects. However, no evident effect was observed when vitamin C was added following Cr (VI) challenge.

Li, Z., et al. (2013). "Effect of cell culture using chitosan membranes on stemness marker genes in mesenchymal stem cells." Mol Med Rep **7**(6): 1945-1949.

Mesenchymal stem cell (MSC) therapy is a promising treatment for diseases of the nervous system. However, MSCs often lose their stemness and homing abilities when cultured in conventional twodimensional (2D) systems. Consequently, it is important to explore novel culture methods for MSC-based therapies in clinical practice. To investigate the effect of a cell culture using chitosan membranes on MSCs, the morphology of MSCs cultured using chitosan membranes was observed and the expression of stemness marker genes was analyzed. We demonstrated that MSCs cultured using chitosan membranes form spheroids. Additionally, the expression of stemness marker genes, including Oct4, Sox2 and Nanog, increased significantly when MSCs were cultured using chitosan membranes compared with 2D culture systems. Finally, MSCs cultured using chitosan membranes were found to have an increased potential to differentiate into nerve cells and chrondrocytes. In conclusion, we demonstrated that MSCs cultured on chitosan membranes maintain their stemness and homing abilities. This finding may be further investigated for the development of novel cell-based therapies for diseases involving neuron-like cells and chondrogenesis.

Lim, W., et al. (2016). "Association between cancer stem cell-like properties and epithelial-to-mesenchymal transition in primary and secondary cancer cells." Int J Oncol **49**(3): 991-1000.

One of the theories on cancer stem cells (CSCs) states that these cells initiate most tumors and give rise to more-or-less differentiated tumor cells. Genetic signatures of CSCs are thought to predict tumor recurrence and metastases, thus, supporting the notion that CSCs may be metastatic precursors and induce epithelial-to-mesenchymal transition (EMT). In this study, we tried to examine the association between CSCs and EMT (using specific markers) in the mucoepidermoid carcinoma cell line YD15 and its derivative cell line YD15M (lymph node metastasis). Relative protein expression levels were analyzed by western blotting, flow cytometry, and immunofluorescence assays. In addition, cell cycle assay and aldehyde dehydrogenase (ALDH) activity assay were carried out. Under growth conditions, YD15M cells formed irregular spherical colonies consistent with a stem cell phenotype. YD15M cells demonstrated the low expression of E-cadherin and beta-catenin but high expression of vimentin than that in YD15 cells. In the metastatic cells (YD15M), the coexpression of vimentin and CD133 was detected. Weak proliferation based on cell cycle analysis and decreased PCNA expression was also observed. In addition, expression levels of ALDHA1, OCT4, and NANOG (CSC-like properties) were significantly increased in YD15M cells. Taken together, these findings should help to elucidate the interplay between EMT and CSC-like properties during metastasis and may provide useful information for the development of a novel classification system and therapeutic strategies against head and neck cancer.

Lin, S., et al. (2017). "Stepwise preconditioning enhances mesenchymal stem cell-based cartilage regeneration through epigenetic modification." Osteoarthritis Cartilage **25**(9): 1541-1550.

OBJECTIVE: This study is to investigate the functions and underlying mechanisms of mesenchymal stem cells (MSCs) underwent stepwise preconditioning in chondrogenic medium before expansion, then further explore their therapeutic effects in a surgically induced osteoarthritis (OA) model. METHODS: MSCs isolated from the adult rats expressing Green Fluorescence Protein (GFP) were incubated in basal medium or primed in chondrogenic medium before expansion. The multipotency including cell proliferation, differentiation, and survivability was compared between chondrogenic manipulated MSCs (M-MSCs) and untreated MSCs. Methylation modification of Nanog and Oct4 were detected by bisulfite genomic sequencing. Loss-of-function phenotype in M-MSCs induced by shNanog was also observed. Then the therapeutic effect of the cells was evaluated in a surgically induced OA rat model by single intraarticular injection. The injected GFP-labeled cells in the joints were monitored in vivo. These rats were sacrificed and subjected to histological examinations and microstructural analysis after 4 weeks. RESULTS: We found that cell clonogenicity, proliferation, survivability, and chondrogenic property were enhanced after stepwise preconditioning. We then further found that the expression level of Nanog and Oct4 was temporarily increased in the M-MSCs. Results of epigenetic analysis revealed that demethylation happened in Nanog and Oct4 after the stepwise preconditioning. Results of in vivo imaging showed more GFP-labeled cells in the M-MSCs-injected group. And results of histology and micro-CT analysis also indicated a superior therapeutic effect of M-MSCs on the surgically induced-OA. CONCLUSION: These findings indicated a feasible method to obtain a cell population with high survivability and chondrogenic commitment for the treatment of OA.

Liu, Y., et al. (2017). "Metabolic Reconfiguration Supports Reacquisition of Primitive Phenotype in Human Mesenchymal Stem Cell Aggregates." Stem Cells **35**(2): 398-410.

Spontaneous aggregation and the associated enhancement of stemness have been observed in many

anchorage dependent cells. Recently, aggregation of human mesenchymal stem cells (hMSCs) in nonadherent culture has been shown to reverse expansion-induced heterogeneity and loss of stemness and reprogram the hMSC to reacquire their primitive phenotype, a phenomenon that can significantly enhance therapeutic applications of hMSC. The objective of this study was to investigate the mechanistic basis underlying the connection between multicellular aggregation and stemness enhancement in hMSC by testing the hypothesis that cellular events induced during three-dimensional aggregation on nonadherent substratum induces changes in mitochondrial metabolism that promote the expression of stem cell genes Oct4, Sox2, and Nanog. Our results show that aggregation changes mitochondrial morphology and reduces mitochondrial membrane potential, resulting in a metabolic reconfiguration characterized by increased glycolytic and anaplerotic flux, and activation of autophagy. We further demonstrate that interrupting mitochondrial respiration in two-dimensional planar culture with small molecule inhibitors partially recapitulates the aggregation-mediated enhancement in stem cell properties, whereas enhancement of mitochondrial oxidative phosphorylation in the aggregated state reduces the aggregation-induced upregulation of Oct4, Sox2, and Nanog. Our findings demonstrate that aggregation-induced metabolic reconfiguration plays a central role in reacquisition of primitive hMSC phenotypic properties. Stem Cells 2017;35:398-410.

Lobello, N., et al. (2016). "Ferritin heavy chain is a negative regulator of ovarian cancer stem cell expansion and epithelial to mesenchymal transition." Oncotarget **7**(38): 62019-62033.

OBJECTIVES: Ferritin is the major intracellular iron storage protein essential for maintaining the cellular redox status. In recent years ferritin heavy chain (FHC) has been shown to be involved also in the control of cancer cell growth. Analysis of public microarray databases in ovarian cancer revealed a correlation between low FHC expression levels and shorter survival. To better understand the role of FHC in cancer, we have silenced the FHC gene in SKOV3 cells. RESULTS: FHC-KO significantly enhanced cell viability and induced a more aggressive behaviour. FHC-silenced cells showed increased ability to form 3D spheroids and enhanced expression of NANOG, OCT4, ALDH and Vimentin. These features were accompanied by augmented expression of SCD1, a major lipid metabolism enzyme. FHC apparently orchestrates part of these changes by regulating a network of miRNAs. METHODS: FHC-silenced and control shScr SKOV3 cells were monitored for changes in proliferation, migration, ability to propagate as 3D spheroids and for the expression of stem cell and epithelial-to-mesenchymal-transition (EMT) markers. The expression of three miRNAs relevant to spheroid formation or EMT was assessed by q-PCR. CONCLUSIONS: In this paper we uncover a new function of FHC in the control of cancer stem cells.

Luo, Z., et al. (2017). "Effect of NR5A2 inhibition on pancreatic cancer stem cell (CSC) properties and epithelial-mesenchymal transition (EMT) markers." Mol Carcinog **56**(5): 1438-1448.

NR5A2 (aka LRH-1) has been identified as a pancreatic cancer susceptibility gene with missing biological link. This study aims to demonstrate expression and potential role of NR5A2 in pancreatic cancer. NR5A2 expression was quantified in resected pancreatic ductal adenocarcinomas and the normal adjacent tissues of 134 patients by immunohistochemistry. The intensity and extent of NR5A2 staining was quantified and analyzed in association with overall survival (OS). The impact of NR5A2 knockdown on pancreatic cancer stem cell (CSC) properties and epithelial-mesenchymal transition (EMT) markers was examined in cancer cells using RT-PCR and Western Blot. NR5A2 was overexpressed in pancreatic tumors, the IHC-staining H score (mean +/- SE) was 96.4 +/- 8.3 in normal versus 137.9 +/- 8.2 in tumor tissues ($P < 0.0001$). Patients with a higher NR5A2 expression had a median survival time 18.4 months compared to 23.7 months for those with low IHC H scores ($P = 0.019$). The hazard ratio of death (95% confidence interval) was 1.60 (1.07-2.41) after adjusting for disease stage and tumor grade ($P = 0.023$). NR5A2 was highly expressed in pancreatic cancer sphere forming cells. NR5A2-inhibition by siRNA was associated with reduced sphere formation and decreased levels of CSCs markers NANOG, OCT4, LIN28B, and NOTCH1. NR5A2 knockdown also resulted in reduced expression of FGB, MMP2, MMP3, MMMP9, SNAIL, and TWIST, increased expression of epithelial markers E-cadherin and beta-catenin, and a lower expression of mesenchymal marker Vimentin. Taken together, our findings suggest that NR5A2 could play a role in CSC stemness and EMT in pancreatic cancer, which may contribute to the worse clinical outcome.

Ma, S. R., et al. (2015). "Anterior gradient protein 2 expression in high grade head and neck squamous cell carcinoma correlated with cancer stem cell and epithelial mesenchymal transition." Oncotarget **6**(11): 8807-8821.

Anterior gradient protein 2 (AGR2) is a novel biomarker with potential oncogenic role. We sought to investigate the diagnostic and prognostic role of AGR2

on head and neck squamous cell carcinoma (HNSCC) with an emphasis on its correlation of cancer stemloid cells (CSC) and epithelial mesenchymal transition (EMT). We found that AGR2 protein levels were higher in HNSCC than in normal oral mucosa. High levels of AGR2 were associated with the T category, pathological grade and lymph node metastasis of HNSCC. Expression of AGR2 increased in recurring HNSCC after radiotherapy and in post cisplatin-based chemotherapeutic tissues. In HNSCC cell lines, knock-down of AGR2 induced apoptosis, reduced sphere formation, and down-regulated Survivin, Cyclin D1, Bcl2, Bcl2l1, Slug, Snail, Nanog and Oct4. In addition, over-expressed AGR2 in transgenic mice with spontaneous HNSCC was associated with lost function of Tgfbr1 and/ or lost function of Pten. In vitro knockdown TGFBR1 in HNSCC cell lines increased AGR2 expression. These results suggest that AGR2 is involved in EMT and self-renewal of CSC and may present a potential therapeutic target (oncotarget) for HNSCC.

Mao, J., et al. (2017). "UBR2 Enriched in p53 Deficient Mouse Bone Marrow Mesenchymal Stem Cell-Exosome Promoted Gastric Cancer Progression via Wnt/beta-Catenin Pathway." Stem Cells **35**(11): 2267-2279.

The deficiency or mutation of p53 has been linked to several types of cancers. The mesenchymal stem cell (MSC) is an important component in the tumor microenvironment, and exosomes secreted by MSCs can transfer bioactive molecules, including proteins and nucleic acid, to other cells in the tumor microenvironment to influence the progress of a tumor. However, whether the state of p53 in MSCs can impact the bioactive molecule secretion of exosomes to promote cancer progression and the regulatory mechanism remains elusive. Our study aimed to investigate the regulation of ubiquitin protein ligase E3 component n-recognin 2 (UBR2) enriched in exosomes secreted by p53 deficient mouse bone marrow MSC (p53(-/-) mBMMSC) in gastric cancer progression in vivo and in vitro. We found that the concentration of exosome was significantly higher in p53(-/-) mBMMSC than that in p53 wild-type mBMMSC (p53(+/+) mBMMSC). In particular, UBR2 was highly expressed in p53(-/-) mBMMSC cells and exosomes. P53(-/-) mBMMSC exosomes enriched UBR2 could be internalized into p53(+/+) mBMMSC and murine foregastric carcinoma (MFC) cells and induce the overexpression of UBR2 in these cells which elevated cell proliferation, migration, and the expression of stemness-related genes. Mechanistically, the downregulation of UBR2 in p53(-/-) mBMMSC exosomes could reverse these actions. Moreover, a majority of Wnt family members, beta-catenin, and its downstream genes (CD44, CyclinD1, CyclinD3, and C-myc) were significantly decreased in MFC knockdown UBR2 and beta-catenin depletion, an additional depletion of UBR2 had no significant difference in the expression of Nanog, OCT4, Vimentin, and E-cadherin. Taken together, our findings indicated that p53(-/-) mBMMSC exosomes could deliver UBR2 to target cells and promote gastric cancer growth and metastasis by regulating Wnt/beta-catenin pathway. Stem Cells 2017;35:2267-2279.

Mohammadian, F. and B. Negahdari (2017). "Isolation and characterization of mesenchymal stem cells and its antitumor application on ovarian cancer cell line." Artif Cells Nanomed Biotechnol: 1-10.

The molecular interaction network of Oct-4 (POU5F1) and NANOG connected to regulation and growth of mesenchymal stem cells (MSCs) were supplemented with information of miRNA to find an important micro-RNAs and supplemented molecular interaction network. Following co-culturing of Bone marrow mesenchymal stem cells (BMMSCs) with SKOV3 ovarian cancer cell lines and undifferentiated BMMSCs, MTT was analyzed for cell cytotoxicity. The analyses of the expression of miRNA were performed either after oesteogenesis (hsa-miR-34 and hsa-miR-335) or chondrogenic (hsa-miR-145 and hsa-miR-455) differentiation. This molecular interaction network was imaged in using software. The results from these findings gave an understanding of the main molecular mechanisms regulating MSCs therapeutic activity and their undifferentiated state maintenance. We recommend that the downregulation of miR-335 is crucial role for tissue homeostasis.

Nanta, R., et al. (2013). "NVP-LDE-225 (Erismodegib) inhibits epithelial-mesenchymal transition and human prostate cancer stem cell growth in NOD/SCID IL2Rgamma null mice by regulating Bmi-1 and microRNA-128." Oncogenesis **2**: e42.

Prostate cancer stem cells (CSCs) are defined by their extensive self-renewal, differentiation and tumor initiation properties. It is now clear that CSCs are involved in tumor growth and recurrence, and resistance to conventional treatments. The sonic hedgehog (Shh) pathway has a crucial role in stemness and tumorigenesis. Thus, the strategy that suppresses stemness and consequently tumorigenic potential of CSCs could be considered for the management of prostate cancer. The objectives of this study were to examine the molecular mechanisms, by which NVP-LDE-225/Erismodegib (smoothened inhibitor) regulates stem cell characteristics and tumor growth in prostate cancer. The effects of NVP-LDE-225 on CSC's viability, sphere formation, apoptosis, epithelial-mesenchymal transition (EMT) and tumor

growth in NOD/SCID IL2Rgamma null mice were examined. NVP-LDE-225 inhibited cell viability and spheroid formation, and induced apoptosis by activation of caspase-3 and cleavage of poly-ADP ribose polymerase (PARP). NVP-LDE-225 induced expression of Bax and Bak, and inhibited the expression of Bcl-2, Bcl-XL, XIAP, cIAP1, cIAP2 and survivin. NVP-LDE-225 inhibited Gli transcriptional activity, Gli-DNA interaction and the expression of Gli1, Gli2, Patched1 and Patched-2 in prostate CSCs. Interestingly, NVP-LDE-225 induced PDCD4 and apoptosis and inhibited cell viability by suppressing miR-21. Furthermore, NVP-LDE-225 inhibited pluripotency-maintaining factors Nanog, Oct-4, c-Myc and Sox-2. The inhibition of Bmi-1 by NVP-LDE-225 was regulated by upregulation of miR-128. NVP-LDE-225 suppressed EMT by upregulating E-cadherin and inhibiting N-cadherin, Snail, Slug and Zeb1 by regulating the miR-200 family. Finally, NVP-LDE-225 inhibited CSC tumor growth, which was associated with the suppression of Gli1, Gli2, Patched-1, Patched-2, Cyclin D1, Bmi-1 and PCNA and cleavage of caspase-3 and PARP in tumor tissues derived from NOD/SCID IL2Rgamma null mice. Overall, our findings suggest that inhibition of the Shh signaling pathway could therefore be a novel therapeutic option in treating prostate cancer.

Niibe, K., et al. (2011). "Purified mesenchymal stem cells are an efficient source for iPS cell induction." PLoS One **6**(3): e17610.

BACKGROUND: Induced pluripotent stem (iPS) cells are generated from mouse and human somatic cells by the forced expression of defined transcription factors. Although most somatic cells are capable of acquiring pluripotency with minimal gene transduction, the poor efficiency of cell reprogramming and the uneven quality of iPS cells are still important problems. In particular, the choice of cell type most suitable for inducing high-quality iPS cells remains unclear. METHODOLOGY/PRINCIPAL FINDINGS: Here, we generated iPS cells from PDGFRalpha+ Sca-1+ (PalphaS) adult mouse mesenchymal stem cells (MSCs) and PDGFRalpha (-) Sca-1(-) osteo-progenitors (OP cells), and compared the induction efficiency and quality of individual iPS clones. MSCs had a higher reprogramming efficiency compared with OP cells and Tail Tip Fibroblasts (TTFs). The iPS cells induced from MSCs by Oct3/4, Sox2, and Klf4 appeared to be the closest equivalent to ES cells by DNA microarray gene profile and germline-transmission efficiency. CONCLUSIONS/SIGNIFICANCE: Our findings suggest that a purified source of undifferentiated cells from adult tissue can produce high-quality iPS cells. In this context, prospectively enriched MSCs are a promising candidate for the efficient generation of high-quality iPS cells.

Ning, X., et al. (2018). "Ectopic expression of miR-147 inhibits stem cell marker and epithelial-mesenchymal transition (EMT)-related protein expression in colon cancer cells." Oncol Res.

Colon cancer is one of the most common cancers in the world. Epithelial-to-mesenchymal transition (EMT) is a crucial step in tumor progression and also involves in the acquisition of stem cell-like properties. Some miRNAs have been shown to function as either tumor suppressors or oncogenes in colon cancer. Here, we investigated the role of miR-147 in the regulation of stem cell-like traits of colon cancer cells. We observed that miR-147 was down-regulated in several colon cancer cell lines and overexpressed miR-147 decreased the expression of cancer stem cell (CSC) markers OCT4, SOX2 and NANOG in colon cancer cells (HCT116, SW480). Besides that, overexpressed miR-147 inhibited EMT by increasing the expression of epithelial markers Ecadherin and alpha-catenin while decreasing the expression of mesenchymal markers fibronectin and vimentin. Moreover, activation of EMT by TGF-beta1 treatment counteracted the inhibitive effect of miR-147 on the expression of CSC markers OCT4, SOX2 and NANOG significantly, supporting that overexpressed miR-147 inhibited stem cell-like traits by suppressing EMT in colon cancer. In addition, we found that overexpressed miR-147 down-regulated the expression of beta-Catenin, c-myc and Survivin which were related to Wnt/beta-Catenin pathway. Moreover, treatment with Wnt/beta-Catenin pathway activator Licl in miR-147 mimic transfected cells attenuated the inhibitive effect of miR-147 mimic on EMT and stem cell-like traits of colon cancer cells, indicating that ectopic expression of miR-147 inhibited stem cell-like traits in colon cancer cells through suppressing EMT via the Wnt/beta-Catenin pathway. In summary, our present study highlighted the crucial role of miR-147 in the inhibition of stem cell-like traits of colon cancer cells and indicated that miR-147 could be a promising therapeutic target for colon cancer treatment.

Obara, C., et al. (2016). "Characteristics of three-dimensional prospectively isolated mouse bone marrow mesenchymal stem/stromal cell aggregates on nanoculture plates." Cell Tissue Res **366**(1): 113-127.

Three-dimensional (3-D) aggregate culturing is useful for investigating the functional properties of mesenchymal stem/stromal cells (MSCs). For 3-D MSC analysis, however, pre-expansion of MSCs with two-dimensional (2-D) monolayer culturing must first be performed, which might abolish their endogenous properties. To avoid the need for 2-D expansion, we

used prospectively isolated mouse bone marrow (BM)-MSCs and examined the differences in the biological properties of 2-D and 3-D MSC cultures. The BM-MSCs self-assembled into aggregates on nanoculture plates (NCP) that have nanoimprinted patterns with a low-cellular binding texture. The 3-D MSCs proliferated at the same rate as 2-D-cultured cells by only diffusion culture and secreted higher levels of pro-angiogenic factors such as vascular endothelial growth factor and hepatocyte growth factor (HGF). Conditioned medium from 3-D MSC cultures promoted more capillary formation than that of 2-D MSCs in an in vitro tube formation assay. Matrigel-implanted 3-D MSC aggregates tended to induce angiogenesis in host mice. The 3-D culturing on NCP induced alpha-fetoprotein (AFP) expression in MSCs without the application of AFP- or endodermal-inducible factors, possibly via an HGF-autocrine mechanism, and maintained their differentiation ability for adipocytes, osteocytes, and chondrocytes. Prospectively isolated mouse BM-MSCs expressed low/negative stemness-related genes including Oct3/4, Nanog, and Sox2, which were not enhanced by NCP-based 3-D culturing, suggesting that some of these cells differentiate into meso-endodermal layer cells. Culturing of prospectively isolated MSCs on NCP in 3-D allows the analysis of the biological properties of more closely endogenous BM-MSCs and might contribute to tissue engineering and repair.

Oh, J. E., et al. (2011). "DeltaNp63alpha protein triggers epithelial-mesenchymal transition and confers stem cell properties in normal human keratinocytes." J Biol Chem **286**(44): 38757-38767.

p63 is a p53 family protein required for morphogenesis and postnatal regeneration of epithelial tissues. Here we demonstrate that DeltaNp63alpha, a p63 isoform lacking the N-terminal transactivation domain, induces epithelial-mesenchymal transition (EMT) in primary human keratinocytes in a TGF-beta-dependent manner. Rapidly proliferating normal human epidermal keratinocytes (NHEK) were infected with retroviral vector expressing DeltaNp63alpha or empty vector and serially subcultured until replicative senescence. No phenotypic changes were observed until the culture reached senescence. Then the DeltaNp63alpha-transduced cells underwent morphological changes resembling mesenchymal cells and acquired the EMT phenotype. Treatment with exogenous TGF-beta accelerated EMT in presenescent DeltaNp63alpha-transduced cells, whereas the inhibition of TGF-beta signaling reversed the EMT phenotype. TGF-beta treatment alone led to growth arrest in control NHEK with no evidence of EMT, indicating that DeltaNp63alpha altered the cellular response to TGF-beta treatment. DeltaNp63alpha-transduced cells acquiring EMT gained the ability to be differentiated to osteo-/odontogenic and adipogenic pathways, resembling mesenchymal stem cells. Furthermore, these cells expressed enhanced levels of Nanog and Lin28, which are transcription factors associated with pluripotency. These data indicate that EMT required DeltaNp63alpha transduction and intact TGF-beta signaling in NHEK.

P, M., et al. (2011). "Adult mesenchymal stem cells and cell surface characterization - a systematic review of the literature." Open Orthop J **5**(Suppl 2): 253-260.

Human adult mesenchymal stem cells (MSCs) were first identified by Friedenstein et al. when observing a group of cells that developed into fibroblastic colony forming cells (CFU-F). Ever since, the therapeutic uses and clinical applications of these cells have increased research and interest in this field. MSCs have the potential to be used in tissue engineering, gene therapy, transplants and tissue injuries. However, identifying these cells can be a challenge. Moreover, there are no articles bringing together and summarizing the cell surface markers of MSCs in adults. The purpose of this study is to summarize all the available information about the cell surface characterization of adult human MSCs by identifying and evaluating all the published literature in this field. We have found that the most commonly reported positive markers are CD105, CD90, CD44, CD73, CD29, CD13, CD34, CD146, CD106, CD54 and CD166. The most frequently reported negative markers are CD34, CD14, CD45, CD11b, CD49d, CD106, CD10 and CD31. A number of other cell surface markers including STRO-1, SH2, SH3, SH4, HLA-A, HLA-B, HLA-C, HLA-DR, HLA-I, DP, EMA, DQ (MHC Class II), CDIO5, Oct 4, Oct 4A, Nanog, Sox-2, TERT, Stat-3, fibroblast surface antigen, smooth muscle alpha-actin, vimentin, integrin subunits alpha4, alpha5, beta1, integrins alphavbeta3 and alphavbeta5 and ICAM-1 have also been reported. Nevertheless, there is great discrepancy and inconsistency concerning the information available on the cell surface profile of adult MSCs and we suggest that further research is needed in this field to overcome the problem.

Pavon, L. F., et al. (2016). "Mesenchymal stem cell-like properties of CD133+ glioblastoma initiating cells." Oncotarget **7**(26): 40546-40557.

Glioblastoma is composed of dividing tumor cells, stromal cells and tumor initiating CD133+ cells. Recent reports have discussed the origin of the glioblastoma CD133+ cells and their function in the tumor microenvironment. The present work sought to investigate the multipotent and mesenchymal

properties of primary highly purified human CD133+ glioblastoma-initiating cells. To accomplish this aim, we used the following approaches: i) generation of tumor subspheres of CD133+ selected cells from primary cell cultures of glioblastoma; ii) analysis of the expression of pluripotency stem cell markers and mesenchymal stem cell (MSC) markers in the CD133+ glioblastoma-initiating cells; iii) side-by-side ultrastructural characterization of the CD133+ glioblastoma cells, MSC and CD133+ hematopoietic stem cells isolated from human umbilical cord blood (UCB); iv) assessment of adipogenic differentiation of CD133+ glioblastoma cells to test their MSC-like in vitro differentiation ability; and v) use of an orthotopic glioblastoma xenograft model in the absence of immune suppression. We found that the CD133+ glioblastoma cells expressed both the pluripotency stem cell markers (Nanog, Mush-1 and SSEA-3) and MSC markers. In addition, the CD133+ cells were able to differentiate into adipocyte-like cells. Transmission electron microscopy (TEM) demonstrated that the CD133+ glioblastoma-initiating cells had ultrastructural features similar to those of undifferentiated MSCs. In addition, when administered in vivo to non-immunocompromised animals, the CD133+ cells were also able to mimic the phenotype of the original patient's tumor. In summary, we showed that the CD133+ glioblastoma cells express molecular signatures of MSCs, neural stem cells and pluripotent stem cells, thus possibly enabling differentiation into both neural and mesodermal cell types.

Potdar, P. and R. Subedi (2011). "Defining Molecular Phenotypes of Mesenchymal and hematopoietic Stem Cells derived from Peripheral blood of Acute Lymphocytic Leukemia patients for regenerative stem cell therapy." J Stem Cells Regen Med **7**(1): 29-40.

Acute Lymphocytic Leukemia (ALL) is a clonal myeloid disorder affecting all age groups, characterized by accumulation of immature blast cells in bone marrow and in peripheral blood. Autologous Bone Marrow Transplantation is a present treatment for cure of ALL patients, which is very expensive, invasive process and may have possibility of transplantation of malignant stem cells to patients. In the present study, we hypothesized to isolate large number of normal Mesenchymal & Hematopoietic stem cells from peripheral blood of ALL patients, which will be further characterized for their normal phenotypes by using specific molecular stem cell markers. This is the first study, which defines the existing phenotypes of isolated MSCs and HSCs from peripheral blood of ALL patients. We have established three cell lines in which two were Mesenchymal stem cells designated as MSCALL and MSCnsALL and one was suspension cell line designated as HSCALL. The HSCALL cell line was developed from the lymphocyte like cells secreted by MSCALL cells. Our study also showed that MSCALL from peripheral blood of ALL patient secreted hematopoietic stem cells in vitro culture. We have characterized all three-cell lines by 14 specific stem cell molecular markers. It was found that both MSC cell lines expressed CD105, CD13, and CD73 with mixed expression of CD34 and CD45 at early passage whereas, HSCALL cell line expressed prominent feature of hematopoietic stem cells such as CD34 and CD45 with mild expression of CD105 and CD13. All three-cell lines expressed LIF, OCT4, NANOG, SOX2, IL6, and DAPK. These cells mildly expressed COX2 and did not express BCR-ABL. Overall it was shown that isolated MSCs and HSCs can be use as a model system to study the mechanism of leukemia at stem cell level and their use in stem cell regeneration therapy for Acute Lymphocytic Leukemia.

Potdar, P. and J. Sutar (2010). "Establishment and molecular characterization of mesenchymal stem cell lines derived from human visceral & subcutaneous adipose tissues." J Stem Cells Regen Med **6**(1): 26-35.

Mesenchymal stem cells (MSCs), are multipotent stem cells that can differentiate into osteoblasts, chondrocytes, myocytes and adipocytes. We utilized adipose tissue as our primary source, since it is a rich source of MSCs as well as it can be harvested using a minimally invasive surgical procedure. Both visceral and subcutaneous adipose tissue (VSAT, SCAT respectively) samples were cultured using growth medium without using any substratum for their attachment. We observed growth of mesenchymal like cells within 15 days of culturing. In spite of the absence of any substratum, the cells adhered to the bottom of the petri dish, and spread out within 2 hours. Presently VSAT cells have reached at passage 10 whereas; SCAT cells have reached at passage 14. Morphologically MSCs obtained from visceral adipose tissue were larger in shape than subcutaneous adipose tissue. We checked these cells for presence or absence of specific stem cell molecular markers. We found that VSAT and SCAT cells confirmed their MSC phenotype by expression of specific MSC markers CD 105 and CD 13 and absence of CD34 and CD 45 markers which are specific for haematopoietic stem cells. These cells also expressed SOX2 gene confirming their ability of self-renewal as well as expressed OCT4, LIF and NANOG for their properties for pluripotency & plasticity. Overall, it was shown that adipose tissue is a good source of mesenchymal stem cells. It was also shown that MSCs, isolated from adipose tissue are multipotent stem cells that can

differentiate into osteoblasts, chondrocytes, cardiomyocytes, adipocytes and liver cells which may open a new era for cell based regenerative therapies for bone, cardiac and liver disorders.

Potdar, P. D. and S. B. D'Souza (2010). "Ascorbic acid induces in vitro proliferation of human subcutaneous adipose tissue derived mesenchymal stem cells with upregulation of embryonic stem cell pluripotency markers Oct4 and SOX 2." Hum Cell **23**(4): 152-155.

Mesenchymal stem cells (MSCs) have immense therapeutic potential because of their ability to self-renew and differentiate into various connective tissue lineages. The in vitro proliferation and expansion of these cells is necessary for their use in stem cell therapy. Recently our group has developed and characterized mesenchymal stem cells from subcutaneous and visceral adipose tissue. We observed that these cells show a slower growth rate at higher passages and therefore decided to develop a supplemented medium, which will induce proliferation. Choi et al. have recently shown that the use of ascorbic acid enhances the proliferation of bone marrow derived MSCs. We therefore studied the effect of ascorbic acid on the proliferation of MSCs and characterized their phenotypes using stem cell specific molecular markers. It was observed that the use of 250 muM ascorbic acid promoted the significant growth of MSCs without loss of phenotype and differentiation potential. There was no considerable change in gene expression of cell surface markers CD105, CD13, Nanog, leukemia inhibitory factor (LIF) and Keratin 18. Moreover, the MSCs maintained in the medium supplemented with ascorbic acid for a period of 4 weeks showed increase in pluripotency markers Oct4 and SOX 2. Also cells in the experimental group retained the typical spindle shaped morphology. Thus, this study emphasizes the development of suitable growth medium for expansion of MSCs and maintenance of their undifferentiated state for further therapeutic use.

Pouryazdanpanah, N., et al. (2018). "Use of Some Additives for Improving Mesenchymal Stem Cell Isolation Outcomes in Non-Mobilized Peripheral Blood." Arch Iran Med **21**(8): 362-367.

BACKGROUND: The mesenchymal stem cells (MSCs) of peripheral blood (PB) have been recognized as a promising source for allogeneic cell therapy. The objective of the present study was to isolate and characterize MSCs derived from non-mobilized PB, and evaluate their differentiation potential. METHODS: The buffy coat mononuclear fractions of the PB were concentrated using the Ficoll-Paque density gradient centrifugation and were grown on primary and secondary culture media, respectively. The isolated cells were characterized using a multidisciplinary approach which was based on morphology, immunophenotyping, gene expression, and multipotentiality. Flow cytometry and Reverse transcription polymerase chain reaction (RT-PCR) were used to identify the expression of different MSC markers. Finally, after culturing in osteogenic and adipogenic induction media, the isolated cells were stained by Alizarin red and Oil-Red O. RESULTS: In spite of absence of any bone marrow stimulating factor, the isolation approach in this study yielded a rather homogeneous and spindle-shaped mononuclear cell population (the yield of passage 0 was 0.65 +/- 0.15) that stained positive for CD90, CD105, and CD73, and were negative for CD45 and CD34. These cells have high proliferative capacity (confirmed by the expression of Oct-4, Nucleostemin, and Nanog genes) and were able to differentiate into lineage-specific committed cells, when exposed to the appropriate medium. CONCLUSION: Overall, it can be concluded that conventional, labour-intensive and time-consuming approaches are not necessary in isolating MSCs from PB. This relatively accessible and minimally invasive source, PB, represents a good alternative reservoir of homogeneous MSCs that could open a new era for practical exploitation in regenerative medicine.

Pringle, S., et al. (2011). "Mesenchymal differentiation propensity of a human embryonic stem cell line." Cell Prolif **44**(2): 120-127.

OBJECTIVES: To characterize basal differentiation tendencies of a human embryonic stem (hES) cell line, KCL-002. MATERIALS AND METHODS: In vitro specification and differentiation of hES cells were carried out using embryoid body (EB) cultures and tests of pluripotency and in vivo differentiation were performed by teratoma assays in SCID mice. Real-time PCR, immunohistochemistry, flow cytometry and histological analyses were used to identify expression of genes and proteins associated with the ectodermal, endodermal and mesodermal germ layers. RESULTS: Undifferentiated KCL-002 cells expressed characteristic markers of pluripotent stem cells such as Nanog, Sox-2, Oct-4 and TRA 1-60. When differentiated in vitro as EB cultures, expression of pluripotency, endodermal and ectodermal markers decreased rapidly. In contrast, mesodermal and mesenchymal markers such as VEGFR-2, alpha-actin and vimentin increased during EB differentiation as shown by qPCR, immunostaining and flow cytometric analyses. Teratoma formation in SCID mice demonstrated the potential to form all germ layers in vivo with a greater proportion of the tumours containing mesenchymal derivatives.

CONCLUSIONS: The data presented suggest that the KCL-002 hES cell line is pluripotent and harbours a bias in basal differentiation tendencies towards mesodermal and mesenchymal lineage cells. Characterizing innate differentiation propensities of hES cell lines is important for understanding heterogeneity between different cell lines and for further studies aimed at deriving specific lineages from hES cells.

Qiao, B., et al. (2012). "Epithelial-mesenchymal transition and mesenchymal-epithelial transition are essential for the acquisition of stem cell properties in hTERT-immortalised oral epithelial cells." Biol Cell **104**(8): 476-489.

BACKGROUND INFORMATION: Evidence has shown that mesenchymal-epithelial transition (MET) and epithelial-mesenchymal transition (EMT) are linked to stem cell properties. We currently lack a model showing how the occurrence of MET and EMT in immortalised cells influences the maintenance of stem cell properties. Thus, we established a project aiming to investigate the roles of EMT and MET in the acquisition of stem cell properties in immortalised oral epithelial cells. RESULTS: In this study, a retroviral transfection vector (pLXSN-hTERT) was used to immortalise oral epithelial cells by insertion of the hTERT gene (hTERT (+)-oral mucosal epithelial cell line [OME]). The protein and RNA expression of EMT transcriptional factors (Snail, Slug and Twist), their downstream markers (E-cadherin and N-cadherin) and embryonic stem cell markers (OCT4, Nanog and Sox2) were studied by reverse transcription PCR and Western blots in these cells. Some EMT markers were detected at both mRNA and protein levels. Adipocytes and bone cells were noted in the multi-differentiation assay, showing that the immortal cells underwent EMT. The differentiation assay for hTERT (+)-OME cells revealed the recovery of epithelial phenotypes, implicating the presence of MET. The stem cell properties were confirmed by the detection of appropriate markers. Altered expression of alpha-tubulin and gamma-tubulin in both two-dimensional-cultured (without serum) and three-dimensional-cultured hTERT (+)-OME spheroids indicated the re-programming of cytoskeleton proteins which is attributed to MET processes in hTERT (+)-OME cells. CONCLUSIONS: EMT and MET are essential for hTERT-immortalised cells to maintain their epithelial stem cell properties.

Qin, Y. and H. Degroot Iii (2009). "[Mesenchymal stem cell-like properties of the fibroblasts derived from the interface membrane related with total hip prosthesis]." Nan Fang Yi Ke Da Xue Xue Bao **29**(8): 1647-1650.

OBJECTIVE: By examining arthroplasty interface membrane-derived fibroblasts in vitro, we observed continuous morphological changes in cultured cells similar to the spontaneous differentiation of adult stem cells. This investigation is aimed to study whether the cells possess mesenchymal stem cell-like properties. METHODS: Tissue culture, immunohistochemistry and flow cytometry were used to characterize the cultured arthroplasty membrane-derived fibroblasts for their fibroblast and stem cell properties. The plasticity of these cells was also analyzed using osteogenic, adipogenic medium culture and histological techniques. RESULTS: All the cells in culture expressed vimentin. We found that 0.1% of the cultured interface membrane-derived fibroblasts possessed mesenchymal stem cell markers (SSEA (4) (+)/CD45(-)), 4.5% expressed CD34, and 2.4% were positive for Nanog. These cells did not contain cells positive for such hematopoietic stem cell markers as CD133, Thy-1 or SCF. After exposure to osteogenic differentiation cocktails, calcium deposition was found in many of the arthroplasty membrane-derived fibroblasts, and (24.5-/+5.5)% of the fibroblasts expressed the mineralization precursor enzyme (alkaline phosphatase). When cultured in adipogenic media, (16.0-/+6.5)% of the cells differentiated into lipid droplet- containing adipocytes. CONCLUSION: A portion of arthroplasty interface membrane-derived fibroblasts express mesenchymal stem cell-related surface antigens. Under certain conditions, these cells can differentiate into osteoblasts or adipocytes, suggesting the properties of mesenchymal stem cells.

Rahn, S., et al. (2018). "Diabetes as risk factor for pancreatic cancer: Hyperglycemia promotes epithelial-mesenchymal-transition and stem cell properties in pancreatic ductal epithelial cells." Cancer Lett **415**: 129-150.

Type 2 diabetes mellitus (T2DM) is associated with hyperglycemia and a risk to develop pancreatic ductal adenocarcinoma (PDAC), one of the most fatal malignancies. Cancer stem cells (CSC) are essential for initiation and maintenance of tumors, and acquisition of CSC-features is linked to epithelial-mesenchymal-transition (EMT). The present study investigated whether hyperglycemia promotes EMT and CSC-features in premalignant and malignant pancreatic ductal epithelial cells (PDEC). Under normoglycemia (5 mM d-glucose), Panc1 PDAC cells but not premalignant H6c7-kras cells exhibited a mesenchymal phenotype along with pronounced colony formation. While hyperglycemia (25 mM d-glucose) did not impact the mesenchymal phenotype of Panc1 cells, CSC-properties were aggravated exemplified by increased Nanog expression and Nanog-dependent formation of holo- and meroclones.

In H6c7-kras cells, high glucose increased secretion of Transforming-Growth-Factor-beta1 (TGF-beta1) as well as TGF-beta1 signaling, and in a TGF-beta1-dependent manner reduced E-cadherin expression, increased Nestin expression and number of meroclones. Finally, reduced E-cadherin expression was detected in pancreatic ducts of hyperglycemic but not normoglycemic mice. These data suggest that hyperglycemia promotes the acquisition of mesenchymal and CSC-properties in PDEC by activating TGF-beta signaling and might explain how T2DM facilitates pancreatic tumorigenesis.

Reumann, M. K., et al. (2018). "Donor Site Location Is Critical for Proliferation, Stem Cell Capacity, and Osteogenic Differentiation of Adipose Mesenchymal Stem/Stromal Cells: Implications for Bone Tissue Engineering." Int J Mol Sci **19**(7).

Human adipose mesenchymal stem/stromal cells (Ad-MSCs) have been proposed as a suitable option for bone tissue engineering. However, donor age, weight, and gender might affect the outcome. There is still a lack of knowledge of the effects the donor tissue site might have on Ad-MSCs function. Thus, this study investigated proliferation, stem cell, and osteogenic differentiation capacity of human Ad-MSCs obtained from subcutaneous fat tissue acquired from different locations (abdomen, hip, thigh, knee, and limb). Ad-MSCs from limb and knee showed strong proliferation despite the presence of osteogenic stimuli, resulting in limited osteogenic characteristics. The less proliferative Ad-MSCs from hip and thigh showed the highest alkaline phosphatase (AP) activity and matrix mineralization. Ad-MSCs from the abdomen showed good proliferation and osteogenic characteristics. Interestingly, the observed differences were not dependent on donor age, weight, or gender, but correlated with the expression of Sox2, Lin28A, Oct4 & alpha;, and Nanog. Especially, low basal Sox2 levels seemed to be pivotal for osteogenic differentiation. Our data clearly show that the donor tissue site affects the proliferation and osteogenic differentiation of Ad-MSCs significantly. Thus, for bone tissue engineering, the donor site of the adipose tissue from which the Ad-MSCs are derived should be adapted depending on the requirements, e.g., cell number and differentiation state.

Riekstina, U., et al. (2009). "Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis." Stem Cell Rev **5**(4): 378-386.

Mesenchymal stem cells (MSCs) have been isolated from a variety of human tissues, e.g., bone marrow, adipose tissue, dermis, hair follicles, heart, liver, spleen, dental pulp. Due to their immunomodulatory and regenerative potential MSCs have shown promising results in preclinical and clinical studies for a variety of conditions, such as graft versus host disease (GvHD), Crohn's disease, osteogenesis imperfecta, cartilage damage and myocardial infarction. MSC cultures are composed of heterogeneous cell populations. Complications in defining MSC arise from the fact that different laboratories have employed different tissue sources, extraction, and cultivation methods. Although cell-surface antigens of MSCs have been extensively explored, there is no conclusive evidence that unique stem cells markers are associated with these adult cells. Therefore the aim of this study was to examine expression of embryonic stem cell markers Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4 in adult mesenchymal stem cell populations derived from bone marrow, adipose tissue, dermis and heart. Furthermore, we tested whether human mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions. We found that bone marrow MSCs express embryonic stem cell markers Oct4, Nanog, alkaline phosphatase and SSEA-4, adipose tissue and dermis MSCs express Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4, whereas heart MSCs express Oct4, Nanog, SOX2 and SSEA-4. Our results also indicate that human adult mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions during early passages, as shown by distinct germ layer and embryonic stem cell marker expression patterns. Studies are now needed to determine the functional role of embryonic stem cell markers Oct4, Nanog and SOX2 in adult human MSCs.

Riggi, N., et al. (2010). "EWS-FLI-1 modulates miRNA145 and SOX2 expression to initiate mesenchymal stem cell reprogramming toward Ewing sarcoma cancer stem cells." Genes Dev **24**(9): 916-932.

Cancer stem cells (CSCs) display plasticity and self-renewal properties reminiscent of normal tissue stem cells, but the events responsible for their emergence remain obscure. We recently identified CSCs in Ewing sarcoma family tumors (ESFTs) and showed that they retain mesenchymal stem cell (MSC) plasticity. In the present study, we addressed the mechanisms that underlie ESFT CSC development. We show that the EWS-FLI-1 fusion gene, associated with 85%-90% of ESFTs and believed to initiate their pathogenesis, induces expression of the embryonic stem cell (ESC) genes OCT4, SOX2, and NANOG in human pediatric MSCs (hpMSCs) but not in their adult counterparts. Moreover, under appropriate culture conditions, hpMSCs expressing EWS-FLI-1 generate a cell subpopulation displaying ESFT CSC features in vitro. We further demonstrate that induction of the ESFT CSC phenotype is the result of the combined

effect of EWS-FLI-1 on its target gene expression and repression of microRNA-145 (miRNA145) promoter activity. Finally, we provide evidence that EWS-FLI-1 and miRNA-145 function in a mutually repressive feedback loop and identify their common target gene, SOX2, in addition to miRNA145 itself, as key players in ESFT cell differentiation and tumorigenicity. Our observations provide insight for the first time into the mechanisms whereby a single oncogene can reprogram primary cells to display a CSC phenotype.

Savickiene, J., et al. (2016). "Senescence-Associated Molecular and Epigenetic Alterations in Mesenchymal Stem Cell Cultures from Amniotic Fluid of Normal and Fetus-Affected Pregnancy." Stem Cells Int **2016**: 2019498.

Human amniotic-fluid-derived mesenchymal stem cells (AF-MSCs) are interesting for their multilineage differentiation potential and wide range of therapeutic applications due to the ease of culture expansion. However, MSCs undergo replicative senescence. So far, the molecular mechanisms that underlie fetal diseases and cell senescence are still poorly understood. Here, we analyzed senescence-associated morphologic, molecular, and epigenetic characteristics during propagation of MSCs derived from AF of normal and fetus-affected pregnancy. AF-MSCs cultures from both cell sources displayed quite similar morphology and expression of specific cell surface (CD44, CD90, and CD105) and stemness (Oct4, Nanog, Sox2, and Rex1) markers but had interindividual variability in proliferation capability and time to reach senescence. Within passages 4 and 8, senescent cultures exhibited typical morphological features, senescence-associated beta-galactosidase activity, increased levels of p16, and decreased levels of miR-17 and miR-21 but showed differential expression of p21, p53, and ATM dependently on the onset of cell senescence. These differences correlated with changes in the level of chromatin modifiers (DNMT1 and HDAC1) and polycomb group proteins (EZH2, SUZ12, and BMI1) paralleling with changes in the expression of repressive histone marks (H3K9me3 and H3K27me3) and stemness markers (Oct4, Nanog, Sox2, and Rex1). Therefore epigenetic factors are important for AF-MSCs senescence process that may be related with individuality of donor or a fetus malignancy status.

Savickiene, J., et al. (2017). "Histone Modifications Pattern Associated With a State of Mesenchymal Stem Cell Cultures Derived From Amniotic Fluid of Normal and Fetus-Affected Gestations." J Cell Biochem **118**(11): 3744-3755.

Human amniotic fluid (AF)-derived mesenchymal stem cells (MSCs) sharing embryonic and adult stem cells characteristics are interesting by their multipotency and the usage for regenerative medicine. However, the usefulness of these cells for revealing the fetal diseases still needs to be assessed. Here, we have analyzed the epigenetic environment in terms of histone modifications in cultures of MSCs derived from AF of normal pregnancies and those with fetal abnormalities. The comparison of MSCs samples from AF of normal pregnancies (N) and fetus-affected (P) revealed two distinct cultures by their proliferation potential (P I and P II). Cell populations from N and P I samples had similar growth characteristics and exhibited quite similar cell surface (CD44, CD90, CD105) and stemness markers (Oct4, Nanog, Sox2, Rex1) profile that was distinct in slower growing and faster senescent P II cultures. Those differences were associated with changes in 5-Cyt DNA methylation and alterations in the expression levels of chromatin modifiers (DNMT1, HDAC1/2), activating (H4ac, H3K4me3), and repressive (H3K9me2/me3, H3K27me3) histone marks. MSCs isolated from AF with the genetic or multifactorial fetal diseases (P II samples) were enriched with repressive histone marks and H4K16ac, H3K9ac, H3K14ac modifications. This study indicates that differential epigenetic environment reflects a state of AF-MSCs dependently on their growth, phenotype, and stemness characteristics suggesting a way for better understanding of epigenetic regulatory mechanisms in AF-MSCs cultures in normal and diseased gestation conditions. J. Cell. Biochem. 118: 3744-3755, 2017. (c) 2017 Wiley Periodicals, Inc.

Seino, S., et al. (2016). "CD44(high) /ALDH1(high) head and neck squamous cell carcinoma cells exhibit mesenchymal characteristics and GSK3beta-dependent cancer stem cell properties." J Oral Pathol Med **45**(3): 180-188.

BACKGROUND: CD44 and aldehyde dehydrogenase 1 (ALDH1) have been shown to be useful markers for identification of cancer stem cells (CSCs). We previously reported that glycogen synthase kinase 3beta (GSK3beta) is involved in regulation of the self-renewal ability of head and neck squamous cell carcinoma (HNSCC) CSCs. The purpose of the present study was to clarify the role of GSK3beta in CD44(high) /ALDH1(high) HNSCC cells. METHODS: Cells with greater expression of CD44 and higher ALDH1 enzymatic activity were FACS sorted from the OM-1 HNSCC cell line. The self-renewal ability of CD44(high) /ALDH1(high) cells was then examined using a tumor sphere formation assay. mRNA expressions of the stem cell markers Sox2, Oct4, and Nanog, as well as GSK3beta were evaluated by real-time RT-PCR. RESULTS: CD44(high) /ALDH1(high) cells exhibited higher

tumor sphere forming ability and increased expression of stem cell markers as compared with CD44(high) /ALDH1(low) cells. Interestingly, spindle-shaped cells positive for vimentin were found in the CD44(high) /ALDH1(high) but not the CD44(high) /ALDH1(low) cell population. In addition, the ALDH1 activity and sphere forming ability of CD44(high) /ALDH1(high) cells was significantly inhibited by GSK3beta knockdown. On the other hand, CD44(high) /ALDH1(low) cells exhibited high epidermal growth factor receptor (EGFR) expression and increased cell growth. CONCLUSIONS: Our results show that GSK3beta plays a major role in maintenance of stemness of CD44(high) /ALDH1(high) HNSCC cells. Additionally, they indicate a close relationship between CSC and mesenchymal characteristics in HNSCC.

Shahini, A., et al. (2017). "NANOG Restores Contractility of Mesenchymal Stem Cell-Based Senescent Microtissues." Tissue Eng Part A **23**(11-12): 535-545.

Mesenchymal stem cells (MSCs) have been extensively used in the field of tissue engineering as a source of smooth muscle cells (SMCs). However, recent studies showed deficits in the contractile function of SMCs derived from senescent MSCs and there are no available strategies to restore the contractile function that is impaired due to cellular or organismal senescence. In this study, we developed a tetracycline-regulatable system and employed micropost tissue arrays to evaluate the effects of the embryonic transcription factor, NANOG, on the contractility of senescent MSCs. Using this system, we show that expression of NANOG fortified the actin cytoskeleton and restored contractile function that was impaired in senescent MSCs. NANOG increased the expression of smooth muscle alpha-actin (ACTA2) as well as the contractile force generated by cells in three-dimensional microtissues. Interestingly, NANOG worked together with transforming growth factor-beta1 to further enhance the contractility of senescent microtissues. The effect of NANOG on contractile function was sustained for about 10 days after termination of its expression. Our results show that NANOG could reverse the effects of stem cell senescence and restore the myogenic differentiation potential of senescent MSCs. These findings may enable development of novel strategies to restore the function of senescent cardiovascular and other SMC-containing tissues.

Shankar, S., et al. (2011). "Resveratrol inhibits pancreatic cancer stem cell characteristics in human and KrasG12D transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition." PLoS One **6**(1): e16530.

BACKGROUND: Cancer stem cells (CSCs) can proliferate and self-renew extensively due to their ability to express anti-apoptotic and drug resistant proteins, thus sustaining tumor growth. Therefore, the strategy to eradicate CSCs might have significant clinical implications. The objectives of this study were to examine the molecular mechanisms by which resveratrol inhibits stem cell characteristics of pancreatic CSCs derived from human primary tumors and Kras (G12D) transgenic mice. METHODOLOGY/PRINCIPAL FINDINGS: Human pancreatic CSCs (CD133(+)CD44(+)CD24(+)ESA (+)) are highly tumorigenic and form subcutaneous tumors in NOD/SCID mice. Human pancreatic CSCs expressing high levels of CD133, CD24, CD44, ESA, and aldehyde dehydrogenase also express significantly more Nanog, Oct-4, Notch1, MDR1 and ABCG2 than normal pancreatic tissues and primary pancreatic cancer cells. Similarly, CSCs from Kras (G12D) mice express significantly higher levels of Nanog and Oct-4 than pancreatic tissues from Pdx-Cre mice. Resveratrol inhibits the growth (size and weight) and development (PanIN lesions) of pancreatic cancer in Kras (G12D) mice. Resveratrol inhibits the self-renewal capacity of pancreatic CSCs derived from human primary tumors and Kras (G12D) mice. Resveratrol induces apoptosis by activating capase-3/7 and inhibiting the expression of Bcl-2 and XIAP in human CSCs. Resveratrol inhibits pluripotency maintaining factors (Nanog, Sox-2, c-Myc and Oct-4) and drug resistance gene ABCG2 in CSCs. Inhibition of Nanog by shRNA enhances the inhibitory effects of resveratrol on self-renewal capacity of CSCs. Finally, resveratrol inhibits CSC's migration and invasion and markers of epithelial-mesenchymal transition (Zeb-1, Slug and Snail). CONCLUSIONS/SIGNIFICANCE: These data suggest that resveratrol inhibits pancreatic cancer stem cell characteristics in human and Kras (G12D) transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition. In conclusion, resveratrol can be used for the management of pancreatic cancer.

Shofuda, T., et al. (2013). "Human Decidua-Derived Mesenchymal Cells Are a Promising Source for the Generation and Cell Banking of Human Induced Pluripotent Stem Cells." Cell Med **4**(3): 125-147.

Placental tissue is a biomaterial with remarkable potential for use in regenerative medicine. It has a three-layer structure derived from the fetus (amnion and chorion) and the mother (decidua), and it contains huge numbers of cells. Moreover, placental tissue can be collected without any physical danger to the donor

and can be matched with a variety of HLA types. The decidua-derived mesenchymal cells (DMCs) are highly proliferative fibroblast-like cells that express a similar pattern of CD antigens as bone marrow-derived mesenchymal cells (BM-MSCs). Here we demonstrated that induced pluripotent stem (iPS) cells could be efficiently generated from DMCs by retroviral transfer of reprogramming factor genes. DMC-hiPS cells showed equivalent characteristics to human embryonic stem cells (hESCs) in colony morphology, global gene expression profile (including human pluripotent stem cell markers), DNA methylation status of the OCT3/4 and NANOG promoters, and ability to differentiate into components of the three germ layers in vitro and in vivo. The RNA expression of XIST and the methylation status of its promoter region suggested that DMC-iPSCs, when maintained undifferentiated and pluripotent, had three distinct states: (1) complete X-chromosome reactivation, (2) one inactive X-chromosome, or (3) an epigenetic aberration. Because DMCs are derived from the maternal portion of the placenta, they can be collected with the full consent of the adult donor and have considerable ethical advantages for cell banking and the subsequent generation of human iPS cells for regenerative applications.

Soong, Y. K., et al. (2015). "The use of human amniotic fluid mesenchymal stem cells as the feeder layer to establish human embryonic stem cell lines." J Tissue Eng Regen Med **9**(12): E302-307.

Human embryonic stem cells (hESCs) are pluripotent cells that have the potential to differentiate into the three germ layers and possibly all tissues of the human body. To fulfil the clinical potentials for cell-based therapy, banks of hESC lines that express different combinations of the major histocompatibility genes should be established, preferably without exposing such cells to animal cells and proteins. In this study, we tested human amniotic fluid mesenchymal stem cells (AFMSCs) as feeder cells to support the growth of hESCs. Our results indicated that mitomycin-treated AFMSCs were able to support the newly established hESC lines CGLK-1 and CGLK-2. The hESC colonies cultured on AFMSCs expressed alkaline phosphatase (ALK-P), SSEA-4, TRA-1-60, TRA-1-81, Oct-4, Nanog and Sox-2, which are markers for undifferentiated hESCs. Chromosomal analyses of both hESC lines, CGLK-1 and CGLK-2, which were cultured on AFMSC feeders for 22 and 14 passages, respectively, were confirmed to be normal karyotypes (46, XX). The ability of AFMSCs as feeder cells to maintain the undifferentiated growth and pluripotency of hESCs was confirmed by in vivo formation of teratomas derived on AFMSC hESCs in severe combined immune-compromised mice. The use of AFMSCs for feeder cells to culture hESCs has several advantages, in that AFMSCs are not tumourigenic and can be expanded extensively with a short doubling time.

Sun, Z., et al. (2015). "MicroRNA-34a regulates epithelial-mesenchymal transition and cancer stem cell phenotype of head and neck squamous cell carcinoma in vitro." Int J Oncol **47**(4): 1339-1350.

MicroRNAs (miRs) are short non-coding single stranded RNAs regulating the translation of target mRNAs in normal and cancer cells in which they are frequently dysregulated promoting tumor progression. Cancer stem cells (CSCs) of head and neck squamous cell carcinoma (HNSCC), identified by aldehyde-dehydrogenase expression (ALDH), are a cell subset within the tumor cell population that takes part in the genesis and progression of cancer. The relevance of epithelial-mesenchymal transition (EMT) has recently been recognized for tumor development and metastasis. Several studies have illustrated that miRs regulate EMT of CSC. CSC from 8 HNSCC lines, 4 of which are human papillomavirus (HPV)positive, were enriched by spheroid culture (spheroid-derived cells, SDC) and compared to their parental monolayer-derived cells (MDC) to analyze expression patterns of miR34a, CSC-related transcription factors (CSC-TFs: Sox2, Nanog, Oct3/4) and EMT-related TFs (EMT-TFs: Twist, Snail1, Snail2) by RT-qPCR. Flow cytometry was used to quantify and enrich ALDH+ CSCs. Transfection of miR34a mimics was used to evaluate its regulatory potential for CSC marker profiles as well as CSC- and EMT-TFs expression in HNSCC-SDC. Invasive, colony-forming and clonogenic capability of the miR34a mimics transfected SDC after sorting for ALDH+ and ALDH- cells was assessed by Matrigel invasion, clonogenicity and spheroid formation assay, respectively. miR34a expression levels were significantly downregulated in the majority of SDC derived from HNSCC-lines as compared to parental MDC (-1.6-16.4-fold). For EMT- and CSC-related TF expression, all HNSCC-derived SDC showed a significantly increased level compared to parental MDC (</=36.8-fold). Significantly increased expression of ALDH was found in SDC (2-3-fold). Compared to the HPV+, the HPV- group showed a significantly higher mean expression level of EMT-TFs, CSCs-TFs and ALDH (30.3 v.s. 12.8%). Transfection of miR34a mimics significantly reduced the EMT- and CSC-related TF expression level in UM-SCC9 (HPV-) and UM-SCC47 (HPV+) SDC. Simultaneously, the ALDH expression was reduced significantly (1.5-2-fold) and the invasive capacity (</=30%) and clonogenicity of HNSCC-SDC was also inhibited by transfection of miR34a mimics compared to controls. Restoration of miR34a significantly

inhibited the capability for EMT formation of CSC-phenotype and functionally reduced clonogenic and invasive capacity in HNSCC cell lines. Therapeutic modulation of miR34a in HNSCC and CSCs may reduce the rate of metastasis and recurrence of tumors after therapy.

Tong, C. K., et al. (2011). "Generation of mesenchymal stem cell from human umbilical cord tissue using a combination enzymatic and mechanical disassociation method." Cell Biol Int **35**(3): 221-226.

MSCs (mesenchymal stem cells) promise a great potential for regenerative medicine due to their unique properties of self-renewal, high plasticity, modulation of immune response and the flexibility for genetic modification. Therefore, the increasing demand for cellular therapy necessitates a larger-scale production of MSC; however, the technical and ethical issues had put a halt on it. To date, studies have shown that MSC could be derived from human UC (umbilical cord), which is once considered as clinical waste. We have compared the two conventional methods which are classic enzymatic digestion and explant method with our newly tailored enzymatic-mechanical disassociation method to generate UC-MSC. The generated UC-MSCs from the methods above were characterized based on their immunophenotyping, early embryonic transcription factors expression and mesodermal differentiation ability. Our results show that enzymatic-mechanical disassociation method increase the initial nucleated cell yield greatly (approximately 160-fold) and maximized the successful rate of UC-MSC generation. Enzymatic-mechanical disassociation-derived UC-MSC exhibited fibroblastic morphology and surface markers expression of CD105, CD73, CD29, CD90 and MHC class I. Furthermore, these cells constitutively express early embryonic transcription factors (Nanog, Oct-4, Sox-2 and Rex-1), as confirmed by RT-PCR, indicating their multipotency and high self-renewal capacity. They are also capable of differentiating into osteoblasts and adipocytes when given an appropriate induction. The present study demonstrates a new and efficient approach in generating MSC from UC, hence serving as ideal alternative source of mesenchymal stem cell for clinical and research use.

Tooi, M., et al. (2016). "Placenta Mesenchymal Stem Cell Derived Exosomes Confer Plasticity on Fibroblasts." J Cell Biochem **117**(7): 1658-1670.

Mesenchymal stem cell (MSC)-conditioned medium (MSC-CM) has been reported to enhance wound healing. Exosomes contain nucleic acids, proteins, and lipids, and function as an intercellular communication vehicle for mediating some paracrine effects. However, the function of MSC-derived exosomes (MSC-exo) remains elusive. In this study, we isolated human placenta MSC (PlaMSC)-derived exosomes (PlaMSC-exo) and examined their function in vitro. PlaMSCs were isolated from human term placenta using enzymatic digestion. PlaMSC-exo were prepared from the conditioned medium of PlaMSC (PlaMSC-CM) by ultracentrifugation. The expression of stemness-related genes, such as OCT4 and NANOG, in normal adult human dermal fibroblasts (NHDF) after incubation with PlaMSC-exo was measured by real-time reverse transcriptase PCR analysis (real-time PCR). The effect of PlaMSC-exo on OCT4 transcription activity was assessed using Oct4-EGFP reporter mice-derived dermal fibroblasts. The stimulating effects of PlaMSC-exo on osteoblastic and adipocyte-differentiation of NHDF were evaluated by alkaline phosphatase (ALP), and Alizarin red S- and oil red O-staining, respectively. The expression of osteoblast- and adipocyte-related genes was also assessed by real-time PCR. The treatment of NHDF with PlaMSC-exo significantly upregulated OCT4 and NANOG mRNA expression. PlaMSC-exo also enhanced OCT4 transcription. The NHDF treated with PlaMSC-exo exhibited osteoblastic and adipocyte-differentiation in osteogenic and adipogenic induction media. PlaMSC-exo increase the expression of OCT4 and NANOG mRNA in fibroblasts. As a result, PlaMSC-exo influence the differentiation competence of fibroblasts to both osteoblastic and adipocyte-differentiation. It shows a new feature of MSCs and the possibility of clinical application of MSC-exo. J. Cell. Biochem. 117: 1658-1670, 2016. (c) 2015 Wiley Periodicals, Inc.

Trohatou, O., et al. (2014). "Sox2 suppression by miR-21 governs human mesenchymal stem cell properties." Stem Cells Transl Med **3**(1): 54-68.

MicroRNAs (miRNAs) have recently been shown to act as regulatory signals for maintaining stemness and for determining the fate of adult and fetal stem cells, such as human mesenchymal stem cells (hMSCs). hMSCs constitute a population of multipotent stem cells that can be expanded easily in culture and are able to differentiate into many lineages. We have isolated two subpopulations of fetal mesenchymal stem cells (MSCs) from amniotic fluid (AF) known as spindle-shaped (SS) and round-shaped (RS) cells and characterized them on the basis of their phenotypes, pluripotency, proliferation rates, and differentiation potentials. In this study, we analyzed the miRNA profile of MSCs derived from AF, bone marrow (BM), and umbilical cord blood (UCB). We initially identified 67 different miRNAs that were expressed in all three types of MSCs but at different levels, depending on the source. A more detailed analysis revealed that miR-21 was expressed at higher

levels in RS-AF-MSCs and BM-MSCs compared with SS-AF-MSCs. We further demonstrated for the first time a direct interaction between miR-21 and the pluripotency marker Sox2. The induction of miR-21 strongly inhibited Sox2 expression in SS-AF-MSCs, resulting in reduced clonogenic and proliferative potential and cell cycle arrest. Strikingly, the opposite effect was observed upon miR-21 inhibition in RS-AF-MSCs and BM-MSCs, which led to an enhanced proliferation rate. Finally, miR-21 induction accelerated osteogenesis and impaired adipogenesis and chondrogenesis in SS-AF-MSCs. Therefore, these findings suggest that miR-21 might specifically function by regulating Sox2 expression in human MSCs and might also act as a key molecule determining MSC proliferation and differentiation.

Varga, N., et al. (2011). "Mesenchymal stem cell like (MSCl) cells generated from human embryonic stem cells support pluripotent cell growth." Biochem Biophys Res Commun **414**(3): 474-480.

Mesenchymal stem cell like (MSCl) cells were generated from human embryonic stem cells (hESC) through embryoid body formation, and isolated by adherence to plastic surface. MSCl cell lines could be propagated without changes in morphological or functional characteristics for more than 15 passages. These cells, as well as their fluorescent protein expressing stable derivatives, efficiently supported the growth of undifferentiated human embryonic stem cells as feeder cells. The MSCl cells did not express the embryonic (Oct4, Nanog, ABCG2, PODXL, or SSEA4), or hematopoietic (CD34, CD45, CD14, CD133, HLA-DR) stem cell markers, while were positive for the characteristic cell surface markers of MSCs (CD44, CD73, CD90, CD105). MSCl cells could be differentiated toward osteogenic, chondrogenic or adipogenic directions and exhibited significant inhibition of mitogen-activated lymphocyte proliferation, and thus presented immunosuppressive features. We suggest that cultured MSCl cells can properly model human MSCs and be applied as efficient feeders in hESC cultures.

Violini, S., et al. (2009). "Horse bone marrow mesenchymal stem cells express embryo stem cell markers and show the ability for tenogenic differentiation by in vitro exposure to BMP-12." BMC Cell Biol **10**: 29.

BACKGROUND: Mesenchymal stem cells (MSCs) have been recently investigated for their potential use in regenerative medicine. MSCs, in particular, have great potential, as in various reports they have shown pluripotency for differentiating into many different cell types. However, the ability of MSCs to differentiate into tendon cells in vitro has not been fully investigated. RESULTS: In this study, we show that equine bone marrow mesenchymal stem cells (BM-MSCs), defined by their expression of markers such as Oct4, Sox-2 and Nanog, have the capability to differentiate in tenocytes. These differentiated cells express tendon-related markers including tenomodulin and decorin. Moreover we show that the same BM-MSCs can differentiate in osteocytes, as confirmed by alkaline phosphatase and von Kossa staining. CONCLUSION: As MSCs represent an attractive tool for tendon tissue repair strategies, our data suggest that bone marrow should be considered the preferred MSC source for therapeutic approaches.

Watson, A. T. D., et al. (2018). "Experimental evidence for an inhibitory role of AhR activation in human mesenchymal stem cell differentiation." Toxicol Sci.

Multipotent mesenchymal stem cells maintain the ability to differentiate into adipogenic, chondrogenic, or osteogenic cell lineages. There is increasing concern that exposure to environmental agents such as aryl hydrocarbon receptor (AhR) ligands, may perturb the osteogenic pathways responsible for normal bone formation. The objective of the current study was to evaluate the potential of the prototypic AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to disrupt osteogenic differentiation of human bone-derived mesenchymal stem cells (hBMSCs) in vitro. Primary hBMSCs from three donors were exposed to 10 nM TCDD and differentiation was interrogated using select histological, biochemical, and transcriptional markers of osteogenesis. Exposure to 10 nM TCDD resulted in an overall consistent attenuation of alkaline phosphatase (ALP) activity and matrix mineralization at terminal stages of differentiation in primary hBMSCs. At the transcriptional level, the transcriptional regulator DLX5 and additional osteogenic markers (ALP, OPN, and IBSP) displayed attenuated expression; conversely, FGF9 and FGF18 were consistently upregulated in each donor. Expression of stem cell potency markers SOX2, NANOG, and SALL4 decreased in the osteogenic controls, while expression in TCDD-treated cells resembled that of undifferentiated cells. Co-exposure with the AhR antagonist GNF351 blocked TCDD-mediated attenuation of matrix mineralization, and either fully or partially rescued expression of genes associated with osteogenic regulation, ECM, and/or maintenance of multipotency. Thus, experimental evidence from this study suggests that AhR transactivation likely attenuates osteoblast differentiation in multipotent hBMSCs. This study also underscores the use of primary human MSCs to

evaluate osteoinductive or osteotoxic potential of chemical and pharmacologic agents in vitro.

Wei, F., et al. (2012). "Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity." J Cell Physiol **227**(9): 3216-3224.

Cell sheet engineering has been developed as an alternative approach to improve mesenchymal stem cell-mediated tissue regeneration. In this study, we found that vitamin C (Vc) was capable of inducing telomerase activity in periodontal ligament stem cells (PDLSCs), leading to the up-regulated expression of extracellular matrix type I collagen, fibronectin, and integrin beta1, stem cell markers Oct4, Sox2, and Nanog as well as osteogenic markers RUNX2, ALP, OCN. Under Vc treatment, PDLSCs can form cell sheet structures because of increased cell matrix production. Interestingly, PDLSC sheets demonstrated a significant improvement in tissue regeneration compared with untreated control dissociated PDLSCs and offered an effective treatment for periodontal defects in a swine model. In addition, bone marrow mesenchymal stem cell sheets and umbilical cord mesenchymal stem cell sheets were also well constructed using this method. The development of Vc-mediated mesenchymal stem cell sheets may provide an easy and practical approach for cell-based tissue regeneration.

Wen, L., et al. (2016). "Role of Endothelial Progenitor Cells in Maintaining Stemness and Enhancing Differentiation of Mesenchymal Stem Cells by Indirect Cell-Cell Interaction." Stem Cells Dev **25**(2): 123-138.

A hot issue in current research regarding stem cells for regenerative medicine is the retainment of the stemness and multipotency of stem cell. Endothelial progenitor cells (EPCs) are characterized by an angiogenic switch that induces angiogenesis and further ameliorates the local microenvironment in ischemic organs. This study investigated whether EPCs could modulate the multipotent and differential abilities of mesenchymal stem cells (MSCs) in vitro and in vivo. We established an EPC/MSC indirect Transwell coculture system and then examined the effects of EPCs on the regulation of MSC biological properties in vitro and bone formation in vivo. The in vitro studies showed that cocultured MSCs (coMSCs) display no overt changes in cell morphology but an enhanced MSC phenotype compared with monocultured MSCs (monoMSCs). Our studies regarding the cellular, molecular, and protein characteristics of coMSCs and monoMSCs demonstrated that EPCs greatly promote the proliferation and differentiation potentials of coMSCs under indirect coculture condition. The expression of the pluripotency factors OCT4, SOX2, Nanog, and Klf4 was also upregulated in coMSCs. Furthermore, coMSCs combined with fibrin glue showed improved bone regeneration when used to repair rat alveolar bone defects compared with monoMSC grafts in vivo. This study is the first to demonstrate that EPCs have dynamic roles in maintaining MSC stemness and regulating MSC differentiation potential.

Windmolders, S., et al. (2014). "Mesenchymal stem cell secreted platelet derived growth factor exerts a pro-migratory effect on resident Cardiac Atrial appendage Stem Cells." J Mol Cell Cardiol **66**: 177-188.

Mesenchymal stem cells (MSCs) modulate cardiac healing after myocardial injury through the release of paracrine factors, but the exact mechanisms are still unknown. One possible mechanism is through mobilization of endogenous cardiac stem cells (CSCs). This study aimed to test the pro-migratory effect of MSC conditioned medium (MSC-CM) on endogenous CSCs from human cardiac tissue. By using a three-dimensional collagen assay, we found that MSC-CM improved migration of cells from human cardiac tissue. Cell counts, perimeter and area measurements were utilized to quantify migration effects. To examine whether resident stem cells were among the migrating cells, specific stem cell properties were investigated. The migrating cells displayed strong similarities with resident Cardiac Atrial appendage Stem Cells (CASCs), including a clonogenic potential of ~21.5% and expression of pluripotency associated genes like Oct-4, Nanog, c-Myc and Klf-4. Similar to CASCs, migrating cells demonstrated high aldehyde dehydrogenase activity and were able to differentiate towards cardiomyocytes. Receptor tyrosine kinase analysis and collagen assays performed with recombinant platelet derived growth factor (PDGF)-AA and Imatinib Mesylate, a PDGF receptor inhibitor, suggested a role for the PDGF-AA/PDGF receptor alpha axis in enhancing the migration process of CASCs. In conclusion, our findings demonstrate that factors present in MSC-CM improve migration of resident stem cells from human cardiac tissue. These data open doors towards future therapies in which MSC secreted factors, like PDGF-AA, can be utilized to enhance the recruitment of CASCs towards the site of myocardial injury.

Xu, M. H., et al. (2014). "EMT and acquisition of stem cell-like properties are involved in spontaneous formation of tumorigenic hybrids between lung cancer and bone marrow-derived mesenchymal stem cells." PLoS One **9**(2): e87893.

The most deadly phase in cancer progression is metastatic conversion. Epithelial-to-mesenchymal transition (EMT) is a key process by which cancer cells acquire invasive and metastatic phenotypes. In order to spawn macroscopic metastases, disseminated cancer cells would seem to require self-renewal capability. However, the underlying mechanism defining these processes is poorly understood. One possible mechanism underlying metastasis is fusion between myeloid cells and cancer cells. In this study, we found that spontaneously-formed tumorigenic hybrids between bone marrow-derived mesenchymal stem cells (MSCs) and three different non-small cell lung cancer (NSCLC) cell lines contributed to highly malignant subpopulations with both EMT and stem cell-like properties. Hybrids lost their epithelial morphology and assumed a fibroblast-like appearance. Up-regulation of vimentin, alpha-smooth muscle actin (alpha-SMA), and fibronectin, and down-regulation of E-cadherin and pancytokeratin were observed in tumorigenic hybrids. These cells also exhibited increased expression of the stem cell marker prominin-1 (CD133) and over-expression of transcription factors OCT4, Nanog, BMI1, Notch1, ALDH1 as well as Sox2, all genes responsible for regulating and maintaining the stem cell phenotype. In addition, in spontaneously-formed tumorigenic hybrids, increased pneumosphere-forming capacity and tumor-forming ability in NOD/SCID mice were detectable. Thus, cell fusion between lung cancer cells and MSCs provides a nonmutational mechanism that could contribute to aberrant gene expression patterns and give rise to highly malignant subpopulations both capable of EMT and with properties of cancer stem cells (CSCs).

Yan, X., et al. (2017). "Clinical significance of Fusobacterium nucleatum, epithelial-mesenchymal transition, and cancer stem cell markers in stage III/IV colorectal cancer patients." Onco Targets Ther **10**: 5031-5046.

Colorectal cancer (CRC) is a common digestive malignancy and emerging studies have closely linked its initiation and development with gut microbiota changes. Fusobacterium nucleatum (Fn) has been recently identified as a pathogenic bacteria for CRC; however, its prognostic significance for patients is poorly investigated and is less for patients within late stage. Therefore, in this study, we made efforts to analyze its level and prognostic significance in a retrospective cohort of 280 stage III/IV CRC patients. We found that the Fn level was abnormally high in tumor tissues and correlated with tumor invasion, lymph node metastasis status, and distant metastasis. We also identified it as an independent adverse prognostic factor for cancer-specific survival (CSS) and disease-free survival (DFS). The following subgroup analysis indicated that Fn level could stratify CSS and DFS in stage IIIB/C and IV patients but failed in stage IIIA patients. In addition, stage III/IV patients with low Fn level were found to benefit more from adjuvant chemotherapy than those with high Fn level, in terms of DFS. Finally, we analyzed the expression and clinical significance of epithelial-to-mesenchymal transition (EMT) markers (E-cadherin and N-cadherin) and cancer stem cell (CSC) markers (Nanog, Oct-4, and Sox-2) in CRC tissues. The results indicated that N-cadherin, Nanog, Oct-4, and Sox-2 were adverse prognostic factors in these patients, while the opposite was true for E-cadherin. More importantly, expression of E-cadherin, N-cadherin, and Nanog was significantly correlated with Fn level in tumor tissues, suggesting the potential involvement of Fn in EMT-CSC cross talk during CRC progression. Taken together, these findings indicate that Fn is a novel predictive biomarker for clinical management in stage III/IV patients, and targeting Fn may be an effective adjuvant approach for preventing CRC metastasis and chemotherapy resistance.

Yang, C. K., et al. (2018). "C-terminus of Hsc70-interacting protein (CHIP) enhances stemness properties of human Wharton's jelly mesenchymal stem cell." Biotech Histochem: 1-8.

Mesenchymal stem cells are an attractive source of multipotent cells in part because they are easy to obtain. Several E3 ligases regulate the stability and functions of various factors in different adult stem cells through the ubiquitylation pathway. We investigated the C-terminus of Hsc70-interacting protein (CHIP) E3 ligase that regulates pluripotency of human Wharton's jelly mesenchymal stem cells (hWJMSC). We found that CHIP increases protein kinase B (Akt) phosphorylation by decreased expression of phosphatase and tensin homolog (PTEN), which suggests improvement of the survival pathway by CHIP over-expression. We also found that increased CHIP expression induced Sox2 and NANOG, which can promote stem cell self-renewal and prevent oxidative stress-induced senescence of hWJMSC by decreased p21. We found that CHIP could be used to enhance the multiple functions of hWJMSC.

Yin, X., et al. (2015). "Coexpression of gene Oct4 and Nanog initiates stem cell characteristics in hepatocellular carcinoma and promotes epithelial-mesenchymal transition through activation of Stat3/Snail signaling." J Hematol Oncol **8**: 23.

BACKGROUND: Oct4 and Nanog are key regulatory genes that maintain the pluripotency and self-renewal properties of embryonic stem cells. We previously reported that the two stemness markers

were tightly associated with cancer progression and poor outcomes of hepatocellular carcinoma. In this study, we demonstrate that coexpression of Oct4/Nanog modulates activation of signal transducer and activator of transcription 3 (Stat3), an oncogenic transcription factor that is activated in many human malignancies including hepatocellular carcinoma (HCC), as well as the expression of Snail, a key regulator implicated in epithelial-mesenchymal transition and tumor metastasis. METHODS: Oct4 and Nanog were ectopic expressed in MHCC97-L cell lines via lentiviral gene transfection. The stemness characteristics including self-renewal, proliferation, chemoresistance, and tumorigenicity were assessed. The effect of coexpression of Oct4 and Nanog on epithelial-mesenchymal transition change, and the underlying molecular signaling was investigated. RESULTS: Ectopic coexpression of Oct4 and Nanog empowered MHCC97-L cells with cancer stem cell (CSC) properties, including self-renewal, extensive proliferation, drug resistance, and high tumorigenic capacity. Significantly, Oct4 and Nanog encouraged epithelial-mesenchymal transition change contributing to tumor migration, invasion/metastasis in vitro and in vivo. Following molecular mechanism investigation indicated Oct4/Nanog-regulated epithelial-mesenchymal transition change through Stat3-dependent Snail activation. Moreover, silencing Stat3 abrogates Oct4/Nanog-mediated epithelial-mesenchymal transition (EMT) change and invasion/metastasis in HCC. CONCLUSIONS: We delineate Oct4 and Nanog initiate stem cell characteristics in hepatocellular carcinoma and promote epithelial-mesenchymal transition through activation of Stat3/Snail signaling. Our findings propose Stat3/Snail pathway as a novel therapeutic target for the treatment of progression and metastasis of HCC with CSC-like signatures and epithelial-mesenchymal transition phenotype.

Yoon, D. S., et al. (2011). "Importance of Sox2 in maintenance of cell proliferation and multipotency of mesenchymal stem cells in low-density culture." Cell Prolif **44**(5): 428-440.

OBJECTIVES: This study has aimed to repopulate 'primitive' cells from late-passage mesenchymal stem cells (MSCs) of poor multipotentiality and low cell proliferation rate, by simply altering plating density. MATERIALS AND METHODS: Effects of low density culture compared t high density culture on late-passage bone marrow (BM)-derived MSCs and pluripotency markers of multipotentiality were investigated. Cell proliferation, gene expression, RNA interference and differentiation potential were assayed. RESULTS AND CONCLUSIONS: We repopulated 'primitive' cells by replating late-passage MSCs at low density (17 cells/cm (2)) regardless of donor age. Repopulated MSCs from low-density culture were smaller cells with spindle shaped morphology compared to MSCs from high-density culture. The latter had enhanced colony-forming ability, proliferation rate, and adipogenic and chondrogenic potential. Strong expression of osteogenic-related genes (Cbfa1, Dlx5, alkaline phosphatase and type Iota collagen) in late-passage MSCs was reduced by replating at low density, whereas expression of three pluripotency markers (Sox2, Nanog and Oct-4), Osterix and Msx2 reverted to levels of early-passage MSCs. Knockdown of Sox2 and Msx2 but not Nanog, using RNA interference, showed significant decrease in colony-forming ability. Specifically, knockdown of Sox2 significantly inhibited multipotentiality and cell proliferation. Our data suggest that plating density should be considered to be a critical factor for enrichment of 'primitive' cells from heterogeneous BM and that replicative senescence and multipotentiality of MSCs during in vitro expansion may be predominantly regulated through Sox2.

Yu, C. Y. and H. C. Kuo (2016). "The Trans-Spliced Long Noncoding RNA tsRMST Impedes Human Embryonic Stem Cell Differentiation Through WNT5A-Mediated Inhibition of the Epithelial-to-Mesenchymal Transition." Stem Cells **34**(8): 2052-2062.

The trans-spliced noncoding RNA RMST (tsRMST) is an emerging regulatory lncRNA in the human pluripotency circuit. Previously, we found that tsRMST represses lineage-specific transcription factors through the PRC2 complex and NANOG in human pluripotent stem cells (hESCs). Here, we demonstrate that tsRMST also modulates noncanonical Wnt signaling to suppress the epithelial-to-mesenchymal transition (EMT) and in vitro differentiation of embryonic stem cells (ESCs). Our results demonstrate that disruption of tsRMST expression in hESCs results in the upregulation of WNT5A, EMT, and lineage-specific genes/markers. Furthermore, we found that the PKC inhibitors Go6983 and Go6976 inhibited the effects of WNT5A, indicating that WNT5A promotes the EMT and in vitro differentiation although conventional and novel PKC activation in hESCs. Finally, we showed that either antiserum neutralization of WNT5A or Go6983 treatment in tsRMST knockdown cells decreased the expression of mesenchymal and lineage-specific markers. Together, these findings indicate that tsRMST regulates Wnt and EMT signaling pathways in hESCs by repressing WNT5A, which is a potential EMT inducer for promoting in vitro differentiation of hESCs through PKC activation. Our findings provide

further insights into the role of trans-spliced RNA and WNT5A in hESC differentiation, in which EMT plays an important role. Stem Cells 2016;34:2052-2062.

Yu, M. A., et al. (2012). "Nicotine promotes acquisition of stem cell and epithelial-to-mesenchymal properties in head and neck squamous cell carcinoma." PLoS One **7**(12): e51967.

The ability of nicotine to enhance the malignancy of cancer cells is known; however, the possibility that nicotine could regulate a cancer stem cell phenotype remains to be well-established. In this study we sought to determine whether long-term exposure to nicotine could promote cancer stem cell-like properties in two head and neck squamous cell carcinoma cell lines, UMSCC-10B and HN-1. Nicotine treatment induced epithelial-to-mesenchymal transition (EMT) in both cell lines by repressing E-cadherin expression, and led to the induction of stem cell markers Oct-4, Nanog, CD44 and BMI-1, which was reversed upon ectopic re-expression of E-cadherin. Nicotine-treated cells formed spheres at a higher efficiency than non-treated cells, formed larger tumors when injected into mice, and formed tumors with 4-fold greater efficiency compared to control cells when injected at limiting doses. Consistent with previous literature, nicotine-treated cells demonstrated a greater capacity for survival and also a higher tendency to invade. Comparison of microRNA profiles between nicotine and control cells revealed the upregulation of miR-9, a repressor of E-cadherin, and the downregulation of miR-101, a repressor of EZH2. Taken together, these results suggest that nicotine may play a critical role in the development of tobacco-induced cancers by regulating cancer stem cell characteristics, and that these effects are likely mediated through EMT-promoting, microRNA-mediated pathways. Further characterization of such pathways remains a promising avenue for the understanding and treatment of tobacco-related cancers.

Zhang, L., et al. (2017). "Doxycycline inhibits the cancer stem cell phenotype and epithelial-to-mesenchymal transition in breast cancer." Cell Cycle **16**(8): 737-745.

Experimental evidence suggest that breast tumors originate from breast cancer stem cells (BCSCs), and that mitochondrial biogenesis is essential for the anchorage-independent clonal expansion and survival of CSCs, thus rendering mitochondria a significant target for novel treatment approaches. One of the recognized side effects of the FDA-approved drug, doxycycline is the inhibition of mitochondrial biogenesis. Here we investigate the mechanism by which doxycycline exerts its inhibitory effects on the properties of breast cancer cells and BCSCs, such as mammosphere forming efficiency, invasion, migration, apoptosis, the expression of stem cell markers and epithelial-to-mesenchymal transition (EMT) related markers of breast cancer cells. In addition, we explored whether autophagy plays a role in the inhibitory effect of doxycycline on breast cancer cells. We find that doxycyline can inhibit the viability and proliferation of breast cancer cells and BCSCs, decrease mammosphere forming efficiency, migration and invasion, and EMT of breast cancer cells. Expression of stem cell factors Oct4, Sox2, Nanog and CD44 were also significantly downregulated after doxycycline treatment. Moreover, doxycycline could down-regulate the expression of the autophagy marker LC-3BI and LC-3BII, suggesting that inhibiting autophagy may be responsible in part for the observed effects on proliferation, EMT and stem cell markers. The potent inhibition of EMT and cancer stem-like characteristics in breast cancer cells by doxycycline treatment suggests that this drug can be repurposed as an anti-cancer drug in the treatment of breast cancer patients in the clinic.

Zhao, P., et al. (2017). "Bone marrow mesenchymal stem cells regulate stemness of multiple myeloma cell lines via BTK signaling pathway." Leuk Res **57**: 20-26.

Bone marrow mesenchymal stem cells (BM-MSCs) are key components of bone marrow microenvironment. Although the importances of BM-MSCs activation in myeloma cells growth, development, progression, angiogenesis are well known, their role in the regulation of myeloma stemness is unclear. In this study, myeloma cell lines (LP-1, U266) were co-cultured with BM-MSCs, we found that BM-MSCs could up-regulate the expression of key stemness genes and proteins (OCT4, SOX2, NANOG) and increase clonogenicity. Similarly, the mechanisms underlying the BM-MSC activation of myeloma stemness remain unclear. Here, we found that PCI-32765, a Bruton tyrosine kinase (BTK) inhibitor, treatment significantly down- regulate expression of key stemness genes and proteins in vitro co-culture system. Together, our results revealed that BM-MSCs could increase myeloma stemness via activation of the BTK signal pathway.

Zhao, X. F., et al. (2013). "[The research of fibroblasts from human hypertrophic scar showing a mesenchymal stem cell phenotype and multilineage differentiation potentialities]." Zhonghua Zheng Xing Wai Ke Za Zhi **29**(4): 273-279.

OBJECTIVE: To explore the CD phenotypic, protein expression and pluripotent differentiation of human hypertrophic scar fibroblasts cultured in vitro, so as to study the mechanisms of scar formation.

METHODS: Fibroblasts were isolated and cultured from human hypertrophic scar of 3 cases. The cells morphology was observed by inverted microscope, and the growing state of the third passage was detected by the cell counting meter of Vi-CELL. The cell surface markers CD105, CD14, CD73, CD34, CD44, CD45 and CD90 were identified by flow cytometry. The expression of CK19, Oct-4, Nanog and vimentin was detected by immunocytochemistry, and the expression of alpha-smooth muscle actin (alpha-SMA) was tested by immunofluorescence. The differentiated potential of fibroblasts of the third passage into adipogenic, osteogenic and chondrogenic lineages was assayed. RESULTS: The primary passage fibroblasts showed the shape of spindle shaped or irregular polygon with a radiated or circinate of growing arrangement. The growth curve showed the cells growth was slow on the first and second day, and quick during the third to fifth day, which reached platform stage on the sixth or seventh day. The fibroblasts highly expressed mesenchymal stem cell surface markers-CD73, CD105, CD44, CD90, but not expressed hematopoietic stem cell surface markers-CD14, CD34, CD45 by flow cytometry. And positive expression of vimentin, Oct-4 and negative expression of CK19 were detected by Immunocytochemistry. Positive expression of alpha-SMA was also detected by immunofluorescence. Multidirectional differentiation induction indicated that the third passage cells could differentiate into adipogenic, osteogenic and chondrogenic lineages. CONCLUSIONS: Human hypertrophic scar-derived fibroblasts show the biologic characteristics of mesenchymal stem cells, which may play an important role in wound healing and hypertrophic scar formation.

Zhu, Y., et al. (2009). "Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1." Leukemia **23**(5): 925-933.

Mesenchymal stem cells (MSCs) have an inhibitory effect on tumor proliferation, but the precise mechanisms are not fully understood. Here, we identified DKK-1 (dickkopf-1), secreted by MSCs and acting as a negative regulator of WNT signaling pathway, to be one of the molecules responsible for the inhibitory effect. When DKK-1 was neutralized by anti-DKK-1 antibodies, or when the expression of DKK-1 was downregulated by RNA interference (RNAi), the inhibitory effects of MSCs on K562 cell proliferation were attenuated. We also provide evidence that the expression of DKK-1 by MSCs is regulated by NANOG, a transcriptional factor ubiquitously expressed in some stem cells. Using the Cellmax artificial capillary modules that eliminate the immunosuppressive properties of MSCs, we further showed that MSCs were able to inhibit proliferation of K562 cells in a humoral microenvironment. Meanwhile, we recapture this effect of MSCs on primary leukemic hematopoietic progenitors from patients. MSCs probably have a general inhibitory effect on their neighboring cells, including malignant cells, en route to achieving tissue homeostasis.

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