Stem Cell and Sox2- 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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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.

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.

Blaber, E. A., et al. (2014). "Mechanical unloading of bone in microgravity reduces mesenchymal and hematopoietic stem cell-mediated tissue regeneration." Stem Cell Res **13**(2): 181-201.

Mechanical loading of mammalian tissues is a potent promoter of tissue growth and regeneration, whilst unloading in microgravity can cause reduced tissue regeneration, possibly through effects on stem cell tissue progenitors. To test the specific hypothesis that mechanical unloading alters differentiation of bone marrow mesenchymal and hematopoietic stem cell lineages, we studied cellular and molecular aspects of how bone marrow in the mouse proximal femur responds to unloading in microgravity. Trabecular and cortical endosteal bone surfaces in the

femoral head underwent significant bone resorption in microgravity, enlarging the marrow cavity. Cells isolated from the femoral head marrow compartment showed significant down-regulation of gene expression markers for early mesenchymal and hematopoietic differentiation, including FUT1(-6.72), CSF2(-3.30), CD90(-3.33), PTPRC(-2.79), and GDF15(-2.45), but not stem cell markers, such as SOX2. At the cellular level, in situ histological analysis revealed decreased megakaryocyte numbers whilst erythrocytes were increased 2.33 fold. Furthermore, erythrocytes displayed elevated fucosylation and clustering adjacent to sinuses forming the marrow-blood barrier, possibly providing a mechanistic basis for explaining spaceflight anemia. Culture of isolated bone marrow cells immediately after microgravity exposure increased the marrow progenitor's potential for mesenchymal differentiation into in-vitro mineralized bone nodules, and hematopoietic differentiation into osteoclasts, suggesting an accumulation of undifferentiated progenitors during exposure to microgravity. These results support the idea that mechanical unloading of mammalian tissues in microgravity is a strong inhibitor of tissue growth and regeneration mechanisms, acting at the level of early mesenchymal and hematopoietic stem cell differentiation.

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.

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.

Gomez-Casal, R., et al. (2013). "Non-small cell lung cancer cells survived ionizing radiation treatment display cancer stem cell and epithelial-mesenchymal transition phenotypes." Mol Cancer **12**(1): 94.

Ionizing radiation (IR) is used for patients diagnosed with unresectable non small cell lung cancer (NSCLC), however radiotherapy remains largely palliative due to radioresistance. Cancer stem cells (CSCs), as well as epithelial-mesenchymal transition (EMT), may contribute to drug and radiation resistance mechanisms in solid tumors. Here we investigated the molecular phenotype of A549 and H460 NSCLC cells that survived treatment with IR (5Gy) and are growing as floating tumor spheres and cells that are maintained in a monolayer after irradiation.Non-irradiated and irradiated cells were collected after one week, seeded onto ultra low attachment plates and propagated as tumor spheres. Bulk NSCLC cells which survived radiation and grew in spheres express cancer stem cell surface and embryonic stem cell markers and are able to self-renew, and generate differentiated progeny. These cells also have a mesenchymal phenotype. Particularly, the radiation survived sphere cells express significantly higher levels of CSC markers (CD24 and CD44), nuclear beta-catenin and EMT markers (Snail1, Vimentin, and N-cadherin) than non-irradiated lung tumor sphere cells. Upregulated levels of Oct-4, Sox2 and beta-catenin were detected in H460 cells maintained in a monolayer after irradiation, but not in radiation survived adherent A459 cells.PDGFR-beta was upregulated in radiation survived sphere cells and in radiation survived adherent cells in both A549 and H460 cell lines. Combining IR treatment with axitinib or dasatinib, inhibitors with anti-PDFGR activity, potentiates the efficacy of NSCLC radiotherapy in vitro.Our findings suggest that radiation survived cells have a complex phenotype combining the properties of CSCs and EMT. CD44, SNAIL and PDGFR-beta are dramatically upregulated in radiation survived cells and might be considered as markers of radiotherapy response in NSCLC.

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.

Hussain, I., et al. (2012). "New approach to isolate mesenchymal stem cell (MSC) from human umbilical cord blood." Cell Biol Int **36**(7): 595-600.

HUCB (human umbilical cord blood) has been frequently used in clinical allogeneic HSC (haemopoietic stem cell) transplant. However, HUCB is poorly recognized as a rich source of MSC (mesenchymal stem cell). The aim of this study has been to establish a new method for isolating large number of MSC from HUCB to recognize it as a good source of MSC. HUCB samples were collected from women following their elective caesarean section. The new method (Clot Spot method) was carried out by explanting HUCB samples in mesencult complete medium and maintained in 37 degrees C, in a 5% CO2 and air incubator. MSC presence was established by quantitative and qualitative immunophenotyping of cells and using FITC attached to MSC phenotypic markers (CD29, CD73, CD44 and CD105). Haematopoietic antibodies (CD34 and CD45) were used as negative control. MSC differentiation was examined in neurogenic and adipogenic media. Immunocytochemistry was carried out for the embryonic markers: SOX2 (sex determining region Y-box 2), OLIG-4 (oligodendrocyte-4) and FABP-4 (fatty acid binding protein-4). The new method was compared with the conventional Rosset Sep method. MSC cultures using the Clot Spot method showed 3-fold increase in proliferation rate compared with conventional method. Also, the cells showed high expression of MSC markers CD29, CD73, CD44 and CD105, but lacked the expression of specific HSC markers (CD34 and CD45). The isolated MSC showed some differentiation by expressing the neurogenic (SOX2 and Olig4) and adipogenic (FABP-4) markers respectively. In conclusion, HUCB is a good source of MSC using this new technique.

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.

Kim, Y. J., et al. (2017). "Ursodeoxycholic acid suppresses epithelial-mesenchymal transition and cancer stem cell formation by reducing the levels of

peroxiredoxin II and reactive oxygen species in pancreatic cancer cells." Oncol Rep **38**(6): 3632-3638.

Reactive oxygen species (ROS) play a key role in cancer development and progression. Ursodeoxycholic acid (UDCA) may possess antioxidant, anti-inflammatory and chemoprophylatic effects. Therefore, we aimed to investigate the effects and mechanisms of UDCA treatment on pancreatic cancer cells. The pancreatic cancer cell lines HPAC and Capan-1 were treated with 0.2 mM UDCA. To examine alterations in the levels of intracellular ROS, the DCF-DA stain was used and both stemness and epithelial-mesenchymal transition (EMT)-related genes were quantified using qRT-PCR and western blot analysis. The pancreatic cancer sphere culture was performed following seven days of treatment with 0.2 mM UDCA, as an indicator of stemness. Following treatment with UDCA, the level of intracellular ROS was decreased in the pancreatic cancer cells. UDCA decreased both the phosphorylation of STAT3 and the expression of peroxiredoxin II (Prx2). Furthermore, the treatment resulted in the upregulation of E-cadherin and in the downregulation of N-cadherin. In addition, UDCA decreased the expression of sex determining region Y-box 2 (Sox2) and it diminished the number of pancreatic cancer spheres formed. In conclusion UDCA suppressed the levels of intracellular ROS and Prx2 and it decreased EMT and stem cell formation in pancreatic cancer cells. Therefore, UDCA may provide favorable therapeutic benefits, through its antioxidant effects, for patients with pancreatic cancer.

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.

Lee, H. K., et al. (2013). "Mesenchymal stem cells deliver synthetic microRNA mimics to glioma cells and glioma stem cells and inhibit their cell migration and self-renewal." Oncotarget **4**(2): 346-361.

MicroRNAs (miRNAs) have emerged as potential cancer therapeutics; however, their clinical use is hindered by lack of effective delivery mechanisms to tumor sites. Mesenchymal stem cells (MSCs) have been shown to migrate to experimental glioma and to exert anti-tumor effects by delivering cytotoxic compounds. Here, we examined the ability of MSCs derived from bone marrow, adipose tissue, placenta and umbilical cord to deliver synthetic miRNA mimics to glioma cells and glioma stem cells (GSCs). We examined the delivery of miR-124 and miR-145 mimics as glioma cells and GSCs express very low levels of these miRNAs. Using fluorescently labeled miRNA mimics and in situ hybridization, we demonstrated that all the MSCs examined delivered miR-124 and miR-145 mimics to co-cultured glioma cells and GSCs via gap junction-dependent and independent processes. The delivered miR-124 and miR-145 mimics significantly decreased the luciferase activity of their respected reporter target genes, SCP-1 and Sox2, and decreased the migration of glioma cells and the self-renewal of GSCs. Moreover, MSCs delivered Cy3-miR-124 mimic to glioma xenografts when administered intracranially. These results suggest that MSCs can deliver synthetic exogenous miRNA mimics to glioma cells and GSCs and may provide an efficient route of therapeutic miRNA delivery in vivo.

Lee, W. J., et al. (2015). "Cell source-dependent in vivo immunosuppressive properties of mesenchymal stem cells derived from the bone marrow and synovial fluid of minipigs." Exp Cell Res **333**(2): 273-288.

The in vitro differentiation and immunosuppressive capacity of mesenchymal stem cells (MSCs) derived from synovial fluid (SF-MSCs) and bone marrow extract (BM-MSCs) in an isogenic background of minipigs were comparatively analyzed in a collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis (RA). The proliferation capacity and expression of pluripotent transcription factors (Oct3/4 and Sox2) were significantly (P<0.05) higher in SF-MSCs than in BM-MSCs. The differentiation capacity of SF-MSCs into adipocytes, osteocytes and neurocytes was significantly (P<0.05) lower than that of BM-MSCs, and the differentiation capacity of SF-MSCs into chondrocytes was significantly (P<0.05) higher than that of BM-MSCs. Systemic injection of BM- and SF-MSCs significantly (P<0.05) ameliorated the clinical symptoms of CIA mice, with SF-MSCs having significantly (P<0.05) higher clinical and histopathological recovery scores than BM-MSCs. Furthermore, the immunosuppressive properties of SF-MSCs in CIA mice were associated with increased levels of the anti-inflammatory cytokine interleukin (IL)-10, and decreased levels of the pro-inflammatory cytokine IL-1beta and osteoclast-related sRANKL. In conclusion, SF-MSCs exhibited eminent pluripotency and differentiation capacity into chondrocytes, addition to substantial in vivo immunosuppressive capacity by elevating IL-10 and reducing IL-1beta levels in CIA mice.

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, Z., et al. (2011). "Epigenetic dysregulation in mesenchymal stem cell aging and spontaneous differentiation." PLoS One **6**(6): e20526.

BACKGROUND: Mesenchymal stem cells (MSCs) hold great promise for the treatment of difficult diseases. As MSCs represent a rare cell population, ex vivo expansion of MSCs is indispensable to obtain sufficient amounts of cells for therapies and tissue engineering. However, spontaneous differentiation and aging of MSCs occur during expansion and the molecular mechanisms involved have been poorly understood. METHODOLOGY/PRINCIPAL FINDINGS: Human MSCs in early and late passages were examined for their expression of genes involved in osteogenesis to determine their spontaneous differentiation towards osteoblasts in vitro, and of genes involved in self-renewal and proliferation for multipotent differentiation potential. In parallel, promoter DNA methylation and hostone H3 acetylation levels were determined. We found that MSCs underwent aging and spontaneous osteogenic differentiation upon regular culture expansion, with progressive downregulation of TERT and upregulation of osteogenic genes such as Runx2 and ALP. Meanwhile, the expression of genes associated with stem cell self-renewal such as Oct4 and Sox2 declined markedly. Notably, the altered expression of these genes were closely associated with epigenetic dysregulation of histone H3 acetylation in K9 and K14, but not with methylation of CpG islands in the promoter regions of most of these genes. bFGF promoted MSC proliferation and suppressed its spontaneous osteogenic differentiation, with corresponding changes in histone H3 acetylation in TERT, Oct4, Sox2, Runx2 and ALP genes. CONCLUSIONS/SIGNIFICANCE: Our results indicate that histone H3 acetylation, which can be modulated by extrinsic signals, plays a key role in regulating MSC aging and differentiation.

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.

Liu, L. J., et al. (2014). "Three-dimensional collagen scaffold enhances the human adenoid cystic carcinoma cancer stem cell and epithelial-mesenchymal transition properties." J Biomed Mater Res B Appl Biomater **102**(4): 772-780.

Three-dimensional (3D) cell culture may provide the architectures similar to the in vivo natural extracellular matrix condition for in vitro cultured cells. In this work, a 3D collagen scaffold was used to culture the adenoid cystic carcinoma (ACC) cells. The results showed that the 3D scaffold not only induced the diversification of cell morphologies but

also increased the cell proliferation. The transcription of matrix metalloproteinase and epithelial-mesenchymal transition were significantly increased in the cells cultured in 3D collagen scaffolds. In addition, the expression of cancer stem cell (CSC) markers of Sox2 and Oct4 were higher than that in 2D cultured cells. The 3D cultured ACC-83 cells showed more resistance to chemotherapeutic drugs. Thus, the 3D collagen scaffold could provide a useful model for CSCs study and anticancer therapeutics research in vitro.

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.

Matsumoto, T., et al. (2018). "TGF-beta-mediated LEFTY/Akt/GSK-3beta/Snail axis modulates epithelial-mesenchymal transition and cancer stem cell properties in ovarian clear cell carcinomas." Mol Carcinog **57**(8): 957-967.

Advanced ovarian clear cell carcinoma (OCCCa) shows poor prognosis with chemoresistance, which is associated with epithelial-mesenchymal transition (EMT)/cancer stem cell (CSC) features. The left-right determination factor (LEFTY), a novel member of the TGF-beta superfamily, is a marker of stemness. Here we focused on the functional roles of LEFTY in OCCCas. OCCCa cell lines that were cultured in STK2, a serum-free medium for mesenchymal stem cells, or treated with TGF-beta1 underwent morphological changes toward an EMT appearance, along with increased expression of LEFTY and Snail. The cells also showed CSC properties, as demonstrated by increases in the aldehyde dehydrogenase (ALDH)1(high) activity population, number of spheroid formation, and expression of several CSC markers. Inhibition of LEFTY expression induced decreases in the number of spindle-shaped cells and CSC features, while cells stably overexpressing LEFTY exhibited enhancement of such EMT/CSC properties. Finally, treatment of cells with TGF-beta1 led to increased LEFTY expression and activation of Akt, which subsequently induced inactivation of GSK-3beta, while inhibition of GSK-3beta resulted in increased expression of both LEFTY and Snail. In clinical samples, LEFTY expression showed a tendency for positive associations with expression of vimentin, as well as Sox2 and ALDH1, in OCCCas with epithelial-like morphology, indicating a possible relationship between LEFTY and the epithelial-mesenchymal hybrid stage of the tumors. In conclusion, TGF-beta-mediated LEFTY/Akt/GSK-3beta/Snail axis may contribute to the establishment and maintenance of phenotypic characteristics of OCCCas through modulation of EMT/CSC properties.

Nam, K. H., et al. (2017). "Predictive value for lymph node metastasis of epithelial-mesenchymal transition and cancer stem cell marker expression in early gastric cancer." Pathol Res Pract **213**(9): 1221-1226.

BACKGROUND: Minimally invasive therapies, including endoscopic mucosal resection or sentinel node navigation surgery, have been widely applied in early gastric cancer because of their benefits in promoting patient quality of life. However, lymph node dissection is beyond the capability of endoscopic therapy, and in sentinel node navigation surgery, the potential for skip metastasis is not negligible. Therefore, the possibility of lymph node metastasis is the most important factor to consider when deciding whether to apply the minimally invasive therapies. In the present study, the significance of epithelial mesenchymal transition and stem cell marker expression in lymph node metastasis

in early gastric cancer was investigated. METHODS: We evaluated the significance of the expression of 5 epithelial mesenchymal transition-related markers (E-cadherin, MMP7, S100A, Snail-1, and HGF) and 6 stem cell markers (ALDH1, SOX2, CD24, CD44, CD54, and CD133) in 119 early gastric cancer specimens using immunohistochemistry. Because protein expression is heterogeneous in gastric cancer, we analyzed the expression of these markers in two selected regions (one each at the superficial zone and the deep invasive front). RESULTS: Expression of E-cadherin, MMP7, HGF, and CD133 at the deep invasive front was associated with the absence of lymph node metastasis (P=0.013, 0.018, <0.001, and 0.026, respectively). Presence of diffuse-type component, lymphatic invasion, and lack of expression of HGF and CD133 at the deep invasive front were independent predictive markers of lymph node metastasis (P=0.019, <0.001, 0.015, and 0.047, respectively). CONCLUSIONS: Lymph node metastasis is strongly associated with expression status of HGF and CD133 at the deep invasive front, suggesting the usefulness of these proteins as independent predictive markers of lymph node metastasis in early gastric 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, H. J., et al. (2012). "Comparison of cell proliferation and epigenetic modification of gene expression patterns in canine foetal fibroblasts and adipose tissue-derived mesenchymal stem cells." Cell Prolif **45**(5): 438-444.

OBJECTIVES: This study compared rate of cell proliferation, viability, cell size, expression patterns of genes related to pluripotency and epigenetic modification between canine foetal fibroblasts (cFF) and canine adipose tissue-derived mesenchymal stem cells (cAd-MSC). MATERIALS AND METHODS: Proliferation pattern, cell viability as well as cell size at each passage of cFF and cAd-MSC were measured when cultures reached confluence. In addition, real-time PCR was performed to investigate expression of Dnmt1, HDAC1, OCT4, SOX2, BAX, BCL2 genes with reference to beta-actin gene expression as an endogenous control in both cell lines. RESULTS: cFF and cAd-MSC differed in number of generations, but not in doubling times, at all passages. Mean cell size of cAd-MSC was significantly smaller than that of cFF. Cell viability was significantly lower in cFFs and apoptotic level was significantly lower in cAd-MSC compared to passage-matched cFF. In the expression of genes related to pluripotency and epigenetic modification, level of HDAC1 in cAd-MSC was significantly higher than in cFF, but expression of Dnmt1 did not differ between the two groups. OCT4 and SOX2 were significantly more highly expressed in cAd-MSC compared to cFF. CONCLUSIONS: cAd-MSC have higher stem-cell potential than cFF in terms of proliferation patterns, epigenetic modification and pluripotency, thus cAd-MSC could be more appropriate than cFF as donors of nuclei in somatic cell nuclear transfer for transgenesis.

Oh, H. J., et al. (2014). "Analysis of cell growth and gene expression of porcine adipose tissue-derived mesenchymal stem cells as nuclear donor cell." Dev Growth Differ **56**(9): 595-604.

In several laboratory animals and humans, adipose tissue-derived mesenchymal stem cells (ASC) are of considerable interest because they are easy to harvest and can generate a huge proliferation of cells from a small quantity of fat. In this study, we investigated: (i) the expression patterns of reprogramming-related genes in porcine ASC; and (ii) whether ASC can be a suitable donor cell type for generating cloned pigs. For these experiments, ASC, adult skin fibroblasts (AF) and fetal fibroblasts (FF) were derived from a 4-year-old female miniature pig. The ASC expressed cell-surface markers characteristic of stem cells, and underwent in vitro differentiation when exposed to specific differentiation-inducing conditions. Expression of DNA methyltransferase (DNMT)1 in ASC was similar to that in AF, but the highest expression of the DNMT3B gene was observed in ASC. The expression of OCT4 was significantly higher in FF and ASC than in AF ($P < 0.05$), and SOX2 showed significantly higher expression in ASC than in the other two cell types ($P < 0.05$). After somatic cell nuclear transfer (SCNT), the development rate of cloned embryos derived from ASC was comparable to the development of those derived using FF. Total cell numbers of blastocysts derived using ASC and FF were significantly higher than in embryos made with AF. The results demonstrated that ASC used for SCNT have a potential comparable to those of AF and FF in terms of embryo in vitro development and blastocyst formation.

Peng, X., et al. (2018). "SOX4 contributes to TGF-beta-induced epithelial-mesenchymal transition and stem cell characteristics of gastric cancer cells." Genes Dis **5**(1): 49-61.

SOX4 is highly expressed in gastric cancer (GC) and is associated with tumor grade, metastasis and prognosis, however the mechanism is not clear. We report herein that SOX4 was upregulated and overexpression of SOX4 was associated with increased expression of the markers of Epithelial-mesenchymal transition (EMT) and stemness in clinic patient samples. In vitro, overexpression of SOX4 promoted the invasion as showed by Transwell assay and stemness of GC cells as assessed by sphere formation assay, which was suppressed by silencing SOX4 with shRNA. Further studies showed that SOX4 up-regulated the expression of EMT transcription factors Twist1, snail1 and zeb1 and stemness transcription factors SOX2 and OCT4, and promoted the nuclear translocation of beta-catenin. Moreover, we revealed that TGF-beta treatment significantly up-regulated the expression of SOX4 and silencing SOX4 reversed TGF-beta induced invasion and sphere formation ability of GC cells. Finally, we showed that SOX4 promoted the lung metastasis and tumor formation ability of gastric cancer cells in nude mice. Our results suggest that SOX4 is a target TGF-beta signaling and mediates TGF-beta-induced EMT and stem cell characteristics of GC cells, revealing a novel role of TGF-beta/SOX4 axis in the regulation of malignant behavior of GC.

Pore, M., et al. (2016). "Cancer Stem Cells, Epithelial to Mesenchymal Markers, and Circulating Tumor Cells in Small Cell Lung Cancer." Clin Lung Cancer **17**(6): 535-542.

BACKGROUND: Small cell lung cancer (SCLC) has a poor prognosis, and even with localized (limited) disease, the 5-year survival has only been around 20%. Elevated levels of circulating tumor cells (CTCs) have been associated with a worse prognosis, and markers of cancer stem cells (CSCs) and epithelial to mesenchymal transition have been associated with increased chemoresistance and metastatic spread in SCLC. PATIENTS AND METHODS: The biopsy specimens of 38 SCLC patients were used for marker evaluation by immunohistochemistry. The markers for CSCs were CD44 and SOX2. The markers for epithelial to mesenchymal transition were E-cadherin, epithelial cell adhesion molecule, cytokeratins 8, 18, and 19, vimentin, and c-MET. Staining was scored as low (weak) or high (strong) intensity for SOX2, epithelial cell adhesion molecule, cytokeratins 8, 18, and 19, and c-MET and using the immunoreactive score for CD44, E-cadherin, and vimentin, expressed as low or high expression. RESULTS: High expression of c-MET (c-MET(H)) and low expression of E-cadherin (E-cad(L)) showed a trend toward a better prognosis (P = .07 and P = .09, respectively). The combination of c-MET(H) and E-cad(L) resulted in significantly better survival (P = .007). The tested markers were not associated with CTCs, although a trend was seen for c-MET(H)E-cad(L) (P = .09) with low CTCs. The CSC markers SOX2 and CD44 were not associated with overall survival in this patient cohort. CONCLUSION: SCLC with a mesenchymal-like phenotype (c-MET(H)E-cad(L)) is associated with longer survival and showed a trend toward lower CTCs.

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.

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., et al. (2015). "Conversion of Adipose Tissue-Derived Mesenchymal Stem Cells to Neural Stem Cell-Like Cells by a Single Transcription Factor, Sox2." Cell Reprogram **17**(3): 221-226.

Adipose tissue is an attractive source of easily accessible adult candidate cells for regenerative medicine. Adipose tissue-derived mesenchymal stem cells (ADSCs) have multipotency and strong proliferation and differentiation capabilities in vitro. However, as mesodermal multipotent stem cells, whether the ADSCs can convert into induced neural stem cells (NSCs) has so far not been demonstrated. In this study, we found that normally the naive ADSCs cultured as either monolayer or spheres in NSC medium did not express Sox2 and Pax6 genes and proteins, and could not differentiate to neuron-like cells. However, when we introduced the Sox2 gene into ADSCs by retrovirus, they exhibited a typical NSC-like morphology, and could be passaged continuously, and expressed NSC specific markers Sox2 and Pax6. In addition, the ADSC-derived NSC-like cells displayed the ability to differentiate into neuron-like cells when switched to the differentiation culture medium, expressing neuronal markers, including Tuj1 and MAP2 genes and proteins. Our results suggest the ADSCs can be converted into induced NSC-like cells with a single transcription factor Sox2. This finding could provide another alternative cell source for cell therapy of neurological disorders.

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, Oct4α, 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.

Rustad, K. C., et al. (2012). "Enhancement of mesenchymal stem cell angiogenic capacity and stemness by a biomimetic hydrogel scaffold." Biomaterials **33**(1): 80-90.

In this study, we examined the capacity of a biomimetic pullulan-collagen hydrogel to create a functional biomaterial-based stem cell niche for the delivery of mesenchymal stem cells (MSCs) into wounds. Murine bone marrow-derived MSCs were seeded into hydrogels and compared to MSCs grown in standard culture conditions. Hydrogels induced MSC secretion of angiogenic cytokines and expression of transcription factors associated with maintenance of pluripotency and self-renewal (Oct4, Sox2, Klf4) when compared to MSCs grown in standard conditions. An excisonal wound healing model was used to compare the ability of MSC-hydrogel constructs versus MSC injection alone to accelerate wound healing. Injection of MSCs did not significantly improve time to wound closure. In contrast, wounds treated with MSC-seeded hydrogels showed significantly accelerated healing and a return of skin appendages. Bioluminescence imaging and FACS analysis of luciferase+/GFP+ MSCs indicated that stem cells delivered within the hydrogel remained viable longer and demonstrated enhanced engraftment efficiency than those delivered via injection. Engrafted MSCs were found to differentiate into fibroblasts, pericytes and endothelial cells but did not contribute to the epidermis. Wounds treated with MSC-seeded hydrogels demonstrated significantly enhanced angiogenesis, which was associated with increased levels of VEGF and other angiogenic cytokines within the wounds. Our data suggest that biomimetic hydrogels provide a functional niche capable of augmenting MSC regenerative potential and enhancing wound healing.

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.

Shanmugasundaram, S., et al. (2011). "Microscale versus nanoscale scaffold architecture for mesenchymal stem cell chondrogenesis." Tissue Eng Part A **17**(5-6): 831-840.

Nanofiber scaffolds, produced by the electrospinning technique, have gained widespread attention in tissue engineering due to their morphological similarities to the native extracellular matrix. For cartilage repair, studies have examined their feasibility; however these studies have been limited, excluding the influence of other scaffold design features. This study evaluated the effect of scaffold design, specifically examining a range of nano to micron-sized fibers and resulting pore size and mechanical properties, on human mesenchymal stem cells (MSCs) derived from the adult bone marrow during chondrogenesis. MSC differentiation was examined on these scaffolds with an emphasis on temporal gene expression of chondrogenic markers

and the pluripotent gene, Sox2, which has yet to be explored for MSCs during chondrogenesis and in combination with tissue engineering scaffolds. Chondrogenic markers of aggrecan, chondroadherin, sox9, and collagen type II were highest for cells on micron-sized fibers (5 and 9 mum) with pore sizes of 27 and 29 mum, respectively, in comparison to cells on nano-sized fibers (300 nm and 600 to 1400 nm) having pore sizes of 2 and 3 mum, respectively. Undifferentiated MSCs expressed high levels of the Sox2 gene but displayed negligible levels on all scaffolds with or without the presence of inductive factors, suggesting that the physical features of the scaffold play an important role in differentiation. Micron-sized fibers with large pore structures and mechanical properties comparable to the cartilage ECM enhanced chondrogenesis, demonstrating architectural features as well as mechanical properties of electrospun fibrous scaffolds enhance differentiation.

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.

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.

Wang, G., et al. (2013). "Critical regulation of miR-200/ZEB2 pathway in Oct4/Sox2-induced mesenchymal-to-epithelial transition and induced pluripotent stem cell generation." Proc Natl Acad Sci U S A **110**(8): 2858-2863.

Fibroblasts can be reprogrammed to induced pluripotent stem cells (iPSCs) by application of transcription factors octamer-binding protein 4 (Oct4), SRY-box containing gene 2 (Sox2), Kruppel-like factor 4 (Klf4), and c-Myelocytomatosis oncogene (c-Myc) (OSKM), but the underlying mechanisms remain unclear. Here, we report that exogenous Oct4 and Sox2 can bind at the promoter regions of mir-141/200c and mir-200a/b/429 cluster, respectively, and induce the transcription activation of miR-200 family during the OSKM-induced reprogramming. Functional suppression of miR-200s with specific inhibitors significantly represses the OSKM-caused mesenchymal-to-epithelial transition (MET, an early event in reprogramming of fibroblasts to iPSCs) and iPSC generation, whereas overexpression of miR-200s promotes the MET and iPSC generation. Mechanistic studies showed that miR-200s significantly repress the expression of zinc finger E-box binding homeobox 2 (ZEB2) through directly targeting its 3' UTR and direct inhibition of ZEB2 can mimic the effects of miR-200s on iPSC generation and MET process. Moreover, the effects of miR-200s during iPSC generation can be blocked by ZEB2 overexpression. Collectively, our findings not only reveal that members of the miR-200 family are unique mediators of the reprogramming factors Oct4/Sox2, but also demonstrate that the miR-200/ZEB2 pathway as one critical mechanism of Oct4/Sox2 to induce somatic cell reprogramming at the early stage.

Wang, M., et al. (2016). "N-cadherin promotes epithelial-mesenchymal transition and cancer stem cell-like traits via ErbB signaling in prostate cancer cells." Int J Oncol **48**(2): 595-606.

N-cadherin has been reported to be upregulated and associated with metastasis and poor prognosis in prostate cancer patients, however the underlying mechanism still remains puzzling. In the present study, we found that upregulation of N-cadherin enhanced, while downregulation of N-cadherin impaired the invasion, migration, and epithelial to mesenchymal transition (EMT) of prostate cancer (PCa) cells. Overexpression of N-cadherin increased the efficiency of colony and tumor spheroid formation and the stemness factor expression (including c-Myc, Klf4, Sox2 and Oct4), and vice versa. Furthermore, microarray analysis and western blot analysis mechanistically proved that N-cadherin activated ErbB signaling pathway by upregulating the expression of Grb2, pShc and pERK1/2. Importantly, the regulation of N-cadherin on EMT and stemness was counteracted by lapatinib, a specific ErbB signaling pathway inhibitor. Collectively, these findings demonstrate that N-cadherin regulates EMT and stemness of PCa cells via activating ErbB signaling pathway, which indicates the pivotal role of N-cadherin/ErbB axis in the metastasis of prostate cancer.

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.

Wei, H., et al. (2017). "The mechanisms for lung cancer risk of PM2.5 : Induction of epithelial-mesenchymal transition and cancer stem cell properties in human non-small cell lung cancer cells." Environ Toxicol **32**(11): 2341-2351.

Fine particulate matter (PM2.5) is a major component of air pollutions that are closely associated with increased risk of lung cancer. However, the role of PM2.5 in the etiology of lung cancer is largely unknown. In this study, we performed acute (24 hours) and chronic (five passages) exposure models to investigate the carcinogenetic mechanisms of PM2.5 by targeting the induction of epithelial-mesenchymal transition (EMT) and cancer stem cells (CSC) properties in human non-small cell lung cancer cell line A549. We found that both acute and chronic PM2.5 exposure enhanced cell migration and invasion, decreased mRNA expression of epithelial markers and increased mRNA expression of mesenchymal markers. Chronic PM2.5 exposure further induced notable EMT morphology and CSC properties, indicating the developing process of cell malignant behaviors from acute to chronic PM2.5 exposure. CSC properties induced by chronic PM2.5 exposure characterized with increased cell-surface markers (CD44, ABCG2), self-renewal genes (SOX2 and OCT4), side population cells and neoplastic capacity. Furthermore, the levels of three stemness-associated microRNAs, Let-7a, miR-16 and miR-34a, were found to be significantly downregulated by chronic PM2.5 exposure, with microarray data analysis from TCGA database showing their lower expression in human lung adenocarcinoma tissues than that in the adjacent normal lung tissues. These data revealed that the induction of EMT and CSC properties were involved in the lung cancer risk of PM2.5 , and implicated CSC properties and related microRNAs as possible biomarkers for carcinogenicity prediction of PM2.5 .

Wei, Y., et al. (2016). "Primordial germ cell-like cells derived from canine adipose mesenchymal stem cells." Cell Prolif **49**(4): 503-511.

OBJECTIVES: Previous studies have shown that adipose mesenchymal stem cells (AMSCs) share the potency of typical bone marrow mesenchymal stem cells (MSCs); however, there is little information concerning characteristics of canine AMSCs (CAMSCs); it has not previously been made clear whether CAMSCs would be able to differentiate into other cell types. MATERIALS AND METHODS: In this study, typical AMSC lines were established, and their characteristics including morphology, typical markers and differentiation potentiality were tested. RESULTS: The cells exhibited typical MSC morphology and were positive for CD90, CD44 and CD166, considered to be MSCs surface markers. They were negative for CD34 and CD45. The CAMSCs also exhibited embryonic stem cell (ESC) markers, including Oct4 and Sox2, at passage 2. In an appropriate microenvironment, CAMSCs differentiated into EBs and were able to produce cells of the three germ layers. These results indicate that established cells were putative adipocyte-derived MSCs, which also displayed properties of ESCs. Moreover, when the CAMSCs were induced by bone morphogenetic protein 4 (BMP4), they differentiated into PGC-like cells (PGCLCs) and male germ-like cells, which were positive for PR domain-containing 1 (Prdm1), PR domain-containing 14 (Prdm14), doublesex and mab-3 related transcription factor (Dmrt1), as well as for promyelocytic leukaemia zinc finger (Plzf). Quantitative real-time PCR (qRT-PCR) and western blotting analysis verified higher expression levels of these markers. CONCLUSION: This study provides an efficient approach to study germ cell development using CAMSCs.

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.

West, N. R., et al. (2014). "Oncostatin-M promotes phenotypic changes associated with mesenchymal and stem cell-like differentiation in breast cancer." Oncogene **33**(12): 1485-1494.

Cancer stem cell (CSC) biology and the epithelial-to-mesenchymal transition (EMT) are thought to be mechanistically linked and may be key components of cancer development and progression. However, stimuli that induce EMT and CSC-like features ('stemness') are poorly defined. We and others have shown that the inflammatory cytokine oncostatin-M (OSM) mediates phenotypic changes in breast cancer that are consistent with EMT and dedifferentiation, including enhanced migration and loss of hormone receptors. In this study, we have expanded on these prior observations to determine whether OSM is a cell-extrinsic driver of EMT and/or stemness. OSM stimulation of the luminal breast cancer cell lines MCF7 and T47D induced EMT features including loss of membranous E-cadherin and induction of snail and slug expression. OSM treatment markedly enhanced the formation of mammospheres (up to 20-fold, $P<0.001$), which displayed high expression of the pluripotency factor SOX2. The proportion of cells with a CD44(high)CD24(-/low) phenotype was similarly increased by OSM ($P<0.001$). OSM-induced mammosphere formation and CD44(high)CD24(-/low) induction was dependent on PI3K signalling. In silico analysis of human breast tumours (from a publicly available data set, n=322) confirmed that co-expression of a PI3K transcriptional signature, but not MAPK or STAT3 signatures, was necessary to detect an association between OSMR and poor prognosis. Assessment of a second in silico data set (n=241 breast tumours) confirmed a significant relationship between OSMR, markers of EMT and CSCs, and chemotherapy resistance. Direct analysis of mRNA expression by RT-PCR in a third cohort (n=72 breast tumours) demonstrated that high expression of OSM is associated positively with indicators of EMT (SNAI1, $P<0.001$) and stemness (SOX2, $P<0.05$). Our data suggest for the first time that OSM may promote a clinically relevant EMT/CSC-like phenotype in human breast cancer via a PI3K-dependent mechanism.

Wittig, D., et al. (2013). "Immunohistochemical localization and characterization of putative mesenchymal stem cell markers in the retinal capillary network of rodents." Cells Tissues Organs **197**(5): 344-359.

Perivascular cells of microvascular niches are the prime candidates for being a reservoire of mesenchymal stem cell (MSC)-like cells in many tissues and organs that could serve as a potential source of cells and a target of novel cell-based therapeutic approaches. In the present study, by utilising typical markers of pericytes (neuronal-glial antigen 2, NG2, a chondroitin sulphate proteoglycan) and those of MSCs (CD146 and CD105) and primitive pluripotent cells (sex-determining region Y-box 2, Sox2), the phenotypic traits and the distribution of murine and rat retinal perivascular cells were investigated in situ. Our findings indicate that retinal microvessels of juvenile rodents are highly covered by NG2-positive branching processes of pericytic (perivascular) cells that are less prominent in mature capillary networks of the adult retina. In the adult rodent retinal vascular bed, NG2 labeling is mainly confined to membranes of the cell body resulting in a pearl-chain-like distribution along the vessels. Retinal pericytes, which were identified by their morphology and NG2 expression, simultaneously express CD146. Furthermore,

CD146-positive cells located at small arteriole-to-capillary branching points appear more intensely stained than elsewhere. Evidence for a differential expression of the two markers around capillaries that would hint at a clonal heterogeneity among pericytic cells, however, is lacking. In contrast, the expression of CD105 is exclusively restricted to vascular endothelial cells and Sox2 is detected neither in perivascular nor in endothelial cells. In dissociated retinal cultures, however, simultaneous expression of NG2 and CD105 was observed. Collectively, our data indicate that vascular wall resident retinal pericytes share some phenotypic features (i.e. CD146 expression) with archetypal MSCs, which is even more striking in dissociated retinal cultures (i.e. CD105 expression). These findings might have implications for the treatment of retinal pathologies.

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).

Xu, Y., et al. (2012). "Efficient commitment to functional CD34+ progenitor cells from human bone marrow mesenchymal stem-cell-derived induced pluripotent stem cells." PLoS One **7**(4): e34321.

The efficient commitment of a specialized cell type from induced pluripotent stem cells (iPSCs) without contamination from unknown substances is crucial to their use in clinical applications. Here, we propose that CD34+ progenitor cells, which retain hematopoietic and endothelial cell potential, could be efficiently obtained from iPSCs derived from human bone marrow mesenchymal stem cells (hBMMSC-iPSCs) with defined factors. By treatment with a cocktail containing mesodermal, hematopoietic, and endothelial inducers (BMP4, SCF, and VEGF, respectively) for 5 days, hBMMSC-iPSCs expressed the mesodermal transcription factors Brachyury and GATA-2 at higher levels than untreated groups ($P<0.05$). After culturing with another hematopoietic and endothelial inducer cocktail, including SCF, Flt3L, VEGF and IL-3, for an additional 7-9 days, CD34+ progenitor cells, which were undetectable in the initial iPSC cultures, reached nearly 20% of the total culture. This was greater than the relative number of progenitor cells produced from human-skin-fibroblast-derived iPSCs (hFib-iPSCs) or from the spontaneous differentiation groups ($P<0.05$), as assessed by flow cytometry analysis. These induced cells expressed hematopoietic transcription factors TAL-1 and GATA-2 [corrected]. They developed into various hematopoietic colonies when exposed to semisolid media with hematopoietic cytokines such as EPO and G-CSF. Hematopoietic cell lineages were identified by phenotype analysis with Wright-Giemsa staining. The endothelial potential of the cells was also verified by the confirmation of the formation of vascular tube-like structures and the expression of endothelial-specific markers CD31 and VE-CADHERIN. Efficient induction of CD34+ progenitor cells, which retain hematopoietic and endothelial cell potential with defined factors, provides an opportunity to obtain patient-specific cells for iPSC therapy and a useful model for the study of the mechanisms of hematopoiesis and drug screening.

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.

Yoon, C. H., et al. (2012). "PTTG1 oncogene promotes tumor malignancy via epithelial to mesenchymal transition and expansion of cancer stem cell population." J Biol Chem **287**(23): 19516-19527.

The prognosis of breast cancer patients is related to the degree of metastasis. However, the mechanisms by which epithelial tumor cells escape from the primary tumor and colonize at a distant site are not entirely understood. Here, we analyzed expression levels of pituitary tumor-transforming gene-1 (PTTG1), a relatively uncharacterized oncoprotein, in patient-derived breast cancer tissues with corresponding normal breast tissues. We found that PTTG1 is highly expressed in breast cancer patients, compared with normal tissues. Also, PTTG1 expression levels were correlated with the degree of malignancy in breast cancer cell lines; the more migratory and invasive cancer cell lines MDA-MB-231 and BT549 displayed the higher expression levels of PTTG1 than the less migratory and invasive MCF7 and SK-BR3 and normal MCF10A cell lines. By modulating PTTG1 expression levels, we found that PTTG1 enhances the migratory and invasive properties of breast cancer cells by inducing epithelial to mesenchymal transition, as evidenced by altered morphology and epithelial/mesenchymal cell marker expression patterns and up-regulation of the transcription factor Snail. Notably, down-regulation of PTTG1 also suppressed cancer stem cell population in BT549 cells by decreasing self-renewing ability and tumorigenic capacity, accompanying decreasing CD44(high) CD24(low) cells and Sox2 expression. Up-regulation of PTTG1 had the opposite effects, increasing sphere-forming ability and Sox2 expression. Importantly, PTTG1-mediated malignant tumor properties were due, at least in part, to activation of AKT, known to be a key regulator of both EMT and stemness in cancer cells. Collectively, these results suggest that PTTG1 may represent a new therapeutic target for malignant breast cancer.

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.

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.

Zheng, Y. L., et al. (2015). "Mesenchymal Stem Cells Obtained from Synovial Fluid Mesenchymal Stem Cell-Derived Induced Pluripotent Stem Cells on a Matrigel Coating Exhibited Enhanced Proliferation and Differentiation Potential." PLoS One **10**(12): e0144226.

Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) serve as a promising source for cell-based therapies in regenerative medicine. However, optimal methods for transforming iPSCs into MSCs and the characteristics of iPSC-MSCs obtained from different methods remain poorly understood. In this study, we developed a one-step method for obtaining iPSC-MSCs (CD146+STRO-1+ MSCs) from human synovial fluid MSC-derived induced iPSCs (SFMSC-iPSCs). CD146-STRO-1-SFMSCs were reprogrammed into iPSCs by transduction with lentivirus-mediated Sox2, Oct-3/4, klf4, and c-Myc. SFMSC-iPSCs were maintained with mTeSR1 medium in Matrigel-coated culture plates. Single dissociated cells were obtained by digesting the SFMSC-iPSCs with trypsin. The dissociated cells were then plated into Matrigel-coated culture plate with alpha minimum essential medium supplemented with 10% fetal bovine serum, 1x Glutamax, and the ROCK inhibitor Y-27632. Cells were then passaged in standard cell culture plates with alpha minimum essential medium supplemented with 10% fetal bovine serum and 1x Glutamax. After passaging in vitro, the cells showed a homogenous spindle-shape similar to their ancestor cells (SFMSCs), but with more robust proliferative activity. Flow cytometric analysis revealed typical MSC surface markers, including expression of CD73, CD90, CD105, and CD44 and lack of CD45, CD34, CD11b, CD19, and HLA-DR. However, these cells were positive for CD146 and stro-1, which the ancestor cells were not. Moreover, the cells could also be induced to differentiate in osteogenic, chondrogenic, and adipogenic lineages in vitro. The differentiation potential was improved compared with the ancestor cells in vitro. The cells were not found to exhibit oncogenicity in vivo. Therefore, the method presented herein facilitated the generation of STRO-1+CD146+ MSCs from SFMSC-iPSCs exhibiting enhanced proliferation and differentiation potential.

Zubeldia, I. G., et al. (2013). "Epithelial to mesenchymal transition and cancer stem cell phenotypes leading to liver metastasis are abrogated by the novel TGFbeta1-targeting peptides P17 and P144." Exp Cell Res **319**(3): 12-22.

Colorectal cancer (CRC) frequently metastasizes to the liver, a phenomenon that involves the participation of transforming-growth-factor-beta(1) (TGFbeta(1)). Blockade of the protumorigenic effects elicited by TGFbeta(1) in advanced CRC could attenuate liver metastasis. We aimed in the present study to assess the antimetastatic

effect of TGFbeta(1)-blocking peptides P17 and P144, and to study mechanisms responsible for this activity in a mouse model. Colon adenocarcinoma cells expressing luciferase were pretreated with TGFbeta(1) (Mc38-luc(TGFbeta1) cells), injected into the spleen of mice and monitored for tumor development. TGFbeta(1) increased primary tumor growth and liver metastasis, whereas systemic treatment of mice with either P17 or P144 significantly reduced tumor burden ($p<0.01$). In metastatic nodules, mitotic/apoptotic ratio, mesenchymal traits and angiogenesis (evaluated by CD-31, as well as circulating endothelial and progenitor cells) induced by TGFbeta(1) were consistently reduced following injection of peptides. In vitro experiments revealed a direct effect of TGFbeta(1) in Mc38 cells, which resulted in activation of Smad2, Smad3 and Smad1/5/8, and increased invasion and transendothelial migration, whereas blockade of TGFbeta(1)-signaling reverted these features. Because TGFbeta(1)-mediated epithelial-mesenchymal transition (EMT) has been suggested to induce a cancer stem cell (CSC) phenotype, we analyzed the ability of this cytokine to induce tumorsphere formation and the expression of CSC markers. In TGFbeta(1)-treated cells, tumorspheres were enriched in CD44 and SOX2, which were diminished in the presence of P17. Our data provide a preclinical rationale to evaluate P17 and P144 as potential therapeutic options for the treatment of metastatic CRC.

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