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Stem Cell

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# Stem Cell Research Literatures (2)

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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.
[Dr. Mark Herbert. **Stem Cell Research Literatures (2).** Stem Cell. 2024;15(1):6-35] ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 2. doi:10.7537/marsscj150124.02.

**Key words**: stem cell; life; research; literature

## Introduction

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

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

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

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

Ball, S. G., et al. (2012). "Inhibition of platelet-derived growth factor receptor signaling regulates Oct4 and

Nanog expression, cell shape, and mesenchymal stem cell potency." Stem Cells **30**(3): 548-560.

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

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

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

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

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

Crobu, F., et al. (2012). "Differentiation of single cell derived human mesenchymal stem cells into cells with a neuronal phenotype: RNA and microRNA expression profile." Mol Biol Rep **39**(4): 3995-4007.

The adult bone marrow contains a subset of non-haematopoietic cells referred to as bone marrow mesenchymal stem cells (BMSCs). Mesenchymal stem cells (MSCs) have attracted immense research interest in the field of regenerative medicine due to their ability to be cultured for successive passages and multi-lineage differentiation. The molecular mechanisms governing the self-renewal and differentiation of MSCs remain largely unknown. In a previous paper we

demonstrated the ability to induce human clonal MSCs to differentiate into cells with a neuronal phenotype (DMSCs). In the present study we evaluated gene expression profiles by Sequential Analysis of Gene Expression (SAGE) and microRNA expression profiles before and after the neuronal differentiation process. Various tissue-specific genes were weakly expressed in MSCs, including those of non-mesodermal origin, suggesting multiple potential tissue-specific differentiation, as well as stemness markers. Expression of OCT4, KLF4 and c-Myc cell reprogramming factors, which are modulated during the differentiation process, was also observed. Many peculiar nervous tissue genes were expressed at a high level in DMSCs, along with genes related to apoptosis. MicroRNA profiling and correlation with mRNA expression profiles allowed us to identify putative important genes and microRNAs involved in the differentiation of MSCs into neuronal-like cells. The profound difference in gene and microRNA expression patterns between MSCs and DMSCs indicates a real functional change during differentiation from MSCs to DMSCs.

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.

Enciso, N., et al. (2018). "Stem cell factor supports migration in canine mesenchymal stem cells." Vet Res Commun **42**(1): 29-38.

Adult Mesenchymal Stem Cells (MSC) are cells that can be defined as multipotent cells able to differentiate into diverse lineages, under appropriate conditions. These cells have been widely used in regenerative medicine, both in preclinical and clinical settings. Initially discovered in bone marrow, MSC can now be isolated from a wide spectrum of adult and foetal tissues. Studies to evaluate the therapeutic potential of these cells are based on their ability to arrive to damaged tissues. In this paper we have done a comparative study analyzing proliferation, surface markers and OCT4, SOX9, RUNX2, PPARG genes expression in MSC cells from Bone marrow

(BMMSC) and Adipose tissue (ASC). We also analyzed the role of Stem Cell Factor (SCF) on MSC proliferation and on ASCs metalloproteinases MMP-2, MMP-9 secretion. Healthy dogs were used as BMMSC donors, and ASC were collected from omentum during elective ovariohysterectomy surgery. Both cell types were cultured in IMDM medium with or without SCF, 10% Dog Serum (DS), and incubated at 38 degrees C with 5% CO2. Growth of BMMSCs and ASCs was exponential until 25-30 days. Flow citometry of MSCs revealed positive results for CD90 and negative for CD34, CD45 and MCH-II. Genes were evaluated by RT-PCR and metalloproteinases by zymografy. Our findings indicate morphological and immunological similarities as well as expression of genes from both origins on analyzed cells. Furthermore, SCF did not affect proliferation of MSCs, however it up-regulated MMP-2 and MMP-9 secretion in ASCs. These results suggest that metalloproteinases are possibly essential molecules pivoting migration.

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.

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

Fetal membranes are abundant; the yolk sac is a source of cell lineages that do not express MHCs and are mainly free from immunological incompatibles when transferred to a recipient. Although data are available especially for hematopoietic stem cells in human and murine; whereas other cell types and species are dramatically unnoticed. Here, we studied the nature and differentiation potential of yolk sac-derived mesenchymal stem cells from a chicken embryo. In this study, we observed the gene expression of pluripotent markers in yolk sac mesenchymal stem cells (YS-MSCs) and the capacity of YS-MSCs to differentiate into neural-like cells using quantitative RT-PCR, immunocytochemistry, and western blotting. YS-MSCs have a spindle shape and revealed the expression of the MSC-related proteins beta-integrin, CD44, CD71, and CD73, but not CD34. YS-MSCs express pluripotent markers such as octamer-binding transcription factor 4 (Oct4) and Nanog at the protein and mRNA levels. QRT-PCR analyses revealed that YS-MSCs expressed nestin. Immunocytochemical and western blotting data showed that the cells expressed Nestin and microtubule-associated protein 2 (Map-2) for neurons, respectively, after induction of neural differentiation. These findings demonstrate the plasticity of YS-MSCs and their potential for use in cellular replacement therapy for neural diseases.

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

Human amniotic mesenchymal stem cells (hAMSCs) demonstrated partially pluripotent characteristics with a strong expression of Oct4 and Nanog genes and immunomodulatory properties characterized

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

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

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

Ghasemzadeh-Hasankolaei, M., et al. (2015). "Male and female rat bone marrow-derived mesenchymal stem cells are different in terms of the expression of germ cell specific genes." Anat Sci Int **90**(3): 187-196.

Recent studies have shown that mesenchymal stem cells (MSCs), under appropriate conditions, can differentiate into cell types including germ cells (GCs). These studies also show that MSCs without any induction express some GC-specific genes innately. Moreover, one report suggests that female MSCs have a greater tendency to differentiate into female instead of male GCs. Therefore, for the first time, this study attempts to assay and determine the differences between the expression levels of some important GC-specific genes (Stra8, Vasa, Dazl, Stella, Piwil2, Oct4, Fragilis, Rnf17 and c-Kit) in male and female bone marrow (BM)-MSCs of rats. BM sampling of the rate was performed by a newly established method. We cultured rat BM samples, then characterized male and female MSCs according to their adhesion onto the culture dish, their differentiation potential into bone, cartilage and fat cells, and phenotype analysis by flow cytometry. The expression of GC-specific genes and their expression levels were evaluated with reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR. Our results showed that Dazl and Rnf17 did not express in the cells. The majority of examined genes, except Piwil2, expressed at almost the same levels in male and female MSCs. Piwil2 had higher expression in male MSCs which was

probably related to the more prominent role of Piwil2 in the male GC development process. Male BM-MSCs appeared more prone to differentiate into male rather than female GCs. Additional research should be performed to determine the exact role of different genes in the male and female GC development process.

Ghasemzadeh-Hasankolai, M., et al. (2012). "Effect of zinc ions on differentiation of bone marrow-derived mesenchymal stem cells to male germ cells and some germ cell-specific gene expression in rams." Biol Trace Elem Res **150**(1-3): 137-146.

This is the first report to describe the effects of zinc (Zn) ions on the expression of germ cell (GC) genes from bone marrow-derived mesenchymal stem cells (BM-MSCs). Zn plays an important role in germinal epithelium maintenance, testosterone secretion, differentiation of GCs, and spermatogenesis. In addition, several studies have suggested that MSCs have the potential for differentiation into numerous cells types, including male GCs. In this study, we have treated passage-3 ram BM-MSCs with 0.14 mug/ml Zn sulfate (ZnSO(4)) for a period of 21 days with the intent to determine whether Zn treatment can stimulate MSCs to differentiate into male GCs in vitro. We also sought to determine the type of changes seen in MSCs by Zn treatment. Differentiation into male GCs was evaluated by the assessment of expressions of the following GC-specific markers: VASA, PIWIL2, OCT4, beta1 INTEGRIN (ITG b1), DAZL (by reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR), and PGP 9.5 (by immunocytochemistry). Also studied were morphological characteristics and changes in alkaline phosphatase activity. Interestingly, Zn upregulated the expressions of VASA and ITG b1 but downregulated PIWIL2 and OCT4. DAZL and PGP 9.5 were not expressed in the treatment group. According to our results, Zn ions did not stimulate BM-MSCs to transdifferentiate into male GCs; however, it changed the expression of GC genes in BM-MSCs. It can be concluded that a possible mechanism by which Zn ions can increase male fertility is by regulation of the expression of testis GC-specific genes in the differentiation process and spermatogenesis.

Goncalves Ndo, N., et al. (2016). "Effect of Melatonin in Epithelial Mesenchymal Transition Markers and Invasive Properties of Breast Cancer Stem Cells of Canine and Human Cell Lines." PLoS One **11**(3): e0150407.

Cancer stem cells (CSCs) have been associated with metastasis and therapeutic resistance and can be generated via epithelial mesenchymal transition (EMT). Some studies suggest that the hormone melatonin acts in CSCs and may participate in the inhibition of the EMT. The objectives of this study were to evaluate the formation of mammospheres from the canine and human breast cancer cell lines, CMT-U229 and MCF-7, and the effects of melatonin treatment on the modulation of stem cell and EMT molecular markers: OCT4, E-cadherin, N-cadherin and vimentin, as well as on cell viability and invasiveness of the cells from mammospheres. The CMT-U229 and MCF-7 cell lines were subjected to three-dimensional culture in special medium for stem cells. The phenotype of mammospheres was first evaluated by flow cytometry (CD44(+)/CD24(low/-) marking). Cell viability was measured by MTT colorimetric assay and the expression of the proteins OCT4, E-cadherin, N-cadherin and vimentin was evaluated by immunofluorescence and quantified by optical densitometry. The analysis of cell migration and invasion was performed in Boyden Chamber. Flow cytometry proved the stem cell phenotype with CD44(+)/CD24(low/-) positive marking for both cell lines. Cell viability of CMT-U229 and MCF-7 cells was reduced after treatment with 1mM melatonin for 24 h ($P<0.05$). Immunofluorescence staining showed increased E-cadherin expression ($P<0.05$) and decreased expression of OCT4, N-cadherin and vimentin ($P<0.05$) in both cell lines after treatment with 1 mM melatonin for 24 hours. Moreover, treatment with melatonin was able to reduce cell migration and invasion in both cell lines when compared to control group ($P<0.05$). Our results demonstrate that melatonin shows an inhibitory role in the viability and invasiveness of breast cancer mammospheres as well as in modulating the expression of proteins related to EMT in breast CSCs, suggesting its potential anti-metastatic role in canine and human breast cancer cell lines.

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

It has been recently reported that side population (SP) cells in nasopharyngeal carcinoma (NPC) cell lines display characteristics of cancer stem-like cells. However, the biological behavior and the significance of these cells for NPC progression remain unclear. In this study, we isolated SP cells from the NPC cell line CNE-2 by flow cytometry and investigated their biological characteristics. We discovered that SP cells had stronger colony forming abilities compared to the non-side population (NSP) cells, and observed that some SP cells looked more like the shape of mesenchymal cells when cultured in the common polyHEMA-coated flask. When checked by quantitative real-time PCR, the SP cells expressed higher levels of stemness-related genes Oct4, Sox2 and Nanog, and mesenchymal cell-related genes N-cadherin, vimentin and Snail, while they expressed lower levels of the epithelial cell-related gene, E-cadherin. Western blot and immunofluorescence staining methods further verified that SP cells expressed higher vimentin and expressed lower E-cadherin levels. Finally, Transwell invasion assay results indicated that the SP cells had higher invasive potential compared to NSP cells. Collectively, our data reveal that SP cells in the CNE-2 cell line not only possess the properties of cancer stem cells, but also have more mesenchymal cell characteristics which are associated with epithelial mesenchymal transition (EMT) and cancer cell invasion and metastasis. These findings are helpful for developing novel targets for effective clinical treatment of NPC.

Han, S. H., et al. (2014). "Effect of ectopic OCT4 expression on canine adipose tissue-derived mesenchymal stem cell proliferation." Cell Biol Int **38**(10): 1163-1173.

Enhancing the proliferative capacity of mesenchymal stem cells (MSCs) is critical for increasing their therapeutic potential in a variety of diseases. We hypothesized that lentivirus-mediated overexpression of canine octamer-binding transcription factor 4 (OCT4) might influence the proliferation of canine adipose tissue-derived MSCs (cATMSCs). cOCT4-cATMSCs were generated by transducing cATMSCs with a cOCT4-lentiviral vector. Increased expression of cOCT4 was confirmed using RT-PCR and immunoblotting. Immunophenotypic characterization using flow cytometry indicated that the CD29, CD44, CD73, CD90, and CD105 surface markers were highly expressed by both cOCT4- and mock-transduced cATMSCs (mock-cATMSCs), whereas the CD31 and CD45 markers were absent. We performed the osteogenic differentiation assay to evaluate the effects of cOCT4 overexpression on the osteogenic differentiation potential of cATMSCs. The results showed that cOCT4-cATMSCs had a much higher potential for osteogenic differentiation than mock-cATMSCs. Next, the proliferative capacities of cOCT4- and mock-cATMSCs were evaluated using a WST-1 cell proliferation assay and trypan blue exclusion. cOCT4-cATMSCs showed a higher proliferative capacity than mock-cATMSCs. Cell cycle analysis indicated that overexpression of cOCT4 in cATMSCs induced an increase in the proportion of cells in S and G2/M phases. Consistent with this, immunoblot analysis showed that cyclin D1 expression was increased in cOCT4-cATMSCs. In conclusion, our results indicate that lentivirus-mediated overexpression of cOCT4 increased the proliferative capacity of cATMSCs. OCT4-mediated enhancement of cell proliferation may be a useful method for expanding MSC population rapidly without loss of stemness.

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

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

Matrigel invasion activity of apoptosis-resistant Panc-1 cells was greater than that of control Panc-1 cells. CONCLUSION: Apoptosis-resistant cancer cells have CSC-like properties, i.e., able to initiate sphere formation, express stem cell genes, and respond to EMT stimulation.

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

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

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

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

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

Human embryonic stem (hES) cells have been successfully maintained using human-cell feeder systems or feeder-free systems. However, despite advances in culture techniques, hES cells require supplementation with fibroblast growth factor 2 (FGF-2), an exogenous stemness factor, which is needed to sustain the authentic undifferentiated status. We developed a new culture system for hES cells; this system does not require supplementation with FGF-2 to obtain hES cells that are suitable for tissue engineering and regenerative medicine. This culture system employed mesenchymal stem cells derived from hES cells (hESC-MSCs) as autologous human feeder cells in the absence of FGF-2. The hES cell line SNUhES3 cultured in this new autologous feeder culture system maintained the typical morphology of hES cells and expression of pluripotency-related proteins, SSEA-4, TRA-1-60, OCT4, and alkaline phosphatase, without development of abnormal karyotypes after

more than 30 passages. RNA expression of the pluripotency-related genes OCT4 and NANOG was similar to the expression in SNUhES3 cells maintained on xenofeeder STO cells. To identify the mechanism that enables the cells to be maintained without exogenous FGF-2, we checked the secretion of FGF-2 from the mitomycin-C treated autofeeder hESC-MSCs versus xenofeeder STO cells, and confirmed that hESC-MSCs secreted FGF-2 whereas STO cells did not. The level of FGF-2 in the media from the autofeeder system without exogenous FGF-2 was comparable to that from the xenofeeder system with addition of FGF-2. In conclusion, our new culture system for hES cells, which employs a feeder layer of autologous hESC-MSCs, supplies sufficient amounts of secreted FGF-2 to eliminate the requirement for exogenous FGF-2.

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.

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

One of the theories on cancer stem cells (CSCs) states that these cells initiate most tumors and give rise to more-or-less differentiated tumor cells. Genetic signatures of CSCs are thought to predict tumor recurrence and metastases, thus, supporting

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

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

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

Liu, F., et al. (2015). "TGF-beta1 acts through miR-155 to down-regulate TP53INP1 in promoting epithelial-mesenchymal transition and cancer stem cell phenotypes." Cancer Lett **359**(2): 288-298.

It has been shown that acquisition of epithelial-mesenchymal transition (EMT) and induction of cancer stem cell (CSC)-like properties contribute to metastasis of cancers in many studies; however, the molecular mechanisms underlying EMT and CSC phenotypes in liver cancer cells remain to be elucidated. MiR-155 is an important microRNA associated with tumour progression. Here, we report that miR-155 regulates not only the epithelial-mesenchymal transition but also the stem-like transition in liver cancer cells. Utilizing quantitative RT-PCR, we found that the expression of miR-155 is positively related to the levels of CD90, CD133 and Oct4 in enriched spheres. Up-regulated miR-155 significantly increases the population of stem-like CSCs among liver cancer cells and the ability to form tumour spheres. Additionally, miR-155 overexpression in cells significantly increases cell motility and invasion, as well as the epithelial-mesenchymal transition process. Conversely, suppression of miR-155 in cells had an opposite effect, which was partially

rescued by the down-regulation of TP53INP1. Collectively, miR-155 promotes liver cancer cell EMT and CSCs, in part, via silencing TP53INP1. In addition, we found that TGF-beta1 indirectly regulates TP53INP1 expression via miR-155 in liver cancer cells. Taken together, our findings suggest that miR-155 regulates TP53INP1 expression, to induce the epithelial-mesenchymal transition and acquisition of a stem cell phenotype.

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.

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

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

(EMT) markers. The expression of three miRNAs relevant to spheroid formation or EMT was assessed by q-PCR. CONCLUSIONS: In this paper we uncover a new function of FHC in the control of cancer stem cells.

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

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

Luyckx, A., et al. (2011). "Oct4-negative multipotent adult progenitor cells and mesenchymal stem cells as regulators of T-cell alloreactivity in mice." Immunol Lett **137**(1-2): 78-81.

Multipotent adult progenitor cells (MAPC) are clinically being explored as an alternative to mesenchymal stem cells (MSC) for the immunomodulatory control of graft-versus-host disease (GvHD). Here, we performed an explorative study of the immunomodulatory potential of mouse MAPC (mMAPC), in comparison with that of MSC (mMSC) using experimental models of T-cell alloreactivity. Suppressive effects of Oct4-expressing mMAPC have been described previously; here, we studied mMAPC expressing low to no Oct4 ('mClone-3'), recently shown to be most representative for the human MAPC counterpart. mClone-3 and mMSC exhibited similar immunophenotype and in vitro immunogenic behavior. Allogeneic T-cell<-->dendritic cell-proliferation assays showed strong dose-dependent T-cell-suppressive effects of both mClone-3 and mMSC. In a popliteal lymph node assay, mClone-3 and mMSC equally suppressed in vivo alloreactive T-cell expansion. We conclude that mouse MAPC and MSC exhibit similar immunosuppressive behavior in in vitro and local in vivo GvHD assays.

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

Anterior gradient protein 2 (AGR2) is a novel biomarker with potential oncogenic role. We sought to investigate the diagnostic and prognostic role of AGR2 on head and neck squamous cell carcinoma (HNSCC) with an emphasis on its correlation of cancer stemloid cells (CSC) and epithelial mesenchymal transition (EMT). We found that AGR2 protein levels were higher in HNSCC than in normal oral mucosa. High levels of AGR2 were associated with the T category, pathological grade and lymph node metastasis of HNSCC. Expression of AGR2 increased in recurring HNSCC after radiotherapy and in post cisplatin-based chemotherapeutic tissues. In HNSCC cell lines, knock-down of AGR2 induced apoptosis, reduced sphere formation, and down-regulated Survivin, Cyclin D1, Bcl2, Bcl2l1, Slug, Snail, Nanog and Oct4. In addition, over-expressed AGR2 in transgenic mice with spontaneous HNSCC was associated with lost function of Tgfbr1 and/ or

lost function of Pten. In vitro knockdown TGFBR1 in HNSCC cell lines increased AGR2 expression. These results suggest that AGR2 is involved in EMT and self-renewal of CSC and may present a potential therapeutic target (oncotarget) for HNSCC.

Manotham, K., et al. (2015). "Generation of CCR5-defective CD34 cells from ZFN-driven stop codon-integrated mesenchymal stem cell clones." J Biomed Sci **22**: 25.

BACKGROUNDS: Homozygous 32-bp deletion of the chemokine receptor 5 gene (CCR5) is associated with resistance to human immunodeficiency virus (HIV) infection, while heterozygosity delays HIV progression. Bone marrow transplantation (BMT) from a 32/32 donor has been shown to cure an HIV-infected patient. However, the rarity of this mutation and the safety risks associated with current BMT protocols are the major obstacles to this treatment. Zinc finger nuclease (ZFN) targeting is a powerful method for achieving genomic disruption at specific DNA sites of interest. RESULTS: Taking advantage of the self-renewal and plasticity properties of stem cells, in this study, we successfully generated isogenic and six-cell clones of bone marrow-derived mesenchymal stem cells that carry the stop codon of the CCR5 gene by using a ZFN-mediated homology-directed repair technique. These cells were expandable for more than 5 passages, and thus show potential to serve as an individual's cell factory. When Oct4 was overexpressed, the mutated cells robustly converted to CD34+ progenitor cells. CONCLUSION: We here reported the novel approach on generation of patients own CD34 cells from high fidelity ZFN-mediated HDR MSC clones. We believe that our approach will be beneficial in future HIV treatment.

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

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

Morata-Tarifa, C., et al. (2016). "Low adherent cancer cell subpopulations are enriched in tumorigenic and metastatic epithelial-to-mesenchymal transition-induced cancer stem-like cells." Sci Rep **6**: 18772.

Cancer stem cells are responsible for tumor progression, metastasis, therapy resistance and cancer recurrence, doing their identification and isolation of special relevance. Here we show that low adherent breast and colon cancer cells subpopulations have stem-like properties. Our results demonstrate that trypsin-sensitive (TS) breast and colon cancer cells subpopulations show increased ALDH activity, higher ability to exclude Hoechst 33342, enlarged proportion of cells with a cancer stem-like cell phenotype and are enriched in sphere- and colony-forming cells

in vitro. Further studies in MDA-MB-231 breast cancer cells reveal that TS subpopulation expresses higher levels of SLUG, SNAIL, VIMENTIN and N-CADHERIN while show a lack of expression of E-CADHERIN and CLAUDIN, being this profile characteristic of the epithelial-to-mesenchymal transition (EMT). The TS subpopulation shows CXCL10, BMI-1 and OCT4 upregulation, differing also in the expression of several miRNAs involved in EMT and/or cell self-renewal such as miR-34a-5p, miR-34c-5p, miR-21-5p, miR-93-5p and miR-100-5p. Furthermore, in vivo studies in immunocompromised mice demonstrate that MDA-MB-231 TS cells form more and bigger xenograft tumors with shorter latency and have higher metastatic potential. In conclusion, this work presents a new, non-aggressive, easy, inexpensive and reproducible methodology to isolate prospectively cancer stem-like cells for subsequent biological and preclinical studies.

Mueller, T., et al. (2009). "Analysis of OCT4 expression in an extended panel of human tumor cell lines from multiple entities and in human mesenchymal stem cells." Cell Mol Life Sci **66**(3): 495-503.

OCT4 is considered a main regulator of embryonic stem cell pluripotency and self renewal capacity. It was shown that relevant OCT4 expression only occurs in cells of embryonic pluripotent nature. However, several recent publications claimed to have demonstrated OCT4 expression in human somatic tumor cells, human adult stem or progenitor cells and differentiated cells.We analysed 42 human tumor cell lines from 13 entities and human bone marrow-derived mesenchymal stem cells (MSC). To validate OCT4 expression we used germ cell tumor (GCT) cell lines, derived xenografts and GCT samples. Analysis by RT-PCR, western blotting, immunocytochemistry and immunohistochemistry was performed. With exception of typical embryonal carcinoma cells, we did not observe reliable OCT4 expression in somatic tumor cell lines and MSC. We suggest that a high level of expression of the OCT4 protein together with its nuclear localization still remains a reliable and definitive feature of cells with embryonic pluripotent nature.

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.

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.

Parameswaran, V., et al. (2012). "ESSA1 embryonic stem like cells from gilthead seabream: a new tool to study mesenchymal cell lineage differentiation in fish." Differentiation **84**(3): 240-251.

Embryonic stem (ES) cells are a promising tool for generation of transgenic animals and an ideal experimental model for in vitro studies of embryonic cell development, differentiation and gene manipulation. Here we report the development and initial characterization of a pluripotent embryonic stem like cell line, designated as ESSA1, derived from blastula stage embryos of the gilthead seabream (Sparus aurata, L). ESSA1 cells are cultured in Leibovitz's L-15 medium supplemented with 5% fetal bovine serum and, unlike other ES cells, without a feeder layer. They have a round or polygonal morphology, grow exponentially in culture and form dense colonies. ESSA1 cells also exhibit intense alkaline phosphatase activity, normal karyotype and are positive for stage-specific embryonic antigen-1 (SSEA1) and octamer-binding transcription factor 4 (Oct4) markers for up to 30 passages. Upon treatment with all-trans retinoic acid, ESSA1 cells differentiate into neuron-like, oligodendritic, myocyte and melanocyte cells; they can also form embryoid bodies when seeded in bacteriological plates, a characteristic usually associated with pluripotency. The capacity of ESSA1 cells to differentiate into osteoblastic, chondroblastic or osteoclastic cell lineages and to produce a mineralized extracellular matrix in vitro was demonstrated through histochemical techniques and further confirmed by immunocytochemistry using lineage-specific markers. Furthermore,

ESSA1 cells can be used to produce chimera, where they contribute to the development of a variety of tissues including the trunk and gut of zebrafish embryos and fry. Thus, ESSA1 cells represent a promising model for investigating bone-lineage cell differentiation in fish and also highlight the potential of piscine stem cell research.

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.

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.

Quint, K., et al. (2012). "Pancreatic cancer cells surviving gemcitabine treatment express markers of stem cell differentiation and epithelial-mesenchymal transition." Int J Oncol **41**(6): 2093-2102.

Objective response rates to standard chemotherapeutic regimens remain low in pancreatic cancer. Subpopulations of cells have been identified in various solid tumors which express stem cell-associated markers and are associated with increased resistance against radiochemotherapy. We investigated the expression of stem cell genes and markers of epithelial-mesenchymal transition in pancreatic cancer cells that survived high concentrations of gemcitabine treatment. Capan-1 and Panc-1 cells were continuously incubated with 1 and 10 microM gemcitabine. Surviving cells were collected after 1, 3 and 6 days. Expression of PDX-1, SHH, CD24, CD44, CD133, EpCAM, CBX7, OCT4, SNAIL, SLUG, TWIST, Ki-67, E-cadherin, beta-catenin and vimentin were quantified by qPCR or immunocytochemistry. Migration was assessed by woundhealing assay. SHH was knocked down using RNA interference. Five primary pancreatic cancer cell lines were used to validate the qPCR results. All investigated genes were upregulated after 6 days of gemcitabine incubation. Highest relative expression levels were observed for OCT4 (13.4-fold), CD24 (47.3-fold) and EpCAM (15.9-fold) in Capan-1 and PDX-1 (13.3fold), SHH (24.1-fold), CD44 (17.4-fold), CD133 (20.2-fold) and SLUG (15.2-fold) in Panc-1 cells. Distinct upregulation patterns were observed in the primary cells. Migration was increased in Panc-1 cells and changes in the expression of E-cadherin and beta-catenin were typical of epithelial-mesenchymal transition in both cell lines. SHH knockdown reduced IC(50) from 30.1 to 27.6 nM in Capan-1 while it strongly inhibited proli-feration in Panc-1 cells. Cells surviving high-dose gemcitabine treatment express increased levels of stem cell genes, show characteristics associated with epithelial-mesenchymal transition and retain their proliferative capacity.

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.

Shou, K., et al. (2018). "Induction of mesenchymal stem cell differentiation in the absence of soluble inducer for cutaneous wound regeneration by a chitin nanofiber-based hydrogel." J Tissue Eng Regen Med **12**(2): e867-e880.

Transplantation of bone marrow mesenchymal stem cells (BMSCs) has been considered to be a promising strategy for wound healing. However, poor viability of engrafted BMSCs and limited capabilities of differentiation into the desired cell types in wounds often hinder its application. Few studies report the induction of BMSC differentiation into the skin regeneration-related cell types using natural biopolymer, e.g. chitin and its derivative. Here we utilized a chitin nanofiber (CNF) hydrogel as a directive cue to induce BMSC differentiation for enhancing cutaneous wound regeneration in the absence of cell-differentiating factors. First, a 'green' fabrication of CNF hydrogels encapsulating green fluorescence protein (GFP)-transfected rat BMSCs was performed via in-situ physical gelation without chemical cross-linking. Without soluble differentiation inducers, CNF hydrogels decreased the expression of BMSC transcription factors (Oct4 and Klf4) and concomitantly induced their differentiation into the angiogenic cells and fibroblasts, which are indispensable for wound regeneration. In vivo, rat full-thickness cutaneous wounds treated with BMSC hydrogel exhibited better viability of the cells than did local BMSC injection-treated wounds. Similar to that of the in vitro result, CNF hydrogels induced BMSCs to differentiate into beneficial cell types, resulting in accelerated wound repair characterized by granulation tissue formation. Our data suggest that three-dimensional CNF hydrogel may not only serve as a 'protection' to improve the viability of exogenous BMSCs, but also provide a functional scaffold capable of enhancing BMSC regenerative potential to promote wound healing. This may help to overcome the current limitations to stem cell therapy that are faced in the field of wound regeneration. Copyright (c) 2017 John Wiley & Sons, Ltd.

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

Mesenchymal stem cell (MSC)-conditioned medium (MSC-CM) has been reported to enhance wound healing. Exosomes contain nucleic acids, proteins, and lipids, and function as an intercellular communication vehicle for mediating some paracrine effects. However, the function of MSC-derived exosomes (MSC-exo) remains elusive. In this study, we isolated human placenta MSC (PlaMSC)-derived exosomes (PlaMSC-exo) and examined their function in vitro. PlaMSCs were isolated from human term placenta using enzymatic digestion. PlaMSC-exo were prepared from the conditioned medium of PlaMSC (PlaMSC-CM) by ultracentrifugation. The expression of stemness-related genes, such as OCT4 and NANOG, in normal adult human dermal fibroblasts (NHDF) after incubation with PlaMSC-exo was measured by real-time reverse transcriptase PCR analysis (real-time PCR). The effect of PlaMSC-exo on OCT4 transcription activity was assessed using Oct4-EGFP reporter mice-derived dermal fibroblasts. The stimulating

effects of PlaMSC-exo on osteoblastic and adipocyte-differentiation of NHDF were evaluated by alkaline phosphatase (ALP), and Alizarin red S- and oil red O-staining, respectively. The expression of osteoblast- and adipocyte-related genes was also assessed by real-time PCR. The treatment of NHDF with PlaMSC-exo significantly upregulated OCT4 and NANOG mRNA expression. PlaMSC-exo also enhanced OCT4 transcription. The NHDF treated with PlaMSC-exo exhibited osteoblastic and adipocyte-differentiation in osteogenic and adipogenic induction media. PlaMSC-exo increase the expression of OCT4 and NANOG mRNA in fibroblasts. As a result, PlaMSC-exo influence the differentiation competence of fibroblasts to both osteoblastic and adipocyte-differentiation. It shows a new feature of MSCs and the possibility of clinical application of MSC-exo. J. Cell. Biochem. 117: 1658-1670, 2016. (c) 2015 Wiley Periodicals, Inc.

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

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

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

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

Visciano, C., et al. (2015). "Mast cells induce epithelial-to-mesenchymal transition and stem cell features in human thyroid cancer cells through an IL-8-Akt-Slug pathway." Oncogene **34**(40): 5175-5186.

There is increasing evidence that mast cells (MCs) and their mediators are involved in the remodeling of the tumor microenvironment and promote tumor growth, angiogenesis and metastasis. We have found that an increased density of MCs in thyroid cancer (TC) correlates with enhanced invasiveness. However, the MC-derived factors responsible for this activity and the mechanisms by which they enhance TC invasiveness remain unidentified. Here, we report that MCs, when activated by TC cells, produce soluble factors that induce epithelial-to-mesenchymal transition (EMT) and stemness features of TC cells. We identified CXCL8/interleukin (IL)-8 as the main mediator contained in activated MC conditioned media (CM) capable of inducing both EMT and stemness of TC cells. Mechanistically, MC CM or exogenous IL-8 stimulated Akt phosphorylation and Slug expression in TC cells. The inhibition of the Akt pathway or depletion of the Slug

transcription factor by RNA interference, reverted EMT and stemness responses. TC cells stably transfected with exogenous IL-8 underwent EMT, displayed increased stemness and enhanced tumorigenicity with respect to control cells. The analysis of TC surgical specimens by immunohistochemical analysis demonstrated a positive correlation between MC density (Tryptase(+) cells) and stemness features (OCT4 staining). Taken together, our data identify an MC-dependent IL-8-Akt-Slug pathway that sustains EMT/stemness of TC cells. The blockade of this circuit might be exploited for the therapy of advanced TC.

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.

Wang, Y. C. and Y. Zhang (2008). "[Proliferative capacity of mesenchymal stem cells from human fetal bone marrow and their ability to differentiate into the derivative cell types of three embryonic germ layers]." Sheng Li Xue Bao **60**(3): 425-430.

Strong proliferative capacity and the ability to differentiate into the derivative cell types of three embryonic germ layers are the two important characteristics of embryonic stem cells. To study whether the mesenchymal stem cells from human fetal bone marrow (hfBM-MSCs) possess these embryonic stem cell-like biological characteristics, hfBM-MSCs were isolated from bone barrows and further purified according to the different adherence of different kinds of cells to the wall of culture flask. The cell cycle of hfBM-MSCs and MSC-specific surface markers such as CD29, CD44, etc were identified using flow cytometry. The expressions of human telomerase reverse transcriptase (hTERT), the

embryonic stem cell-specific antigens, such as Oct4 and SSEA-4 were detected with immunocytochemistry at the protein level and were also tested by RT-PCR at the mRNA level. Then, hfBM-MSCs were induced to differentiate toward neuron cells, adipose cells, and islet B cells under certain conditions. It was found that 92.3% passage-4 hfBM-MSCs and 96.1% passage-5 hfBM-MSCs were at G(0)/G(1) phase respectively. hfBM-MSCs expressed CD44, CD106 and adhesion molecule CD29, but not antigens of hematopoietic cells CD34 and CD45, and almost not antigens related to graft-versus-host disease (GVHD), such as HLA-DR, CD40 and CD80. hfBM-MSCs expressed the embryonic stem cell-specific antigens such as Oct4, SSEA-4, and also hTERT. Exposure of these cells to various inductive agents resulted in morphological changes towards neuron-like cells, adipose-like cells, and islet B-like cells and they were tested to be positive for related characteristic markers. These results suggest that there are plenty of MSCs in human fetal bone marrow, and hfBM-MSCs possess the embryonic stem cell-like biological characteristics, moreover, they have a lower immunogenic nature. Thus, hfBM-MSCs provide an ideal source for tissue engineering and cellular therapeutics.

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.

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).

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

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

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.

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