Mesenchymal Stem Cell Research Literatures

Mark Herbert, PhD

39-06 Main Street, Flushing, Queens, New York 11354, USA, ma8080@gmail.com

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

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Key words: stem cell; mesenchymal; 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.

Abdelrazik, H., et al. (2011). "Mesenchymal stem cells expanded in human platelet lysate display a decreased inhibitory capacity on T- and NK-cell proliferation and function." Eur J Immunol **41**(11): 3281-3290.

The use of fetal bovine serum (FBS) for the culture and expansion of mesenchymal stromal cells (MSCs) limits their possible clinical applications. Although some recent studies recommended substituting FBS with human platelet lysate (HPL) for the expansion of MSCs for clinical use, the functional capacity of the expanded cells has only been partially explored. 10% FBS and two other commercial FBS-containing media (MesenCult and MesenPro) were compared with 10% HPL-containing medium for their ability to support MSCs expansion and immunomodulation. We demonstrate that HPL sustained MSC proliferation and expansion in vitro. However, the cumulative cell numbers recovered were comparable with those obtained in MesenPro medium. Moreover, we show that HPL alters the expression of some relevant MSC surface molecules, namely the DNAM-1 ligands PVR and Nectin-2, the NKG2D ligand ULBP3, the adhesion molecules CD49d and alphavbeta3 and the fibroblast-associated protein. In addition, MSCs cultured in HPL displayed impaired inhibitory capacity on T-cell proliferation to alloantigen and NK-cell proliferation and cytotoxicity. Finally, they showed decreased constitutive PGE2 production while IL-6, IL-8 and RANTES secretion were upregulated. These results imply some limitations in the use of HPL for the expansion of MSCs to be used as immunomodulators in clinical applications.

Abumaree, M. H., et al. (2017). "Human chorionic villous mesenchymal stem/stromal cells modify the effects of oxidative stress on endothelial cell functions." Placenta **59**: 74-86.

Mesenchymal stem/stromal cells derived from chorionic villi of human term placentae (pMSCs) produce a unique combination of molecules, which modulate important cellular functions of their target cells while concurrently suppressing their immune responses. These properties make MSCs advantageous candidates for cell-based therapy. Our first aim was to examine the effect of high levels of oxidative stress on pMSC functions. pMSCs were exposed to hydrogen peroxide (H2O2) and their ability to proliferate and adhere to an endothelial cell monolayer was determined. Oxidatively stressed pMSCs maintained their proliferation and adhesion potentials. The second aim was to measure the ability of pMSCs to prevent oxidative stress-related damage to endothelial cells. Endothelial cells were exposed to H2O2, then co-cultured with pMSCs, and the effect on endothelial cell adhesion, proliferation and migration was

determined. pMSCs were able to reverse the damaging effects of oxidative stress on the proliferation and migration but not on the adhesion of endothelial cells. These data indicate that pMSCs are not only inherently resistant to oxidative stress, but also protect endothelial cell functions from oxidative stress-associated damage. Therefore, pMSCs could be used as a therapeutic tool in inflammatory diseases by reducing the effects of oxidative stress on endothelial cells.

Acquistapace, A., et al. (2011). "Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer." Stem Cells **29**(5): 812-824.

Because stem cells are often found to improve repair tissue including heart without evidence of engraftment or differentiation, mechanisms underlying wound healing are still elusive. Several studies have reported that stem cells can fuse with cardiomyocytes either by permanent or partial cell fusion processes. However, the respective physiological impact of these two processes remains unknown in part because of the lack of knowledge of the resulting hybrid cells. To further characterize cell fusion, we cocultured mouse fully differentiated cardiomyocytes with human multipotent adipose-derived stem (hMADS) cells as a model of adult stem cells. We found that heterologous cell fusion promoted cardiomyocyte reprogramming back to a progenitor-like state. The resulting hybrid cells expressed early cardiac commitment and proliferation markers such as GATA-4, myocyte enhancer factor 2C, Nkx2.5, and Ki67 and exhibited a mouse genotype. Interestingly, human bone marrow-derived stem cells shared similar reprogramming properties than hMADS cells but not human fibroblasts, which suggests that these features might be common to multipotent cells. Furthermore, cardiac hybrid cells were preferentially generated by partial rather than permanent cell fusion and that intercellular structures composed of f-actin and microtubule filaments were involved in the process. Finally, we showed that stem cell mitochondria were transferred into cardiomyocytes, persisted in hybrids and were required for somatic cell reprogramming. In conclusion, by providing new insights into previously reported cell fusion processes, our data might contribute to a better understanding of stem cell-mediated regenerative mechanisms and thus, the development of more efficient stem cell-based heart therapies.

Aggarwal, S. and M. F. Pittenger (2005). "Human mesenchymal stem cells modulate allogeneic immune cell responses." Blood **105**(4): 1815-1822.

Mesenchymal stem cells (MSCs) are multipotent cells found in several adult tissues. Transplanted allogeneic MSCs can be detected in recipients at extended time points, indicating a lack of immune recognition and clearance. As well, a role for bone marrow-derived MSCs in reducing the incidence and severity of graft-versus-host disease (GVHD) during allogeneic transplantation has recently been reported; however, the mechanisms remain to be investigated. We examined the immunomodulatory functions of human MSCs (hMSCs) by coculturing them with purified subpopulations of immune cells and report here that hMSCs altered the cytokine secretion profile of dendritic cells (DCs), naive and effector T cells (T helper 1 [T (H)1] and T (H)2), and natural killer (NK) cells to induce a more anti-inflammatory or tolerant phenotype. Specifically, the hMSCs caused mature DCs type 1 (DC1) to decrease tumor necrosis factor alpha (TNF-alpha) secretion and mature DC2 to increase interleukin-10 (IL-10) secretion; hMSCs caused T (H)1 cells to decrease interferon gamma (IFN-gamma) and caused the T (H)2 cells to increase secretion of IL-4; hMSCs caused an increase in the proportion of regulatory T cells (T (Regs)) present; and hMSCs decreased secretion of IFN-gamma from the NK cells. Mechanistically, the hMSCs produced elevated prostaglandin E2 (PGE (2)) in co-cultures, and inhibitors of PGE (2) production mitigated hMSC-mediated immune modulation. These data offer insight into the interactions between allogeneic MSCs and immune cells and provide mechanisms likely involved with the in vivo MSC-mediated induction of tolerance that could be therapeutic for reduction of GVHD, rejection, and modulation of inflammation.

Ahn, J. O., et al. (2014). "Human adipose tissue-derived mesenchymal stem cells inhibit T-cell lymphoma growth in vitro and in vivo." Anticancer Res **34**(9): 4839-4847.

BACKGROUND/AIM: Human mesenchymal stem cells (hMSCs) are thought to be one of the most reliable stem cell sources for a variety of cell therapies. This study investigated the anti-tumor effect of human adipose tissue-derived mesenchymal stem cells (hAT-MSCs) on EL4 murine T-cell lymphoma in vitro and in vivo. MATERIALS AND METHODS: The growth-inhibitory effect of hAT-MSCs on EL4 tumor cells was evaluated using a WST-1 cell proliferation assay. Cell-cycle arrest and apoptosis were investigated by flow cytometry and western blot. To evaluate an anti-tumor effect of hAT-MSCs on T-cell lymphoma in vivo, CM-DiI-labeled hAT-MSCs were circumtumorally injected in tumor-bearing nude mice, and tumor size was measured. RESULTS: hAT-MSCs inhibited T-cell lymphoma growth by altering cell-cycle progression and inducing apoptosis in vitro.

hAT-MSCs inhibited tumor growth in tumor-bearing nude mice and prolonged survival time. Immunofluorescence analysis showed that hAT-MSCs migrated to tumor sites. CONCLUSION: hAT-MSCs suppress the growth of T-cell lymphoma, suggesting a therapeutic option for T-cell lymphoma.

Akimov, S., et al. (2009). "Murine bone marrow stromal cell culture with features of mesenchymal stem cells susceptible to mouse-adapted human TSE agent, Fukuoka-1." Folia Neuropathol **47**(2): 205-214.

Transmission of transmissible spongiform encephalopathies (TSEs)/prion diseases through transplantation of bone marrow (BM) has never been reported in humans. However, the use of fetal bovine serum in current protocols for generating mesenchymal stem cells (MSCs) carries the risk of iatrogenic spread. We developed a cell model from murine BM-derived MSCs and tested its susceptibility to Fukuoka-1 (Fu) strain of TSEs. The adherent cells expressed significant levels of normal prion protein, PrPC, at the time when they became immortalized. The cell culture underwent spontaneous transformation following inoculation with Fu-infected brain homogenate and became persistently infected after reinoculation with Fu agent. Extensive analysis of the original and two Fu-exposed cell cultures revealed a phenotype characteristic of MSCs with a majority of cells being positive for stem cell antigen, Sca-1. Taken together, our results demonstrate that BM-derived MSCs can be infected with TSE agents under certain conditions ex vivo. Comprehensive studies should be undertaken to address the safety of cell-based therapies in regard to iatrogenic transmission of TSEs. BM-derived cell cultures can be used for studies of molecular mechanisms underlying the cells' susceptibility to various strains of TSEs, their propagation ex vivo, and for screening of potential anti-TSEs therapeutics.

Almeida, C. R., et al. (2016). "NAP-2 Secreted by Human NK Cells Can Stimulate Mesenchymal Stem/Stromal Cell Recruitment." Stem Cell Reports **6**(4): 466-473.

Strategies for improved homing of mesenchymal stem cells (MSCs) to a place of injury are being sought and it has been shown that natural killer (NK) cells can stimulate MSC recruitment. Here, we studied the chemokines behind this recruitment. Assays were performed with bone marrow human MSCs and NK cells freshly isolated from healthy donor buffy coats. Supernatants from MSC-NK cell co-cultures can induce MSC recruitment but not to the same extent as when NK cells are present. Antibody arrays and ELISA assays confirmed that NK cells secrete RANTES (CCL5) and revealed that human NK cells secrete NAP-2 (CXCL7), a chemokine that can induce MSC migration. Inhibition with specific antagonists of CXCR2, a receptor that recognizes NAP-2, abolished NK cell-mediated MSC recruitment. This capacity of NK cells to produce chemokines that stimulate MSC recruitment points toward a role for this immune cell population in regulating tissue repair/regeneration.

Alshabibi, M. A., et al. (2017). "Mesenchymal Stem/Multipotent Stromal Cells from Human Decidua Basalis Reduce Endothelial Cell Activation." Stem Cells Dev **26**(18): 1355-1373.

Recently, we reported the isolation and characterization of mesenchymal stem cells from the decidua basalis of human placenta (DBMSCs). These cells express a unique combination of molecules involved in many important cellular functions, which make them good candidates for cell-based therapies. The endothelium is a highly specialized, metabolically active interface between blood and the underlying tissues. Inflammatory factors stimulate the endothelium to undergo a change to a proinflammatory and procoagulant state (ie, endothelial cell activation). An initial response to endothelial cell activation is monocyte adhesion. Activation typically involves increased proliferation and enhanced expression of adhesion and inflammatory markers by endothelial cells. Sustained endothelial cell activation leads to a type of damage to the body associated with inflammatory diseases, such as atherosclerosis. In this study, we examined the ability of DBMSCs to protect endothelial cells from activation through monocyte adhesion, by modulating endothelial proliferation, migration, adhesion, and inflammatory marker expression. Endothelial cells were cocultured with DBMSCs, monocytes, monocyte-pretreated with DBMSCs and DBMSC-pretreated with monocytes were also evaluated. Monocyte adhesion to endothelial cells was examined following treatment with DBMSCs. Expression of endothelial cell adhesion and inflammatory markers was also analyzed. The interaction between DBMSCs and monocytes reduced endothelial cell proliferation and monocyte adhesion to endothelial cells. In contrast, endothelial cell migration increased in response to DBMSCs and monocytes. Endothelial cell expression of adhesion and inflammatory molecules was reduced by DBMSCs and DBMSC-pretreated with monocytes. The mechanism of reduced endothelial proliferation involved enhanced phosphorylation of the tumor suppressor protein p53. Our study shows for the first time that DBMSCs protect endothelial cells from activation by inflammation triggered by monocyte adhesion and increased endothelial cell proliferation. These events are manifest in inflammatory diseases, such as atherosclerosis. Therefore, our results suggest

that DBMSCs could be usefully employed as a therapeutic strategy for atherosclerosis.

Alshatwi, A. A., et al. (2012). "Al (2)O (3) nanoparticles induce mitochondria-mediated cell death and upregulate the expression of signaling genes in human mesenchymal stem cells." J Biochem Mol Toxicol **26**(11): 469-476.

An increase in the broad usage of Al (2)O (3) nanoparticles (ANPs) in the food and agricultural sectors may produce rare hazards for human health. The objective of this study was to assess the acute toxicity of ANPs in human mesenchymal stem cells (hMSCs) in vitro. Cell viability, cellular uptake, morphology, and gene expression using quantitative real-time polymerase chain reaction (qRT-PCR) were analyzed. The results indicate that ANPs have a significant and dose-dependent effect on cytotoxicity. Control cells showed a characteristic, homogeneous nuclear staining pattern, whereas ANP-exposed cells showed abnormal nuclear morphological changes such as condensation or fragmentation. An early characteristic of apoptosis was observed in ANP-treated cells. Further confirmation of cell death in hMSCs was observed through increased expression of chosen signaling genes and also decreased expression of Bcl-2 during mitochondria-mediated cell death. Although they provide great advantages in food and agricultural products, the chronic and acute toxicity of ANPs still needs to be assessed carefully.

Altanerova, U., et al. (2017). "Human mesenchymal stem cell-derived iron oxide exosomes allow targeted ablation of tumor cells via magnetic hyperthermia." Int J Nanomedicine **12**: 7923-7936.

Magnetic hyperthermia, or the heating of tissues using magnetic materials, is a promising approach for treating cancer. We found that human mesenchymal stem cells (MSCs) isolated from various tissues and MSCs expressing the yeast cytosine deaminaseColon, two colonsuracil phosphoribosyl transferase suicide fusion gene (yCDColon, two colonsUPRT) can be labeled with Venofer, an iron oxide carbohydrate nanoparticle. Venofer labeling did not affect cell proliferation or the ability to home to tumors. All Venofer-labeled MSCs released exosomes that contained iron oxide. Furthermore, these exosomes were efficiently endocytosed by tumor cells. Exosomes from Venofer-labeled MSCs expressing the yCDColon, two colonsUPRT gene in the presence of the prodrug 5-fluorocytosine inhibited tumor growth in a dose-dependent fashion. The treated tumor cells were also effectively ablated following induction of hyperthermia using an external alternating magnetic field. Cumulatively, we found that magnetic nanoparticles packaged into MSC exosomes are efficiently endocytosed by tumor cells, facilitating targeted tumor cell ablation via magnetically induced hyperthermia.

Alvarez, R., et al. (2015). "Characterization of the osteogenic potential of mesenchymal stem cells from human periodontal ligament based on cell surface markers." Int J Oral Sci **7**(4): 213-219.

Mesenchymal stem cell (MSC)-mediated therapy has been shown to be clinically effective in regenerating tissue defects. For improved regenerative therapy, it is critical to isolate homogenous populations of MSCs with high capacity to differentiate into appropriate tissues. The utilization of stem cell surface antigens provides a means to identify MSCs from various tissues. However, few surface markers that consistently isolate highly regenerative MSCs have been validated, making it challenging for routine clinical applications and making it all the more imperative to identify reliable surface markers. In this study, we used three surface marker combinations: CD51/CD140alpha, CD271, and STRO-1/CD146 for the isolation of homogenous populations of dental mesenchymal stem cells (DMSCs) from heterogeneous periodontal ligament cells (PDLCs). Fluorescence-activated cell sorting analysis revealed that 24% of PDLCs were CD51(+)/CD140alpha (+), 0.8% were CD271(+), and 2.4% were STRO-1(+)/CD146(+). Sorted cell populations were further assessed for their multipotent properties by inducing osteogenic and chondrogenic differentiation. All three subsets of isolated DMSCs exhibited differentiation capacity into osteogenic and chondrogenic lineages but with varying degrees. CD271(+) DMSCs demonstrated the greatest osteogenic potential with strong induction of osteogenic markers such as DLX5, RUNX2, and BGLAP. Our study provides evidence that surface marker combinations used in this study are sufficient markers for the isolation of DMSCs from PDLCs. These results provide important insight into using specific surface markers for identifying homogenous populations of DMSCs for their improved utilization in regenerative medicine.

Ame-Thomas, P., et al. (2007). "Human mesenchymal stem cells isolated from bone marrow and lymphoid organs support tumor B-cell growth: role of stromal cells in follicular lymphoma pathogenesis." Blood **109**(2): 693-702.

Accumulating evidence indicates that the cellular microenvironment plays a key role in follicular lymphoma (FL) pathogenesis, both within tumor lymph nodes (LNs) and in infiltrated bone marrow where ectopic LN-like reticular cells are integrated within malignant B-cell nodular aggregates. In normal secondary lymphoid organs, specific stromal cell

subsets provide a highly specialized microenvironment that supports immune response. In particular, fibroblastic reticular cells (FRCs) mediate immune cell migration, adhesion, and reciprocal interactions. The role of FRCs and their postulated progenitors, that is, bone marrow mesenchymal stem cells (MSCs), in FL remains unexplored. In this study, we investigated the relationships between FRCs and MSCs and their capacity to sustain malignant B-cell growth. Our findings strongly suggest that secondary lymphoid organs contain MSCs able to give rise to adipocytes, chondrocytes, osteoblasts, as well as fully functional B-cell supportive FRCs. In vitro, bone marrow-derived MSCs acquire a complete FRC phenotype in response to a combination of tumor necrosis factor-alpha and lymphotoxin-alpha1beta2. Moreover, MSCs recruit primary FL cells that, in turn, trigger their differentiation into FRCs, making them able to support malignant B-cell survival. Altogether, these new insights into the cross talk between lymphoma cells and their microenvironment could offer original therapeutic strategies.

Anderson, D. G., et al. (2013). "Human umbilical cord blood-derived mesenchymal stem cells in the cultured rabbit intervertebral disc: a novel cell source for disc repair." Am J Phys Med Rehabil **92**(5): 420-429.

OBJECTIVE: Back pain associated with symptomatic disc degeneration is a common clinical condition. Intervertebral disc (IVD) cell apoptosis and senescence increase with aging and degeneration. Repopulating the IVD with cells that could produce and maintain extracellular matrix would be an alternative therapy to surgery. The objective of this study was to determine the potential of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) as a novel cell source for disc repair. In this study, we intended to confirm the potential for hUCB-MSCs to differentiate and display a chondrocyte-like phenotype after culturing in micromass and after injection into the rabbit IVD explant culture. We also wanted to confirm hUCB-MSC survival after transplantation into the IVD explant culture. DESIGN: This study consisted of micromass cultures and in vitro rabbit IVD explant cultures to assess hUCB-MSC survival and differentiation to display chondrocyte-like phenotype. First, hUCB-MSCs were cultured in micromass and stained with Alcian blue dye. Second, to confirm cell survival, hUCB-MSCs were labeled with an infrared dye and a fluorescent dye before injection into whole rabbit IVD explants (host). IVD explants were then cultured for 4 wks. Cell survival was confirmed by two independent techniques: an imaging system detecting the infrared dye at the organ level and fluorescence microscopy detecting fluorescent dye at the cellular level. Cell viability was assessed by staining the explant with CellTracker green, a membrane-permeant tracer specific for live cells. Human type II collagen gene expression (from the graft) was assessed by polymerase chain reaction. RESULTS: We have shown that hUCB-MSCs cultured in micromass are stained blue with Alcian blue dye, which suggests that proteoglycan-rich extracellular matrix is produced. In the cultured rabbit IVD explants, hUCB-MSCs survived for at least 4 wks and expressed the human type II collagen gene, suggesting that the injected hUCB-MSCs are differentiating into a chondrocyte-like lineage. CONCLUSIONS: This study demonstrates the abiity of hUBC-MSCs to survive and assume a chondrocyte-like phenotype when injected into the rabbit IVD. These data support the potential for hUBC-MSCs as a cell source for disc repair. Further measures of the host response to the injection and studies in animal models are needed before trials in humans.

Anderson, S. B., et al. (2011). "The performance of human mesenchymal stem cells encapsulated in cell-degradable polymer-peptide hydrogels." Biomaterials **32**(14): 3564-3574.

Thiol-ene photopolymerization offers a unique platform for the formation of peptide-functionalized poly (ethylene glycol) hydrogels and the encapsulation, culture and differentiation of cells. Specifically, this photoinitiated polymerization scheme occurs at neutral pH and can be controlled both spatially and temporally. Here, we have encapsulated human mesenchymal stem cells (hMSCs) in matrix metalloproteinase (MMP) degradable and cell-adhesive hydrogels using thiol-ene photopolymerization. We find that hMSCs survive equally well in this system, regardless of MMP-degradability. When hMSCs are encapsulated in these cell-degradable hydrogels, they survive and are able to proliferate. In classic hMSC differentiation medias, hMSCs locally remodel their microenvironment and take on characteristic morphologies; hMSCs cultured in growth or osteogenic differentiation media are less round, as measured by elliptical form factor, and are smaller than hMSCs cultured in chondrogenic or adipogenic differentiation media. In addition, hMSCs encapsulated in completely cell-degradable hydrogels and cultured in osteogenic, chondrogenic, or adipogenic differentiation media generally express increased levels of specific differentiation markers as compared to cells in hydrogels that are not cell-degradable. These studies demonstrate the ability to culture and differentiate hMSCs in MMP-degradable hydrogels polymerized via a thiol-ene reaction scheme and that increased cell-mediated hydrogel

degradability facilitates directed differentiation of hMSCs.

Ardeshirylajimi, A., et al. (2017). "Renal Differentiation of Mesenchymal Stem Cells Seeded on Nanofibrous Scaffolds Improved by Human Renal Tubular Cell Lines-Conditioned Medium." ASAIO J **63**(3): 356-363.

Kidney injuries and renal dysfunctions are one of the most important clinical problems, and tissue engineering could be a valuable method for solving it. The objective of this study was to investigate the synergistic effect of renal cell line-conditioned medium and Polycaprolactone (PCL) nanofibers on renal differentiation of human mesenchymal stem cells (MSCs). In the current study, after stem cells isolation and characterization, PCL nanofibrous scaffold was fabricated using electrospinning methods and characterized morphologically, mechanically, and for biocompatibility. The renal differentiation of seeded MSCs on the surface of PCL nanofibers with and without human renal tubular cell lines-conditioned medium was investigated by evaluation of eight important renal-related genes expression by real-time reverse transcription polymerase chain reaction (RT-PCR) and immunocytochemistry. Fabricated nanofibrous scaffolds were good in all characterized items. Almost highest expression of all genes was detected in stem cells seeded on PCL under conditioned media in comparison with the stem cells seeded on PCL, tissue culture polystyrene (TCPS) under renal induction medium, and TCPS under conditioned medium. According to the results, PCL nanofibers in contribution with conditioned medium can provide the optimal conditions for renal differentiation of MSCs and could be a promising candidate for renal tissue engineering application.

Arpornmaeklong, P., et al. (2009). "Phenotypic characterization, osteoblastic differentiation, and bone regeneration capacity of human embryonic stem cell-derived mesenchymal stem cells." Stem Cells Dev **18**(7): 955-968.

To enhance the understanding of differentiation patterns and bone formation capacity of hESCs, we determined (1) the temporal pattern of osteoblastic differentiation of human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs), (2) the influence of a three-dimensional matrix on the osteogenic differentiation of hESC-MSCs in long-term culture, and (3) the bone-forming capacity of osteoblast-like cells derived from hESC-MSCs in calvarial defects. Incubation of hESC-MSCs in osteogenic medium induced osteoblastic differentiation of hESC-MSCs into mature osteoblasts in a similar chronological pattern to human bone marrow stromal cells and primary osteoblasts. Osteogenic differentiation was enhanced by culturing the cells on three-dimensional collagen scaffolds. Fluorescent-activated cell sorting of alkaline phosphatase expressing cells was used to obtain an enriched osteogenic cell population for in vivo transplantation. The identification of green fluorescence protein and expression of human-specific nuclear antigen in osteocytes in newly formed bone verified the role of transplanted human cells in the bone regeneration process. The current cell culture model and osteogenic cell enrichment method could provide large numbers of osteoprogenitor cells for analysis of differentiation patterns and cell transplantation to regenerate skeletal defects.

Arpornmaeklong, P., et al. (2017). "Effects of Titanium Surface Microtopography and Simvastatin on Growth and Osteogenic Differentiation of Human Mesenchymal Stem Cells in Estrogen-Deprived Cell Culture." Int J Oral Maxillofac Implants **32**(1): e35-e46.

PURPOSE: This study aimed to investigate the effects of titanium surface topography and simvastatin on growth and osteogenic differentiation of human bone marrow stromal cells (hBMSCs) in estrogen-deprived (ED) cell culture. MATERIALS AND METHODS: Human BMSCs were seeded on cell culture plates, smooth-surface titanium (Ti) disks, and sandblasted with large grits and acid etched (SLA)-surface Ti disks; and subsequently cultured in regular (fetal bovine serum [FBS]), ED, and ED-with 100 nM simvastatin (ED-SIM) culture media for 14 to 21 days. Live/dead cell staining, scanning electron microscope examination, and cell viability assay were performed to determine cell attachment, morphology, and growth. Expression levels of osteoblast-associated genes, Runx2 and bone sialoprotein and levels of alkaline phosphatase (ALP) activity, calcium content, and osteocalcin in culture media were measured to determine osteoblastic differentiation. Expression levels of bone morphogenetic protein-2 (BMP-2) were investigated to examine stimulating effects of simvastatin (n = 4 to 5, mean +/- SD). In vitro mineralization was verified by calcein staining. RESULTS: Human BMSCs exhibited different attachment and shapes on smooth and SLA titanium surfaces. Estrogen-deprived cell culture decreased cell attachment and growth, particularly on the SLA titanium surface, but cells were able to grow to reach confluence on day 21 in the ED-osteogenic (OS) culture medium. Promoting effects of the SLA titanium surface in ED-OS were significantly decreased. Simvastatin significantly increased osteogenic differentiation of human BMSCs on the SLA titanium surface in the ED-OS medium, and the

promoting effects of simvastatin corresponded with the increasing of BMP-2 gene expression on the SLA titanium surface in ED-OS-SIM culture medium. CONCLUSION: The ED cell culture model provided a well-defined platform for investigating the effects of hormones and growth factors on cells and titanium surface interaction. Titanium, the SLA surface, and simvastatin synergistically promoted osteoblastic differentiation of hBMSCs in ED condition and might be useful to promote osteointegration in osteoporotic bone.

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

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

Askar, S. F., et al. (2013). "Engraftment patterns of human adult mesenchymal stem cells expose electrotonic and paracrine proarrhythmic mechanisms in myocardial cell cultures." Circ Arrhythm Electrophysiol **6**(2): 380-391.

BACKGROUND: After intramyocardial injection, mesenchymal stem cells (MSCs) may engraft and influence host myocardium. However, engraftment rate and pattern of distribution are difficult to control in vivo, hampering assessment of potential adverse effects. In this study, the role of the engraftment patterns of MSCs on arrhythmicity in controllable in vitro models is investigated. METHODS AND RESULTS: Cocultures of 4x10(5) neonatal rat cardiomyocytes and 7% or 28% adult human MSCs (hMSCs) in diffuse or clustered distribution patterns were prepared. Electrophysiological effects were studied by optical mapping and patch-clamping. In diffuse cocultures, hMSCs dose-dependently decreased neonatal rat cardiomyocyte excitability, slowed conduction, and prolonged action potential duration until 90% repolarization (APD90). Triggered activity (14% versus 0% in controls) and increased inducibility of re-entry (53% versus 6% in controls) were observed in 28% hMSC cocultures. MSC clusters increased APD90, slowed conduction locally, and increased re-entry inducibility (23%), without increasing triggered activity. Pharmacological heterocellular electric uncoupling increased excitability and conduction velocity to 133% in 28% hMSC cocultures, but did not alter APD90. Transwell experiments showed that hMSCs dose-dependently increased APD90, APD dispersion, inducibility of re-entry and affected specific ion channel protein levels, whereas excitability was unaltered. Incubation with hMSC-derived exosomes did not increase APD in neonatal rat cardiomyocyte cultures. CONCLUSIONS: Adult hMSCs affect arrhythmicity of neonatal rat cardiomyocyte cultures by heterocellular coupling leading to depolarization-induced conduction slowing and by direct release of paracrine factors that negatively affect repolarization rate. The extent of these detrimental effects depends on the number and distribution pattern of hMSCs. These results suggest that caution should be urged against potential adverse effects of myocardial hMSC engraftment.

Astori, G., et al. (2007). ""In vitro" and multicolor phenotypic characterization of cell subpopulations identified in fresh human adipose tissue stromal vascular fraction and in the derived mesenchymal stem cells." J Transl Med **5**: 55.

BACKGROUND: The stromal vascular fraction (SVF) is a heterogeneous cell population derived from the adipose tissue. There is still a lack of information concerning the characterization of the cell subpopulations constituting the SVF as well as its mesenchymal and haematopoietic potential. Furthermore there are great variations in its phenotypical characterization. METHODS: Composition of SVF was investigated by FACS analysis, cytological and "in vitro" assays. We studied CD34+ population by combining FACS with human CFC (colony-forming-cell haematopoietic assay). The endothelial fraction was investigated by quantifying the co-expression of specific markers (CD146, CD105, CD31 and UEA-1). Mesenchymal potential was assessed by CFU-F assay and cultured AT-MSC were characterized by a 5-color FACS analysis. The multipotent differentiation potential (osteogenic, adipogenic and chondrogenic) was investigated both at cellular and molecular level. RESULTS: We identified in the SVF two CD34+ populations with a marked difference in the intensity of antigen expression, the majority of the cells expressing CD34 at low intensity. Moreover, two CD146+ cell populations were clearly distinguishable in the SVF:a CD146 dim accounting for 9.9% of the total SVF cells and a CD146+ bright cell population accounting for about 39.3%. The frequency of CFC clones was comparable with the one reported for peripheral blood. Endothelial cells account for about 7.7% of the SVF cells. AT-MSC differenced in the osteogenic adipogenic and chondrogenic lineage. CONCLUSION: The SVF is not a homogeneous cell population, and its final composition could be influenced both by the flow cytometric technique analysis and the SVF extraction steps. The CFU-F frequency in the SVF was 1/4880, a value about seven times greater than the data reported for bone marrow. The antigenic profile of AT-MSC was comparable with bone-marrow derived MSC. AT-MSC were able to differentiate along the osteogenic adipogenic and chondrogenic lineages. The data here reported, further contribute to the characterization of SVF, a tissue providing an alternative as a source of MSC for clinical applications.

Bagheri-Mohammadi, S., et al. (2018). "Stem cell-based therapy for Parkinson's disease with a focus on human endometrium-derived mesenchymal stem cells." J Cell Physiol.

Parkinson's disease (PD) as an increasing clinical syndrome is a multifunctional impairment with systemic involvement. At present, therapeutic approaches such as l-3,4-dihydroxy-phenylalanine replacement therapy, dopaminergic agonist administration, and neurosurgical treatment intend to relieve PD symptoms which are palliative and incompetent in counteracting PD progression. These mentioned therapies have not been able to replace the lost cells and they could not effectively slow down the relentless neurodegenerative process. Till now, there is a lack of eligible treatment for PD, and stem cells therapy recently has been considered for PD treatment. In this review, we demonstrate how human stem cell technology especially human endometrium-derived stem cells have made advancement as a therapeutic source for PD compared with other treatments.

Baharaghdam, S., et al. (2018). "Effects of Hypoxia on Biology of Human Leukemia T-cell Line (MOLT-4 cells) Co-cultured with Bone Marrow Mesenchymal Stem Cells." Avicenna J Med Biotechnol **10**(2): 62-68.

Background: One of the most significant problems in the treatment of leukemia is the expansion of resistance to chemotherapeutic agents. Therefore, assessing the drug resistance and especially the drug resistance genes of leukemic cells is important in any treatment. The impact of Mesenchymal Stem Cells (MSCs) and hypoxic condition have been observed in the biological performance of majority of leukemic cells. Methods: MOLT-4 cells were co-cultured with MSCs in the hypoxic condition induced by Cobalt Chloride (CoCl2) for 6 and 24 hr. Then, apoptosis of cells was analyzed using annexin-V/PI staining and expression of the drug resistance genes including MDR1, MRP, and BCRP along with apoptotic and anti-apoptotic genes, including BAX and BCL2, was evaluated by real-time PCR. Results: The hypoxic condition for MOLT-4 cells co-cultured with MSCs could significantly increase the expression of MDR1 and BCRP genes ($p<0.05$) which are involved in drug resistance. Also, the results indicated that this condition significantly increases the expression of BCL2 ($p<0.05$) and reduces the apoptosis in MOLT-4 cells co-cultured with MSCs in the hypoxic condition. Conclusion: These effects can demonstrate the important role of hypoxia and MSCs on the biological behavior of Acute Lymphoblastic Leukemia (ALL) cells that may lead to particular treatment outcomes.

Bai, L., et al. (2007). "Human mesenchymal stem cells signals regulate neural stem cell fate." Neurochem Res **32**(2): 353-362.

Neural stem cells (NSCs) differentiate into neurons, astrocytes and oligodendrocytes depending

on their location within the central nervous system (CNS). The cellular and molecular cues mediating end-stage cell fate choices are not completely understood. The retention of multipotent NSCs in the adult CNS raises the possibility that selective recruitment of their progeny to specific lineages may facilitate repair in a spectrum of neuropathological conditions. Previous studies suggest that adult human bone marrow derived mesenchymal stem cells (hMSCs) improve functional outcome after a wide range of CNS insults, probably through their trophic influence. In the context of such trophic activity, here we demonstrate that hMSCs in culture provide humoral signals that selectively promote the genesis of neurons and oligodendrocytes from NSCs. Cell-cell contacts were less effective and the proportion of hMSCs that could be induced to express neural characteristics was very small. We propose that the selective promotion of neuronal and oligodendroglial fates in neural stem cell progeny is responsible for the ability of MSCs to enhance recovery after a wide range of CNS injuries.

Bajetto, A., et al. (2017). "Different Effects of Human Umbilical Cord Mesenchymal Stem Cells on Glioblastoma Stem Cells by Direct Cell Interaction or Via Released Soluble Factors." Front Cell Neurosci **11**: 312.

Glioblastoma (GBM), the most common primary brain tumor in adults, is an aggressive, fast-growing and highly vascularized tumor, characterized by extensive invasiveness and local recurrence. In GBM and other malignancies, cancer stem cells (CSCs) are believed to drive invasive tumor growth and recurrence, being responsible for radio- and chemo-therapy resistance. Mesenchymal stem cells (MSCs) are multipotent progenitors that exhibit tropism for tumor microenvironment mediated by cytokines, chemokines and growth factors. Initial studies proposed that MSCs might exert inhibitory effects on tumor development, although, to date, contrasting evidence has been provided. Different studies reported either MSC anti-tumor activity or their support to tumor growth. Here, we examined the effects of umbilical cord (UC)-MSCs on in vitro GBM-derived CSC growth, by direct cell-to-cell interaction or indirect modulation, via the release of soluble factors. We demonstrate that UC-MSCs and CSCs exhibit reciprocal tropism when co-cultured as 3D spheroids and their direct cell interaction reduces the proliferation of both cell types. Contrasting effects were obtained by UC-MSC released factors: CSCs, cultured in the presence of conditioned medium (CM) collected from UC-MSCs, increased proliferation rate through transient ERK1/2 and Akt phosphorylation/activation. Analysis of the profile of the cytokines released by UC-MSCs in the CM revealed a strong production of molecules involved in inflammation, angiogenesis, cell migration and proliferation, such as IL-8, GRO, ENA-78 and IL-6. Since CXC chemokine receptor 2 (CXCR2), a receptor shared by several of these ligands, is expressed in GBM CSCs, we evaluated its involvement in CSC proliferation induced by UC-MSC-CM. Using the CXCR2 antagonist SB225002, we observed a partial but statistically significant inhibition of CSC proliferation and migration induced by the UC-MSC-released cytokines. Conversely, CXCR2 blockade did not reduce the reciprocal tropism between CSCs and UC-MSCs grown as spheroids. In conclusion, we show that direct (cell-to-cell contact) or indirect (via the release of soluble factors) interactions between GBM CSCs and UC-MSCs in co-culture produce divergent effects on cell growth, invasion and migration, with the former mainly causing an inhibitory response and the latter a stimulatory one, involving a paracrine activation of CXCR2.

Bajpai, V. K., et al. (2012). "Functional vascular smooth muscle cells derived from human induced pluripotent stem cells via mesenchymal stem cell intermediates." Cardiovasc Res **96**(3): 391-400.

AIMS: Smooth muscle cells (SMC) play an important role in vascular homeostasis and disease. Although adult mesenchymal stem cells (MSC) have been used as a source of contractile SMC, they suffer from limited proliferation potential and culture senescence, particularly when originating from older donors. By comparison, human induced pluripotent stem cells (hiPSC) can provide an unlimited source of functional SMC for autologous cell-based therapies and for creating models of vascular disease. Our goal was to develop an efficient strategy to derive functional, contractile SMC from hiPSC. METHODS AND RESULTS: We developed a robust, stage-wise, feeder-free strategy for hiPSC differentiation into functional SMC through an intermediate stage of multipotent MSC, which could be coaxed to differentiate into fat, bone, cartilage, and muscle. At this stage, the cells were highly proliferative and displayed higher clonogenic potential and reduced senescence when compared with parental hair follicle mesenchymal stem cells. In addition, when exposed to differentiation medium, the myogenic proteins such as alpha-smooth muscle actin, calponin, and myosin heavy chain were significantly upregulated and displayed robust fibrillar organization, suggesting the development of a contractile phenotype. Indeed, tissue constructs prepared from these cells exhibited high levels of contractility in response to receptor- and non-receptor-mediated agonists. CONCLUSION: We developed an efficient stage-wise strategy that enabled

hiPSC differentiation into contractile SMC through an intermediate population of clonogenic and multipotent MSC. The high yield of MSC and SMC derivation suggests that our strategy may facilitate an acquisition of the large numbers of cells required for regenerative medicine or for studying vascular disease pathophysiology.

Bak, X. Y., et al. (2011). "Human embryonic stem cell-derived mesenchymal stem cells as cellular delivery vehicles for prodrug gene therapy of glioblastoma." Hum Gene Ther **22**(11): 1365-1377.

Mesenchymal stem cells (MSCs) possess tumor-tropic properties and consequently have been used to deliver therapeutic agents for cancer treatment. Their potential in cancer therapy highlights the need for a consistent and renewable source for the production of uniform human MSCs suitable for clinical applications. In this study, we seek to investigate whether human embryonic stem cells can be used as a cell source to fulfill this goal. We generated MSC-like cells from two human embryonic stem cell lines, HuES9 and H1, and observed that MSC-like cells derived from human embryonic stem cells were able to migrate into human glioma intracranial xenografts after being injected into the cerebral hemisphere contralateral to the tumor inoculation site. We engineered these cells with baculoviral and lentiviral vectors, respectively, for transient and stable expression of the herpes simplex virus thymidine kinase gene. In tumor-bearing mice the engineered MSC-like cells were capable of inhibiting tumor growth and prolonging survival in the presence of ganciclovir after they were injected either directly into the xenografts or into the opposite hemisphere. Our findings suggest that human embryonic stem cell-derived MSCs may be a viable and attractive alternative for large-scale derivation of targeting vehicles for cancer therapy.

Balci, D. and A. Can (2013). "The assessment of cryopreservation conditions for human umbilical cord stroma-derived mesenchymal stem cells towards a potential use for stem cell banking." Curr Stem Cell Res Ther **8**(1): 60-72.

Human umbilical cord stroma-derived mesenchymal stem cells (hUCS-MSCs) are considered as a remarkable and promising stem cell source to be potentially used in cellular therapies. While no graft rejection has been reported in the recipient organism even in xeno-transplantation studies, attenuate tumor cell growth and gene transfers have been experimentally shown. In this study, we have demonstrated a reliable, reproducible and efficient cryopreservation method of hUCS-MSCs resulting in one of the highest cell survival rates reported so far. Conventional, computer-controlled multistep slow freezing (MSSF), and vitrification methods were comparatively tested using cell permeable [dimethylsulfoxide (DMSO), ethylene glycol] and impermeable [trehalose, sucrose, hydroxyethyl starch (HES), human serum albumin] cryoprotectant agents (CPAs). After determining the ice nucleation point for each solution, latent heat evolution was suppressed during freezing, followed by a cooling process to -40 degrees C at 1 degrees C/min or 0.3 degrees C/min. The efficiency of the cryopreservation techniques used was determined by cell viability and proliferation assays, the expression of cell surface markers, cytoskeletal proteins and chromosome alignments. The cell survival rate was found to be highest (87 +/- 5%) by MSSF with sucrose (0.1 M) +DMSO (10%) at 1 degrees C/min freezing rate. In this group, no significant difference was noted before and after the cryopreservation in cell morphology, cytokeratin, vimentin, and alpha-smooth muscle actin profiles and the expressions of CD105, CD90, CD73, CD29 and HLA-DR. Second highest cell survival ratio (85 +/- 6%) was obtained in DMSO (10%) alone at 1 degrees C/min freezing rate. Interestingly, poor (18 +/- 15%) cell survival rates were obtained after vitrification. Cumulatively, results indicated that MSSF favors the other freezing protocols with an addition of sucrose or DMSO alone depending on the freezing rate used.

Barbet, R., et al. (2012). "Expression of the 49 human ATP binding cassette (ABC) genes in pluripotent embryonic stem cells and in early- and late-stage multipotent mesenchymal stem cells: possible role of ABC plasma membrane transporters in maintaining human stem cell pluripotency." Cell Cycle **11**(8): 1611-1620.

The 49-member human ATP binding cassette (ABC) gene family encodes 44 membrane transporters for lipids, ions, peptides or xenobiotics, four translation factors without transport activity, as they lack transmembrane domains, and one pseudogene. To understand the roles of ABC genes in pluripotency and multipotency, we performed a sensitive qRT-PCR analysis of their expression in embryonic stem cells (hESCs), bone marrow-derived mesenchymal stem cells (hMSCs) and hESC-derived hMSCs (hES-MSCs). We confirm that hES-MSCs represent an intermediate developmental stage between hESCs and hMSCs. We observed that 44 ABCs were significantly expressed in hESCs, 37 in hES-MSCs and 35 in hMSCs. These variations are mainly due to plasma membrane transporters with low but significant gene expression: 18 are expressed in hESCs compared with 16 in hES-MSCs and 8 in hMSCs, suggesting important roles in pluripotency. Several of these ABCs shared similar substrates but differ regarding gene regulation. ABCA13 and ABCB4, similarly to ABCB1, could be

new markers to select primitive hMSCs with specific plasma membrane transporter (low) phenotypes. ABC proteins performing basal intracellular functions, including translation factors and mitochondrial heme transporters, showed the highest constant gene expression among the three populations. Peptide transporters in the endoplasmic reticulum, Golgi and lysosome were well expressed in hESCs and slightly upregulated in hMSCs, which play important roles during the development of stem cell niches in bone marrow or meningeal tissue. These results will be useful to study specific cell cycle regulation of pluripotent stem cells or ABC dysregulation in complex pathologies, such as cancers or neurological disorders.

Basu, J., et al. (2011). "Expansion of the human adipose-derived stromal vascular cell fraction yields a population of smooth muscle-like cells with markedly distinct phenotypic and functional properties relative to mesenchymal stem cells." Tissue Eng Part C Methods **17**(8): 843-860.

Adipose tissue contains a heterogeneous cell population composed of endothelial cells, adipocytes, smooth muscle cells (SMC), and mesenchymal progenitors and stromal cells that meet the criteria put forth by the International Society for Cellular Therapy as defining mesenchymal stem cells (MSC). In this study, we expanded the stromal vascular fraction (SVF) of human adipose tissue and characterized the resulting adherent primary cell cultures by quantitative reverse transcription-polymerase chain reaction, antigen expression, protein fingerprinting, growth kinetics, in vitro tri-lineage differentiation bioactivity, and functional responses to small molecules modulating SMC-related developmental pathways and compared the results to those obtained with functionally validated MSC cultures. SVF-derived initial cultures (P0) were expanded in a defined medium that was not optimized for MSC growth conditions, neither were recombinant cytokines or growth factors added to the media to direct differentiation. The adherent cell cultures derived from SVF expansion under these conditions had markedly distinct phenotypic and biological properties relative to functionally validated MSC cultures. SVF-derived adherent cell cultures retained characteristics consistent with the SMC subpopulation within adipose tissue--phenotype, gene, and protein expression--that were independent of passage number and source of SVF (n=4 independent donors). SVF-derived cells presented significantly less robust in vitro tri-lineage differentiation bioactivity relative to validated MSC. Expanded SVF cells and MSC had opposite responses to the thromboxane A2 mimetic U46619, demonstrating an unambiguous functional distinction between the two cell types. Taken together, these data support the conclusions that SVF cells expanded under the conditions described in these studies are accurately described as adipose-derived SMC and represent a cellular subpopulation of adipose SVF that is separate and distinct from other classes of adipose-derived cells.

Batten, P., et al. (2006). "Human mesenchymal stem cells induce T cell anergy and downregulate T cell allo-responses via the TH2 pathway: relevance to tissue engineering human heart valves." Tissue Eng **12**(8): 2263-2273.

To generate an "off the shelf" tissue-engineered heart valve, the cells would need to be of allogeneic origin. Here, we report the possibility of using human bone marrow-derived mesenchymal stem cells (MSCs) as a suitable allogeneic cell source for tissue-engineered heart valves. Proliferative responses of primary and primed CD4+ T cells to allogeneic MSCs were examined. A protein microarray system was used to detect soluble factors from supernatants collected from the T cell assays. MSCs are poor stimulators of primary and primed CD4+ T cell proliferation, despite provision of B7-1 trans-co-stimulation. MSCs not only directly inhibited primary and primed T cell responses to allogeneic peripheral blood mononuclear cells (PBMCs), but 24-h pre-culture of T cells with MSCs suppressed subsequent T cell proliferative responses to allogeneic PBMCs in a contact-dependent manner. Analysis of supernatants revealed a distinctly different cytokine profile after co-culture of T cells with MSCs than with PBMCs or endothelial cells. Pro-inflammatory Th1 cytokines interleukin (IL)-1alpha and beta, interferon (IFN)gamma, and tumor necrosis factor (TNF)alpha were downregulated, whereas, anti-inflammatory Th2 cytokines IL-3, IL-5, IL-10, and IL-13 and the Th2 chemokine I-309, a chemoattractant for regulatory T cells, were upregulated. Further analysis revealed that after co-culture with MSCs, the T cells exhibited a regulatory phenotype (CD4+ CD25(lo) CD69(lo) FoxP3+). MSCs downregulate T cell responses through direct contact and secretion of anti-inflammatory and tolerogenic cytokines, which may involve the recruitment of regulatory T cells. This implies that allogeneic MSCs could be a suitable cell source for tissue engineering a heart valve.

Bensidhoum, M., et al. (2004). "Homing of in vitro expanded Stro-1- or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment." Blood **103**(9): 3313-3319.

The Stro-1 antigen potentially defines a mesenchymal stem cell (MSC) progenitor subset. We here report on the role of human ex vivo-expanded selected Stro-1(+) or Stro-1(-) MSC subsets on the

engraftment of human CD34(+) cord blood cells in the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mouse model. The data show that cotransplantation of expanded Stro-1(-) cells with CD34(+) cells resulted in a significant increase of human CD45, CD34, CD19, and CD11b cells detected in blood or in bone marrow (BM) and spleen as compared with the infusion of CD34(+) cells alone. Infusion into mice of expanded Stro-1(+) and Stro-1(-) cells (without CD34(+) cells) showed that the numbers of Stro-1(+)-derived (as assessed by DNA analysis of human beta-globin with quantitative polymerase chain reaction [PCR]) were higher than Stro-1(-)-derived cells in spleen, muscles, BM, and kidneys, while more Stro-1(-)-derived than Stro-1(+)-derived cells were found in lungs. The transduction of expanded Stro-1(+) cells with an enhanced green fluorescent protein (eGFP) gene did not modify their cytokine release and their homing in NOD/SCID mouse tissues. The difference between the hematopoietic support and the homing capabilities of expanded Stro-1(+) and Stro-1(-) cells may be of importance for clinical therapeutic applications: Stro-1(+) cells may rather be used for gene delivery in tissues while Stro-1(-) cells may rather be used to support hematopoietic engraftment.

Benvenuto, F., et al. (2015). "Human mesenchymal stem cells target adhesion molecules and receptors involved in T cell extravasation." Stem Cell Res Ther **6**: 245.

INTRODUCTION: Systemic delivery of bone marrow-derived mesenchymal stem cells (MSC) seems to be of benefit in the treatment of multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS) sustained by migration of T cells across the brain blood barrier (BBB) and subsequent induction of inflammatory lesions into CNS. MSC have been found to modulate several effector functions of T cells. In this study, we investigated the effects of MSC on adhesion molecules and receptors on T cell surface that sustain their transendothelial migration. METHODS: We used different co-culture methods combined with real-time PCR and flow cytometry to evaluate the expression both at the mRNA and at the plasma-membrane level of alpha4 integrin, beta2 integrin, ICAM-1 and CXCR3. In parallel, we assessed if MSC are able to modulate expression of adhesion molecules on the endothelial cells that interact with T cells during their transendothelial migration. RESULTS: Our in vitro analyses revealed that MSC: (i) inhibit proliferation and activation of both peripheral blood mononuclear cells (PBMC) and CD3(+)-selected lymphocytes through the release of soluble factors; (ii) exert suppressive effects on those surface molecules highly expressed by activated lymphocytes and involved in transendothelial migration; (iii) inhibit CXCL10-driven chemotaxis of CD3(+) cells; (iv) down-regulated expression of adhesion molecules on endothelial cells. CONCLUSIONS: Taken together, these data demonstrate that the immunosuppressive effect of MSC does not exclusively depends on their anti-proliferative activity on T cells, but also on the impairment of leukocyte migratory potential through the inhibition of the adhesion molecules and receptors that are responsible for T cell trafficking across BBB. This could suggest a new mechanism through which MSC modulate T cell responses.

Bergante, S., et al. (2014). "Gangliosides as a potential new class of stem cell markers: the case of GD1a in human bone marrow mesenchymal stem cells." J Lipid Res **55**(3): 549-560.

Owing to their exposure on the cell surface and the possibility of being directly recognized with specific antibodies, glycosphingolipids have aroused great interest in the field of stem cell biology. In the search for specific markers of the differentiation of human bone marrow mesenchymal stem cells (hBMSCs) toward osteoblasts, we studied their glycosphingolipid pattern, with particular attention to gangliosides. After lipid extraction and fractionation, gangliosides, metabolically (3)H-labeled in the sphingosine moiety, were separated by high-performance TLC and chemically characterized by MALDI MS. Upon induction of osteogenic differentiation, a 3-fold increase of ganglioside GD1a was observed. Therefore, the hypothesis of GD1a involvement in hBMSCs commitment toward the osteogenic phenotype was tested by comparison of the osteogenic propensity of GD1a-highly expressing versus GD1a-low expressing hBMSCs and direct addition of GD1a in the differentiation medium. It was found that either the high expression of GD1a in hBMSCs or the addition of GD1a in the differentiation medium favored osteogenesis, providing a remarkable increase of alkaline phosphatase. It was also observed that ganglioside GD2, although detectable in hBMSCs by immunohistochemistry with an anti-GD2 antibody, could not be recognized by chemical analysis, likely reflecting a case, not uncommon, of molecular mimicry.

Bertoncini, P., et al. (2012). "Early adhesion of human mesenchymal stem cells on TiO (2) surfaces studied by single-cell force spectroscopy measurements." J Mol Recognit **25**(5): 262-269.

Understanding the interactions involved in the adhesion of living cells on surfaces is essential in the field of tissue engineering and biomaterials. In this study, we investigate the early adhesion of living human mesenchymal stem cells (hMSCs) on flat

titanium dioxide (TiO (2)) and on nanoporous crystallized TiO (2) surfaces with the use of atomic force microscopy-based single-cell force spectroscopy measurements. The choice of the substrate surfaces was motivated by the fact that implants widely used in orthopaedic and dental surgery are made in Ti and its alloys. Nanoporous TiO (2) surfaces were produced by anodization of Ti surfaces. In a typical force spectroscopy experiment, one living hMSC, immobilized onto a fibronectine-functionalized tipless lever is brought in contact with the surface of interest for 30 s before being detached while recording force-distance curves. Adhesion of hMSCs on nanoporous TiO (2) substrates having inner pore diameter of 45 nm was lower by approximately 25% than on TiO (2) flat surfaces. Force-distance curves exhibited also force steps that can be related to the pulling of membrane tethers from the cell membrane. The mean force step was equal to 35 pN for a given speed independently of the substrate surface probed. The number of tethers observed was substrate dependent. Our results suggest that the strength of the initial adhesion between hMSCs and flat or nanoporous TiO (2) surfaces is driven by the adsorption of proteins deposited from serum in the culture media.

Beyth, S., et al. (2005). "Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness." Blood **105**(5): 2214-2219.

Infusion of either embryonic or mesenchymal stem cells prolongs the survival of organ transplants derived from stem cell donors and prevents graft-versus-host-disease (GVHD). An in-depth mechanistic understanding of this tolerization phenomenon could lead to novel cell-based therapies for transplantation. Here we demonstrate that while human mesenchymal stem cells (hMSCs) can promote superantigen-induced activation of purified T cells, addition of antigen-presenting cells (APCs; either monocytes or dendritic cells) to the cultures inhibits the T-cell responses. This contact- and dose-dependent inhibition is accompanied by secretion of large quantities of interleukin (IL)-10 and aberrant APC maturation, which can be partially overridden by the addition of factors that promote APC maturation (ie, lipopolysaccharide [LPS] or anti-CD40 monoclonal antibody [mAb]). Thus, our data support an immunoregulatory mechanism wherein hMSCs inhibit T cells indirectly by contact-dependent induction of regulatory APCs with T-cell-suppressive properties. Our data may reveal a physiologic phenomenon whereby the development of a distinct APC population is regulated by the tissue's cellular microenvironment.

Binato, R., et al. (2013). "Stability of human mesenchymal stem cells during in vitro culture: considerations for cell therapy." Cell Prolif **46**(1): 10-22.

Ex vivo expansion and manipulation of human mesenchymal stem cells are important approaches to immunoregulatory and regenerative cell therapies. Although these cells show great potential for use, issues relating to their overall nature emerge as problems in the field. The need for extensive cell quantity amplification in vitro to obtain sufficient cell numbers for use, poses a risk of accumulating genetic and epigenetic abnormalities that could lead to sporadic malignant cell transformation. In this study, we have examined human mesenchymal stem cells derived from bone marrow, over extended culture time, using cytogenetic analyses, mixed lymphocyte reactions, proteomics and gene expression assays to determine whether the cultures would retain their potential for use in subsequent passages. Results indicate that in vitro cultures of these cells demonstrated chromosome variability after passage 4, but their immunomodulatory functions and differentiation capacity were maintained. At the molecular level, changes were observed from passage 5 on, indicating initiation of differentiation. Together, these results lead to the hypothesis that human mesenchymal stem cells cultures can be used successfully in cell therapy up to passage 4. However, use of cells from higher passages would have to be analysed case by case.

Bindslev, L., et al. (2006). "Labelling of human mesenchymal stem cells with indium-111 for SPECT imaging: effect on cell proliferation and differentiation." Eur J Nucl Med Mol Imaging **33**(10): 1171-1177.

PURPOSE: Stem cell therapy seems to be a new treatment option within cardiac diseases to improve myocardial perfusion and function. However, the delivery and traceability of the cells represent a problem. Radioactive labelling with 111In could be a method for tracking mesenchymal stem cells (MSCs). However, 111In could influence the viability and differentiation capacity of MSCs, which would limit its use. Therefore, the aim of this study was to evaluate the influence of 111In labelling in doses relevant for SPECT imaging in humans on the viability and differentiation capacity of human MSCs. METHODS AND RESULTS: Human MSCs isolated from bone marrow were incubated with 111In-tropolone (15-800 Bq/cell). The labelling efficiency was approximately 25% with 30 Bq/cell 111In. The MSC doubling time was 1.04+/-0.1 days and was not influenced by 111In within the range 15-260 Bq/cell. Using 30 Bq 111In/cell it was possible to label MSCs to a level

relevant for clinical scintigraphic use. With this dose, 111In had no effect on characteristic surface and intracellular markers of cultured MSCs analysed both by flow cytometry and by real-time polymerase chain reaction. Further, the labelled MSCs differentiated towards endothelial cells and formed vascular structures. CONCLUSION: It is possible to label human MSCs with 111In for scintigraphic tracking of stem cells delivered to the heart in clinical trials without affecting the viability and differentiation capacity of the MSCs. This creates an important tool for the control of stem cell delivery and dose response in clinical cardiovascular trials.

Block, T. J., et al. (2017). "Restoring the quantity and quality of elderly human mesenchymal stem cells for autologous cell-based therapies." Stem Cell Res Ther **8**(1): 239.

BACKGROUND: Degenerative diseases are a major public health concern for the aging population and mesenchymal stem cells (MSCs) have great potential for treating many of these diseases. However, the quantity and quality of MSCs declines with aging, limiting the potential efficacy of autologous MSCs for treating the elderly population. METHODS: Human bone marrow (BM)-derived MSCs from young and elderly donors were obtained and characterized using standard cell surface marker criteria (CD73, CD90, CD105) as recommended by the International Society for Cellular Therapy (ISCT). The elderly MSC population was isolated into four subpopulations based on size and stage-specific embryonic antigen-4 (SSEA-4) expression using fluorescence-activated cell sorting (FACS), and subpopulations were compared to the unfractionated young and elderly MSCs using assays that evaluate MSC proliferation, quality, morphology, intracellular reactive oxygen species, beta-galactosidase expression, and adenosine triphosphate (ATP) content. RESULTS: The ISCT-recommended cell surface markers failed to detect any differences between young and elderly MSCs. Here, we report that elderly MSCs were larger in size and displayed substantially higher concentrations of intracellular reactive oxygen species and beta-galactosidase expression and lower amounts of ATP and SSEA-4 expression. Based on these findings, cell size and SSEA-4 expression were used to separate the elderly MSCs into four subpopulations by FACS. The original populations (young and elderly MSCs), as well as the four subpopulations, were then characterized before and after culture on tissue culture plastic and BM-derived extracellular matrix (BM-ECM). The small SSEA-4-positive subpopulation representing ~ 8% of the original elderly MSC population exhibited a "youthful" phenotype that was similar to that of young MSCs. The biological activity of this elderly subpopulation was inhibited by senescence-associated factors produced by the unfractionated parent population. After these "youthful" cells were isolated and expanded (three passages) on a "young microenvironment" (i.e., BM-ECM produced by BM cells from young donors), the number of cells increased approximately 17,000-fold to 3 x 10(9) cells and retained their "youthful" phenotype. CONCLUSIONS: These results suggest that it is feasible to obtain large numbers of high-quality autologous MSCs from the elderly population and establish personal stem cell banks that will allow serial infusions of "rejuvenated" MSCs for treating age-related diseases.

Bochev, I., et al. (2008). "Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production in vitro." Cell Biol Int **32**(4): 384-393.

Mesenchymal stem cells (MSC) have been characterized as multipotent cells which are able to differentiate into several mesodermal and nonmesodermal lineage cells and this feature along with their extensive growth and comprehensive immunomodulatory properties establish them as a promising tool for therapeutic applications, including cell-based tissue engineering and treatment of immune-mediated disorders. Although bone marrow (BM) is the most common MSC source, cells with similar characteristics have been shown to be present in several other adult tissues. Adipose tissue (AT), large quantities of which can be easily obtained, represents an attractive alternative to BM in isolating adipose tissue-derived MSC (AT-MSC). BM-MSCs and AT-MSCs share some immunomodulatory properties as they are both not inherently immunogenic and suppress the proliferation of alloantigen- or mitogen-stimulated T-cells. Our purpose was to comparatively examine under appropriate in vitro conditions, phenotypes, morphology and some functional properties of BM-MSCs and AT-MSCs, such as differentiation potential and especially the ability to suppress the immunoglobulin production by mitogen-stimulated B-cells. While the morphological, immunophenotypical, colony-forming and adipogenic characteristics of both types of cells were almost identical, AT-MSCs showed less potential for osteogenic differentiation than BM-MSCs. We found that AT-MSCs not only inhibited the Ig-production but also suppressed this B-cell function to a much greater extent compared to BM-MSC. This finding supports the potential role of AT-MSCs as an alternative to BM-MSCs for clinical purposes.

Boxall, S. and E. Jones (2015). "The use of multiparameter flow cytometry and cell sorting to characterize native human bone marrow mesenchymal stem cells (MSC)." Methods Mol Biol **1235**: 121-130.

This chapter describes a method for identification, phenotypic analysis, and cell sorting of rare mesenchymal stem cells (MSCs) from human bone marrow (BM) aspirates. The native BM MSC population is identified based on the CD45(-/low)CD271(+) phenotype. The method consists of three related procedures: Procedure 1 involves a microbead-based pre-enrichment step. Two other procedures describe direct flow cytometric analysis of MSCs following the isolation of the mononuclear cell (MNC) fraction (Procedure 2) or more rapidly, following a simple ammonium chloride-based red cell lysis (Procedure 3). Recently described multi-lineage transcript expression in the CD45(-/low)CD271(+) cells suggests that the native BM MSC fraction could be further subdivided into functionally distinct subpopulations. The present protocols are hoped to help MSC biologists to enter this exciting field of research and to take it forward towards a better understanding of MSC biology in vivo.

Boyd, N. L., et al. (2011). "Microvascular mural cell functionality of human embryonic stem cell-derived mesenchymal cells." Tissue Eng Part A **17**(11-12): 1537-1548.

Microvascular mural or perivascular cells are required for the stabilization and maturation of the remodeling vasculature. However, much less is known about their biology and function compared to large vessel smooth muscle cells. We have developed lines of multipotent mesenchymal cells from human embryonic stem cells (hES-MC); we hypothesize that these can function as perivascular mural cells. Here we show that the derived cells do not form teratomas in SCID mice and independently derived lines show similar patterns of gene expression by microarray analysis. When exposed to platelet-derived growth factor-BB, the platelet-derived growth factor receptor beta is activated and hES-MC migrate in response to a gradient. We also show that in a serum-free medium, transforming growth factor beta1 (TGFbeta1) induces robust expression of multiple contractile proteins (alpha smooth muscle actin, smooth muscle myosin heavy chain, smooth muscle 22alpha, and calponin). TGFbeta1 signaling is mediated through the TGFbetaR1/Alk5 pathway as demonstrated by inhibition of alpha smooth muscle actin expression by treatment of the Alk5-specific inhibitor SB525334 and stable retroviral expression of the Alk5 dominant negative (K232R). Coculture of human umbilical vein endothelial cell (HUVEC) with hES-MC maintains network integrity compared to HUVEC alone in three-dimensional collagen I-fibronectin by paracrine signaling. Using high-resolution laser confocal microscopy, we show that hES-MC also make direct contact with HUVEC. This demonstrates that hESC-derived mesenchymal cells possess the molecular machinery expected in a perivascular progenitor cells and can play a functional role in stabilizing EC networks in in vitro three-dimensional culture.

Boyd, N. L., et al. (2013). "Dissecting the role of human embryonic stem cell-derived mesenchymal cells in human umbilical vein endothelial cell network stabilization in three-dimensional environments." Tissue Eng Part A **19**(1-2): 211-223.

The microvasculature is principally composed of two cell types: endothelium and mural support cells. Multiple sources are available for human endothelial cells (ECs) but sources for human microvascular mural cells (MCs) are limited. We derived multipotent mesenchymal progenitor cells from human embryonic stem cells (hES-MC) that can function as an MC and stabilize human EC networks in three-dimensional (3D) collagen-fibronectin culture by paracrine mechanisms. Here, we have investigated the basis for hES-MC-mediated stabilization and identified the pleiotropic growth factor hepatocyte growth factor/scatter factor (HGF/SF) as a putative hES-MC-derived regulator of EC network stabilization in 3D in vitro culture. Pharmacological inhibition of the HGF receptor (Met) (1 mum SU11274) inhibits EC network formation in the presence of hES-MC. hES-MC produce and release HGF while human umbilical vein endothelial cells (HUVEC) do not. When HUVEC are cultured alone the networks collapse, but in the presence of recombinant human HGF or conditioned media from human HGF-transduced cells significantly more networks persist. In addition, HUVEC transduced to constitutively express human HGF also form stable networks by autocrine mechanisms. By enzyme-linked immunosorbent assay, the coculture media were enriched in both angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2), but at significantly different levels (Ang1=159+/-15 pg/mL vs. Ang2=30,867+/-2685 pg/mL) contributed by hES-MC and HUVEC, respectively. Although the coculture cells formed stabile network architectures, their morphology suggests the assembly of an immature plexus. When HUVEC and hES-MC were implanted subcutaneously in immune compromised Rag1 mice, hES-MC increased their contact with HUVEC along the axis of the vessel. This data suggests that HUVEC and hES-MC form an immature plexus mediated in part by HGF and angiopoietins that is capable of maturation under the correct environmental conditions (e.g., in vivo). Therefore, hES-MC can function as

microvascular MCs and may be a useful cell source for testing EC-MC interactions.

Boyd, N. L., et al. (2009). "Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells." Tissue Eng Part A **15**(8): 1897-1907.

Human embryonic stem cells (hESC) have the potential to produce all of the cells in the body. They are able to self-renew indefinitely, potentially making them a source for large-scale production of therapeutic cell lines. Here, we developed a monolayer differentiation culture that induces hESC (WA09 and BG01) to form epithelial sheets with mesodermal gene expression patterns (BMP4, RUNX1, and GATA4). These E-cadherin+ CD90low cells then undergo apparent epithelial-mesenchymal transition for the derivation of mesenchymal progenitor cells (hESC-derived mesenchymal cells [hES-MC]) that by flow cytometry are negative for hematopoietic (CD34, CD45, and CD133) and endothelial (CD31 and CD146) markers, but positive for markers associated with mesenchymal stem cells (CD73, CD90, CD105, and CD166). To determine their functionality, we tested their capacity to produce the three lineages associated with mesenchymal stem cells and found they could form osteogenic and chondrogenic, but not adipogenic lineages. The derived hES-MC were able to remodel and contract collagen I lattice constructs to an equivalent degree as keloid fibroblasts and were induced to express alpha-smooth muscle actin when exposed to transforming growth factor (TGF)-beta1, but not platelet derived growth factor-B (PDGF-B). These data suggest that the derived hES-MC are multipotent cells with potential uses in tissue engineering and regenerative medicine and for providing a highly reproducible cell source for adult-like progenitor cells.

Bravo, B., et al. (2016). "Restrained Th17 response and myeloid cell infiltration into the central nervous system by human decidua-derived mesenchymal stem cells during experimental autoimmune encephalomyelitis." Stem Cell Res Ther **7**: 43.

BACKGROUND: Multiple sclerosis is a widespread inflammatory demyelinating disease. Several immunomodulatory therapies are available, including interferon-beta, glatiramer acetate, natalizumab, fingolimod, and mitoxantrone. Although useful to delay disease progression, they do not provide a definitive cure and are associated with some undesirable side-effects. Accordingly, the search for new therapeutic methods constitutes an active investigation field. The use of mesenchymal stem cells (MSCs) to modify the disease course is currently the subject of intense interest. Decidua-derived MSCs (DMSCs) are a cell population obtained from human placental extraembryonic membranes able to differentiate into the three germ layers. This study explores the therapeutic potential of DMSCs. METHODS: We used the experimental autoimmune encephalomyelitis (EAE) animal model to evaluate the effect of DMSCs on clinical signs of the disease and on the presence of inflammatory infiltrates in the central nervous system. We also compared the inflammatory profile of spleen T cells from DMSC-treated mice with that of EAE control animals, and the influence of DMSCs on the in vitro definition of the Th17 phenotype. Furthermore, we analyzed the effects on the presence of some critical cell types in central nervous system infiltrates. RESULTS: Preventive intraperitoneal injection of DMSCs resulted in a significant delay of external signs of EAE. In addition, treatment of animals already presenting with moderate symptoms resulted in mild EAE with reduced disease scores. Besides decreased inflammatory infiltration, diminished percentages of CD4(+)IL17(+), CD11b (+)Ly6G (+) and CD11b (+)Ly6C (+) cells were found in infiltrates of treated animals. Early immune response was mitigated, with spleen cells of DMSC-treated mice displaying low proliferative response to antigen, decreased production of interleukin (IL)-17, and increased production of the anti-inflammatory cytokines IL-4 and IL-10. Moreover, lower RORgammaT and higher GATA-3 expression levels were detected in DMSC-treated mice. DMSCs also showed a detrimental influence on the in vitro definition of the Th17 phenotype. CONCLUSIONS: DMSCs modulated the clinical course of EAE, modified the frequency and cell composition of the central nervous system infiltrates during the disease, and mediated an impairment of Th17 phenotype establishment in favor of the Th2 subtype. These results suggest that DMSCs might provide a new cell-based therapy for the control of multiple sclerosis.

Brenner, A. K., et al. (2016). "The Complexity of Targeting PI3K-Akt-mTOR Signalling in Human Acute Myeloid Leukaemia: The Importance of Leukemic Cell Heterogeneity, Neighbouring Mesenchymal Stem Cells and Immunocompetent Cells." Molecules **21**(11).

Therapeutic targeting of PI3K-Akt-mTOR is considered a possible strategy in human acute myeloid leukaemia (AML); the most important rationale being the proapoptotic and antiproliferative effects of direct PI3K/mTOR inhibition observed in experimental studies of human AML cells. However, AML is a heterogeneous disease and these effects caused by direct pathway inhibition in the leukemic cells are observed only for a subset of patients. Furthermore,

the final effect of PI3K-Akt-mTOR inhibition is modulated by indirect effects, i.e., treatment effects on AML-supporting non-leukemic bone marrow cells. In this article we focus on the effects of this treatment on mesenchymal stem cells (MSCs) and monocytes/macrophages; both these cell types are parts of the haematopoietic stem cell niches in the bone marrow. MSCs have unique membrane molecule and constitutive cytokine release profiles, and mediate their support through bidirectional crosstalk involving both cell-cell contact and the local cytokine network. It is not known how various forms of PI3K-Akt-mTOR targeting alter the molecular mechanisms of this crosstalk. The effect on monocytes/macrophages is also difficult to predict and depends on the targeted molecule. Thus, further development of PI3K-Akt-mTOR targeting into a clinical strategy requires detailed molecular studies in well-characterized experimental models combined with careful clinical studies, to identify patient subsets that are likely to respond to this treatment.

Brohlin, M., et al. (2009). "Characterisation of human mesenchymal stem cells following differentiation into Schwann cell-like cells." Neurosci Res **64**(1): 41-49.

Cell-based therapies provide a clinically applicable and available alternative to nerve autografts. Our previous studies have characterised rat-derived mesenchymal stem cells (MSC) and here we have investigated the phenotypic, molecular and functional characteristics of human-derived MSC (hMSC) differentiated along a Schwann cell lineage. The hMSC were isolated from healthy human donors and the identity of the undifferentiated hMSC was confirmed by the detection of MSC specific cells surface markers. The hMSC were differentiated along a glial cell lineage using an established cocktail of growth factors including glial growth factor-2. Following differentiation, the hMSC expressed the key Schwann cell (SC) markers at both the transcriptional and translational level. More importantly, we show the functional effect of hMSC on neurite outgrowth using an in vitro co-culture model system with rat-derived primary sensory neurons. The number of DRG sprouting neurites was significantly enhanced in the presence of differentiated hMSC; neurite length and density (branching) were also increased. These results provide evidence that hMSC can undergo molecular, morphological and functional changes to adopt a SC-like behaviour and, therefore, could be suitable as SC substitutes for nerve repair in clinical applications.

Caja, L., et al. (2011). "The transforming growth factor-beta (TGF-beta) mediates acquisition of a mesenchymal stem cell-like phenotype in human liver cells." J Cell Physiol **226**(5): 1214-1223.

Transforming growth factor-beta (TGF-beta) mediates several and sometime opposite effects in epithelial cells, inducing growth inhibition, and apoptosis but also promoting an epithelial to mesenchymal transition (EMT) process, which enhances cell migration and invasion. TGF-beta plays relevant roles in different liver pathologies; however, very few is known about its specific signaling and cellular effects in human primary hepatocytes. Here we show that TGF-beta inhibits proliferation and induces pro-apoptotic genes (such as BMF or BIM) in primary cultures of human fetal hepatocytes (HFH), but also up-regulates anti-apoptotic genes, such as BCL-XL and XIAP. Inhibition of the epidermal growth factor receptor (EGFR), using gefitinib, abrogates the increase in the expression of the anti-apoptotic genes and significantly enhances cell death. Simultaneously, TGF-beta is able to induce an EMT process in HFH, coincident with Snail up-regulation and a decrease in E-cadherin levels, cells showing mesenchymal proteins and reorganization of the actin cytoskeleton in stress fibers. Interestingly, these cells show loss of expression of specific hepatic genes and increased expression of stem cell markers. Chronic treatment with TGF-beta allows selection of a population of mesenchymal cells with a de-differentiated phenotype, reminiscent of progenitor-like cells. Process is reversible and the mesenchymal stem-like cells re-differentiate to hepatocytes under controlled experimental conditions. In summary, we show for the first time that human hepatocytes may respond to TGF-beta inducing different signals, some of them might contribute to tumor suppression (growth inhibition and apoptosis), but others should mediate liver tumor progression and invasion (EMT and acquisition of a stem-like phenotype).

Castilho-Fernandes, A., et al. (2011). "Human hepatic stellate cell line (LX-2) exhibits characteristics of bone marrow-derived mesenchymal stem cells." Exp Mol Pathol **91**(3): 664-672.

The LX-2 cell line has characteristics of hepatic stellate cells (HSCs), which are considered pericytes of the hepatic microcirculatory system. Recent studies have suggested that HSCs might have mesenchymal origin. We have performed an extensive characterization of the LX-2 cells and have compared their features with those of mesenchymal cells. Our data show that LX-2 cells have a phenotype resembling activated HSCs as well as bone marrow-derived mesenchymal stem cells (BM-MSCs). Our immunophenotypic analysis showed that LX-2 cells are positive for activated HSC markers (alphaSMA, GFAP, nestin and CD271) and classical mesenchymal

makers (CD105, CD44, CD29, CD13, CD90, HLA class-I, CD73, CD49e, CD166 and CD146) but negative for the endothelial marker CD31 and endothelial progenitor cell marker CD133 as well as hematopoietic markers (CD45 and CD34). LX-2 cells also express the same transcripts found in immortalized and primary BM-MSCs (vimentin, annexin 5, collagen 1A, NG2 and CD140b), although at different levels. We show that LX-2 cells are capable to differentiate into multilineage mesenchymal cells in vitro and can stimulate new blood vessel formation in vivo. LX-2 cells appear not to possess tumorigenic potential. Thus, the LX-2 cell line behaves as a multipotent cell line with similarity to BM-MSCs. This line should be useful for further studies to elucidate liver regeneration mechanisms and be the foundation for development of hepatic cell-based therapies.

Chang, J. C., et al. (2013). "Cell orientation and regulation of cell-cell communication in human mesenchymal stem cells on different patterns of electrospun fibers." Biomed Mater **8**(5): 055002.

Cell behavior can be manipulated by the topography of the culture surface. In this study, we examined the intercellular communication and osteogenic differentiation of mesenchymal stem cells (MSCs) grown on electrospun fibers with different orientations and densities. Human bone marrow-derived MSCs (hMSCs) were seeded on poly (epsilon-caprolactone) (PCL) electrospun scaffolds composed of aligned (1D) or cross-aligned (2D) fibers (1.0-1.2 microm diameter) with high, medium, or low fiber densities. It was found that cells preferred to adhere onto electrospun PCL fibers rather than on the flat substrate. The immunofluorescence staining showed that the expression of vinculin, a focal adhesion protein, was limited to the periphery and the two extremities of aligned cells on the edge of the fibers. Electron microscopy showed that cells extended their lamellipodia across the adjacent fibers and proliferated along the direction of fibers. Cells grown on 1D fibrous scaffolds at all fiber densities had an obvious alignment. On 2D fibers, a higher degree of cell alignment was observed at the higher fiber density. On 1D scaffolds, the gap junction intercellular communication (GJIC) quantified by the lucifer yellow dye transfer assay was significantly promoted in the aligned cells in the direction parallel to the fibers but was abolished in the direction perpendicular to the fibers. The expression of osteogenic marker genes (RUNX2, ALP, and OCN) was significantly enhanced in seven days by culture on 1D but not 2D fibers. It was thus proposed that the promoted osteogenic differentiation of hMSCs may be associated with the fiber-guided and directional induction of GJIC.

Chao, K. C., et al. (2012). "Human umbilical cord mesenchymal stem cells suppress breast cancer tumourigenesis through direct cell-cell contact and internalization." J Cell Mol Med **16**(8): 1803-1815.

The purpose of this study was to investigate how human umbilical cord mesenchymal stem cells (HUMSCs) affect breast cancer tumourigenesis. To observe the influence of HUMSCs on tumourigenesis in vitro, we performed a co-culture of MDA MB-231 breast cancer cells with HUMSCs, and a result of HUMSCs on tumourigenesis in vivo was achieved by injection of HUMSCs into nonobese diabetic/severe combined immunodeficient mice following tumour establishment with MDA-MB231. During the co-culture, apoptosis of MDA-MB231 was noted, which was driven either by binding with HUMSC through direct cell-cell contact or by formation of a novel cell-in-cell phenomenon after internalization of HUMSC. Also, treatment with HUMSC injection was efficacious in both in situ and metastatic breast cancers in the animal models. Since HUMSCs were proved to efficaciously suppress breast cancer tumourigenesis both in vitro and in vivo, it is our expectation that treatment with HUMSCs can be a viable therapy for breast cancer in the near future. In addition, we share a new point of view on the role of HUMSCs in foetal development during pregnancy.

Chijimatsu, R., et al. (2017). "Characterization of Mesenchymal Stem Cell-Like Cells Derived From Human iPSCs via Neural Crest Development and Their Application for Osteochondral Repair." Stem Cells Int **2017**: 1960965.

Mesenchymal stem cells (MSCs) derived from induced pluripotent stem cells (iPSCs) are a promising cell source for the repair of skeletal disorders. Recently, neural crest cells (NCCs) were reported to be effective for inducing mesenchymal progenitors, which have potential to differentiate into osteochondral lineages. Our aim was to investigate the feasibility of MSC-like cells originated from iPSCs via NCCs for osteochondral repair. Initially, MSC-like cells derived from iPSC-NCCs (iNCCs) were generated and characterized in vitro. These iNCC-derived MSC-like cells (iNCMSCs) exhibited a homogenous population and potential for osteochondral differentiation. No upregulation of pluripotent markers was detected during culture. Second, we implanted iNCMSC-derived tissue-engineered constructs into rat osteochondral defects without any preinduction for specific differentiation lineages. The implanted cells remained alive at the implanted site, whereas they failed to repair the defects,

with only scarce development of osteochondral tissue in vivo. With regard to tumorigenesis, the implanted cells gradually disappeared and no malignant cells were detected throughout the 2-month follow-up. While this study did not show that iNCMSCs have efficacy for repair of osteochondral defects when implanted under undifferentiated conditions, iNCMSCs exhibited good chondrogenic potential in vitro under appropriate conditions. With further optimization, iNCMSCs may be a new source for tissue engineering of cartilage.

Chikhovskaya, J. V., et al. (2012). "Human testis-derived embryonic stem cell-like cells are not pluripotent, but possess potential of mesenchymal progenitors." Hum Reprod **27**(1): 210-221.

BACKGROUND: Spontaneous in vitro transition of undifferentiated spermatogonia into the pluripotent cell state has been achieved using neonatal and adult mouse testis tissue. In an effort to establish an analogous source of human patient-specific pluripotent stem cells, several research groups have described the derivation of embryonic stem cell-like cells from primary cultures of human testis. These cells are characterized in all studies as growing in compact colonies, expressing pluripotency-associated markers and possessing multilineage differentiation capabilities in vitro, but only one study claimed their ability to induce teratomas. This controversy initiated a debate about the pluripotent state and origin of human testis-derived ES-like cells (htES-like cells). METHODS: htES-like cell colonies were obtained from primary testicular cultures of three individuals and selectively expanded using culture conditions known to support the propagation of blastocyst-derived human embryonic stem cells (ESCs), mouse epiblast stem cells and 'naive' human ESCs. The stem cell properties of htES-like cells were subsequently assessed by testing the expression of ESC-specific markers, differentiation abilities in vitro and in vivo, and microarray profiling. RESULTS: The expression of pluripotency-associated markers in htES-like cells and their differentiation abilities differed significantly from those of ESCs. Gene expression microarray analysis revealed that htES-like cells possess a transcriptome distinct from human ESCs and fibroblasts, but closely resembling the transcriptome of mesenchymal stem cells (MSCs). The similarity to MSCs was confirmed by detection of SSEA4/CD146 expressing cells within htES-like colonies and efficient in vitro differentiation toward three mesodermal lineages (adipogenic, osteogenic, chondrogenic). CONCLUSIONS: Taken together, these results indicate that htES-like cells, in contrast to pluripotent stem cells derived from adult mouse testis, are not pluripotent and most likely not of germ cell but of mesenchymal origin.

Chikhovskaya, J. V., et al. (2014). "Mesenchymal origin of multipotent human testis-derived stem cells in human testicular cell cultures." Mol Hum Reprod **20**(2): 155-167.

In contrast to mouse germ cell-derived pluripotent stem cells, the pluripotent state of human testis-derived embryonic stem cell (ESC)-like that spontaneously arise in primary testicular cell cultures remains controversial. Recent studies have shown that these cells closely resemble multipotent mesenchymal stem cells (MSCs), raising the question of their origin and designating these cell populations as multipotent human testis-derived stem cells (mhtSCs) rather than truly ESC-like cells. Here, we evaluate the origin of mhtSCs in vitro by culturing selected testicular cell types. We demonstrate that mhtSCs can be obtained equally efficiently in cultures of pure testicular somatic cells devoid of germ cells. Conversely, cultures with a purified population of germ cells/spermatogonia do not produce any mhtSCs. Based on common molecular characteristics of the somatic starting population and mhtSCs, we conclude that mhtSCs colonies originate from somatic mesenchymal progenitors present in primary testicular cell cultures and do not arise from germ cells undergoing incomplete reprogramming in vitro.

Chistiakova, I. A. and G. G. Polianskaia (2014). "[Influence of human fetal mesenchymal stem cells on glioma cell proliferation. A consequence of cellular crosstalk]." Tsitologiia **56**(11): 800-808.

The effect of mesenchymal stem cells (MSCs) on the growth of various tumors is ambiguous. MSCs derived from different tissues stimulate growth of some tumor types and exert antitumor effects on the other. Several recent reports have shown that crosstalk between tumor cells and MSCs contribute to these effects. The aim of this work was to study the effects of MSCs derived from fetal tissues on the proliferative activity of glioma cells in conditions of prolonged co-cultivation. We have analyzed the proliferative activity of glioma cells exposed to conditioned medium (CM) from MSCs derived from fetal bone marrow (FetMSC) and fetal muscle (M-FetMSC) as well as to CM from co-cultivation of the fetal MSCs with U251MG glioma cells. As a comparison, the influence of CM from adult dermal fibroblasts (DFs) was examined in identical experiments. Using MTT assay, we have found that CM from both the fetal MSCs and adult DFs (without their co-culturing with glioma cells) had no effect on U251MG and A172 glioma cell proliferation. However, CM from early co-cultures (3-9 days) of U251MG cells with FetMSC or M-FetMSC exerted stimulatory effect on U251MG cell proliferation up to 2.3-fold increase, while CM obtained later from the

same co-cultures (15-21 days) had inhibitory effect on the proliferation up to arrest of cell division. Analogous experiments with adult DFs have revealed a persistent stimulation of U251MG cell proliferative activity for all 21 days of co-culturing. Immunofluorescence analysis revealed a reduction in the expression of cell cycle protein cyclin D1 in U251MG cells after their treatment with CM taken from 21-days co-cultures of U251MG cells with FetMSC or M-FetMSC. In contrast, CM from 21-days co-cultures of U251MG cells with DFs did not decrease the expression of cyclin D1. These results show that fetal MSCs have dual effect on glioma cell proliferation. In spite of the earlier stimulatory effect on the proliferative activity, prominent inhibition of glioma cell proliferation was observed after three week co-culturing of glioma cells with fetal MSCs. This is the first report demonstrating reversion of tumor cell proliferative program during co-culturing with MSCs for a long time. These data suggest that CM obtained at different time points of cell co-culturing can be used in the modeling of prolonged cellular crosstalk.

Cho, J. A., et al. (2009). "Hyperthermia-treated mesenchymal stem cells exert antitumor effects on human carcinoma cell line." Cancer **115**(2): 311-323.

BACKGROUND: Mesenchymal stem cells (MSCs) possess the potential for differentiation into multilineages. MSCs have been reported to play a role as precursors for tumor stroma in providing a favorable environment for tumor progression. Hyperthermia destroys cancer cells by raising the temperature of tumor-loaded tissue to 40 degrees C to 43 degrees C and causes indirect sensitizing effects when combined with chemo- and/or radiotherapy. However, how hyperthermia affects the tumor-supportive stroma is unknown. Here, the authors investigated the effects of hyperthermia-treated MSCs, from different sources, on the human ovarian cancer cell line SK-OV-3. METHODS: MSCs from adipose tissue and amniotic fluid were untreated or heat-treated (HS-MSCs). The culture supernatant of each treatment group was collected and transferred to the SK-OV-3 cells. RESULTS: The morphological analysis and cell proliferation assay showed a reduced viability of the tumor cells in the conditioned medium with the HS-MSCs. Further investigations revealed that the conditioned medium of the HS-MSCs induced a higher nuclear condensation and a greater number of sub-G1 cells among the tumor cells. Analysis of the mRNA expression demonstrated that the conditioned medium of the HS-MSCs induced up-regulation or down-regulation of several tumor-associated molecules. Finally, the cytokine array of each conditioned medium showed that angiogenin, insulin-like growth factor binding protein 4, neurotrophin 3, and chemokine (C-C motif) ligand 18 are involved as main factors. CONCLUSIONS: This study showed that the conditioned medium of the HS-MSCs exerted a suppressive effect on tumor progression and malignancy, suggesting that hyperthermia enables tumor stromal cells to provide a sensitizing environment for tumor cells to undergo cell death.

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.

Choi, Y. J., et al. (2018). "Phthalazinone Pyrazole Enhances the Hepatic Functions of Human Embryonic Stem Cell-Derived Hepatocyte-Like Cells via Suppression of the Epithelial-Mesenchymal Transition." Stem Cell Rev **14**(3): 438-450.

During liver development, nonpolarized hepatic progenitor cells differentiate into mature hepatocytes with distinct polarity. This polarity is essential for maintaining the intrinsic properties of hepatocytes. The balance between the epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) plays a decisive role in differentiation of polarized hepatocytes. In this study, we found that phthalazinone pyrazole (PP), a selective inhibitor of Aurora-A kinase (Aurora-A), suppressed the EMT during the differentiation of hepatocyte-like cells (HLCs) from human embryonic stem cells. The differentiated HLCs treated with PP at the hepatoblast stage showed enhanced hepatic morphology and functions, particularly with regard to the expression of drug metabolizing enzymes. Moreover, we found that these effects were mediated though suppression of the AKT pathway, which is involved in induction of the EMT, and upregulation of hepatocyte nuclear factor 4alpha expression rather than Aurora-A inhibition. In conclusion, these findings provided insights into the regulatory role of the EMT on in vitro hepatic maturation, suggesting that inhibition of the EMT may

drive transformation of hepatoblast cells into mature and polarized HLCs.

Chung, N., et al. (2009). "HOX gene analysis of endothelial cell differentiation in human bone marrow-derived mesenchymal stem cells." Mol Biol Rep **36**(2): 227-235.

Human bone marrow-derived mesenchymal stem cells (hMSCs) have been shown to possess multilineage differentiation potential. HOX genes function in transcriptional regulators, and are involved in stem cell differentiation. The aim of the present study was to demonstrate HOX genes that are related to angiogenesis. To identify the expression patterns of 37 HOX genes in the endothelial cell differentiation of hMSCs, we analyzed HOX genes through profiling with multiplex RT-PCR. The results showed that the expression patterns of four HOX genes, HOXA7, HOXB3, HOXA3, and HOXB13, significantly changed during angiogenesis. The expression levels of HOXA7 and HOXB3 were dramatically increased, whereas those of HOXA3 and HOXB13 were decreased during endothelial cell differentiation. When further analysis of the expressions of these HOX genes was performed with real-time PCR and an immunoblot assay, the expression patterns were also found to be well-matched with the results of multiplex RT-PCR. Here, we report that HOXA7, HOXB3, HOXA3, and HOXB13 might be involved in the angiogenesis of hMSCs.

Citro, L., et al. (2014). "Comparison of human induced pluripotent stem-cell derived cardiomyocytes with human mesenchymal stem cells following acute myocardial infarction." PLoS One **9**(12): e116281.

INTRODUCTION: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have recently been shown to express key cardiac proteins and improve in vivo cardiac function when administered following myocardial infarction. However, the efficacy of hiPSC-derived cell therapies, in direct comparison to current, well-established stem cell-based therapies, is yet to be elucidated. The goal of the current study was to compare the therapeutic efficacy of human mesenchymal stem cells (hMSCs) with hiPSC-CMs in mitigating myocardial infarction (MI). METHODS: Male athymic nude hyrats were subjected to permanent ligation of the left-anterior-descending (LAD) coronary artery to induce acute MI. Four experimental groups were studied: 1) control (non-MI), 2) MI, 3) hMSCs (MI+MSC), and 4) hiPSC-CMs (MI+hiPSC-derived cardiomyocytes). The hiPSC-CMs and hMSCs were labeled with superparamagnetic iron oxide (SPIO) in vitro to track the transplanted cells in the ischemic heart by high-field cardiac MRI. These cells were injected into the ischemic heart 30-min after LAD ligation. Four-weeks after MI, cardiac MRI was performed to track the transplanted cells in the infarct heart. Additionally, echocardiography (M-mode) was performed to evaluate the cardiac function. Immunohistological and western blot studies were performed to assess the cell tracking, engraftment and cardiac fibrosis in the infarct heart tissues. RESULTS: Echocardiography data showed a significantly improved cardiac function in the hiPSC-CMs and hMSCs groups, when compared to MI. Immunohistological studies showed expression of connexin-43, alpha-actinin and myosin heavy chain in engrafted hiPSC-CMs. Cardiac fibrosis was significantly decreased in hiPSC-CMs group when compared to hMSCs or MI groups. Overall, this study demonstrated improved cardiac function with decreased fibrosis with both hiPSC-CMs and hMSCs groups when compared with MI group.

Cobo, F., et al. (2008). "Electron microscopy reveals the presence of viruses in mouse embryonic fibroblasts but neither in human embryonic fibroblasts nor in human mesenchymal cells used for hESC maintenance: toward an implementation of microbiological quality assurance program in stem cell banks." Cloning Stem Cells **10**(1): 65-74.

Human embryonic stem cells (hESCs) are expected to open up new avenues in regenerative medicine by allowing the generation of transplantable cells to be used in future cell replacement therapies. Maintenance of hESCs in the presence of xenogenic compounds is likely to prevent their use in future therapeutic applications in humans. Recently, it has been claimed that human foreskin-derived human embryonic fibroblast (HEFs) and human adult marrow cells have the ability to support prolonged expansion of hESCs in culture similar to murine feeders. Here, to minimize the use of xenogenic components for hESC maintenance, we performed transmission electron microscopy-based microbiological studies in an attempt to implement a microbiological Quality Assurance Program in Stem Cell Banks by determining the potential presence of viral particles in MEFs compared with human HEFs and bone marrow-derived mesenchymal cells. We observed in three out of nine MEF samples (33.3%) viruses belonging to the Retroviridae family. Within the Retroviridae family, these viruses have a C morphology, which indicates they belong to the subfamily Orthoretroviridae. In contrast, no viral particles could be observed in either the HEF samples (n = 5) or the human BM-derived mesenchymal cells (n = 9) analyzed. Based on these experimental microbiological data, we recommend the implementation of microbiological Quality Assurance Programs by means of transmission electron microscopy as a routine technique to assess the

potential presence of viral particles in any feeder cell used in stem cell banks and support the use of human cells rather than murine cells as feeders to maintain hESC cultures in an undifferentiated state.

Colletti, E. J., et al. (2006). "The time course of engraftment of human mesenchymal stem cells in fetal heart demonstrates that Purkinje fiber aggregates derive from a single cell and not multi-cell homing." Exp Hematol **34**(7): 926-933.

OBJECTIVE: To study the early time course of engraftment of human mesenchymal stem cells in fetal sheep heart and determine the relative roles of proliferation and homing in formation of aggregates of human Purkinje fiber cells. METHODS: The human sheep xenograft model was utilized for these studies. Prior to injection in the preimmune fetus, human cells were labeled with fluorescent dyes to be able to track human cells at early times of engraftment. RESULTS: Human stem cells were detected in fetal hearts between 29 and 39 hours after intraperitoneal injection. Engraftment was primarily in the Purkinje fiber system. By 45 hours engrafted human cells had a cardiac phenotype. When two groups of human mesenchymal stem cells, each labeled with a different fluorescent dye, were combined prior to injection, aggregates of human Purkinje fiber cells contained cells labeled with either one dye or the other, no aggregate contained cells labeled with both dyes. CONCLUSIONS: Human mesenchymal stem cells introduced into fetal sheep rapidly enter the myocardium. The swift differentiation into a cardiac phenotype indicates that the cardiac milieu has a strong influence on the fate of engrafting human mesenchymal stem cells. The absence of any aggregates of human Purkinje fiber cells containing both fluorescent dyes demonstrates that each aggregate of human Purkinje fiber cells is derived from a single mesenchymal stem cell and not from homing of multiple cells to a hotspot.

Coppi, E., et al. (2007). "ATP modulates cell proliferation and elicits two different electrophysiological responses in human mesenchymal stem cells." Stem Cells **25**(7): 1840-1849.

Bone marrow-derived human mesenchymal stem cells (hMSCs) have the potential to differentiate into several cell lines. Extracellular adenosine 5'-triphosphate (ATP) acts as a potent signaling molecule mediating cell-to-cell communication. Particular interest has been focused in recent years on the role of ATP in stem cell proliferation and differentiation. In the present work, we demonstrate that hMSCs at early stages of culture (P0-P5) spontaneously release ATP, which decreases cell proliferation. Increased hMSC proliferation is induced by the unselective P2 antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonate (PPADS) and by the selective P2Y1 antagonist 2'-deoxy-N6-methyladenosine3',5'-bisphosphate (MRS 2179). A functional role of extracellular ATP in modulating ionic conductances with the whole-cell and/or perforated patch-clamp techniques was also investigated. Exogenous ATP increased both the voltage-sensitive outward and inward currents in 47% of cells, whereas, in 31% of cells, only an increase in inward currents was found. Cells responding in this dual manner to ATP presented different resting membrane potentials. Both ATP-induced effects had varying sensitivity to the P2 antagonists PPADS and MRS 2179. Outward ATP-sensitive currents are carried by potassium ions, since they are blocked by cesium replacement and are Ca2+ -dependent because they are eliminated in the presence of 1,2-bis (2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid. On the basis of different electrophysiological and pharmacological characteristics, we conclude that outward ATP-sensitive currents are due to Ca2+ -dependent K+ -channel activation following stimulation of P2Y receptors, whereas inward ATP-sensitive currents are mediated by P2X receptor activation. In summary, ATP released in early life stages of hMSCs modulates their proliferation rate and likely acts as one of the early factors determining their cell fate. Disclosure of potential conflicts of interest is found at the end of this article.

Corcione, A., et al. (2006). "Human mesenchymal stem cells modulate B-cell functions." Blood **107**(1): 367-372.

Human mesenchymal stem cells (hMSCs) suppress T-cell and dendritic-cell function and represent a promising strategy for cell therapy of autoimmune diseases. Nevertheless, no information is currently available on the effects of hMSCs on B cells, which may have a large impact on the clinical use of these cells. hMSCs isolated from the bone marrow and B cells purified from the peripheral blood of healthy donors were cocultured with different B-cell tropic stimuli. B-cell proliferation was inhibited by hMSCs through an arrest in the G0/G1 phase of the cell cycle and not through the induction of apoptosis. A major mechanism of B-cell suppression was hMSC production of soluble factors, as indicated by transwell experiments. hMSCs inhibited B-cell differentiation because IgM, IgG, and IgA production was significantly impaired. CXCR4, CXCR5, and CCR7 B-cell expression, as well as chemotaxis to CXCL12, the CXCR4 ligand, and CXCL13, the CXCR5 ligand, were significantly down-regulated by hMSCs, suggesting that these cells affect chemotactic properties of B cells. B-cell costimulatory molecule expression and cytokine production were unaffected

by hMSCs. These results further support the potential therapeutic use of hMSCs in immune-mediated disorders, including those in which B cells play a major role.

Costa, M. H. G., et al. (2017). "Tridimensional configurations of human mesenchymal stem/stromal cells to enhance cell paracrine potential towards wound healing processes." J Biotechnol **262**: 28-39.

This study proposes to use alginate encapsulation as a strategy to assess the paracrine activity of 3D- and 2D-cultured human bone marrow mesenchymal stem/stromal cells (BM MSC) in the setting of wound repair and regeneration processes. A side-by-side comparison of MSC culture in three different 3D configurations (spheroids, encapsulated spheroids and encapsulated single cells) versus 2D monolayer cell culture is presented. The results reveal enhanced resistance to oxidative stress and paracrine potential of 3D spheroid-organized BM MSC. MSC spheroids (148+/-2mum diameter) encapsulated in alginate microbeads evidence increased angiogenic and chemotactic potential relatively to encapsulated single cells, as supported by higher secreted levels of angiogenic factors and by functional assays showing the capability of encapsulated MSC to promote formation of tubelike structures and migration of fibroblasts into a wounded area. In addition, a higher expression of the anti-inflammatory factor tumor necrosis factor alpha-induced protein 6 (TSG-6) was demonstrated by RT-PCR for encapsulated and non-encapsulated spheroids. Culture of spheroids within an alginate matrix maintains low aggregation levels below 5% and favors resistance to oxidative stress. These are important factors towards the establishment of more standardized and controlled systems, crucial to explore the paracrine effects of 3D-cultured MSC in therapeutic settings.

Cournil-Henrionnet, C., et al. (2008). "Phenotypic analysis of cell surface markers and gene expression of human mesenchymal stem cells and chondrocytes during monolayer expansion." Biorheology **45**(3-4): 513-526.

Both chondrocytes and mensenchymal stem cells (MSCs) are the most used cell sources for cartilage tissue engineering. However, monolayer expansion to obtain sufficient cells leads to a rapid chondrocyte dedifferentiation and a subsequent ancillary reduced ability of MSCs to differentiate into chondrocytes, thus limiting their application in cartilage repair. The aim of this study was to investigate the influence of the monolayer expansion on the immunophenotype and the gene expression profile of both cell types, and to find the appropriate compromise between monolayer expansion and the remaining chondrogenic characteristics. To this end, human chondrocytes, isolated enzymatically from femoral head slice, and human MSCs, derived from bone marrow, were maintained in monolayer culture up to passage 5. The respective expressions of cell surface markers (CD34, CD45, CD73, CD90, CD105, CD166) and several chondrogenic-related genes for each passage (P0-P5) of those cells were then analyzed using flow cytometry and quantitative real-time PCR, respectively. Flow cytometry analyses showed that, during the monolayer expansion, some qualitative and quantitative regulations occur for the expression of cell surface markers. A rapid increase in mRNA expression of type 1 collagen occurs whereas a significant decrease of type 2 collagen and Sox 9 was observed in chondrocytes through the successive passages. On the other hand, the expansion did not induced obvious change in MSCs gene expression. In conclusion, our results suggest that passage 1 might be the up-limit for chondrocytes in order to achieve their subsequent redifferentiation in 3D scaffold. Nevertheless, MSCs could be expanded in monolayer until passage 5 without loosing their undifferentiated phenotypes.

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.

Cronwright, G., et al. (2005). "Cancer/testis antigen expression in human mesenchymal stem cells: down-regulation of SSX impairs cell migration and matrix metalloproteinase 2 expression." Cancer Res **65**(6): 2207-2215.

Several families of genes by and large located on the X chromosome encode proteins of unspecified function. Commonly known as cancer/testis (CT) antigens, they are considered, under normal conditions, only to be expressed in cells of the germ line and placenta. CT genes are also often expressed in cancer cells, hence their classification. Here we report that their expression in normal cells is wider spread and can be observed in cells with the potential for self-renewal and pleuripotency, namely, stem cells. Several CT genes and their products, CT antigens, including SSX, NY-ESO-1, and N-RAGE, were expressed in undifferentiated mesenchymal stem cells (MSCs) and down-regulated after osteocyte and adipocyte differentiation. To elucidate the possible overlapping function played by these genes in cancer and stem cells, a comparative analysis of the localization of their proteins was made. In addition, localization relative to other MSC markers was examined. This revealed that SSX localizes in the cytoplasm and overlap occurs in regions where matrix metalloproteinase 2 (MMP2) and vimentin accumulate. Nevertheless, it was found that no protein interactions between these molecules occur. Further investigation revealed that the migration of a melanoma cell line (DFW), which expresses SSX, MMP2, and vimentin, decreases when SSX is down-regulated. This decrease in cell migration was paralleled by a reduction in MMP2 levels. Analogous to this, SSX expression is down-regulated in MSCs after differentiation; concomitantly a reduction in MMP2 levels occurs. In addition, E-cadherin expression increases, mimicking a mesenchymal epithelial transition. These results afford SSX a functional role in normal stem cell migration and suggest a potentially similar function in cancer cell metastases.

Crop, M. J., et al. (2010). "Human adipose tissue-derived mesenchymal stem cells induce explosive T-cell proliferation." Stem Cells Dev **19**(12): 1843-1853.

Mesenchymal stem cells (MSCs) inhibit the proliferation of allo-activated lymphocytes. This effect is primarily dependent on the secretion of anti-inflammatory factors by MSCs and is enhanced under inflammatory conditions. MSCs, however, also produce factors that can potentially activate resting immune cells. Full understanding of the behavior of MSCs under inflammatory and noninflammatory conditions is crucial when clinical application of MSCs is considered. Human adipose tissue-derived MSCs were cultured with nonactivated peripheral blood mononuclear cells (PBMCs) and the activation, proliferation, and function of PBMCs were examined. Seven days of coculture with autologous or allogeneic MSCs significantly increased the proliferation of PBMCs (3-fold). This effect was observed in both direct and transwell coculture systems. MSCs cocultured with PBMCs showed increased mRNA expression of the proinflammatory mediators interleukin-6 (IL-6), IL-8, tumor necrosis factor-alpha, the growth factors basic fibroblast growth factor and vascular endothelial growth factor-alpha, and the anti-inflammatory factor indoleamine 2,3-dioxygenase. After removal of MSCs, PBMCs showed a spectacular further increase in proliferation, with a maximum of 25-fold after 7 days. This increase in proliferation was not seen when PBMCs were kept in the presence of MSCs. The proliferating fraction of PBMCs largely consisted of CD4(+) T-cells with high CD25 expression and the proportion of CD127(neg)FoxP3(+) regulatory T-cells significantly increased from 5.0% to 8.5% of total CD4(+) T-cells. The expanded T-cells demonstrated normal responses to mitogen or alloantigen stimulation. The CD25(positive) fraction of these cells had immunosuppressive capacity. In conclusion, MSCs can stimulate the activation and proliferation of resting T-cells and generate regulatory T-cells. These findings are important when MSCs are applied in the clinic.

Cui, R., et al. (2016). "Human mesenchymal stromal/stem cells acquire immunostimulatory capacity upon cross-talk with natural killer cells and might improve the NK cell function of immunocompromised patients." Stem Cell Res Ther **7**(1): 88.

BACKGROUND: The suppressive effect of mesenchymal stromal/stem cells (MSCs) on diverse immune cells is well known, but it is unclear whether MSCs additionally possess immunostimulatory properties. We investigated the impact of human MSCs on the responsiveness of primary natural killer (NK) cells in terms of cytokine secretion. METHODS: Human MSCs were generated from bone marrow and nasal mucosa. NK cells were isolated from peripheral blood of healthy volunteers or of immunocompromised patients after severe injury. NK cells were cultured with MSCs or with MSC-derived conditioned media in the absence or presence of IL-12 and IL-18. C-C chemokine receptor (CCR) 2, C-C chemokine ligand (CCL) 2, and the interferon (IFN)-gamma receptor was blocked by specific inhibitors or

antibodies. The synthesis of IFN-gamma and CCL2 was determined. RESULTS: In the absence of exogenous cytokines, trace amounts of NK cell-derived IFN-gamma licensed MSCs for enhanced synthesis of CCL2. In turn, MSCs primed NK cells for increased release of IFN-gamma in response to IL-12 and IL-18. Priming of NK cells by MSCs occurred in a cell-cell contact-independent manner and was impaired by inhibition of the CCR2, the receptor of CCL2, on NK cells. CD56(bright) NK cells expressed higher levels of CCR2 and were more sensitive to CCL2-mediated priming by MSCs and by recombinant CCR2 ligands than cytotoxic CD56(dim) NK cells. NK cells from severely injured patients were impaired in cytokine-induced IFN-gamma synthesis. Co-culture with MSCs or with conditioned media from MSCs and MSC/NK cell co-cultures from healthy donors improved the IFN-gamma production of the patients' NK cells in a CCR2-dependent manner. CONCLUSIONS: A positive feedback loop driven by NK cell-derived IFN-gamma and MSC-derived CCL2 increases the inflammatory response of cytokine-stimulated NK cells not only from healthy donors but also from immunocompromised patients. Therapeutic application of MSCs or their soluble factors might thus improve the NK function after severe injury.

Cunha, B., et al. (2017). "Bioprocess integration for human mesenchymal stem cells: From up to downstream processing scale-up to cell proteome characterization." J Biotechnol **248**: 87-98.

To deliver the required cell numbers and doses to therapy, scaling-up production and purification processes (at least to the liter-scale) while maintaining cells' characteristics is compulsory. Therefore, the aim of this work was to prove scalability of an integrated streamlined bioprocess compatible with current good manufacturing practices (cGMP) comprised by cell expansion, harvesting and volume reduction unit operations using human mesenchymal stem cells (hMSC) isolated from bone marrow (BM-MSC) and adipose tissue (AT-MSC). BM-MSC and AT-MSC expansion and harvesting steps were scaled-up from spinner flasks to 2L scale stirred tank single-use bioreactor using synthetic microcarriers and xeno-free medium, ensuring high cellular volumetric productivities (50x10(6)cellL (-1)day (-1)), expansion factors (14-16 fold) and cell recovery yields (80%). For the concentration step, flat sheet cassettes (FSC) and hollow fiber cartridges (HF) were compared showing a fairly linear scale-up, with a need to slightly decrease the permeate flux (30-50 LMH, respectively) to maximize cell recovery yield. Nonetheless, FSC allowed to recover 18% more cells after a volume reduction factor of 50. Overall, at the end of the entire bioprocess more than 65% of viable (>95%) hMSC could be recovered without compromising cell's critical quality attributes (CQA) of viability, identity and differentiation potential. Alongside the standard quality assays, a proteomics workflow based on mass spectrometry tools was established to characterize the impact of processing on hMSC's CQA; These analytical tools constitute a powerful tool to be used in process design and development.

Czaplewski, S. K., et al. (2014). "Tenogenic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells dictated by properties of braided submicron fibrous scaffolds." Biomaterials **35**(25): 6907-6917.

Tendon and ligament (T/L) engineering is a growing area of research with potential to address the inadequacies of current T/L defect treatments. Our group previously developed braided submicron fibrous scaffolds (BSMFSs) and demonstrated the viability of BSMFSs for T/L tissue engineering. The objective of this study was to investigate the effect of fiber chemistry and braiding angle on BSMFS mechanical properties and in turn, tenogenic differentiation of human induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs) seeded on BSMFSs subjected to cyclic tensile stimulation in the absence of tenogenic medium. By varying fiber chemistry and/or braiding angle, BSMFSs with a range of mechanical properties were produced. We found that fiber chemistry dictated cell adhesion while braiding angle dictated the tissue-specific lineage commitment of hiPSC-MSCs. Scaffolds braided with large angles better supported hiPSC-MSC tenogenic differentiation as evidenced by the production of T/L-associated markers, downregulation of osteogenic markers, and expression of fibroblast-like, spindle cell morphology compared to scaffolds braided with small angles. Our results demonstrate the importance of substrate properties and mechanical stimulation on tenogenic differentiation. These results also demonstrate the versatility of BSMFSs and the potential of hiPSC-MSCs for T/L tissue engineering.

Dang, L. T., et al. (2018). "Intravenous Infusion of Human Adipose Tissue-Derived Mesenchymal Stem Cells to Treat Type 1 Diabetic Mellitus in Mice: An Evaluation of Grafted Cell Doses." Adv Exp Med Biol.

Mesenchymal stem cell (MSC) transplantation is a novel treatment for diabetes mellitus, especially type 1 diabetes. Many recent publications have demonstrated the efficacy of MSC transplantation on reducing blood glucose and increasing insulin production in both preclinical and clinical trials. However, the investigation of grafted cell doses has been lacking. Therefore, this study aimed to evaluate

the different doses of MSCs on treatment of type 1 diabetes in mouse models. MSCs were isolated and expanded from human adipose tissue. Streptozotocin (STZ)-induced diabetic mice were divided into two groups that were intravenously transfused with two different doses of human MSCs: 10(6) or 2.10(6) cells/mouse. After transplantation, both grafted and placebo mice were monitored weekly for their blood glucose levels, glucose and insulin tolerance, pancreatic structural changes, and insulin production for 56 days after transplantation. The results showed that the higher dose of MSCs (2.10(6) cells/mouse) remarkably reduced death rate. The death rates were 50%, 66%, and 0% in placebo group, low-dose (1.10(6) MSCs) group, and high-dose (2.10(6) MSCs) group, respectively, after 56 days of treatment. Moreover, blood glucose levels were lower for the high-dose group compared to other groups. Glucose and insulin tolerance, as well as insulin production, were significantly improved in mice transplanted with 2.10(6) cells. The histochemical analyses also support these results. Thus, a higher (e.g., 2.10(6)) dose of MSCs may be an effective dose for treatment of type 1 diabetes mellitus.

de Peppo, G. M., et al. (2010). "Osteogenic potential of human mesenchymal stem cells and human embryonic stem cell-derived mesodermal progenitors: a tissue engineering perspective." Tissue Eng Part A **16**(11): 3413-3426.

INTRODUCTION: Human mesenchymal stem cells (hMSCs) are promising candidates for bone engineering and regeneration with a considerable number of experimental successes reported over the last years. However, hMSCs show several limitations for tissue engineering applications, which can be overcome by using human embryonic stem cell-derived mesodermal progenitors (hES-MPs). The aim of this study was to investigate and compare the osteogenic differentiation potential of hMSCs and hES-MPs. MATERIALS AND METHODS: The osteogenic differentiation and mineralization behavior of both cell types were evaluated at passage 5, 10, 15, and 20. Expression of COL1A1, RUNX2, OPN, and OC was evaluated by reverse transcription (RT)-polymerase chain reaction, whereas mineralization was examined by photospectrometry, von Kossa staining, and time-of-flight secondary ion mass spectrometry. The immunoprofile of both cell types was investigated by flow cytometry. RESULTS: We demonstrated that, under proper stimulation, hES-MPs undergo osteogenic differentiation and exhibit significantly increased mineralization ability compared to hMSCs after protracted expansion. hES-MPs were also found to express lower amount of human leukocyte antigens class II proteins. CONCLUSIONS: The high osteogenic ability of hES-MPs, together with low expression of human leukocyte antigens class II, makes these cells an attractive alternative for bulk production of cells for bone engineering applications.

de Peppo, G. M., et al. (2013). "Human embryonic stem cell-derived mesodermal progenitors display substantially increased tissue formation compared to human mesenchymal stem cells under dynamic culture conditions in a packed bed/column bioreactor." Tissue Eng Part A **19**(1-2): 175-187.

Bone tissue engineering represents a promising strategy to obviate bone deficiencies, allowing the ex vivo construction of bone substitutes with unprecedented potential in the clinical practice. Considering that in the human body cells are constantly stimulated by chemical and mechanical stimuli, the use of bioreactor is emerging as an essential factor for providing the proper environment for the reproducible and large-scale production of the engineered substitutes. Human mesenchymal stem cells (hMSCs) are experimentally relevant cells but, regardless the encouraging results reported after culture under dynamic conditions in bioreactors, show important limitations for tissue engineering applications, especially considering their limited proliferative potential, loss of functionality following protracted expansion, and decline in cellular fitness associated with aging. On the other hand, we previously demonstrated that human embryonic stem cell-derived mesodermal progenitors (hES-MPs) hold great potential to provide a homogenous and unlimited source of cells for bone engineering applications. Based on prior scientific evidence using different types of stem cells, in the present study we hypothesized that dynamic culture of hES-MPs in a packed bed/column bioreactor had the potential to affect proliferation, expression of genes involved in osteogenic differentiation, and matrix mineralization, therefore resulting in increased bone-like tissue formation. The reported findings suggest that hES-MPs constitute a suitable alternative cell source to hMSCs and hold great potential for the construction of bone substitutes for tissue engineering applications in clinical settings.

Deleu, S., et al. (2009). "Human cystic fibrosis embryonic stem cell lines derived on placental mesenchymal stromal cells." Reprod Biomed Online **18**(5): 704-716.

This study describes the production of two new human embryonic stem cell (hESC) lines affected by cystic fibrosis. These cell lines are heterozygous compounds, each a carrier of the DF508 mutations associated either with E585X or with 3849+10 kb C-->T. The derivation process was performed on irradiated human placental mesenchymal stromal cells

and designed to minimize contact with xeno-components. This new source of feeder cells is easy to obtain and devoid of ethical concerns. The cells have a great capacity to proliferate which reduces the need for continuous preparation of new feeder cell lines. In addition, three normal hESC lines were obtained in the same conditions. The five stem cell lines retained hESC-specific features, including an unlimited and undifferentiated proliferation capacity, marker expression and the maintenance of stable karyotype. They also demonstrated pluripotency in vitro, forming cell lineages of the three germ layers, as indicated by immunolocalization of beta-tubulin, alpha-fetoprotein and actin. These new genetic cell lines represent an important in-vitro tool to study the physiological processes underlying this genetic disease, drug screening, and tissue engineering.

Dickinson, S. C., et al. (2017). "The Wnt5a Receptor, Receptor Tyrosine Kinase-Like Orphan Receptor 2, Is a Predictive Cell Surface Marker of Human Mesenchymal Stem Cells with an Enhanced Capacity for Chondrogenic Differentiation." Stem Cells **35**(11): 2280-2291.

Multipotent mesenchymal stem cells (MSCs) have enormous potential in tissue engineering and regenerative medicine. However, until now, their development for clinical use has been severely limited as they are a mixed population of cells with varying capacities for lineage differentiation and tissue formation. Here, we identify receptor tyrosine kinase-like orphan receptor 2 (ROR2) as a cell surface marker expressed by those MSCs with an enhanced capacity for cartilage formation. We generated clonal human MSC populations with varying capacities for chondrogenesis. ROR2 was identified through screening for upregulated genes in the most chondrogenic clones. When isolated from uncloned populations, ROR2+ve MSCs were significantly more chondrogenic than either ROR2-ve or unfractionated MSCs. In a sheep cartilage-repair model, they produced significantly more defect filling with no loss of cartilage quality compared with controls. ROR2+ve MSCs/perivascular cells were present in developing human cartilage, adult bone marrow, and adipose tissue. Their frequency in bone marrow was significantly lower in patients with osteoarthritis (OA) than in controls. However, after isolation of these cells and their initial expansion in vitro, there was greater ROR2 expression in the population derived from OA patients compared with controls. Furthermore, osteoarthritis-derived MSCs were better able to form cartilage than MSCs from control patients in a tissue engineering assay. We conclude that MSCs expressing high levels of ROR2 provide a defined population capable of predictably enhanced cartilage production. Stem Cells 2017;35:2280-2291.

Diederichs, S., et al. (2012). "Interplay between local versus soluble transforming growth factor-beta and fibrin scaffolds: role of cells and impact on human mesenchymal stem cell chondrogenesis." Tissue Eng Part A **18**(11-12): 1140-1150.

Structural extracellular matrix molecules gain increasing attention as scaffolds for cartilage tissue engineering owing to their natural role as a growth factor repository. We recently observed that a collagen-type I/III (Col-I/III) matrix, human recombinant transforming growth factor-beta (TGF-beta) protein, and fibrin hydrogel (FG) combined to a biphasic construct provided sufficient long-term TGF-beta support to drive in vitro chondrogenesis of human mesenchymal stem cells (hMSC). Here we ask whether FG and Col-I/III can both retain TGF-beta, describe the influence of cell seeding on TGF-beta release, and compare the molecular path of hMSC chondrogenic differentiation under soluble versus local TGF-beta supply. Release of growth factor from scaffolds augmented with increasing amounts of TGF-beta was analyzed over 7 days and chondrogenesis was assessed over 42 days. Low TGF-beta release rates from Col-I/III as opposed to higher release from FG indicated that both molecules retained TGF-beta, with Col-I/III being the superior storage component. Cell seeding enhanced TGF-beta retention in FG by about threefold and almost stopped release beyond 24 h. TGF-beta remained bioactive and supported MSC chondrogenesis without impairing the amount of proteoglycan and collagen-type II deposition per cell and per construct compared to standard scaffold-free MSC pellets supplied with soluble TGF-beta. Local TGF-beta, however, mediated lower cell content, less collagen-type X relative to collagen-type II deposition and no matrix metalloproteinase-13 up-regulation. In conclusion, cells quickly halted release of local TGF-beta from FG, turning FG and Col-I/III into attractive TGF-beta repositories capable to drive full hMSC chondrogenesis, but via a modulated differentiation pathway. Since only part of the changes was reproduced by transient soluble TGF-beta supply, release kinetics alone could not explain the molecular differences, suggesting that local TGF-beta acts distinct from its soluble counterpart.

Diederichs, S. and R. S. Tuan (2014). "Functional comparison of human-induced pluripotent stem cell-derived mesenchymal cells and bone marrow-derived mesenchymal stromal cells from the same donor." Stem Cells Dev **23**(14): 1594-1610.

Mesenchymal stem cells (MSCs) have a high potential for therapeutic efficacy in treating diverse

musculoskeletal injuries and cardiovascular diseases, and for ameliorating the severity of graft-versus-host and autoimmune diseases. While most of these clinical applications require substantial cell quantities, the number of MSCs that can be obtained initially from a single donor is limited. Reports on the derivation of MSC-like cells from pluripotent stem cells (PSCs) are, thus, of interest, as the infinite proliferative capacity of PSCs opens the possibility to generate large amounts of uniform batches of MSCs. However, characterization of such MSC-like cells is currently inadequate, especially with regard to the question of whether these cells are equivalent or identical to MSCs. In this study, we have derived MSC-like cells [induced PSC-derived MSC-like progenitor cells (iMPCs)] using four different methodologies from a newly established induced PSC line reprogrammed from human bone marrow stromal cells (BMSCs), and compared the iMPCs directly with the originating parental BMSCs. The iMPCs exhibited typical MSC/fibroblastic morphology and MSC-typical surface marker profile, and they were capable of differentiation in vitro along the osteogenic, chondrogenic, and adipogenic lineages. However, compared with the parental BMSCs, iMPCs displayed a unique expression pattern of mesenchymal and pluripotency genes and were less responsive to traditional BMSC differentiation protocols. We, therefore, conclude that iMPCs generated from PSCs via spontaneous differentiation represent a distinct population of cells which exhibit MSC-like characteristics.

Diez, J. M., et al. (2015). "Culture of human mesenchymal stem cells using a candidate pharmaceutical grade xeno-free cell culture supplement derived from industrial human plasma pools." Stem Cell Res Ther **6**: 28.

INTRODUCTION: Fetal bovine serum (FBS) is an animal product used as a medium supplement. The animal origin of FBS is a concern if cultured stem cells are to be utilized for human cell therapy. Therefore, a substitute for FBS is desirable. In this study, an industrial, xeno-free, pharmaceutical-grade supplement for cell culture (SCC) under development at Grifols was tested for growth of human mesenchymal stem cells (hMSCs), cell characterization, and differentiation capacity. METHODS: SCC is a freeze-dried product obtained through cold-ethanol fractionation of industrial human plasma pools from healthy donors. Bone marrow-derived hMSC cell lines were obtained from two commercial suppliers. Cell growth was evaluated by culturing hMSCs with commercial media or media supplemented with SCC or FBS. Cell viability and cell yield were assessed with an automated cell counter. Cell surface markers were studied by indirect immunofluorescence assay. Cells were cultured then differentiated into adipocytes, chondrocytes, osteoblasts, and neurons, as assessed by specific staining and microscopy observation. RESULTS: SCC supported the growth of commercial hMSCs. Starting from the same number of seeded cells in two consecutive passages of culture with medium supplemented with SCC, hMSC yield and cell population doubling time were equivalent to the values obtained with the commercial medium and was consistent among lots. The viability of hMSCs was higher than 90%, while maintaining the characteristic phenotype of undifferentiated hMSCs (positive for CD29, CD44, CD90, CD105, CD146, CD166 and Stro-1; negative for CD14 and CD19). Cultured hMSCs maintained the potential for differentiation into adipocytes, chondrocytes, osteoblasts, and neurons. CONCLUSIONS: The tested human plasma-derived SCC sustains the adequate growth of hMSCs, while preserving their differentiation capacity. SCC can be a potential candidate for cell culture supplement in advanced cell therapies.

Ding, D. C., et al. (2015). "Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy." Cell Transplant **24**(3): 339-347.

The human umbilical cord is a promising source of mesenchymal stem cells (HUCMSCs). Unlike bone marrow stem cells, HUCMSCs have a painless collection procedure and faster self-renewal properties. Different derivation protocols may provide different amounts and populations of stem cells. Stem cell populations have also been reported in other compartments of the umbilical cord, such as the cord lining, perivascular tissue, and Wharton's jelly. HUCMSCs are noncontroversial sources compared to embryonic stem cells. They can differentiate into the three germ layers that promote tissue repair and modulate immune responses and anticancer properties. Thus, they are attractive autologous or allogenic agents for the treatment of malignant and nonmalignant solid and soft cancers. HUCMCs also can be the feeder layer for embryonic stem cells or other pluripotent stem cells. Regarding their therapeutic value, storage banking system and protocols should be established immediately. This review critically evaluates their therapeutic value, challenges, and future directions for their clinical applications.

Dolatshahi-Pirouz, A., et al. (2014). "A combinatorial cell-laden gel microarray for inducing osteogenic differentiation of human mesenchymal stem cells." Sci Rep **4**: 3896.

Development of three dimensional (3D) microenvironments that direct stem cell differentiation into functional cell types remains a major challenge in the field of regenerative medicine. Here, we describe a new platform to address this challenge by utilizing a robotic microarray spotter for testing stem cell fates inside various miniaturized cell-laden gels in a systematic manner. To demonstrate the feasibility of our platform, we evaluated the osteogenic differentiation of human mesenchymal stem cells (hMSCs) within combinatorial 3D niches. We were able to identify specific combinations, that enhanced the expression of osteogenic markers. Notably, these 'hit' combinations directed hMSCs to form mineralized tissue when conditions were translated to 3D macroscale hydrogels, indicating that the miniaturization of the experimental system did not alter stem cell fate. Overall, our findings confirmed that the 3D cell-laden gel microarray can be used for screening of different conditions in a rapid, cost-effective, and multiplexed manner for a broad range of tissue engineering applications.

Domev, H., et al. (2012). "Efficient engineering of vascularized ectopic bone from human embryonic stem cell-derived mesenchymal stem cells." Tissue Eng Part A **18**(21-22): 2290-2302.

Human mesenchymal stem cells (hMSCs) can be derived from various adult and fetal tissues. However, the quality of tissues for the isolation of adult and fetal hMSCs is donor dependent with a nonreproducible yield. In addition, tissue engineering and cell therapy require large-scale production of a pure population of lineage-restricted stem cells that can be easily induced to differentiate into a specific cell type. Therefore, human embryonic stem cells (hESCs) can provide an alternative, plentiful source for generation of reproducible hMSCs. We have developed efficient differentiation protocols for derivation of hMSCs from hESCs, including coculture with murine OP9 stromal cells and feeder layer-free system. Our protocols have resulted in the generation of up to 49% of hMSCs, which expressed CD105, CD90, CD29, and CD44. The hMSCs exhibited high adipogenic, chondrocytic, and osteogenic differentiation in vitro. The latter correlated with osteocalcin secretion and vascular endothelial growth factor (VEGF) production by the differentiating hMSCs. hMSC-derived osteoblasts further differentiated and formed ectopic bone in vivo, and induced the formation of blood vessels in Matrigel implants. Our protocol enables generation of a purified population of hESC-derived MSCs, with the potential of differentiating into several mesodermal lineages, and particularly into vasculogenesis-inducing osteoblasts, which can contribute to the development of bone repair protocols.

Dos Santos, F., et al. (2010). "Ex vivo expansion of human mesenchymal stem cells: a more effective cell proliferation kinetics and metabolism under hypoxia." J Cell Physiol **223**(1): 27-35.

The low bone marrow (BM) MSC titers demand a fast ex vivo expansion process to meet the clinically relevant cell dosage. Attending to the low oxygen tension of BM in vivo, we studied the influence of hypoxia on human BM MSC proliferation kinetics and metabolism. Human BM MSC cultured under 2% (hypoxia) and 20% O (2) (normoxia) were characterized in terms of proliferation, cell division kinetics and metabolic patterns. BM MSC cultures under hypoxia displayed an early start of the exponential growth phase, and cell numbers obtained at each time point throughout culture were consistently higher under low O (2), resulting in a higher fold increase after 12 days under hypoxia (40 +/- 10 vs. 30 +/- 6). Cell labeling with PKH26 allowed us to determine that after 2 days of culture, a significant higher cell number was already actively dividing under 2% compared to 20% O (2) and BM MSC expanded under low oxygen tension displayed consistently higher percentages of cells in the latest generations (generations 4-6) until the 5th day of culture. Cells under low O (2) presented higher specific consumption of nutrients, especially early in culture, but with lower specific production of inhibitory metabolites. Moreover, 2% O (2) favored CFU-F expansion, while maintaining BM MSC characteristic immunophenotype and differentiative potential. Our results demonstrated a more efficient BM MSC expansion at 2% O (2), compared to normoxic conditions, associated to an earlier start of cellular division and supported by an increase in cellular metabolism efficiency towards the maximization of cell yield for application in clinical settings.

Dozza, B., et al. (2012). "Cell growth inhibition and apoptotic effect of the rexinoid 6-OH-11-O-hydroxyphenantrene on human osteosarcoma and mesenchymal stem cells." Toxicol In Vitro **26**(1): 142-149.

Natural derivatives of vitamin A, including all-trans-retinoic acid (ATRA), commonly known as retinoids, currently produce favorable results in the treatment of many types of tumors. The rexinoid 6-OH-11-O-hydroxyphenantrene (IIF) is a synthetic derivative of ATRA. Previous in vitro and in vivo studies demonstrated that IIF is able to induce growth inhibition of various cancer cells and is a potent apoptosis-inducing agent with clinical potential. Osteosarcoma (OS) is the most common type of bone cancer, characterized by a rising aggressiveness. Recent evidences suggest that mesenchymal stem cells

(MSC) may favour tumor growth and progression. Thus, it is important to investigate whether a compound with potential anti-tumoral properties such as IIF affects not only tumor cells but also MSC. The current study is an attempt to understand the mode of the potential cytotoxicity of IIF on OS cells and MSC. The response to IIF treatment of osteosarcoma SaOS-2, MG63, and U2OS cells and of bone marrow-derived MSC was the subject of investigation. The results showed that IIF significantly inhibited cell growth in OS cell lines and MSC in both a time- and dose-dependent manner, as evaluated by methylene blue assay. This was also associated with altered cell morphology and an increase in cell death with the involvement of apoptosis as demonstrated by NucleoCounter, Hoechst 33342 staining and FACS analysis. No cell death and apoptosis was found in U2OS cells. Analysis of cells treated with 20 and 40muM IIF for 24h by western blot suggests the activation of initiator caspase 9, indicating the involvement of caspases in inducing apoptosis. Furthermore, IIF upregulated the expression of the pro-apoptotic protein Bax and downregulated the anti-apoptotic protein Bcl2. For the first time, our results collectively provide an evidence for cell growth inhibition and activation of apoptosis in human OS cells and MSC by IIF. These results confirm that IIF may be an effective compound for anticancer treatment, including that of OS.

Dregalla, R. C., et al. (2014). "Amide-type local anesthetics and human mesenchymal stem cells: clinical implications for stem cell therapy." Stem Cells Transl Med **3**(3): 365-374.

In the realm of regenerative medicine, human mesenchymal stem cells (hMSCs) are gaining attention as a cell source for the repair and regeneration of tissues spanning an array of medical disciplines. In orthopedics, hMSCs are often delivered in a site-specific manner at the area of interest and may require the concurrent application of local anesthetics (LAs). To address the implications of using hMSCs in combination with anesthetics for intra-articular applications, we investigated the effect that clinically relevant doses of amide-type LAs have on the viability of bone marrow-derived hMSCs and began to characterize the mechanism of LA-induced hMSC death. In our study, culture-expanded hMSCs from three donors were exposed to the amide-type LAs ropivacaine, lidocaine, bupivacaine, and mepivacaine. To replicate the physiological dilution of LAs once injected into the synovial capsule, each anesthetic was reduced to 12.5%, 25%, and 50% of the stock solution and incubated with each hMSC line for 40 minutes, 120 minutes, 360 minutes, and 24 hours. At each time point, cell viability assays were performed. We found that extended treatment with LAs for 24 hours had a significant impact on both hMSC viability and adhesion. In addition, hMSC treatment with three of the four anesthetics resulted in cell death via apoptosis following brief exposures. Ultimately, we concluded that amide-type LAs induce hMSC apoptosis in a time- and dose-dependent manner that may threaten clinical outcomes, following a similar trend that has been established between these particular anesthetics and articular chondrocytes both in vitro and in vivo.

Du, J., et al. (2012). "IFN-gamma-primed human bone marrow mesenchymal stem cells induce tumor cell apoptosis in vitro via tumor necrosis factor-related apoptosis-inducing ligand." Int J Biochem Cell Biol **44**(8): 1305-1314.

Human mesenchymal stem cells hold promise as gene therapy vectors for delivery of various genes to solid tumors for either therapeutic or tumor-tracing purposes. However, whether Mesenchymal stem cells support or inhibit tumor growth remains unknown. Herein, we first observed that mesenchymal stem cells primed with IFN-gamma selectively induced the death of tumor cell lines, but not normal cells. We further identified that IFN-gamma-primed mesenchymal stem cells expressed tumor necrosis factor-related apoptosis-inducing ligand. Tumor-suppressive effect of IFN-gamma-primed mesenchymal stem cells could be blocked by activity neutralization or expression reduction of tumor necrosis factor-related apoptosis-inducing ligand. Moreover, mesenchymal stem cells mediated apoptosis of tumor cells by activating caspase-3 in such cells, via a mechanism involving tumor necrosis factor-related apoptosis-inducing ligand. However, when IFN-gamma-primed or non-primed mesenchymal stem cells were co-injected into nude mice along with H460 cells, tumor growth was much faster than that of the group receiving only tumor cells ($p<0.01$) because of the promoting vascularization effect of mesenchymal stem cells, although IFN-gamma-primed mesenchymal stem cells also exerted a certain degree of tumor-suppressive effect compared with non-primed cells (2.79+/-0.9 g versus 2.03+/-0.6 g). Collectively, our findings show that IFN-gamma-primed human mesenchymal stem cells could induce cancer cell apoptosis via TRAIL-mediated pathway. In addition, our data afford a novel explanation of the opposing effects of hMSCs presence on tumor growth in vitro and in vivo. Thus, more attention needs to be paid when seeking to exploit mesenchymal stem cells as a therapeutic option under the condition of malignant tumor.

Du, L., et al. (2015). "[Culturing and characterization of human gingival mesenchymal stem

cells and their chemotactic responses to stromal cell-derived factor-1]." Hua Xi Kou Qiang Yi Xue Za Zhi **33**(3): 238-243.

OBJECTIVE: To investigate the expression of chemokine stromal cell-derived factor-1 (SDF-1) receptor CXCR4 in human gingival mesenchymal stem cells (GMSCs) and the migration potential of GMSCs stimulated with SDF-1. METHODS: Human GMSCs were isolated by single-cell cloning method. Their cell surface markers were characterized by flow cytometry, and the rate of colony formation was evaluated. Differentiation assay was used to detect the differentiation potential of GMSCs. The expression of chemokine SDF-l receptor CXCR4 in GMSCs was detected by immunocytochemical staining. The chemotactic effect of SDF-1 on GMSCs was detected using a 24-multiwell Transwell cell culture chamber. The number of net migrated cells was counted in different microscope fields. RESULTS: Human GMSCs possessed high self-renewal potential and formed single-cell colonies cultured in vitro. GMSCs expressed mesenchymal stem cells-associated markers CD44, CD73, CD90, CD105, and CD166, and the expression of hemopoietic stem cell surface markers CD14, CD34, and CD45 was negative. GMSCs differentiated into osteoblasts and adipocytes under defined culture conditions. The colony forming unit-fibroblastic for GMSCs was 21.4%/+/-2.8%. Immunocytochemical staining demonstrated that GMSCs expressed chemokine SDF-1 receptor CXCR4. The number of GMSCs migrating at concentrations of 100 ng.mL-1 and 200 ng.mL-1 of SDF-l in the Transwell cell culture chamber was significantly higher than that of the negative control (189.3+/-4.4, 164.6+/-4.9 cells/field vs. 47.8+/-2.5 cells/field, $P<0.01$). Treatment with the CXCR4 neutralizing antibody, an antagonist for CXCR4, significantly reduced the migratory effect compared with the negative controls (29.0+/-2.4 cells/field vs. 47.8+/-2.5 cells/field, $P<0.01$). CONCLUSION: Human GMSCs express chemokine SDF-l receptor CXCR4. SDF-1 may participate in regulating chemotaxis of human GMSCs. Results suggest that the migration induced by SDF-1 is mediated by CXCR4.

Du, T., et al. (2014). "Microvesicles derived from human Wharton's jelly mesenchymal stem cells promote human renal cancer cell growth and aggressiveness through induction of hepatocyte growth factor." PLoS One **9**(5): e96836.

In our previous study, microvesicles (MVs) released from human Wharton's jelly mesenchymal stem cells (hWJ-MSCs) retard the growth of bladder cancer cells. We would like to know if MVs have a similar effect on human renal cell carcinoma (RCC). By use of cell culture and the BALB/c nu/nu mice xeno-graft model, the influence of MVs upon the growth and aggressiveness of RCC (786-0) was assessed. Cell counting kit-8 (CCK-8) assay, incidence of tumor, tumor size, Ki-67 or TUNEL staining was used to evaluate tumor cell growth in vitro or in vivo. Flow cytometry assay (in vitro) or examination of cyclin D1 expression (in vivo) was carried out to determine the alteration of cell cycle. The aggressiveness was analyzed by Wound Healing Assay (in vitro) or MMP-2 and MMP-9 expression (in vivo). AKT/p-AKT, ERK1/2/p-ERK1/2 or HGF/c-MET expression was detected by real-time PCR or western blot. Our data demonstrated that MVs promote the growth and aggressiveness of RCC both in vitro and in vivo. In addition, MVs facilitated the progression of cell cycle from G0/1 to S. HGF expression in RCC was greatly induced by MVs, associated with activation of AKT and ERK1/2 signaling pathways. RNase pre-treatment abrogated all effects of MVs. In summary, induction of HGF synthesis via RNA transferred by MVs activating AKT and ERK1/2 signaling is one of crucial contributors to the pro-tumor effect.

Du, Y., et al. (2017). "Exosomes from Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stromal Cells (hiPSC-MSCs) Protect Liver against Hepatic Ischemia/ Reperfusion Injury via Activating Sphingosine Kinase and Sphingosine-1-Phosphate Signaling Pathway." Cell Physiol Biochem **43**(2): 611-625.

BACKGROUND/AIMS: This study aimed to evaluate the effects of exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) on hepatic ischemia-reperfusion (I/R) injury, as well as the underlying mechanisms. METHODS: Exosomes derived from hiPSC-MSCs were isolated and characterized both biochemically and biophysically. hiPSC-MSCs-Exo were injected systemically into a murine ischemia/reperfusion injury model via the inferior vena cava, and then the therapeutic effects were evaluated. The serum levels of transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as histological changes were examined. Primary hepatocytes and human hepatocyte cell line HL7702 were used to test whether exosomes could induce hepatocytes proliferation in vitro. In addition, the expression levels of proliferation markers (proliferation cell nuclear antigen, PCNA; Phosphohistone-H3, PHH3) were measured by immunohistochemistry and Western blot. Moreover, SK inhibitor (SKI-II) and S1P1 receptor antagonist (VPC23019) were used to investigate the role of sphingosine kinase and sphingosine-1-phosphate-dependent pathway in the effects of hiPSC-

MSCs-Exo on hepatocytes. RESULTS: hiPSCs were efficiently induced into hiPSC-MSCs that had typical MSC characteristics. hiPSC-MSCs-Exo had diameters ranging from 100 to 200 nm and expressed exosome markers (Alix, CD63 and CD81). After hiPSC-MSCs-Exo administration, hepatocyte necrosis and sinusoidal congestion were markedly suppressed in the ischemia/reperfusion injury model, with lower histopathological scores. The levels of hepatocyte injury markers AST and ALT were significantly lower in the treatment group compared to control, and the expression levels of proliferation markers (PCNA and PHH3) were greatly induced after hiPSC-MSCs-Exo administration. Moreover, hiPSC-MSCs-Exo also induced primary hepatocytes and HL7702 cells proliferation in vitro in a dose-dependent manner. We found that hiPSC-MSCs-Exo could directly fuse with target hepatocytes or HL7702 cells and increase the activity of sphingosine kinase and synthesis of sphingosine-1-phosphate (S1P). Furthermore, the inhibition of SK1 or S1P1 receptor completely abolished the protective and proliferative effects of hiPSC-MSCs-Exo on hepatocytes, both in vitro and in vivo. CONCLUSIONS: Our results demonstrated that hiPSC-MSCs-Exo could alleviate hepatic I/R injury via activating sphingosine kinase and sphingosine-1-phosphate pathway in hepatocytes and promote cell proliferation. These findings represent a novel mechanism that potentially contributes to liver regeneration and have important implications for new therapeutic approaches to acute liver disease.

Duan, B., et al. (2015). "Comparison of Mesenchymal Stem Cell Source Differentiation Toward Human Pediatric Aortic Valve Interstitial Cells within 3D Engineered Matrices." Tissue Eng Part C Methods **21**(8): 795-807.

Living tissue-engineered heart valves (TEHV) would be a major benefit for children who require a replacement with the capacity for growth and biological integration. A persistent challenge for TEHV is accessible human cell source (s) that can mimic native valve cell phenotypes and matrix remodeling characteristics that are essential for long-term function. Mesenchymal stem cells derived from bone marrow (BMMSC) or adipose tissue (ADMSC) are intriguing cell sources for TEHV, but they have not been compared with pediatric human aortic valve interstitial cells (pHAVIC) in relevant 3D environments. In this study, we compared the spontaneous and induced multipotency of ADMSC and BMMSC with that of pHAVIC using different induction media within three-dimensional (3D) bioactive hybrid hydrogels with material modulus comparable to that of aortic heart valve leaflets. pHAVIC possessed some multi-lineage differentiation capacity in response to induction media, but limited to the earliest stages and much less potent than either ADMSC or BMMSC. ADMSC expressed cell phenotype markers more similar to pHAVIC when conditioned in basic fibroblast growth factor (bFGF) containing HAVIC growth medium, while BMMSC generally expressed similar extracellular matrix remodeling characteristics to pHAVIC. Finally, we covalently attached bFGF to PEG monoacrylate linkers and further covalently immobilized in the 3D hybrid hydrogels. Immobilized bFGF upregulated vimentin expression and promoted the fibroblastic differentiation of pHAVIC, ADMSC, and BMMSC. These findings suggest that stem cells retain a heightened capacity for osteogenic differentiation in 3D culture, but can be shifted toward fibroblast differentiation through matrix tethering of bFGF. Such a strategy is likely important for utilizing stem cell sources in heart valve tissue engineering applications.

Duan, P., et al. (2018). "How cell culture conditions affect the microstructure and nanomechanical properties of extracellular matrix formed by immortalized human mesenchymal stem cells: An experimental and modelling study." Mater Sci Eng C Mater Biol Appl **89**: 149-159.

This paper presents an investigation of how different culture media (i.e. basal and osteogenic media) affect the nanomechanical properties and microstructure of the mineralized matrix produced by the human mesenchymal stem cell line Y201, from both an experimental and theoretical approach. A bone nodule (i.e. mineralized matrix) cultured from basal medium shows a more anisotropic microstructure compared to its counterpart cultured from an osteogenic medium. As confirmed by finite element simulations, this anisotropic microstructure explains the bimodal distribution of the corresponding mechanical properties very well. The overall nanomechanical response of the bone nodule from the osteogenic medium is poorer compared to its counterpart from the basal medium. The bone nodules, from both basal and osteogenic media, have shown reverse aging effects in terms of mechanical properties. These are possibly due to the fact that cell proliferation outcompetes the mineralization process.

English, K., et al. (2009). "Cell contact, prostaglandin E (2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25(High) forkhead box P3+ regulatory T cells." Clin Exp Immunol **156**(1): 149-160.

Adult human mesenchymal stromal or stem cells (MSC) can differentiate into a variety of cell types and are candidate cellular therapeutics in regenerative

medicine. Surprisingly, these cells also display multiple potent immunomodulatory capabilities, including allosuppression, making allogeneic cell therapy a possibility. The exact mechanisms involved in regulatory T cell induction by allogeneic human MSC was examined, using purified CD4+ populations and well-characterized bone marrow-derived adult human MSC. Allogeneic MSC were shown to induce forkhead box P3 (FoxP3)+ and CD25+ mRNA and protein expression in CD4+ T cells. This phenomenon required direct contact between MSC and purified T cells, although cell contact was not required for MSC induction of FoxP3 expression in an unseparated mononuclear cell population. In addition, through use of antagonists and neutralizing antibodies, MSC-derived prostaglandins and transforming growth factor (TGF)-beta1 were shown to have a non-redundant role in the induction of CD4+CD25+FoxP3+ T cells. Purified CD4+CD25+ T cells induced by MSC co-culture expressed TGF-beta1 and were able to suppress alloantigen-driven proliferative responses in mixed lymphocyte reaction. These data clarify the mechanisms of human MSC-mediated allosuppression, supporting a sequential process of regulatory T cell induction involving direct MSC contact with CD4+ cells followed by both prostaglandin E (2) and TGF-beta1 expression. Overall, this study provides a rational basis for ongoing clinical studies involving allogeneic MSC.

Eyckmans, J., et al. (2012). "Adhesive and mechanical regulation of mesenchymal stem cell differentiation in human bone marrow and periosteum-derived progenitor cells." Biol Open **1**(11): 1058-1068.

It has previously been demonstrated that cell shape can influence commitment of human bone marrow-derived mesenchymal stem cells (hBMCs) to adipogenic, osteogenic, chondrogenic, and other lineages. Human periosteum-derived cells (hPDCs) exhibit multipotency similar to hBMCs, but hPDCs may offer enhanced potential for osteogenesis and chondrogenesis given their apparent endogenous role in bone and cartilage repair in vivo. Here, we examined whether hPDC differentiation is regulated by adhesive and mechanical cues comparable to that reported for hBMC differentiation. When cultured in the appropriate induction media, hPDCs at high cell seeding density demonstrated enhanced levels of adipogenic or chondrogenic markers as compared with hPDCs at low cell seeding density. Cell seeding density correlated inversely with projected area of cell spreading, and directly limiting cell spreading with micropatterned substrates promoted adipogenesis or chondrogenesis while substrates promoting cell spreading supported osteogenesis. Interestingly, cell seeding density influenced differentiation through both changes in cell shape and non-shape-mediated effects: density-dependent adipogenesis and chondrogenesis were regulated primarily by cell shape whereas non-shape effects strongly influenced osteogenic potential. Inhibition of cytoskeletal contractility by adding the Rho kinase inhibitor Y27632 further enhanced adipogenic differentiation and discouraged osteogenic differentiation of hPDCs. Together, our results suggest that multipotent lineage decisions of hPDCs are impacted by cell adhesive and mechanical cues, though to different extents than hBMCs. Thus, future studies of hPDCs and other primary stem cell populations with clinical potential should consider varying biophysical metrics for more thorough optimization of stem cell differentiation.

Fan, D., et al. (2017). "Human mesenchymal stem cell homing induced by SKOV3 cells." Am J Transl Res **9**(2): 230-246.

Human mesenchymal stem cell (hMSC) homing is the migration of endogenous and exogenous hMSCS to the target organs and the subsequent colonization under the action chemotaxic factors. This is an important process involved in the repair of damaged tissues. However, we know little about the mechanism of hMSC homing. Stromal cell derived factor-1 (SDF-1) is a cytokine secreted by stromal cells. Its only receptor CXCR4 is widely expressed in blood cells, immune cells and cells in the central nervous system. SDF-1/CXCR4 signaling pathway plays an important role in hMSC homing and tissue repair. Human cbll1 gene encodes E3 ubiquitin-protein ligase Hakai (also known as CBLL1) consisting of RING-finger domain that is involved in ubiquitination, endocytosis and degradation of epithelial cadherin (E-cadherin) as well as in the regulation of cell proliferation. We successfully constructed LV3-CXCR4 siRNA lentiviral vector, LV3-CBLL1 RNAi lentiviral vector and the corresponding cell systems which were used to induce hMSC homing in the presence of SKOV3 cells. Thus the mechanism of hMSC homing was studied.

Foglietta, F., et al. (2017). "Selective sensitiveness of mesenchymal stem cells to shock waves leads to anticancer effect in human cancer cell co-cultures." Life Sci **173**: 28-35.

AIM: Mesenchymal stem cells (MSC) possess the distinctive feature of homing in on and engrafting into the tumor stroma making their therapeutic applications in cancer treatment very promising. Research into new effectors and external stimuli, which can selectively trigger the release of cytotoxic species from MSC toward the cancer cells, significantly raises their potential. MAIN METHODS: Shock waves (SW) have recently gained recognition for their ability to induce specific biological effects,

such as the local generation of cytotoxic reactive oxygen species (ROS) in a non-invasive and tunable manner. We thus investigate whether MSC are able to generate ROS and, in turn, affect cancer cell growth when in co-culture with human glioblastoma (U87) or osteosarcoma (U2OS) cells and exposed to SW. KEY FINDINGS: MSC were found to be the cell line that was most sensitive to SW treatment as shown by SW-induced ROS production and cytotoxicity. Notably, U87 and U2OS cancer cell growth was unaffected by SW exposure. However, significant decreases in cancer cell growth, 1.8 fold for U87 and 2.3 fold for U2OS, were observed 24h after the SW treatment of MSC co-cultures with cancer cells. The ROS production induced in MSC by SW exposure was then responsible for lipid peroxidation and cell death in U87 and U2OS cells co-cultured with MSC. SIGNIFICANCE: This experiment highlights the unique ability of MSC to generate ROS upon SW treatment and induce the cell death of co-cultured cancer cells. SW might therefore be proposed as an innovative tool for MSC-mediated cancer treatment.

Fonseka, M., et al. (2012). "Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSC) inhibit the proliferation of K562 (human erythromyeloblastoid leukaemic cell line)." Cell Biol Int **36**(9): 793-801.

hUCB-MSC (human umbilical cord blood-derived mesenchymal stem cells) offer an attractive alternative to bone marrow-derived MSC for cell-based therapy by being less invasive a source of biological material. We have evaluated the effect of hUCB-MSC on the proliferation of K562 (an erythromyeloblastoid cell line) and the cytokine secretion pattern of hUCB-MSC. Co-culturing of hUCB-MSC and K562 resulted in inhibition of proliferation of K562 in a dose-dependent manner. However, the anti-proliferative effect was reduced in transwells, suggesting the importance of direct cell-to-cell contact. hUCB-MSC inhibited proliferation of K562, arresting them in the G0 /G1 phase. NO (nitric oxide) was not involved in the hUCB-MSC-mediated tumour suppression. The presence of IL-6 (interleukin 6) and IL-8 were obvious in the hUCB-MSC conditioned media, but no significant increase was found in 29 other cytokines. Th1 cytokines, IFNalpha (interferon alpha), Th2 cytokine IL-4 and Th17 cytokine, IL-17 were not secreted by hUCB-MSC. There was an increase in the number of hUCB-MSC expressing the latent membrane-bound form of TGFbeta1 co-cultured with K562. The anti-proliferative effect of hUCB-MSC was due to arrest of the growth of K562 in the G0 /G1 phase. The mechanisms underlying increased IL-6 and IL-8 secretion and LAP (latency-associated peptide; TGFbeta1) by hUCB-MSC remains unknown.

Fossett, E., et al. (2012). "The effects of ageing on proliferation potential, differentiation potential and cell surface characterisation of human mesenchymal stem cells." Curr Stem Cell Res Ther **7**(4): 282-286.

Mesenchymal stem cells (MSCs) have a great capacity for use in regenerative medicine and other clinical applications. However, one question creating curiosity of their use, is how they are affected by ageing. As we now live within an ageing population, the prevalence of age related disorders is increasing, so it is important to investigate how effectively MSCs from older patients can be expanded and differentiated in vitro before their use in autologous cell transplantation. This paper will look at how ageing effects proliferation potential, differentiation potential and cell surface characterisation of human mesenchymal stem cells.

Freida, D., et al. (2013). "Human bone marrow mesenchymal stem cells regulate biased DNA segregation in response to cell adhesion asymmetry." Cell Rep **5**(3): 601-610.

Biased DNA segregation is a mitotic event in which the chromatids carrying the original template DNA strands and those carrying the template copies are not segregated randomly into the two daughter cells. Biased segregation has been observed in several cell types, but not in human mesenchymal stem cells (hMSCs), and the factors affecting this bias have yet to be identified. Here, we have investigated cell adhesion geometries as a potential parameter by plating hMSCs from healthy donors on fibronectin-coated micropatterns. On symmetric micropatterns, the segregation of sister chromatids to the daughter cells appeared random. In contrast, on asymmetric micropatterns, the segregation was biased. This sensitivity to asymmetric extracellular cues was reproducible in cells from all donors but was not observed in human skin-derived fibroblasts or in a fibroblastic cell line used as controls. We conclude that the asymmetry of cell adhesion is a major factor in the regulation of biased DNA segregation in hMSCs.

Friedman, R., et al. (2007). "Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation." Biol Blood Marrow Transplant **13**(12): 1477-1486.

The Wharton's jelly of the umbilical cord is rich in mesenchymal stem cells (UC-MSCs) that fulfill the criteria for MSCs. Here we describe a novel, simple method of obtaining and cryopreserving UC-MSCs by extracting the Wharton's jelly from a small piece of cord, followed by mincing the tissue and

cryopreserving it in autologous cord plasma to prevent exposure to allogeneic or animal serum. This direct freezing of cord microparticles without previous culture expansion allows the processing and freezing of umbilical cord blood (UCB) and UC-MSCs from the same individual on the same day on arrival in the laboratory. UC-MSCs produce significant concentrations of hematopoietic growth factors in culture and augment hematopoietic colony formation when co-cultured with UCB mononuclear cells. Mice undergoing transplantation with limited numbers of human UCB cells or CD34(+) selected cells demonstrated augmented engraftment when UC-MSCs were co-transplanted. We also explored whether UC-MSCs could be further manipulated by transfection with plasmid-based vectors. Electroporation was used to introduce cDNA and mRNA constructs for GFP into the UC-MSCs. Transfection efficiency was 31% for cDNA and 90% for mRNA. These data show that UC-MSCs represent a reliable, easily accessible, noncontroversial source of MSCs. They can be prepared and cryopreserved under good manufacturing practices (GMP) conditions and are able to enhance human hematopoietic engraftment in SCID mice. Considering their cytokine production and their ability to be easily transfected with plasmid-based vectors, these cells should have broad applicability in human cell-based therapies.

Fu, Q., et al. (2017). "Human decidua mesenchymal stem cells regulate decidual natural killer cell function via interactions between collagen and leukocyteassociated immunoglobulinlike receptor 1." Mol Med Rep **16**(3): 2791-2798.

The development of maternal tolerance to the fetal allograft in critical for the maintenance of the pregnancy, and it is accompanied by the development of a special decidual natural killer (dNK) cell tolerance phenotype. To understand the factors that influence dNK cells during early pregnancy, the present study aimed to identify mesenchymal stem cells (MSCs) from human firsttrimester deciduas, termed decidual MSCs (DMSCs), and to investigate the effect of DMSCs on the regulation of dNK cells via collagen. Decidual samples were collected from women with normal pregnancy that had undergone elective vaginal surgical terminations at 69 weeks gestation. DMSCs derived from human decidual tissues were cultured under differentiation conditions to examine their multipotent differentiation capacities, and the expression of MSCspecific markers, including cluster of differentiation (CD)44, CD73, CD105, CD90, CD34, CD31, CD14, CD45, CD11b and human leukocyte antigenantigen D related, was determined. dNK cells were cocultured with DMSCs in order to examine the effect of DMSCs on the tolerance phenotype of dNK cells. The expression of cell surface molecules, natural cytotoxicity triggering receptor 3 and killer cell immunoglobulinlike receptor (KIR) 2DL1, and the secretion of cytokines, including interferongamma, tumor necrosis factor (TNF)alpha, interleukin (IL)10, IL4 and perforin, were examined by flow cytometry analysis. To determine whether the regulation of dNK cells by DMSCs was mediated by collagen, DMSCs were pretreated with human recombinant leukocyteassociated immunoglobulinlike receptor (LAIR)2 and transfected with pScoRGFPhP4H to inhibit the interaction between LAIR1 and collagen. The present results demonstrated that collagen produced by DMSCs increased the expression of KIR2DL1 and IL4, decrease the expression of NKp30 and TNFalpha. In conclusion, the results of the present study demonstrated that DMSCs may be cultured in vitro for prolonged periods, whilst retaining the ability to differentiate into different cell lineages. In addition, DMSCs may modulate the function of dNK cells via the interaction between collagen and LAIR1.

Fu, Q. L., et al. (2012). "Mesenchymal stem cells derived from human induced pluripotent stem cells modulate T-cell phenotypes in allergic rhinitis." Allergy **67**(10): 1215-1222.

BACKGROUND: Human induced pluripotent stem cells (iPSCs) possess remarkable self-renewal capacity and the potential to differentiate into novel cell types, such as mesenchymal stem cells (MSCs). iPSC-MSCs have been shown to enhance tissue regeneration and attenuate tissue ischaemia; however, their contribution to the immune regulation of Th2-skewed allergic rhinitis (AR) and asthma remains unclear. OBJECTIVE: This study compared the immunomodulatory effects of iPSC-MSCs and bone marrow-derived MSCs (BM-MSCs) on lymphocyte proliferation, T-cell phenotypes and cytokine production in peripheral blood mononuclear cells (PBMCs) in patients with AR, and investigated the possible molecular mechanisms underlying the immunomodulatory properties of iPSC-MSCs. METHODS: In co-cultures of PBMCs with iPSC-MSCs or BM-MSCs, lymphocyte proliferation was evaluated using 3H-thymidine (3H-TdR) uptake, carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) assays; the regulatory T-cell (Treg) phenotype was determined by flow cytometry, and cytokine levels were measured using an enzyme-linked immunosorbent assay. The immunomodulatory properties of both MSCs were further evaluated using NS398 and transwell experiments. RESULTS: Similar to BM-MSCs, we determined that iPSC-MSCs significantly inhibit lymphocyte proliferation and promote Treg response in PBMCs ($P < 0.05$).

Accordingly, the cytokine milieu (IFN-gamma, IL-4, IL-5, IL-10 and IL-13) in the supernatants of PBMCs changed significantly ($P < 0.05$). The immunomodulatory properties of iPSC-MSCs and BM-MSCs were associated with prostaglandin E2 (PGE2) production and cell-cell contact. CONCLUSIONS: These data demonstrate that iPSC-MSCs are capable of modulating T-cell phenotypes towards Th2 suppression through inducing Treg expansion, suggesting that iPSC-MSCs can be used as an alternative candidate to adult MSCs to treat allergic airway diseases.

Fukuchi, Y., et al. (2004). "Human placenta-derived cells have mesenchymal stem/progenitor cell potential." Stem Cells **22**(5): 649-658.

Mesenchymal stem/progenitor cells (MSCs) are widely distributed in a variety of tissues in the adult human body (e.g., bone marrow [BM], kidney, lung, and liver). These cells are also present in the fetal environment (e.g., blood, liver, BM, and kidney). However, MSCs are a rare population in these tissues. Here we tried to identify cells with MSC-like potency in human placenta. We isolated adherent cells from trypsin-digested term placentas and established two clones by limiting dilution. We examined these cells for morphology, surface markers, gene expression patterns, and differentiation potential and found that they expressed several stem cell markers, hematopoietic/ endothelial cell-related genes, and organ-specific genes, as determined by reverse transcription-polymerase chain reaction and fluorescence-activated cell sorter analysis. They also showed osteogenic and adipogenic differentiation potentials under appropriate conditions. We suggest that placenta-derived cells have multilineage differentiation potential similar to MSCs in terms of morphology, cell-surface antigen expression, and gene expression patterns. The placenta may prove to be a useful source of MSCs.

Gadkari, R., et al. (2014). "Human embryonic stem cell derived-mesenchymal stem cells: an alternative mesenchymal stem cell source for regenerative medicine therapy." Regen Med **9**(4): 453-465.

AIM: To enumerate and characterize mesenchymal stem cells (MSC) derived from human embryonic stem cells (hESC) for clinical application. MATERIALS & METHODS: hESC were differentiated into hESC-MSC and characterized by the expression of surface markers using flow cytometry. hESC-MSC were evaluated with respect to growth kinetics, colony-forming potential, as well as osteogenic and adipogenic differentiation capacity. Immunosuppressive effects were assessed using peripheral blood mononuclear cell (PBMC) proliferation and cytotoxicity assays. RESULTS: hESC-MSC showed similar morphology, and cell surface markers as adipose (AMSC) and bone marrow-derived MSC (BMSC). hESC-MSC exhibited a higher growth rate during early in vitro expansion and equivalent adipogenic and osteogenic differentiation and colony-forming potential as AMSC and BMSC. hESC-MSC demonstrated similar immunosuppressive effects as AMSC and BMSC. CONCLUSION: hESC-MSC were comparable to BMSC and AMSC and hence can be used as an alternative source of MSC for clinical applications.

Gaebel, R., et al. (2011). "Cell origin of human mesenchymal stem cells determines a different healing performance in cardiac regeneration." PLoS One **6**(2): e15652.

The possible different therapeutic efficacy of human mesenchymal stem cells (hMSC) derived from umbilical cord blood (CB), adipose tissue (AT) or bone marrow (BM) for the treatment of myocardial infarction (MI) remains unexplored. This study was to assess the regenerative potential of hMSC from different origins and to evaluate the role of CD105 in cardiac regeneration. Male SCID mice underwent LAD-ligation and received the respective cell type (400.000/per animal) intramyocardially. Six weeks post infarction, cardiac catheterization showed significant preservation of left ventricular functions in BM and CD105(+)-CB treated groups compared to CB and nontreated MI group (MI-C). Cell survival analyzed by quantitative real time PCR for human GAPDH and capillary density measured by immunostaining showed consistent results. Furthermore, cardiac remodeling can be significantly attenuated by BM-hMSC compared to MI-C. Under hypoxic conditions in vitro, remarkably increased extracellular acidification and apoptosis has been detected from CB-hMSC compared to BM and CD105 purified CB-derived hMSC. Our findings suggests that hMSC originating from different sources showed a different healing performance in cardiac regeneration and CD105(+) hMSC exhibited a favorable survival pattern in infarcted hearts, which translates into a more robust preservation of cardiac function.

Gagari, E., et al. (2006). "Expression of stem cell factor and its receptor, c-kit, in human oral mesenchymal cells." Eur J Oral Sci **114**(5): 409-415.

Stem cell factor (SCF) is the pleiotropic ligand for the tyrosine kinase receptor, c-kit. Ligand and receptor are usually expressed in different cell types, and binding of SCF to c-kit promotes cell proliferation, differentiation, and recruitment of progenitor cells in various biologic systems. However, the localization of

these two molecules in cells of the oral cavity has not been systematically examined. We investigated the expression of SCF and c-kit in human dental pulp (HDP) cells as well as in human gingival fibroblasts (HGF). Both alternatively spliced isoforms of SCF were detected (through reverse transcription-polymerase chain reaction) in RNA obtained from the two cell types. Western analysis established that both cell types express SCF and/or c-kit, whereas flow cytometry demonstrated distinct cell populations expressing only the ligand (SCF), only the receptor (c-kit), or co-expressing the two. HDP cultures showed higher soluble SCF (sSCF) production associated with faster cell growth, as compared with HGF cultures. In both cell types, however, sSCF levels appeared to increase as a result of in vitro aging and/or differentiation.

Galindo, S., et al. (2017). "Therapeutic Effect of Human Adipose Tissue-Derived Mesenchymal Stem Cells in Experimental Corneal Failure Due to Limbal Stem Cell Niche Damage." Stem Cells **35**(10): 2160-2174.

Limbal stem cells are responsible for the continuous renewal of the corneal epithelium. The destruction or dysfunction of these stem cells or their niche induces limbal stem cell deficiency (LSCD) leading to visual loss, chronic pain, and inflammation of the ocular surface. To restore the ocular surface in cases of bilateral LSCD, an extraocular source of stem cells is needed to avoid dependence on allogeneic limbal stem cells that are difficult to obtain, isolate, and culture. The aim of this work was to test the tolerance and the efficacy of human adipose tissue-derived mesenchymal stem cells (hAT-MSCs) to regenerate the ocular surface in two experimental models of LSCD that closely resemble different severity grades of the human pathology. hAT-MSCs transplanted to the ocular surface of the partial and total LSCD models developed in rabbits were well tolerated, migrated to inflamed tissues, reduced inflammation, and restrained the evolution of corneal neovascularization and corneal opacity. The expression profile of the corneal epithelial cell markers CK3 and E-cadherin, and the limbal epithelial cell markers CK15 and p63 was lost in the LSCD models, but was partially recovered after hAT-MSC transplantation. For the first time, we demonstrated that hAT-MSCs improve corneal and limbal epithelial phenotypes in animal LSCD models. These results support the potential use of hAT-MSCs as a novel treatment of ocular surface failure due to LSCD. hAT-MSCs represent an available, non-immunogenic source of stem cells that may provide therapeutic benefits in addition to reduce health care expenses. Stem Cells 2017;35:2160-2174.

Gao, F., et al. (2008). "In vitro cultivation of islet-like cell clusters from human umbilical cord blood-derived mesenchymal stem cells." Transl Res **151**(6): 293-302.

A major obstacle to successful islet transplantation for both type 1 and 2 diabetes is an inadequate supply of insulin-producing tissue. In vitro transdifferentiation of human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) into insulin-producing cells could provide an abundant source of cells for this procedure. For this study, we isolated and characterized human UCB-MSCs and induced them in vitro to differentiate into islet-like cell clusters using a 15-day protocol based on a combination of high-glucose, retinoic acid, nicotinamide, epidermal growth factor, and exendin-4. These clusters appeared about 9 days after pancreatic differentiation; expressed pancreatic beta-cell markers, including insulin, glucagon, Glut-2, PDX1, Pax4, and Ngn3; and could synthesize and secrete functional islet proteins at the end of the inducing protocol. The insulin-positive cells accounted for (25.2-3.36)% of whole induced cells. Although insulin secretion of those insulin-producing cells did not respond to glucose challenge very well, human UCB-MSCs have the ability to differentiate into islet-like cells in vitro and may be a potential new source for islet transplantation.

Gao, H., et al. (2009). "Activation of signal transducers and activators of transcription 3 and focal adhesion kinase by stromal cell-derived factor 1 is required for migration of human mesenchymal stem cells in response to tumor cell-conditioned medium." Stem Cells **27**(4): 857-865.

Mesenchymal stem cells (MSCs) migrate to tumors both in vitro and in vivo. Gene expression profiling analysis reveals that stromal cell-derived factor 1 (SDF-1) is significantly upregulated in MSCs exposed to tumor cell-conditioned medium, when compared with cells treated with control medium, suggesting that SDF-1 signaling is important in mediating MSC migration. This study investigates downstream signaling during MSC migration in response to tumor cell-conditioned medium and recombinant SDF-1 protein treatments. We observed that both recombinant SDF-1 and tumor cell-conditioned medium were able to activate downstream signaling via signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) as revealed by increased phosphorylation of STAT3 and ERK1/2 in human MSCs (hMSCs). Significant impairment of in vitro migration was observed in the presence of

MAPK/ERK kinase (MEK) inhibitor PD98059, whereas two Janus kinase 2 (Jak2) inhibitors completely abolished migration induced by tumor cell-conditioned medium. Impaired MSC migration correlated with decreased levels of phosphorylated STAT3 and ERK1/2, suggesting that SDF-1 stimulation activates Jak2/STAT3 as well as MEK/ERK1/2 signaling, which in turn promotes migration of MSCs toward tumor cells. Furthermore, stimulation of hMSCs with recombinant SDF-1 and tumor cell-conditioned medium also significantly activated the focal adhesion kinases (FAKs) and paxillin, which correlated with reorganization of F-actin filaments in hMSCs. Decreased phosphorylation of FAK and paxillin as well as disruption of cytoskeleton organization was observed following Jak2 and MEK inhibitor treatment. Taken together, our results provide insight into the molecular pathways responsible for MSC migration toward the tumor microenvironment and may provide the molecular basis for modifying MSCs for therapeutic purposes.

Gao, P., et al. (2010). "Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma." Cancer Lett **290**(2): 157-166.

Mesenchymal stem cells (MSCs) represent a new tool for delivery of therapeutic agents to cancer. The cytokine interleukin-12 (IL-12) has demonstrated a potent anti-tumor activity in a variety of mouse tumor models. In this study, human MSCs were isolated from human bone marrow and identified by phenotype analysis and differentiation assays. The anti-tumor activity of human MSCs stably transduced with a recombinant adenoviral vector expressing the murine IL-12 (MSC/IL-12) were evaluated in a mouse xenograft model of renal cell carcinoma (RCC). Expression and bioactivity of the transgenic protein IL-12 from adenoviral vector were confirmed prior to in vivo studies. A nude mouse model of RCC was developed by subcutaneously injection of 786-0 cells into nude mice. MSC/IL-12 was injected into the lateral tail vein with single dose. Results indicated that systemic administration of MSC/IL-12 reduced the growth of 786-0 RCC and significantly prolonged mouse survival. These transfected cells could home to tumors after intravenous injection and largely produce local IL-12 protein. In contrast, systemic level of IL-12 was modestly elevated. Further studies showed that the anti-tumor activity of the MSC/IL-12 was dependent on the presence of natural killer (NK) cells and IFN-gamma in this experimental setting. These data demonstrate the potential of adult MSC constitutively producing IL-12 to reduce the growth of RCC and enhance the tumor-bearing mouse survival.

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.

Ghafarzadeh, M., et al. (2016). "Human amniotic fluid derived mesenchymal stem cells cause an anti-cancer effect on breast cancer cell line in vitro." Cell Mol Biol (Noisy-le-grand) **62**(6): 102-106.

Human amniotic fluid stem cells (hAFSCs) have the ability to self-renew, and multipotent differentiation into three germ layer cells. We obtained 5 ml amniotic fluid from ten 16-20 week pregnant women undergoing amniocentesis. hAFSCs were isolated from all samples, co-cultured with T47D breast cancer cell line and characterized using flow cytometry and RT-PCR. After 3, 4 and 5 days, T47D and HSFCs viability were evaluated with MTT assay.

After 5 days of co-culture T47D cells viability were decreased. Our findings showed that hAFSCs can release soluble factors in cell culture, causing an efficient anticancer effect.

Ghannam, S., et al. (2010). "Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype." J Immunol **185**(1): 302-312.

Mesenchymal stem cells (MSCs) exert immunomodulatory properties via the inhibition of T cell activation and proliferation. Because of the deleterious role of Th17 cells in the pathogenesis of inflammatory disease, we investigated whether proinflammatory cytokines could modify the expression of adhesion molecules on human MSCs, thereby contributing to increased Th17 cell adhesion to MSCs and, as a consequence, modulating the function of the latter cells. IFN-gamma and TNF-alpha synergistically enhanced the expression of CD54 by MSCs, enabling the CCR6 chemokine ligand CCL20 to induce in vitro adhesion of Th17 cells to MSCs. MSCs prevented the in vitro differentiation of naive CD4(+) T cells into Th17 cells and inhibited the production of IL-17, IL-22, IFN-gamma, and TNF-alpha by fully differentiated Th17 cells; this was mediated, in part, via PGE (2), the production of which was enhanced in cocultures with Th17 cells. Moreover, MSCs induced the production of IL-10 and trimethylation of histone H3K4me3 at the promoter of the FOXP3 gene locus, whereas it suppressed trimethylation of the corresponding region in the RORC gene in Th17 cells. These epigenetic changes were associated with the induction of fork head box p3 and the acquisition by Th17 cells of the capacity to inhibit in vitro proliferative responses of activated CD4(+) T cells, which was enhanced when MSCs were preincubated with IFN-gamma and TNF-alpha. These results showed that, under inflammatory conditions, MSCs mediate the adhesion of Th17 cells via CCR6 and exert anti-inflammatory effects through the induction of a T cell regulatory phenotype in these cells.

Gildehaus, F. J., et al. (2011). "Impact of indium-111 oxine labelling on viability of human mesenchymal stem cells in vitro, and 3D cell-tracking using SPECT/CT in vivo." Mol Imaging Biol **13**(6): 1204-1214.

PURPOSE: This study investigates the effects of (111)In-oxine incorporation on human mesenchymal stem cells' (hMSC) biology and viability, and the applicability of (111)In-oxine for single-photon emission computed tomography/X-ray computed tomography (SPECT/CT) monitoring of hMSC in vivo. PROCEDURES: HMSC were labelled with 10 Bq/cell. Cellular retention of radioactivity, cell survival, and migration were evaluated over 48 h. Metabolic activity was assessed over 14 days and the hMSC's stem cell character was evaluated. Serial SPECT/CT was performed after intra-osseous injection to athymic rats over 48 h. RESULTS: Labelling efficiency was 25%, with 61% of incorporated (111)In remaining in the hMSC at 48 h. The radiolabelling was without effect on cell viability, stem cell character, and plasticity, whereas metabolic activity and migration were significantly reduced. Grafted cells could be imaged in situ with SPECT/CT. CONCLUSIONS: (111)In-oxine labelling moderately impaired hMSC's functional integrity while preserving their stem cell character. Combined SPECT/CT imaging of (111)In-oxine-labelled hMSC opens the possibility for non-invasive sequential monitoring of therapeutic stem cells.

Gindraux, F., et al. (2007). "Human and rodent bone marrow mesenchymal stem cells that express primitive stem cell markers can be directly enriched by using the CD49a molecule." Cell Tissue Res **327**(3): 471-483.

Bone marrow (BM) from human and rodent species contains a population of multipotential cells referred to as mesenchymal stem cells (MSCs). Currently, MSCs are isolated indirectly by using a culture step and then the generation of fibroblast colony-forming units (CFU-fs). Unprocessed or native BM MSCs have not yet been fully characterised. We have previously developed a direct enrichment method for the isolation of MSCs from human BM by using the CD49a protein (alpha1-integrin subunit). As the CD49a gene is highly conserved in mammals, we have evaluated whether this direct enrichment can be employed for BM cells from rodent strains (rat and mouse). We have also studied the native phenotype by using both immunodetection and immunomagnetic methods and have compared MSCs from mouse, rat and human BM. As is the case for human BM, we have demonstrated that all rodent multipotential CFU-fs are contained within the CD49a-positive cell population. However, in the mouse, the number of CFU-fs is strain-dependent. Interestingly, all rat and mouse Sca-1-positive cells are concentrated within the CD49a-positive fraction and also contain all CFU-fs. In human, the colonies have been detected in the CD49a/CD133 double-positive population. Thus, the CD49a protein is a conserved marker that permits the direct enrichment of BM MSCs from various mammalian species; these cells have been phenotyped as true BM stem cells.

Giuliani, M., et al. (2011). "Human mesenchymal stem cells derived from induced pluripotent stem cells

down-regulate NK-cell cytolytic machinery." Blood **118**(12): 3254-3262.

A major issue in immunosuppressive biotherapy is the use of mesenchymal stem cells (MSCs) that harbor regulatory capacity. However, currently used bone marrow-derived MSCs (BM-MSCs) are short-lived and cannot assure long lasting immunoregulatory function both in vitro and in vivo. Consequently, we have generated MSCs from human induced pluripotent stem (IPS-MSCs) cells that share similar properties with embryonic stem cells (ES-MSCs). Herein, we compared the immunoregulatory properties of ES/IPS-MSCs with those of BM-MSCs and showed, for the first time, that IPS-derived MSCs display remarkable inhibition of NK-cell proliferation and cytolytic function in a similar way to ES-MSCs. Both MSCs disrupt NK-cell cytolytic machinery in the same fashion that BM-MSCs, by down-regulating the expression of different activation markers and ERK1/2 signaling, leading to an impairment to form immunologic synapses with target cells and, therefore, secretion of cytotoxic granules. In addition, they are more resistant than adult BM-MSCs to preactivated NK cells. IPS-MSCs could represent an attractive alternative source of immunoregulatory cells, and their capacity to impair NK-cell cytotoxicity constitutes a complex mechanism to prevent allograft rejection.

Glavaski-Joksimovic, A., et al. (2010). "Glial cell line-derived neurotrophic factor-secreting genetically modified human bone marrow-derived mesenchymal stem cells promote recovery in a rat model of Parkinson's disease." J Neurosci Res **88**(12): 2669-2681.

Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive degeneration of nigrostriatal dopaminergic (DA) neurons. The therapeutic potential of glial cell line-derived neurotrophic factor (GDNF), the most potent neurotrophic factor for DA neurons, has been demonstrated in many experimental models of PD. However, chronic delivery of GDNF to DA neurons in the brain remains an unmet challenge. Here, we report the effects of GDNF-releasing Notch-induced human bone marrow-derived mesenchymal stem cells (MSC) grafted into striatum of the 6-hydroxydopamine (6-OHDA) progressively lesioned rat model of PD. Human MSC, obtained from bone marrow aspirates of young, healthy adult volunteers, were transiently transfected with the intracellular domain of the Notch1 gene (NICD) to generate SB623 cells. SB623 cells expressing GDNF and/or humanized Renilla green fluorescent protein (hrGFP) following lentiviral transduction or nontransduced cells were stereotaxically placed into rat striatum 1 week after a unilateral partial 6-OHDA striatal lesion. At 4 weeks, rats that had received GDNF-transduced SB623 cells had significantly decreased amphetamine-induced rotation compared with control rats, although this effect was not observed in rats that received GFP-transduced or nontransduced SB623 cells. At 5 weeks, rejuvenated tyrosine hydroxylase-immunoreactive (TH-IR) fibers that appeared to be host DA axons were observed in and around grafts. This effect was more prominent in rats that received GDNF-secreting cells and was not observed in controls. These observations suggest that human bone-marrow derived MSC, genetically modified to secrete GDNF, hold potential as an allogeneic or autologous stem cell therapy for PD.

Golpanian, S., et al. (2016). "Rationale and design of the allogeneiC human mesenchymal stem cells (hMSC) in patients with aging fRAilTy via intravenoUS delivery (CRATUS) study: A phase I/II, randomized, blinded and placebo controlled trial to evaluate the safety and potential efficacy of allogeneic human mesenchymal stem cell infusion in patients with aging frailty." Oncotarget **7**(11): 11899-11912.

Frailty is a syndrome associated with reduced physiological reserves that increases an individual's vulnerability for developing increased morbidity and/or mortality. While most clinical trials have focused on exercise, nutrition, pharmacologic agents, or a multifactorial approach for the prevention and attenuation of frailty, none have studied the use of cell-based therapies. We hypothesize that the application of allogeneic human mesenchymal stem cells (allo-hMSCs) as a therapeutic agent for individuals with frailty is safe and efficacious. The CRATUS trial comprises an initial non-blinded phase I study, followed by a blinded, randomized phase I/II study (with an optional follow-up phase) that will address the safety and pre-specified beneficial effects in patients with the aging frailty syndrome. In the initial phase I protocol, allo-hMSCs will be administered in escalating doses via peripheral intravenous infusion (n=15) to patients allocated to three treatment groups: Group 1 (n=5, 20 million allo-hMSCs), Group 2 (n=5, 100 million allo-hMSCs), and Group 3 (n=5, 200 million allo-hMSCs). Subsequently, in the randomized phase, allo-hMSCs or matched placebo will be administered to patients (n=30) randomly allocated in a 1:1:1 ratio to one of two doses of MSCs versus placebo: Group A (n=10, 100 million allo-hMSCs), Group B (n=10, 200 million allo-hMSCs), and Group C (n=10, placebo). Primary and secondary objectives are, respectively, to demonstrate the safety and efficacy of allo-hMSCs administered in frail older individuals. This study will determine the safety of intravenous infusion of stem cells and

compare phenotypic outcomes in patients with aging frailty.

Goncalves, M. A., et al. (2006). "Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion." Hum Mol Genet **15**(2): 213-221.

Duchenne muscular dystrophy (DMD) is the most prevalent inheritable muscle disease. It is caused by mutations in the approximately 2.5-megabase dystrophin (Dys) encoding gene. Therapeutic attempts at DMD have relied on injection of allogeneic Dys-positive myoblasts. The immune rejection of these cells and their limited availability have prompted the search for alternative therapies and sources of myogenic cells. Stem cell-based gene therapy aims to restore tissue function by the transplantation of gene-corrected autologous cells. It depends on (i) the capacity of stem cells to participate in tissue regeneration and (ii) the efficient genetic correction of defective autologous stem cells. We explored the potential of bone marrow-derived human mesenchymal stem cells (hMSCs) genetically modified with the full-length Dys-coding sequence to engage in myogenesis. By tagging hMSCs with enhanced green fluorescent protein (EGFP) or the membrane dye PKH26, we demonstrated that they could participate in myotube formation when cultured together with differentiating human myoblasts. Experiments performed with EGFP-marked hMSCs and DsRed-labeled DMD myoblasts revealed that the EGFP-positive DMD myotubes were also DsRed-positive indicating that hMSCs participate in human myogenesis through cellular fusion. Finally, we showed that hMSCs transduced with a tropism-modified high-capacity hybrid viral vector encoding full-length Dys could complement the genetic defect of DMD myotubes.

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.

Gong, Z., et al. (2009). "Influence of culture medium on smooth muscle cell differentiation from human bone marrow-derived mesenchymal stem cells." Tissue Eng Part A **15**(2): 319-330.

Human bone marrow-derived mesenchymal stem cells (hMSCs) represent an appealing source of smooth muscle cells (SMCs) for engineering small-diameter vascular grafts due to the limited availability and replicative capacity of somatic SMCs. However, lack of standardization of hMSC culture conditions has limited some progress in hMSC research. Because, at the moment, a chemically defined, serum-free medium without growth factors is not capable of amplifying hMSCs in vitro, the usage of serum (either human serum or fetal bovine serum [FBS]) continues in hMSC research. The emergence of commercial hMSCs and hMSC media opened a series of questions regarding the compatibility of commercial and homemade hMSCs and hMSC media. In this study, two types of commonly used FBS-containing hMSC media-MSCGM (containing 10% FBS) and MesenPro (containing 2% FBS), along with our homemade medium (low-glucose Dulbecco's modified Eagle's medium plus 10% selected lot FBS)-were compared in their ability to support SMC differentiation from

hMSCs. The effects of FBS level, medium supplements (ascorbic acid, copper, etc.), and growth factors (transforming growth factor beta1) were also examined for their impact on SMC differentiation. It was discovered that MesenPro and transforming growth factor beta1 are the strongest SMC inducers from hMSCs. In contrast, hMSCs grown in homemade (10% Dulbecco's modified Eagle's medium) and commercial MSCGM media remained undifferentiated. FBS concentration did not affect SMC differentiation when 10% FBS was compared with 2%. Finally, the mechanism underlying SMC differentiation from hMSCs grown in FBS-containing medium was explored by following the expression changes of serum response factor during the establishment of hMSC culture.

Gonzalez-Rey, E., et al. (2010). "Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis." Ann Rheum Dis **69**(1): 241-248.

OBJECTIVES: Adult mesenchymal stem cells were recently found to suppress effector T cell and inflammatory responses and have emerged as attractive therapeutic candidates for immune disorders. In rheumatoid arthritis (RA), a loss in the immunological self-tolerance causes the activation of autoreactive T cells against joint components and subsequent chronic inflammation. The aim of this study is to characterise the immunosuppressive activity of human adipose-derived mesenchymal stem cells (hASCs) on collagen-reactive T cells from patients with RA. METHODS: The effects of hASCs on collagen-reactive RA human T cell proliferation and cytokine production were investigated, as well as effects on the production of inflammatory mediators by monocytes and fibroblast-like synoviocytes from patients with RA. RESULTS: hASCs suppressed the antigen-specific response of T cells from patients with RA. hASCs inhibited the proliferative response and the production of inflammatory cytokines by collagen-activated CD4 and CD8 T cells. In contrast, the numbers of IL10-producing T cells and monocytes were significantly augmented upon hASC treatment. The suppressive activity of hASCs was cell-to-cell contact dependent and independent. hASCs also stimulated the generation of FoxP3 protein-expressing CD4(+)CD25(+) regulatory T cells, with the capacity to suppress collagen-specific T cell responses. Finally, hASCs downregulated the inflammatory response and the production of matrix-degrading enzymes by synovial cells isolated from patients with RA. CONCLUSIONS: The present work identifies hASCs as key regulators of immune tolerance, with the capacity to suppress T cell and inflammatory responses and to induce the generation/activation of antigen-specific regulatory T cells.

Gonzalo-Gil, E., et al. (2016). "Human embryonic stem cell-derived mesenchymal stromal cells ameliorate collagen-induced arthritis by inducing host-derived indoleamine 2,3 dioxygenase." Arthritis Res Ther **18**: 77.

BACKGROUND: The immunosuppressive and anti-inflammatory properties of mesenchymal stromal cells (MSC) have prompted their therapeutic application in several autoimmune diseases, including rheumatoid arthritis. Adult MSC are finite and their clinical use is restricted by the need for long-term expansion protocols that can lead to genomic instability. Inhibition of Smad2/3 signaling in human pluripotent stem cells (hPSC) provides an infinite source of MSC that match the phenotype and functional properties of adult MSC. Here, we test the therapeutic potential of hPSC-MSC of embryonic origin (embryonic stem cell-derived mesenchymal stromal cells, hESC-MSC) in the experimental model of collagen-induced arthritis (CIA). METHODS: CIA was induced in DBA/1 mice by immunization with type II collagen (CII) in Complete Freund's Adjuvant (CFA). Mice were treated with either a single dose (10(6) cells/mouse) of hESC-MSC on the day of immunization (prophylaxis) or with three doses of hESC-MSC every other day starting on the day of arthritis onset (therapy). Arthritis severity was evaluated daily for six weeks and ten days, respectively. Frequency of Treg (FoxP3(+)), Th1 (IFNgamma (+)) and Th17 (IL17(+)) CD4(+) T cells in inguinal lymph nodes (ILN) was quantified by flow cytometry. Serum levels of anti-CII antibodies were determined by ELISA. Detection of hESC-MSC and quantification of murine and human indoleamine 2,3 dioxygenase (IDO1) expression was performed by quantitative real-time PCR. Statistical differences were analyzed by ANOVA and the Mann-Whitney U test. RESULTS: Administration of hESC-MSC to mice with established arthritis reduced disease severity compared to control-treated mice. Analysis of CD4 T cell populations in treated mice showed an increase in FoxP3(+) Treg and IFNgamma (+) Th1 cells but not in Th17 cells in the ILN. Anti-CII antibody levels were not affected by treatment. Migration of hESC-MSC to the ILN in treated mice was associated with the induction of murine IDO1. CONCLUSION: Treatment with hESC-MSC ameliorates CIA by inducing IFNgamma (+) Th1 cells and IDO1 in the host. Thus, hESC-MSC can provide an infinite cellular source for treatment of rheumatoid arthritis.

Greco, S. J. and P. Rameshwar (2007). "MicroRNAs regulate synthesis of the

neurotransmitter substance P in human mesenchymal stem cell-derived neuronal cells." Proc Natl Acad Sci U S A **104**(39): 15484-15489.

MicroRNAs (miRNAs) are a class of 19- to 23-nt, small, noncoding RNAs, which bind the 3' UTR of target mRNAs to mediate translational repression in animals. miRNAs have been shown to regulate developmental processes, such as self-renewal of stem cells, neuronal differentiation, myogenesis, and cancer. A functional role of miRNAs in the regulation of neurotransmitter synthesis has yet to be ascribed. We used mesenchymal stem cells (MSCs) as a model to study miRNA-mediated neurotransmitter regulation in developing neuronal cells. MSCs are mesoderm-derived cells, primarily resident in adult bone marrow, which can generate functional neuronal cells. We have previously shown that human MSC-derived neuronal cells express the neurotransmitter gene, Tac1, but do not synthesize the gene's encoded peptide, the neurotransmitter substance P (SP), unless stimulated with the inflammatory mediator IL-1alpha. These findings suggested a potential role for miRNAs in the regulation of SP synthesis. Here, we report on the miRNA profile of undifferentiated human MSCs and MSC-derived neuronal cells by using miRNA-specific bioarrays. miRNAs that were increased in the neuronal cells and decreased after IL-1alpha stimulation were analyzed by the miRanda algorithm to predict Tac1 mRNA targets. Putative miR-130a, miR-206, and miR-302a binding sites were predicted within the 3' UTR of Tac1. Target validation using a luciferase reporter system confirmed the miR-130a and miR-206 sites. Specific inhibition of miR-130a and miR-206 in the neuronal cells resulted in SP synthesis and release. The studies provide a different approach in ascribing a new regulatory role for miRNAs in regulating neurotransmitter synthesis.

Gruber, H. E., et al. (2012). "Human adipose-derived mesenchymal stem cells: serial passaging, doubling time and cell senescence." Biotech Histochem **87**(4): 303-311.

Adult adipose-derived mesenchymal stem cells (AD-MSC) are very interesting to our research group because they are easy to harvest, they are abundant in humans, and they have potential clinical applications in autologous cell therapy for disc degeneration. We examined these cells through sequential serial passages to assess osteogenic and chondrogenic capabilities, mean doubling time and cell senescence. Osteogenic and chondrogenic potencies were maintained through 13 passages. Mean passage doubling time increased significantly with increasing passage number. When donor age was evaluated, passages 1-4 from older donors had significantly longer doubling times compared to cells from younger donors. Passages 5-11 showed similar findings when analyzed by donor age. The mean percent senescence increased significantly with cell passaging, rising from 0% at passage 1 to 3.4% at passage 13. These novel data suggest that caution should be exercised when using AD-MSC with long passage times.

Gu, H., et al. (2016). "Exosomes derived from human mesenchymal stem cells promote gastric cancer cell growth and migration via the activation of the Akt pathway." Mol Med Rep **14**(4): 3452-3458.

Mesenchymal stem cells (MSCs) are a component of the tumor microenvironment and can promote the development of gastric cancer through paracrine mechanism. However, the effects of MSCexosomes (MSCex) on gastric cancer are less clear. The present study reported that MSCex promoted the proliferative and metastatic potential of gastric cancer cells ex vivo. It was found that MSCex enhanced the migration and invasion of HGC27 cells via the induction of the epithelialmesenchymal transition. MSCex increased the expression of mesenchymal markers and reduced the expression of epithelial markers in gastric cancer cells. MSCex also enhanced the tumorigenicity of gastric cancer cells ex vivo. MSCex induced the stemness of gastric cancer cells. The expression of octamerbinding transcription factor 4, ex determining region Ybox 2 and Lin28B significantly increased in gastric cancer cells treated with MSCex. The present study further demonstrated that MSCex elicited these biological effects predominantly via the activation of the protein kinase B signaling pathway. Taken together, the present findings provided novel evidence for the role of MSCex in gastric cancer and a new opportunity for improving the efficiency of gastric cancer treatment by targeting MSCex.

Guerrero, J., et al. (2013). "Cell interactions between human progenitor-derived endothelial cells and human mesenchymal stem cells in a three-dimensional macroporous polysaccharide-based scaffold promote osteogenesis." Acta Biomater **9**(9): 8200-8213.

Several studies have reported the benefits of mesenchymal stem cells (MSCs) for bone tissue engineering. However, vascularization remains one of the main obstacles that must be overcome to reconstruct large bone defects. In vitro prevascularization of the three-dimensional (3-D) constructs using co-cultures of human progenitor-derived endothelial cells (PDECs) with human bone marrow mesenchymal stem cells (HBMSCs) appeared as a potential strategy. However, the crosstalk between the two lineages has been studied in two-dimensional (2-D), but remains unknown in 3-D. The aim of this

study is to investigate the cell interactions between PDECs and HBMSCs in a porous matrix composed of polysaccharides. This biodegradable scaffold promotes cell interactions by inducing multicellular aggregates composed of HBMSCs surrounded by PDECs. Cell aggregation contributes to the formation of junctional proteins composed of Connexin43 (Cx43) and VE-cadherin, and an activation of osteoblastic differentiation of HBMSCs stimulated by the presence of PDECs. Inhibition of Cx43 by mimetic peptide 43GAP27 induced a decrease in mRNA levels of Cx43 and all the bone-specific markers. Finally, subcutaneous implantations for 3 and 8 weeks in NOG mice revealed an increase in osteoid formation with the tissue-engineered constructs seeded with HBMSCs/PDECs compared with those loaded with HBMSCs alone. Taking together, these results demonstrate that this 3-D microenvironment favored cell communication, osteogenesis and bone formation.

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.

Guo, X., et al. (2013). "A novel in vitro model system for smooth muscle differentiation from human embryonic stem cell-derived mesenchymal cells." Am J Physiol Cell Physiol **304**(4): C289-298.

The objective of this study was to develop a novel in vitro model for smooth muscle cell (SMC) differentiation from human embryonic stem cell-derived mesenchymal cells (hES-MCs). We found that hES-MCs were differentiated to SMCs by transforming growth factor-beta (TGF-beta) in a dose- and time-dependent manner as demonstrated by the expression of SMC-specific genes smooth muscle alpha-actin, calponin, and smooth muscle myosin heavy chain. Under normal growth conditions, however, the differentiation capacity of hES-MCs was very limited. hES-MC-derived SMCs had an elongated and spindle-shaped morphology and contracted in response to the induction of carbachol and KCl. KCl-induced calcium transient was also evident in these cells. Compared with the parental cells, TGF-beta-treated hES-MCs sustained the endothelial tube formation for a longer time due to the sustained SMC phenotype. Mechanistically, TGF-beta-induced differentiation was both Smad- and serum response factor/myocardin dependent. TGF-beta regulated myocardin expression via multiple signaling pathways including Smad2/3, p38 MAPK, and PI3K. Importantly, we found that a low level of myocardin was present in mesoderm prior to SMC lineage determination, and a high level of myocardin was not induced until the differentiation process was initiated. Taken together, our study characterized a novel SMC differentiation model that can be used for studying human SMC differentiation from mesoderm during vascular development.

Haack-Sorensen, M., et al. (2008). "Comparison of different culture conditions for human mesenchymal stromal cells for clinical stem cell therapy." Scand J Clin Lab Invest **68**(3): 192-203.

OBJECTIVE: Mesenchymal stromal cells (MSCs) from adult bone marrow (BM) are considered potential candidates for therapeutic neovascularization in cardiovascular disease. When implementing results from animal trials in clinical treatment, it is essential to isolate and expand the MSCs under conditions following good manufacturing practice (GMP). The aims of the study were first to establish culture conditions following GMP quality demands for human MSC expansion and differentiation for use in clinical trials, and second to compare these MSCs with MSCs derived from culture in four media commonly used for MSC cultivation in animal studies simulating clinical stem cell therapy. MATERIAL AND METHODS: Human mononuclear cells (MNCs) were isolated from

BM aspirates by density gradient centrifugation and cultivated in a GMP-accepted medium (EMEA medium) or in one of four other media. RESULTS: FACS analysis showed that the plastic-adherent MSCs cultured in EMEA medium or in the other four media were identically negative for the haematopoietic surface markers CD45 and CD34 and positive for CD105, CD73, CD90, CD166 and CD13, which in combined expression is characteristic of MSCs. MSC stimulation with vascular endothelial growth factor (VEGF) increased expression of the characteristic endothelial genes KDR and von Willebrand factor; the von Willebrand factor and CD31 at protein level as well as the capacity to develop capillary-like structures. CONCLUSIONS: We established culture conditions with a GMP compliant medium for MSC cultivation, expansion and differentiation. The expanded and differentiated MSCs can be used in autologous mesenchymal stromal cell therapy in patients with ischaemic heart disease.

Hajizadeh-Saffar, E., et al. (2015). "Inducible VEGF expression by human embryonic stem cell-derived mesenchymal stromal cells reduces the minimal islet mass required to reverse diabetes." Sci Rep **5**: 9322.

UNLABELLED: Islet transplantation has been hampered by loss of function due to poor revascularization. We hypothesize that co-transplantation of islets with human embryonic stem cell-derived mesenchymal stromal cells that conditionally overexpress VEGF (hESC-MSC:VEGF) may augment islet revascularization and reduce the minimal islet mass required to reverse diabetes in mice. HESC-MSCs were transduced by recombinant lentiviruses that allowed conditional (Dox-regulated) overexpression of VEGF. HESC-MSC: VEGF were characterized by tube formation assay. After co-transplantation of hESC-MSC:VEGF with murine islets in collagen-fibrin hydrogel in the omental pouch of diabetic nude mice, we measured blood glucose, body weight, glucose tolerance and serum C-peptide. As control, islets were transplanted alone or with non-transduced hESC-MSCs. Next, we compared functional parameters of 400 islets alone versus 200 islets co-transplanted with hESC-MSC:VEGF. As control, 200 islets were transplanted alone. Metabolic function of islets transplanted with hESC-MSC:VEGF significantly improved, accompanied by superior graft revascularization, compared with control groups. Transplantation of 200 islets with hESC-MSC:VEGF showed superior function over 400 islets alone. We conclude that co-transplantation of islets with VEGF-expressing hESC-MSCs allowed for at least a 50% reduction in minimal islet mass required to reverse diabetes in mice. This approach may contribute to alleviate the need for multiple donor organs per patient.

Hamouda, H., et al. (2013). "N-glycosylation profile of undifferentiated and adipogenically differentiated human bone marrow mesenchymal stem cells: towards a next generation of stem cell markers." Stem Cells Dev **22**(23): 3100-3113.

Mesenchymal stem cells (MSCs) are multipotent cells that are easy to isolate and expand, develop into several tissues, including fat, migrate to diseased organs, have immunosuppressive properties and secrete regenerative factors. This makes MSCs ideal for regenerative medicine. For application and regulatory purposes, knowledge of (bio)markers characterizing MSCs and their development stages is of paramount importance. The cell surface is coated with glycans that possess lineage-specific nature, which makes glycans to be promising candidate markers. In the context of soft tissue generation, we aimed to identify glycans that could be markers for MSCs and their adipogenically differentiated progeny. MSCs were isolated from human bone marrow, adipogenically stimulated for 15 days and adipogenesis was verified by staining the lipid droplets and quantitative real time polymerase chain reaction of the marker genes peroxisome proliferator-activated receptor gamma (PPARG) and fatty acid binding protein-4 (FABP4). Using matrix-assisted laser desorption-ionization-time of flight mass spectrometry combined with exoglycosidase digestions, we report for the first time the N-glycome of MSCs during adipogenic differentiation. We were able to detect more than 100 different N-glycans, including high-mannose, hybrid, and complex N-glycans, as well as poly-N-acetyllactosamine chains. Adipogenesis was accompanied by an increased amount of biantennary fucosylated structures, decreased amount of fucosylated, afucosylated tri- and tetraantennary structures and increased sialylation. N-glycans H6N5F1 and H7N6F1 were significantly overexpressed in undifferentiated MSCs while H3N4F1 and H5N4F3 were upregulated in adipogenically differentiated MSCs. These glycan structures are promising candidate markers to detect and distinguish MSCs and their adipogenic progeny.

Han, C., et al. (2014). "Human adipose-derived mesenchymal stem cells: a better cell source for nervous system regeneration." Chin Med J (Engl) **127**(2): 329-337.

BACKGROUND: In order to suggest an ideal source of adult stem cells for the treatment of nervous system diseases, MSCs from human adipose tissue and bone marrow were isolated and studied to explore the differences with regard to cell morphology, surface

markers, neuronal differentiation capacity, especially the synapse structure formation and the secretion of neurotrophic factors. METHODS: The neuronal differentiation capacity of human mesenchymal stem cells from adipose tissue (hADSCs) and bone marrow (hBMSCs) was determined based on nissl body and synapse structure formation, and neural factor secretion function. hADSCs and hBMSCs were isolated and differentiated into neuron-like cells with rat brain-conditioned medium, a potentially rich source of neuronal differentiation promoting signals. Specific neuronal proteins and neural factors were detected by immunohistochemistry and enzyme-linked immunosorbent assay analysis, respectively. RESULTS: Flow cytometric analysis showed that both cell types had similar phenotypes. Cell growth curves showed that hADSCs proliferated more quickly than hBMSCs. Both kinds of cells were capable of osteogenic and adipogenic differentiation. The morphology of hADSCs and hBMSCs changed during neuronal differentiation and displayed neuron-like cell appearance after 14 days' differentiation. Both hADSCs and hBMSCs were able to differentiate into neuron-like cells based on their production of neuron specific proteins including beta-tubulin-III, neuron-specific enolase (NSE), nissl bodies, and their ability to secrete brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Assessment of synaptop hysin and growth-associated protein-43 (GAP-43) suggested synapse structure formation in differentiated hADSCs and hBMSCs. CONCLUSIONS: Our results demonstrate that hADSCs have neuronal differentiation potential similar to hBMSC, but with a higher proliferation capacity than hBMSC. Adipose tissue is abundant, easily available and would be a potential ideal source of adult stem cells for neural-related clinical research and application.

Han, K., et al. (2008). "Human amnion-derived mesenchymal stem cells are a potential source for uterine stem cell therapy." Cell Prolif **41**(5): 709-725.

OBJECTIVES: Human amnion is an easy-to-obtain novel source of human mesenchymal stem cells, which poses little or no ethical dilemmas. We have previously shown that human amnion-derived mesenchymal (HAM) cells exhibit certain mesenchymal stem cell-like characteristics with respect to expression of stem cell markers and differentiation potentials. MATERIALS AND METHODS: In this study, we further characterized HAM cells' potential for in vivo therapeutic application. RESULTS: Flow cytometric analyses of HAM cells show that they express several stem cell-related cell surface markers, including CD90, CD105, CD59, CD49d, CD44 and HLA-ABC, but not CD45, CD34, CD31, CD106 or HLA-DR. HAM cells at the 10th passage showed normal karyotype. More interestingly, the AbdB-like HOXA genes HOXA9, HOXA10 and HOXA11 that are expressed in the mesenchyme of the developing female reproductive tract and pregnant uteri are also expressed in HAM cells, suggesting similarities between these two mesenchymal cell types. Progesterone receptor is also highly expressed in HAM cells and expression of genes or proteins in HAM cells could be manipulated with the aid of lentivirus technology or cell-permeable peptides. To test potentials of HAM cells for in vivo application, we introduced enhanced green fluorescence protein (EGFP)-expressing HAM cells to mice by intrauterine infusion (into uteri) or by intravenous injection (into the circulation). Presence of EGFP-expressing cells within the uterine mesenchyme after intrauterine infusion or in lungs after intravenous injection was noted within 1-4 weeks. CONCLUSIONS: Collectively, these results suggest that HAM cells are a potential source of mesenchymal stem cells with therapeutic potential.

Hao, Q., et al. (2015). "Study of Bone Marrow and Embryonic Stem Cell-Derived Human Mesenchymal Stem Cells for Treatment of Escherichia coli Endotoxin-Induced Acute Lung Injury in Mice." Stem Cells Transl Med **4**(7): 832-840.

UNLABELLED:: Mesenchymal stem cells (MSCs) can be derived from multiple tissue sources. However, the optimal source of MSCs for cell-based therapy for acute lung injury (ALI) is unclear. In the present experiments, we studied bone marrow (BM)-derived and embryonic stem cell-derived human MSC (ES-MSCs) as a therapeutic agent in Escherichia coli endotoxin-induced ALI in mice. We hypothesized that ES-MSCs would be more potent than BM-MSCs owing to its more primitive source of origin. ALI was induced by the intratracheal instillation of endotoxin at 4 mg/kg into 10-12-week-old C57BL/6 mice with or without BM-MSCs, ES-MSCs, or normal human lung fibroblasts as a cellular control. Compared with the endotoxin-injured mice at 48 hours, the administration of ES-MSCs provided results similar to those of BM-MSCs, significantly reducing the influx of white blood cells and neutrophils and decreasing the secretion of the inflammatory cytokines, macrophage inflammatory protein-2 and tumor necrosis factor-alpha, in the injured alveolus. BM-MSCs also reduced extravascular lung water, a measure of pulmonary edema, by 60% and the total protein levels, a measure of lung permeability, by 66%. However, surprisingly, ES-MSCs did not have these protective effects, which was partially explained by the increased secretion of matrix metallopeptidase 9 by ES-MSCs, an enzyme known to increase lung protein permeability. In

conclusion, both BM-MSCs and ES-MSCs markedly decreased endotoxin-induced inflammation. However, ES-MSCs did not show any beneficial effect on reducing pulmonary edema and lung protein permeability compared with BM-MSCs, suggesting that not all MSCs behave in a similar fashion. Our results highlight the need perhaps for a disease-specific potency assay for MSCs. SIGNIFICANCE: To determine the optimal source of mesenchymal stem cells (MSCs) for cell-based therapy for acute lung injury, bone marrow (BM)- and embryonic stem cell-derived human MSC (ES-MSCs) were compared as therapeutic agents for Escherichia coli endotoxin-induced lung injury in mice. ES-MSCs behaved similarly to BM-MSCs by markedly decreasing the inflammatory response induced by endotoxin. However, unlike BM-MSCs, ES-MSCs provided no protective effects against increasing lung water and protein permeability, in part because of an increase in expression of matrix metallopeptidase 9 by ES-MSCs. In patients with acute respiratory distress syndrome, impaired alveolar fluid clearance (i.e., no resolution of pulmonary edema fluid) has been associated with higher mortality rates. Although ES-MSCs might ultimately be found to have properties superior to those of BM-MSCs, such as for immunomodulation, these results highlight the need for a disease-specific potency assay for stem cell-based therapy.

Hara, K., et al. (2011). "Potential characteristics of stem cells from human exfoliated deciduous teeth compared with bone marrow-derived mesenchymal stem cells for mineralized tissue-forming cell biology." J Endod **37**(12): 1647-1652.

INTRODUCTION: Tissue engineering and regenerative medicine using stem cell biology has been a promising field for treatment of local and systemic intractable diseases. Recently, stem cells from human exfoliated deciduous teeth (SHED) have been identified as a novel population of stem cells. This study focused on the characterization of SHED as compared with bone marrow-derived mesenchymal stem cells (BMMSCs). METHODS: We investigated potential characteristics of SHED by using DNA microarray, real-time reverse transcriptase polymerase chain reaction, and immunofluorescence analysis. RESULTS: Multiple gene expression profiles indicated that the expression of 2753 genes in SHED had changed by >/=2.0-fold as compared with that in BMMSCs. One of the most significant pathways that accelerated in SHED was that of bone morphogenetic protein (BMP) receptor signaling, which contains several cascades such as PKA, JNK, and ASK1. When the BMP signaling pathway was stimulated by BMP-2, the expression of BMP-2, BMP-4, Runx2, and DSPP was up-regulated significantly in SHED than that in BMMSCs. Furthermore, the BMP-4 protein was expressed much higher in SHED but not in BMMSCs, as confirmed by immunofluorescence. CONCLUSIONS: By using the gene expression profiles, this study indicates that SHED is involved in the BMP signaling pathway and suggests that BMP-4 might play a crucial role in this. These results might be useful for effective cell-based tissue regeneration, including that of bone, pulp, and dentin, by applying the characteristics of SHED.

Hayashi, N., et al. (2009). "Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells support hematopoietic recovery of X-irradiated human CD34+ cells." Life Sci **84**(17-18): 598-605.

AIMS: The potential of human mesenchymal stem cell-like stroma prepared from placental/umbilical cord blood for hematopoietic regeneration by X-irradiated hematopoietic stem cells is herein assessed. MAIN METHODS: Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells were applied to a regenerative ex vivo expansion of X-irradiated human CD34+ cells in a serum-free liquid culture supplemented with a combination of interleukine-3 plus stem cell factor plus thrombopoietin. KEY FINDINGS: The total number of cells and of lineage-committed myeloid hematopoietic progenitor cells generated in the co-culture of both non-irradiated and X-irradiated cells with stromal cells was significantly higher than those in the stroma-free culture. In addition, the number of CD34+ cells and CD34+/CD38- cells, immature hematopoietic stem/progenitor cells also increased more than the stroma-free culture. The stromal cells produced various types of cytokines, although there was little difference between the co-cultures of non-irradiated and X-irradiated cells with stromal cells. Furthermore, when X-irradiated cells came in contact with stromal cells for 16 h before cytokine stimulation, a similar degree of hematopoiesis was observed, thus suggesting the critical role of cell-to-cell interaction. SIGNIFICANCE: The present results showed the potential efficacy of human mesenchymal stem cell-like stroma for hematopoietic regeneration from irradiated hematopoietic stem/progenitor cells.

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He, W., et al. (2010). "[Effect of ginsenoside Rg1 on the microenvironment dependent differentiation of human bone marrow mesenchymal stem cell to vaso-endothelioid formative cells in vitro]." Zhongguo Zhong Xi Yi Jie He Za Zhi **30**(11): 1201-1205.

OBJECTIVE: To investigate the effect of ginsenoside Rg1 on the microenvironment dependent differentiation of human mesenchymal stem cells (hMSCs) to vaso-endothelioid cells (VECs) in vitro. METHODS: The in vitro differentiation of hMSCs to VECs were established adopting the in vivo environment simulated semi-permeable membrane separated non-contact co-culturing method. The mRNA expressions of endothelial markers, such as platelet endothelial adhesive factor-1 (CD31), vascular hemophillia factor (vWF) and vascular endothelial cadherin (VE-cadherin) were analyzed by RT-PCR; the protein expressions of CD31 and vascular endothelial adhesive factor-1 (VCAM1) were detected by fluorescence immunohistochemistry; structural identification for the endothelial characteristics of differentiated hMSCs were made under electron microscopy; and the percentage of CD31 expression in differentiated hMSCs was determined by flow cytometry to explore the effect of ginsenoside Rg1 on the differentiation. RESULTS: The bone marrow mesenchymal stem cells co-cultured with mature endothelial membrane showed a microenvironment dependent capacity for differentiating to endothelium, with the morphological changes revealed starting from the 2nd week, showing cell body contraction, polygonal-shaped change; and at the 3rd week, the markedly speedily cell proliferation with elliptic or slabstone-like change of cells. High levels of classic endothelial cell markers, such as mRNA expressions of CD31, vWF, VE-cadherin, and protein expressions of CD31 and VCAM1, were shown; the typical weibel-palade body of endothelial cell was found in the differentiated cells. Moreover, percentage of CD31 expression in the differentiated hMSCs was increased after Rg1 treatment dose-dependently. CONCLUSION: Under the microenvironment of co-culture, hMSCs could differentiate into cells presenting the characteristics of endothelial cell in aspects of the morphology and ultrastructure of cells, as well as the gene and protein expressions of cell markers; ginsenoside Rg1 can promote the microenvironment dependent differentiation of hMSCs to VECs system in vitro.

Hematti, P. (2011). "Human embryonic stem cell-derived mesenchymal stromal cells." Transfusion **51 Suppl 4**: 138S-144S.

Mesenchymal stromal cells (MSCs) originally isolated from marrow have multipotent differentiation potential and favorable immunomodulatory and anti-inflammatory properties that make them very attractive for regenerative cellular therapy. Cells with similar phenotypic characteristics have now been derived from almost all fetal, neonatal, and adult tissues; furthermore, more recently similar cells have also been generated from human embryonic stem cells (ESCs). Generation of MSCs from human ESCs provides an opportunity to study the developmental biology of human mesenchymal lineage generation in vitro. Generation of bone and cartilage from human ESC-derived MSCs and their functional characterization, both in vitro and in vivo, is also an active area of investigation as ESCs could provide an unlimited source of MSCs for potential repair of bone and cartilage defects. MSCs from adult sources are being investigated in numerous Phase I-III clinical trials for a wide variety of indications, mainly based on their immunomodulatory properties. Our group and others have shown MSCs derived from human ESCs possess immunomodulatory properties similar to marrow-derived MSCs. Immunomodulatory properties of ESC-derived MSCs could prove to be highly valuable for their potential clinical applications in the future as derivatives of human ESCs have already entered clinical arena in the context of Phase I clinical trials.

Hendijani, F., et al. (2015). "Effect of human Wharton's jelly mesenchymal stem cell secretome on proliferation, apoptosis and drug resistance of lung cancer cells." Res Pharm Sci **10**(2): 134-142.

Multipotent mesenchymal stem cells (MSCs) are recently found to alter the tumor condition. However their exact role in tumor development is not yet fully unraveled. MSCs were established to perform many of their actions through paracrine effect. Thus investigation of MSC secretome interaction with tumor cells may provide important information for scientists who are attempting to apply stem cells in the treatment of the disease. In this study we investigated the effect of human Wharton's jelly derived MSC (WJ-MSCs) secretome on proliferation, apoptotic potential of A549 lung cancer cells, and their response to the chemotherapeutic agent doxorubicin. WJ-MSCs were isolated from human umbilical cord and then characterized according to the International Society for Cellular Therapy criteria and WJ-MSC secretome was collected. BrdU cell proliferation assay and Annexin V-PI staining were used for the evaluation of cytotoxic and proapoptotic effects of WJ-MSC secretome on A549 cells. WJ-MSC secretome neither induced proliferation of lung cancer cells nor affected the apoptotic potential of the tumor cells. We also studied the combinatorial effect of WJ-MSC secretome and the anticancer drug doxorubicinwhich showed no induction of drug resistance when A549 cells was treated with combination of WJ-MSC secretome and doxorubicin. Although MSCs did not show antitumor properties, our in vitro results showed that MSC secretome was not tumorigenic and also did not make lung cancer cells resistant to doxorubicin. Thus MSC secretome could be considered safe for other medical purposes such as cardiovascular, neurodegenerative, and autoimmune diseases which may exist or occur in cancer patients.

Henrich, D., et al. (2013). "Human endothelial-like differentiated precursor cells maintain their endothelial characteristics when cocultured with mesenchymal stem cell and seeded onto human cancellous bone." Mediators Inflamm **2013**: 364591.

INTRODUCTION: Cancellous bone is frequently used for filling bone defects in a clinical setting. It provides favourable conditions for regenerative cells such as MSC and early EPC. The combination of MSC and EPC results in superior bone healing in experimental bone healing models. MATERIALS AND METHODS: We investigated the influence of osteogenic culture conditions on the endothelial properties of early EPC and the osteogenic properties of MSC when cocultured on cancellous bone. Additionally, cell adhesion, metabolic activity, and differentiation were assessed 2, 6, and 10 days after seeding. RESULTS: The number of adhering EPC and MSC decreased over time; however the cells remained metabolically active over the 10-day measurement period. In spite of a decline of lineage specific markers, cells maintained their differentiation to a reduced level. Osteogenic stimulation of EPC caused a decline but not abolishment of endothelial characteristics and did not induce osteogenic gene expression. Osteogenic stimulation of MSC significantly increased their metabolic activity whereas collagen-1alpha and alkaline phosphatase gene expressions declined. When cocultured with EPC, MSC's collagen-1alpha gene expression increased significantly. CONCLUSION: EPC and MSC can be cocultured in vitro on cancellous bone under osteogenic conditions, and coculturing EPC with MSC stabilizes the latter's collagen-1alpha gene expression.

Hill, A. J., et al. (2009). "Human umbilical cord blood-derived mesenchymal stem cells do not differentiate into neural cell types or integrate into the retina after intravitreal grafting in neonatal rats." Stem Cells Dev **18**(3): 399-409.

This study investigated the ability of mesenchymal stem cells (MSCs) derived from full-term human umbilical cord blood to survive, integrate and differentiate after intravitreal grafting to the degenerating neonatal rat retina following intracranial optic tract lesion. MSCs survived for 1 week in the absence of immunosuppression. When host animals were treated with cyclosporin A and dexamethasone to suppress inflammatory and immune responses, donor cells survived for at least 3 weeks, and were able to spread and cover the entire vitreal surface of the host retina. However, MSCs did not significantly integrate into or migrate through the retina. They also maintained their human antigenicity, and no indication of neural differentiation was observed in retinas where retinal ganglion cells either underwent severe degeneration or were lost. These results have provided the first in vivo evidence that MSCs derived from human umbilical cord blood can survive for a significant period of time when the host rat response is suppressed even for a short period. These results, together with the observation of a lack of neuronal differentiation and integration of MSCs after intravitreal grafting, has raised an important question as to the potential use of MSCs for neural repair through the replacement of lost neurons in the mammalian retina and central nervous system.

Ho, J. H., et al. (2011). "Cell contact accelerates replicative senescence of human mesenchymal stem cells independent of telomere shortening and p53 activation: roles of Ras and oxidative stress." Cell Transplant **20**(8): 1209-1220.

Mesenchymal stem cells (MSCs) are of great therapeutic potentials due to their multilineage differentiation capabilities. Before transplantation, in vitro culture expansion of MSCs is necessary to get desired cell number. We observed that cell contact accelerated replicative senescence during such process. To confirm the finding as well as to investigate the underlying mechanisms, we cultured both human bone marrow- and umbilical cord blood-derived MSCs under noncontact culture (subculture performed at 60-70% of confluence), or contact culture (cell passage performed at 100% of confluence). It was found that MSCs reached cellular senescence earlier in contact culture, and the doubling time was significantly prolonged. Marked increase of senescence-associated beta-galactosidase-positive staining was also observed as a result of cell contact. Cell cycle analysis revealed increased frequency of cell cycle arrest after contact culture. It was noted, however, that the telomere length was not altered during contact-induced acceleration of senescence. Moreover, cell cycle checkpoint regulator P53 expression was not affected by cell contact. Marked increase in intracellular reactive oxygen species (ROS) and a concomitant decrease in the activities of antioxidative enzymes were also observed during contact-induced senescence. Importantly, increased p16(INK4a) following Ras upregulation was found after contact culture. Taken together, cell contact induced accelerated senescence of MSCs, which is telomere shortening and p53 independent. ROS accumulation due to defective ROS clearance function together with Ras and p16(INK4a) upregulation play an important role in contact-induced senescence of MSCs. Overconfluence should therefore be avoided during in vitro culture expansion of MSCs in order to maintain their qualities for clinical application purposes. The contact-induced senescence model reported in this study will serve as a useful model system that allows further study of the molecular mechanisms of senescence in MSCs.

Ho, J. H., et al. (2010). "Thymosin beta-4 directs cell fate determination of human mesenchymal stem cells through biophysical effects." J Orthop Res **28**(1): 131-138.

Change of actin filament organization at the early stage of cell differentiation directs cell fate commitment of mesenchymal stem cells (MSCs). Thymosin beta-4 (Tbeta (4)), a major G-actin sequestering peptide, is known to regulate the cytoskeleton. The study investigated the ways in which Tbeta (4) regulates cell fate determination in MSCs upon differentiation induction. It was found that Tbeta (4) decreased F-actin formation, reduced the F-actin/G-actin ratio, and inhibited osteogenic differentiation; such actin reorganization was not associated with the change of Runt-related transcription factor 2 gene expression during early osteogenic induction. Besides, Tbeta (4) reciprocally facilitated adipogenic differentiation. Tbeta (4) treatment was found to up-regulate gene as well as promote surface expression of adipocyte adhesion molecule during early adipogenic differentiation, which accompanied acceleration of adipocyte phenotypic maturation but was not associated with differential expression of peroxisome proliferator-activated receptor gamma during the first week of adipogenic induction. In summary, Tbeta (4) initiated cell fate determination of MSCs through biophysical effects exerted by cytoskeleton reorganization and altered cell-cell adhesion rather than direct regulation of lineage-determining transcriptional factors. Such findings suggest that Tbeta (4), a ubiquitous peptide, may be involved in osteoporosis when its intracellular concentration is elevated. Further investigation of targeting Tbeta (4) for future osteoporosis treatment is warranted.

Ho, Y. C., et al. (2006). "Baculovirus transduction of human mesenchymal stem cell-derived progenitor cells: variation of transgene expression with cellular differentiation states." Gene Ther **13**(20): 1471-1479.

We have previously demonstrated that baculovirus can efficiently transduce human mesenchymal stem cells (MSCs). In this study, we further demonstrated, for the first time, that baculovirus can transduce adipogenic, chondrogenic and osteogenic progenitors originating from MSCs. The transduction efficiency (21-90%), transgene expression level and duration (7-41 days) varied widely with the differentiation lineages and stages of the progenitors, as determined by flow cytometry. The variation stemmed from differential transgene transcription (as revealed by real-time reverse transcription-polymerase chain reaction), rather than from variability in virus entry or cell cycle (as determined by quantitative real-time PCR and flow cytometry). Nonetheless, the baculovirus-transduced cells remained capable of differentiating into adipogenic, osteogenic and chondrogenic pathways. The susceptibility to baculovirus transduction was higher for adipogenic and osteogenic progenitors, but was lower for chondrogenic progenitors. In particular, the duration of transgene expression was prolonged in the transduced adipogenic and osteogenic progenitors (as opposed to the MSCs), implicating the possibility of extending transgene expression via a proper transduction strategy design. Taken together, baculovirus may be an attractive alternative to genetically modify adipogenic and osteogenic

progenitors in the ex vivo setting for cell therapy or tissue engineering.

Horita, Y., et al. (2006). "Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat." J Neurosci Res **84**(7): 1495-1504.

Intravenous administration of human mesenchymal stem cells (hMSCs) prepared from adult bone marrow has been reported to ameliorate functional deficits after cerebral artery occlusion in rats. Several hypotheses to account for these therapeutic effects have been suggested, and current thinking is that neuroprotection rather than neurogenesis is responsible. To enhance the therapeutic benefits of hMSCs potentially, we transfected hMSCs with the glial cell line-derived neurotrophic factor (GDNF) gene using a fiber-mutant F/RGD adenovirus vector and investigated whether GDNF gene-modified hMSCs (GDNF-hMSCs) could contribute to functional recovery in a rat permanent middle cerebral artery occlusion (MCAO) model. We induced MCAO by using intraluminal vascular occlusion, and GDNF-hMSCs were intravenously infused into the rats 3 hr later. MRI and behavioral analyses revealed that rats receiving GDNF-hMSCs or hMSCs exhibited increased recovery from ischemia compared with the control group, but the effect was greater in the GDNF-hMSC group. Thus, these results suggest that intravenous administration of hMSCs transfected with the GDNF gene using a fiber-mutant adenovirus vector may be useful in the cerebral ischemia and may represent a new strategy for the treatment of stroke.

Hsieh, J. Y., et al. (2013). "miR-146a-5p circuitry uncouples cell proliferation and migration, but not differentiation, in human mesenchymal stem cells." Nucleic Acids Res **41**(21): 9753-9763.

Administration of mesenchymal stem cells (MSCs) has the potential to ameliorate degenerative disorders and to repair damaged tissues. The homing of transplanted MSCs to injured sites is a critical property of engraftment. Our aim was to identify microRNAs involved in controlling MSC proliferation and migration. MSCs can be isolated from bone marrow and umbilical cord Wharton's jelly (BM-MSCs and WJ-MSCs, respectively), and WJ-MSCs show poorer motility yet have a better amplification rate compared with BM-MSCs. Small RNA sequencing revealed that miR-146a-5p is significantly overexpressed and has high abundance in WJ-MSCs. Knockdown of miR-146a-5p in WJ-MSCs inhibited their proliferation yet enhanced their migration, whereas overexpression of miR-146a-5p in BM-MSCs did not influence their osteogenic and adipogenic potentials. Chemokine (C-X-C motif) ligand 12 (CXCL12), together with SIKE1, which is an I-kappa-B kinase epsilon (IKKepsilon) suppressor, is a direct target of miR-146a-5p in MSCs. Knockdown of miR-146a-5p resulted in the down-regulation of nuclear factor kappa-B (NF-kappaB) activity, which is highly activated in WJ-MSCs and is known to activate miR-146a-5p promoter. miR-146a-5p is also downstream of CXCL12, and a negative feedback loop is therefore formed in MSCs. These findings suggest that miR-146a-5p is critical to the uncoupling of motility and proliferation of MSCs. Our miRNome data also provide a roadmap for further understanding MSC biology.

Hu, G. W., et al. (2015). "Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells attenuate limb ischemia by promoting angiogenesis in mice." Stem Cell Res Ther **6**: 10.

INTRODUCTION: 'Patient-specific' induced pluripotent stem cells (iPSCs) are attractive because they can generate abundant cells without the risk of immune rejection for cell therapy. Studies have shown that iPSC-derived mesenchymal stem cells (iMSCs) possess powerful proliferation, differentiation, and therapeutic effects. Recently, most studies indicate that stem cells exert their therapeutic effect mainly through a paracrine mechanism other than transdifferentiation, and exosomes have emerged as an important paracrine factor for stem cells to reprogram injured cells. The objective of this study was to evaluate whether exosomes derived from iMSCs (iMSCs-Exo) possess the ability to attenuate limb ischemia and promote angiogenesis after transplantation into limbs of mice with femoral artery excision. METHODS: Human iPSCs (iPS-S-01, C1P33, and PCKDSF001C1) were used to differentiate into iMSCs in a modified one-step method. iMSCs were characterized by flow cytometry and multipotent differentiation potential analysis. Ultrafiltration combined with a purification method was used to isolate iMSCs-Exo, and transmission electron microscopy and Western blotting were used to identify iMSCs-Exo. After establishment of mouse hind-limb ischemia with excision of femoral artery and iMSCs-Exo injection, blood perfusion was monitored at days 0, 7, 14, and 21; microvessel density in ischemic muscle was also analyzed. In vitro migration, proliferation, and tube formation experiments were used to analyze the ability of pro-angiogenesis in iMSCs-Exo, and quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay were used to identify expression levels of angiogenesis-related molecules in human umbilical vein endothelial cells (HUVECs) after being

cultured with iMSCs-Exo. RESULTS: iPSCs were efficiently induced into iMSC- with MSC-positive and -negative surface antigens and osteogenesis, adipogenesis, and chondrogenesis differentiation potential. iMSCs-Exo with a diameter of 57 +/- 11 nm and expressed CD63, CD81, and CD9. Intramuscular injection of iMSCs-Exo markedly enhanced microvessel density and blood perfusion in mouse ischemic limbs, consistent with an attenuation of ischemic injury. In addition, iMSCs-Exo could activate angiogenesis-related molecule expression and promote HUVEC migration, proliferation, and tube formation. CONCLUSION: Implanted iMSCs-Exo was able to protect limbs from ischemic injury via the promotion of angiogenesis, which indicated that iMSCs-Exo may be a novel therapeutic approach in the treatment of ischemic diseases.

Hu, J., et al. (2010). "Response of human embryonic stem cell-derived mesenchymal stem cells to osteogenic factors and architectures of materials during in vitro osteogenesis." Tissue Eng Part A **16**(11): 3507-3514.

One of the major challenges to the application of human embryonic stem cells (hESCs) to the repair of defective tissues is the directed differentiation of cells into specific lineages to avoid the formation of inferior heterogeneous tissues. To accomplish this goal, the lineage-specific stem cell population needs to be isolated and optimal differentiation conditions need to be defined. In this study, homogenous hESC-derived mesenchymal stem cells (hESC-MSCs) were generated and used to construct bone tissue. The effect of osteogenic factors, including dexamethasone (Dex) and bone morphogenetic protein-7 (BMP-7), on the osteogenesis of hESC-MSCs was investigated. It was found that BMP-7 itself had little effect on the in vitro osteogenic differentiation of hESC-MSCs; however, there was a synergic effect between BMP-7 and Dex in promoting osteogenesis. The effect of osteoconductive nanofibrous polylactic acid material on osteogenesis of hESC-MSCs was also investigated. It was found that the nanofibrous matrix architecture promoted alkaline phosphatase activity and calcium deposition of cells cultured under osteogenic conditions. Based on these findings, the hESC-MSCs were cultured on three-dimensional nanofibrous scaffolds in combination with Dex and BMP-7 stimulation in vitro to generate bone-like tissues. After 6 weeks of culture, highly mineralized tissues developed with specific bone marker genes expressed. These data illustrate the promise of hESC-MSCs for bone regeneration under optimal conditions.

Hu, S., et al. (2018). "Mesenchymal Stem Cell Microvesicles Restore Protein Permeability Across Primary Cultures of Injured Human Lung Microvascular Endothelial Cells." Stem Cells Transl Med **7**(8): 615-624.

Our previous study demonstrated that mesenchymal stem cell (MSC) microvesicles (MV) reduced lung inflammation, protein permeability, and pulmonary edema in endotoxin-induced acute lung injury in mice. However, the underlying mechanisms for restoring lung protein permeability were not fully understood. In this current study, we hypothesized that MSC MV would restore protein permeability across injured human lung microvascular endothelial cells (HLMVEC) in part through the transfer of angiopoietin-1 (Ang1) mRNA to the injured endothelium. A transwell coculture system was used to study the effect of MSC MV on protein permeability across HLMVECs injured by cytomix, a mixture of IL-1beta, TNF-alpha, and IFN-gamma (50 ng/ml). Our result showed that cytomix significantly increased permeability to FITC-dextran (70 kDa) across HLMVECs over 24 hours. Administration of MSC MVs restored this permeability in a dose dependent manner, which was associated with an increase in Ang1 mRNA and protein secretion in the injured endothelium. This beneficial effect was diminished when MSC MV was pretreated with an anti-CD44 antibody, suggesting that internalization of MV into the HLMVEC was required for the therapeutic effect. Fluorescent microscopy showed that MSC MV largely prevented the reorganization of cytoskeleton protein F-actin into "actin stress fiber" and restored the location of the tight junction protein ZO-1 and adherens junction protein VE-cadherin in injured HLMVECs. Ang1 siRNA pretreatment of MSC MV prior to administration to injured HLMVECs eliminated the therapeutic effect of MV. In summary, MSC MVs restored protein permeability across HLMVEC in part by increasing Ang1 secretion by injured HLMVEC. Stem Cells Translational Medicine 2018;7:615-624.

Hu, W., et al. (2011). "Human umbilical blood mononuclear cell-derived mesenchymal stem cells serve as interleukin-21 gene delivery vehicles for epithelial ovarian cancer therapy in nude mice." Biotechnol Appl Biochem **58**(6): 397-404.

Ovarian cancer causes more deaths than any other cancer of the female reproductive system, and its overall cure rate remains low. The present study investigated human umbilical blood mononuclear cell (UBMC)-derived mesenchymal stem cells (UBMC-MSCs) as interleukin-21 (IL-21) gene delivery vehicles for ovarian cancer therapy in nude mice. MSCs were isolated from UBMCs and the expanded cells were phenotyped by flow cytometry. Cultured UBMCs were differentiated into osteocytes and adipocytes using appropriate media and then the

UBMC-MSCs were transfected with recombinant pIRES2-IL-21-enhancement green fluorescent protein. UBMC-MSCs expressing IL-21 were named as UBMC-MSC-IL-21. Mice with A2780 ovarian cancer were treated with UBMC-MSC-IL-21 intravenously, and the therapeutic efficacy was evaluated by the tumor volume and mouse survival. To address the mechanism of UBMC-MSC-IL-21 against ovarian cancer, the expression of IL-21, natural killer glucoprotein 2 domain and major histocompatibility complex class I chain-related molecules A/B were detected in UBMC-MSC-IL-21 and in the tumor sites. Interferon-gamma-secreting splenocyte numbers and natural killer cytotoxicity were significantly increased in the UBMC-MSC-IL-21-treated mice as compared with the UBMC-MSCs or the UBMC-MSC-mock plasmid-treated mice. Most notably, tumor growth was delayed and survival was prolonged in ovarian-cancer-bearing mice treated with UBMC-MSC-IL-21. Our data provide important evidence that UBMC-MSCs can serve as vehicles for IL-21 gene delivery and inhibit the established tumor.

Hu, X., et al. (2014). "Severe hypoxia exerts parallel and cell-specific regulation of gene expression and alternative splicing in human mesenchymal stem cells." BMC Genomics **15**: 303.

BACKGROUND: The endosteum of the bone marrow provides a specialized hypoxic niche that may serve to preserve the integrity, pluripotency, longevity and stemness of resident mesenchymal stem cells (MSCs). To explore the molecular genetic consequences of such a niche we subjected human (h) MSCs to a pO2 of 4 mmHg and analyzed global gene expression and alternative splicing (AS) by genome-exon microarray and RT-qPCR, and phenotype by western blot and immunostaining. RESULTS: Out of 446 genes differentially regulated by >2.5-fold, down-regulated genes outnumbered up-regulated genes by 243:203. Exon analyses revealed 60 hypoxia-regulated AS events with splice indices (SI) >1.0 from 53 genes and a correlation between high SI and degree of transcript regulation. Parallel analyses of a publicly available AS study on human umbilical vein endothelial cells (HUVECs) showed that there was a strong cell-specific component with only 11 genes commonly regulated in hMSCs and HUVECs and 17 common differentially spliced genes. Only 3 genes were differentially responsive to hypoxia at the gene (>2.0) and AS levels in both cell types. Functional assignments revealed unique profiles of gene expression with complex regulation of differentiation, extracellular matrix, intermediate filament and metabolic marker genes. Antioxidant genes, striated muscle genes and insulin/IGF-1 signaling intermediates were down-regulated. There was a coordinate induction of 9 out of 12 acidic keratins that along with other epithelial and cell adhesion markers implies a partial mesenchymal to epithelial transition. CONCLUSIONS: We conclude that severe hypoxia confers a quiescent phenotype in hMSCs that is reflected by both the transcriptome profile and gene-specific changes of splicosome actions. The results reveal that severe hypoxia imposes markedly different patterns of gene regulation of MSCs compared with more moderate hypoxia. This is the first study to report hypoxia-regulation of AS in stem/progenitor cells and the first molecular genetic characterization of MSC in a hypoxia-induced quiescent immobile state.

Huang, A. H., et al. (2010). "[Culture and characterization of and lentiviral vectors mediated glial cell derived neurotrophic factor expression in mesenchymal stem cells from human umbilical cord blood]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao **32**(1): 39-45.

OBJECTIVE: To isolate and culture mesenchymal stem cells from umbilical cord blood (UCB-MSCs), study its biological characterization in vitro, transfect UCB-MSCs using lentiviral vectors encoding glial cell derived neurotrophic factor (GDNF) gene, evaluate the biological function change of UCB-MSCs, and detect GDNF expression level in vitro. METHODS: We isolated monocyte by Ficoll density gradient, separated two kinds of adherent cells through different trypsin digestion time, and detected the cells surface markers by fluorescence activated cell sorting when it was proliferated for P7 passages. At the same time, we sub-cloned GDNF gene into lentiviral vectors and packaged lentiviral supernatant through three plasmids co-transfection method, then transfected the UCB-MSCs using lentiviral vectors encoding GDNF at different multiplicity of infection, and evaluated the change of biological function by observing the ability of proliferation and differentiation, morphology, and the cells surface markers. We detected the GDNF mRNA and protein expression level by using real-time polymerase chain reaction (real-time PCR) and enzyme-link immunosorbent assay (ELISA). RESULTS: The UCB-MSCs were successfully isolated and cultured in vitro, and induced it to differentiate into fat cells. FACS results showed that the UCB-MSCs expressed CD90, CD73, and CD105 positively, and CD14, CD34, CD45, CD19, HLA-DR, Stro-1, and CD106 negatively. Real-time PCR and ELISA showed that the expressions of GDNF protein and mRNA were correlated with the copy number of transfected cells: high copy number of transfected cells were associated with high GDNF expression. The biological characterization of UCB-MSCs did not obviously change after sub-cloning with GDNF. CONCLUSIONS: UCB-MSCs was successfully

isolated and cultured in vitro. By transfecting UCB-MSCs with GDNF gene-containing lentiviral vectors, the secretion of GDNF protein and mRNA expression level can be controlled by the copy number of transfected cells, and thus make it constantly express GDNF at high level.

Huang, B., et al. (2013). "Generation of human epidermis-derived mesenchymal stem cell-like pluripotent cells (hEMSCPCs)." Sci Rep **3**: 1933.

We isolated human epidermis-derived mesenchymal stem cell-like pluripotent cells (hEMSCPCs) and demonstrate efficient harvesting, maintenance in vitro for at least 30 passages, reprogramming into multiple phenotypes in vivo, and integration into adult host tissues after injection into the mouse blastocyst to create chimeras. Cell phenotype was examined by karyotyping, immunostaining, immunofluorescence, and flow cytometry. A nested PCR protocol using primers specific for human SRY genes was designed to detect hEMSCPC-derived cells in female chimeric mice. FISH was used to validate the results of nested PCR. Results indicated that hEMSCPCs were derived from epidermis but were distinct from epidermal cells; they resembled mesenchymal stem cells (MSCs) morphologically and expressed the main markers of MSCs. About half of all female offspring of mice implanted with embryos injected with hEMSCPCs at the blastocyst stage harbored the human Y chromosome and tissue-specific human protein, thereby demonstrating the transdifferentiation of hEMSCPCs.

Huang, D. M., et al. (2005). "Highly efficient cellular labeling of mesoporous nanoparticles in human mesenchymal stem cells: implication for stem cell tracking." FASEB J **19**(14): 2014-2016.

Tracking the distribution of stem cells is crucial to their therapeutic use. However, the usage of current vectors in cellular labeling is restricted by their low internalizing efficiency. Here, we reported a cellular labeling approach with a novel vector composed of mesoporous silica nanoparticles (MSNs) conjugated with fluorescein isothiocyanate in human bone marrow mesenchymal stem cells and 3T3-L1 cells, and the mechanism about fluorescein isothiocyanate-conjugated MSNs (FITC-MSNs) internalization was studied. FITC-MSNs were efficiently internalized into mesenchymal stem cells and 3T3-L1 cells even in short-term incubation. The process displayed a time- and concentration-dependent manner and was dependent on clathrin-mediated endocytosis. In addition, clathrin-dependent endocytosis seemed to play a decisive role on more internalization and longer stay of FITC-MSNs in mesenchymal stem cells than in 3T3-L1 cells. The internalization of FITC-MSNs did not affect the cell viability, proliferation, immunophenotype, and differentiation potential of mesenchymal stem cells, and 3T3-L1 cells. Finally, FITC-MSNs could escape from endolysosomal vesicles and were retained the architectonic integrity after internalization. We conclude that the advantages of biocompatibility, durability, and higher efficiency in internalization suit MSNs to be a better vector for stem cell tracking than others currently used.

Huang, W., et al. (2006). "Interleukin-17A: a T-cell-derived growth factor for murine and human mesenchymal stem cells." Stem Cells **24**(6): 1512-1518.

Interleukin-17A (IL-17A) is a proinflammatory cytokine expressed in activated T-cells. It is required for microbial host defense and is a potent stimulator of granulopoiesis. In a dose-dependent fashion, IL-17A expanded human mesenchymal stem cells (MSCs) and induced the proliferation of mature stroma cells in bone marrow-derived stroma cultures. Recombinant human interleukin-17A (rhIL-17A) nearly doubled colony-forming unit-fibroblast (CFU-f) frequency and almost tripled the surface area covered by stroma. In a murine transplant model, in vivo murine (m)IL-17A expression enhanced CFU-f by 2.5-fold. Enrichment of the graft with CD4(+) T-cell resulted in a 7.5-fold increase in CFU-f in normal C57BL/6, but only threefold in IL-17Ra (-/-) mice on day 14 post-transplant. In this transplant model, in vivo blockade of IL-17A in C57BL/6 mice resembled the phenotype of IL-17Ra (-/-) mice. Approximately half of the T-cell-mediated effect on MSC recovery following radiation-conditioned transplantation was attributed to the IL-17A/IL-17Ra pathway. Pluripotent MSCs have the potential of regenerating various tissues, and mature stroma cells are critical elements of the hematopoietic microenvironment (HME). The HME is pivotal for formation and maintenance of functional blood cells. As a newly identified stroma cell growth factor, IL-17A might have potential applications for novel treatment approaches involving MSCs, such as tissue graft engineering.

Huang, Y., et al. (2016). "Effects of Human Umbilical Cord Mesenchymal Stem Cells on Human Trophoblast Cell Functions In Vitro." Stem Cells Int **2016**: 9156731.

Trophoblast cell dysfunction is involved in many disorders during pregnancy such as preeclampsia and intrauterine growth restriction. Few treatments exist, however, that target improving trophoblast cell function. Human umbilical cord mesenchymal stem cells (hUCMSCs) are capable of self-renewing, can undergo multilineage differentiation, and have homing

abilities; in addition, they have immunomodulatory effects and paracrine properties and thus are a prospective source for cell therapy. To identify whether hUCMSCs can regulate trophoblast cell functions, we treated trophoblast cells with hUCMSC supernatant or cocultured them with hUCMSCs. Both treatments remarkably enhanced the migration and invasion abilities of trophoblast cells and upregulated their proliferation ability. At a certain concentration, hUCMSCs also modulated hCG, PIGF, and sEndoglin levels in the trophoblast culture medium. Thus, hUCMSCs have a positive effect on trophoblast cellular functions, which may provide a new avenue for treatment of placenta-related diseases during pregnancy.

Hui, T. Y., et al. (2008). "In vitro chondrogenic differentiation of human mesenchymal stem cells in collagen microspheres: influence of cell seeding density and collagen concentration." Biomaterials **29**(22): 3201-3212.

Given the inadequacies of existing repair strategies for cartilage injuries, tissue engineering approach using biomaterials and stem cells offers new hope for better treatments. Recently, we have fabricated injectable collagen-human mesenchymal stem cell (hMSC) microspheres using microencapsulation. Apart from providing a protective matrix for cell delivery, the collagen microspheres may also act as a bio-mimetic matrix facilitating the functional remodeling of hMSCs. In this study, whether the encapsulated hMSCs can be pre-differentiated into chondrogenic phenotype prior to implantation has been investigated. The effects of cell seeding density and collagen concentration on the chondrogenic differentiation potential of hMSCs have been studied. An in vivo implantation study has also been conducted. Fabrication of cartilage-like tissue micro-masses was demonstrated by positive immunohistochemical staining for cartilage-specific extracellular matrix components including type II collagen and aggrecan. The meshwork of collagen fibers was remodeled into a highly ordered microstructure, characterized by thick and parallel bundles, upon differentiation. Higher cell seeding density and higher collagen concentration favored the chondrogenic differentiation of hMSCs, yielding increased matrix production and mechanical strength of the micro-masses. These micro-masses were also demonstrated to integrate well with the host tissue in NOD/SCID mice.

Hwang, N. S., et al. (2008). "In vivo commitment and functional tissue regeneration using human embryonic stem cell-derived mesenchymal cells." Proc Natl Acad Sci U S A **105**(52): 20641-20646.

Development of clinically relevant regenerative medicine therapies using human embryonic stem cells (hESCs) requires production of a simple and readily expandable cell population that can be directed to form functional 3D tissue in an in vivo environment. We describe an efficient derivation method and characterization of mesenchymal stem cells (MSCs) from hESCs (hESCd-MSCs) that have multilineage differentiation potential and are capable of producing fat, cartilage, and bone in vitro. Furthermore, we highlight their in vivo survival and commitment to the chondrogenic lineage in a microenvironment comprising chondrocyte-secreted morphogenetic factors and hydrogels. Normal cartilage architecture was established in rat osteochondral defects after treatment with chondrogenically-committed hESCd-MSCs. In view of the limited available cell sources for tissue engineering applications, these embryonic-derived cells show significant potential in musculoskeletal tissue regeneration applications.

Imamura, H., et al. (2018). "An engineered cell sheet composed of human islets and human fibroblast, bone marrow-derived mesenchymal stem cells, or adipose-derived mesenchymal stem cells: An in vitro comparison study." Islets **10**(3): e1445948.

BACKGROUND: We previously reported the utility of engineered cell sheets composed of human islets and supporting cells in vitro and in vivo. It is unclear which type of supporting cell is most suitable for constructing cell sheets with human islets. The present study aimed to compare human fibroblasts, bone marrow-derived mesenchymal stem cells (BM-MSCs), and adipose-derived mesenchymal stem cells (ADSCs) as a supporting source for cell sheets. METHODS: Engineered cell sheets were fabricated with human islets using human fibroblasts, BM-MSCs, or ADSCs as supporting cells. The islet viability, recovery rate, glucose-stimulated insulin release (determined by the stimulation index), and cytokine secretion (TGF-beta1, IL-6, and VEGF) of groups-including an islet-alone group as a control-were compared. RESULTS: All three sheet groups consistently exhibited higher viability, recovery rate, and stimulation index values than the islet-alone group. The ADSC group showed the highest viability and recovery rate among the three sheet groups. There were no discernible differences in the stimulation index values of the groups. The fibroblast group exhibited significantly higher TGF-beta1 values in comparison to the other groups. The IL-6 level of the ADSC group was more than five times higher than that of the other groups. The ADSC group showed the VEGF level; however, it did not differ from that of the BM-MSC group to a statistically significant extent. CONCLUSION: Engineered cell sheets composed of

islets and supporting cells had a cytoprotective effect on islets. These results suggest that individual cell types could be a more attractive source for crafting engineered cell sheets in comparison to islets alone.

Ishii, T., et al. (2010). "In vitro hepatic maturation of human embryonic stem cells by using a mesenchymal cell line derived from murine fetal livers." Cell Tissue Res **339**(3): 505-512.

Hepatocytes derived from human embryonic stem cells (hESCs) are an attractive cell source for regenerative medicine. We previously reported the differentiation of hESCs into alpha-fetoprotein (AFP)-producing endodermal cells by using extracellular matrix and growth factors. We also reported the establishment of the MLSgt20 cell line, which was derived from mesenchymal cells residing in murine fetal livers and accelerated the hepatic maturation of both murine hepatic progenitor cells and murine ESCs. In this study, hESC-derived AFP-producing cells were isolated by using a flow cytometer and co-cultured with MLSgt20 cells. The co-cultured hESC-derived AFP-producing cells had the immunocytological characteristics of hepatocytes, expressed mature hepatocyte markers (as indicated by reverse transcription and the polymerase chain reaction), and displayed higher hepatocyte functions including ammonia removal, cytochrome P450 3A4/7 activity, and the ability to produce and store glycogen. However, the MLSgt20 cells did not directly cause undifferentiated hESCs to mature into hepatocyte-like cells. The co-culture method was thus successfully shown to induce the differentiation of hESC-derived endodermal cells into functional hepatocyte-like cells.

Ishimine, H., et al. (2013). "N-Cadherin is a prospective cell surface marker of human mesenchymal stem cells that have high ability for cardiomyocyte differentiation." Biochem Biophys Res Commun **438**(4): 753-759.

Mesenchymal stem cells (MSCs) are among the most promising sources of stem cells for regenerative medicine. However, the range of their differentiation ability is very limited. In this study, we explored prospective cell surface markers of human MSCs that readily differentiate into cardiomyocytes. When the cardiomyogenic differentiation potential and the expression of cell surface markers involved in heart development were analyzed using various immortalized human MSC lines, the MSCs with high expression of N-cadherin showed a higher probability of differentiation into beating cardiomyocytes. The differentiated cardiomyocytes expressed terminally differentiated cardiomyocyte-specific markers such as alpha-actinin, cardiac troponin T, and connexin-43. A similar correlation was observed with primary human MSCs derived from bone marrow and adipose tissue. Moreover, N-cadherin-positive MSCs isolated with N-cadherin antibody-conjugated magnetic beads showed an apparently higher ability to differentiate into cardiomyocytes than the N-cadherin-negative population. Quantitative polymerase chain reaction analyses demonstrated that the N-cadherin-positive population expressed significantly elevated levels of cardiomyogenic progenitor-specific transcription factors, including Nkx2.5, Hand1, and GATA4 mRNAs. Our results suggest that N-cadherin is a novel prospective cell surface marker of human MSCs that show a better ability for cardiomyocyte differentiation.

Itokazu, M., et al. (2016). "Transplantation of Scaffold-Free Cartilage-Like Cell-Sheets Made from Human Bone Marrow Mesenchymal Stem Cells for Cartilage Repair: A Preclinical Study." Cartilage **7**(4): 361-372.

OBJECTIVE: The object of this study was to determine culture conditions that create stable scaffold-free cartilage-like cell-sheets from human bone marrow-derived mesenchymal stem cells (hBMSCs) and to assess their effects after transplantation into osteochondral defects in nude rats. DESIGN: (Experiment 1) The hBMSCs were harvested from 3 males, the proliferative and chondrogenic capacities were assessed at passage 1, and the cells were expanded in 3 different culture conditions: (1) 5% fetal bovine serum (FBS), (2) 10% FBS, and (3) 5% FBS with fibroblast growth factor 2 (FGF-2). The cells were harvested and made chondrogenic pellet culture. The cell proliferation rate, glycosaminoglycan/DNA ratio, and safranin-O staining intensity of pellets cultured condition 3 were higher than those of conditions 1 and 2. (Experiment 2) The hBMSCs were expanded and passaged 3 times under culture condition 3, and fabricate the cell-sheets in chondrogenic medium either with or without FBS. The cell-sheets fabricated with FBS maintained their size with flat edges. (Experiment 3) The cell-sheets were transplanted into osteochondral defects in nude rats. Histological analysis was performed at 2, 4, and 12 weeks after surgery. RESULTS: The osteochondral repair was better after sheet transplantation than in the control group and significantly improved Wakitani score. Immunostaining with human-specific vimentin antibody showed that the transplanted cells became fewer and disappeared at 12 weeks. CONCLUSIONS: These results indicate that culture with FGF-2 may help to quickly generate sufficient numbers of cells to create stable and reliable scaffold-free cartilage-like cell-sheets, which contribute to the regeneration of osteochondral defects.

Iwasa, M., et al. (2017). "Bortezomib interferes with adhesion of B cell precursor acute lymphoblastic leukemia cells through SPARC up-regulation in human bone marrow mesenchymal stromal/stem cells." Int J Hematol **105**(5): 587-597.

The poor prognosis of adults with B cell precursor acute lymphoblastic leukemia (BCP-ALL) is attributed to leukemia cells that are protected by the bone marrow (BM) microenvironment. In the present study, we explored the pharmacological targeting of mesenchymal stromal/stem cells in BM (BM-MSCs) to eliminate chemoresistant BCP-ALL cells. Human BCP-ALL cells (NALM-6 cells) that adhered to human BM-MSCs (NALM-6/Ad) were highly resistant to multiple anti-cancer drugs, and exhibited pro-survival characteristics, such as an enhanced Akt/Bcl-2 pathway and increased populations in the G0 and G2/S/M cell cycle stages. Bortezomib, a proteasome inhibitor, interfered with adhesion between BM-MSCs and NALM-6 cells and up-regulated the matricellular protein SPARC (secreted protein acidic and rich in cysteine) in BM-MSCs, thereby reducing the NALM-6/Ad population. Inhibition of SPARC expression in BM-MSCs using a small interfering RNA enhanced adhesion of NALM-6 cells. Conversely, recombinant SPARC protein interfered with adhesion of NALM-6 cells. These results suggest that SPARC disrupts adhesion between BM-MSCs and NALM-6 cells. Co-treatment with bortezomib and doxorubicin prolonged the survival of BCP-ALL xenograft mice, with a significant reduction of leukemia cells in BM. Our findings demonstrate that bortezomib contributes to the elimination of BCP-ALL cells through disruption of their adhesion to BM-MSCs, and offer a novel therapeutic strategy for BCP-ALL through targeting of BM-MSCs.

Jarvinen, L., et al. (2008). "Lung resident mesenchymal stem cells isolated from human lung allografts inhibit T cell proliferation via a soluble mediator." J Immunol **181**(6): 4389-4396.

Development of allograft rejection continues to be the major determinant of morbidity and mortality postlung transplantation. We have recently demonstrated that a population of donor-derived mesenchymal stem cells is present in human lung allografts and can be isolated and expanded ex vivo. In this study, we investigated the impact of lung resident mesenchymal stem cells (LR-MSCs), derived from allografts of human lung transplant recipients, on T cell activation in vitro. Similar to bone marrow-derived MSCs, LR-MSCs did not express MHC II or the costimulatory molecules CD80 or CD86. In vitro, LR-MSCs profoundly suppressed the proliferative capacity of T cells in response to a mitogenic or an allogeneic stimulus. The immunosuppressive function of LR-MSCs was also noted in the absence of direct cell contact, indicating that LR-MSCs mediated their effect predominantly via a soluble mediator. LR-MSCs isolated from lung transplant recipients demonstrated PGE (2) secretion at baseline (385 +/- 375 pg/ml), which increased in response to IL-1beta (1149 +/- 1081 pg/ml). The addition of PG synthesis inhibitors (indomethacin and NS-398) substantially abrogated LR-MSC-mediated immunosuppression, indicating that PGE (2) may be one of the major soluble mediators impacting T cell activity. This is the first report to demonstrate that human tissue-derived MSCs isolated from an allogeneic environment have the potential to mediate immunological responses in vitro.

Jeon, E. S., et al. (2010). "Ovarian cancer-derived lysophosphatidic acid stimulates secretion of VEGF and stromal cell-derived factor-1 alpha from human mesenchymal stem cells." Exp Mol Med **42**(4): 280-293.

Lysophosphatidic acid (LPA) stimulates growth and invasion of ovarian cancer cells and tumor angiogenesis. Cancer-derived LPA induces differentiation of human adipose tissue-derived mesenchymal stem cells (hASCs) to alpha-smooth muscle actin (alpha-SMA)-positive cancer-associated fibroblasts. Presently, we explored whether cancer-derived LPA regulates secretion of pro-angiogenic factors from hASCs. Conditioned medium (CM) from the OVCAR-3 and SKOV3 ovarian cancer cell lines stimulated secretion angiogenic factors such as stromal-derived factor-1 alpha (SDF-1 alpha) and VEGF from hASCs. Pretreatment with the LPA receptor inhibitor Ki16425 or short hairpin RNA lentiviral silencing of the LPA ((1)) receptor abrogated the cancer CM-stimulated expression of alpha-SMA, SDF-1, and VEGF from hASCs. LPA induced expression of myocardin and myocardin-related transcription factor-A, transcription factors involved in smooth muscle differentiation, in hASCs. siRNA-mediated depletion of endogenous myocardin and MRTF-A abrogated the expression of alpha-SMA, but not SDF-1 and VEGF. LPA activated RhoA in hASCs and pretreatment with the Rho kinase inhibitor Y27632 completely abrogated the LPA-induced expression of alpha-SMA, SDF-1, and VEGF in hASCs. Moreover, LPA-induced alpha-SMA expression was abrogated by treatment with the ERK inhibitor U0126 or the phosphoinositide-3-kinase inhibitor LY294002, but not the PLC inhibitor U73122. LPA-induced VEGF secretion was inhibited by LY294002, whereas LPA-induced SDF-1 secretion was markedly attenuated by U0126, U73122, and LY294002. These results suggest that cancer-secreted LPA induces differentiation of hASCs to cancer-associated fibroblasts through multiple signaling

pathways involving Rho kinase, ERK, PLC, and phosphoinositide-3-kinase.

Jeon, E. S., et al. (2005). "Role of MEK-ERK pathway in sphingosylphosphorylcholine-induced cell death in human adipose tissue-derived mesenchymal stem cells." Biochim Biophys Acta **1734**(1): 25-33.

Sphingosylphosphorylcholine (SPC) is a bioactive lipid molecule involved in a variety of cellular responses. In the present study, we demonstrated that treatment of human adipose tissue-derived mesenchymal stem cells (hATSCs) with D-erythro-SPC resulted in apoptosis-like cell death, as demonstrated by decreased cell viability, DNA strand breaks, the increase of sub-G1 fraction, cytochrome c release into cytosol, and activation of caspase-3. In contrast, the exposure of hATSCs to L-threo-SPC did not induce the cell death, suggesting that the SPC-induced cell death was selective for the D-erythro-stereoisomer of SPC. The D-erythro-SPC-induced cell death was prevented by DEVD-CHO, a caspase-3 specific inhibitor, and Z-VAD-FMK, a general caspase inhibitor, suggesting that the SPC-induced cell death of hATSCs occurs through the cytochrome c- and caspase-3-dependent pathways. In addition, D-erythro-SPC treatment stimulated the activation of mitogen-activated protein kinases, such as ERK and c-Jun NH2-terminal protein kinase (JNK), and the D-erythro-SPC-induced cell death was completely prevented by pretreatment with the MEK inhibitor, U0126, but not by pretreatment with the JNK inhibitor, SP600125, and the p38 MAPK inhibitor, SB202190, suggesting a specific involvement of ERK in the D-erythro-SPC-induced cell death. Pretreatment with U0126 attenuated the D-erythro-SPC-induced release of cytochrome c. From these results, we suggest that ERK is involved in the SPC-induced cell death of hATSC through stimulation of the cytochrome c/caspase-3-dependent pathway.

Jeon, Y. J., et al. (2016). "Comparative Analysis of Human Mesenchymal Stem Cells Derived From Bone Marrow, Placenta, and Adipose Tissue as Sources of Cell Therapy." J Cell Biochem **117**(5): 1112-1125.

Various source-derived mesenchymal stem cells (MSCs) with multipotent capabilities were considered for cell therapeutics of incurable diseases. The applicability of MSCs depends on the cellular source and on their different in vivo functions, despite having similar phenotypic and cytological characteristics. We characterized MSCs from different sources, including human bone marrow (BM), placenta (PL), and adipose tissue (AT), in terms of the phenotype, surface antigen expression, differentiation ability, proteome reference map, and blood flow recovery in a hindlimb ischemic disease model. The MSCs exhibit different differentiation potentials depending on the cellular source despite having similar phenotypic and surface antigen expression. We identified approximately 90 differentially regulated proteins. Most up- or down-regulated proteins show cytoskeletal or oxidative stress, peroxiredoxin, and apoptosis roles according to their functional involvement. In addition, the PL-MSCs retained a higher therapeutic efficacy than the BM- and AT-MSCs in the hindlimb ischemic disease model. In summary, we examined differentially expressed key regulatory factors for MSCs that were obtained from several cellular sources and demonstrated their differentially expressed proteome profiles. Our results indicate that primitive PL-MSCs have biological advantages relative to those from other sources, making PL-MSCs a useful model for clinical applications of cell therapy.

Jezierska-Wozniak, K., et al. (2018). "Migration of human mesenchymal stem cells stimulated with pulsed electric field and the dynamics of the cell surface glycosylation." Adv Clin Exp Med **27**(9): 1181-1193.

BACKGROUND: The analysis of the stem cells' glycome dynamics at different stages of differentiation and migration makes possible the exploration of the cell surface glycans as markers of the stem cell functional status, and, in the future, compatibility between transplanted cell and host environment. OBJECTIVES: The objective of our study was to develop novel techniques of investigating cell motility and to assess whether the electric field of the therapeutic spinal cord stimulation system used in vivo contributes to the migration of human mesenchymal stem cells (hMSCs) in vitro. MATERIAL AND METHODS: We have investigated the electrotaxis of bone marrow-derived MSCs using pulsed electric field (PEF) in the range of 16-80 mV/mm and the frequency of 130 Hz and 240 Hz. The PEF-related dynamics of the cell surface glycosylation was evaluated using 6 plant lectins recognizing individual glycans. RESULTS: Pulsed electric field at physiological levels (10 mV/mm; 130 Hz) did not influence cellular motility in vitro, which may correspond to the maintenance of the transplanted cells at the lesion site in vivo. An increase of the PEF intensity and the frequency exceeding physiological levels resulted in an increase in the cellular migration rate in vitro. Pulsed electric field elevated above physiological intensity and frequency (40-80 mV/mm; 240 Hz), but not at physiological levels, resulted in changes of the cell surface glycosylation. CONCLUSIONS: We found the described approach convenient for investigations and for the in vitro modeling of the cellular systems intended for the regenerative cell

transplantations in vivo. Probing cell surface glycomes may provide valuable biomarkers to assess the competence of transplanted cells.

Ji, Y., et al. (2017). "Microvesicles released from human embryonic stem cell derived-mesenchymal stem cells inhibit proliferation of leukemia cells." Oncol Rep **38**(2): 1013-1020.

Human embryonic stem cell derived-mesenchymal stem cells (hESCMSCs) are able to inhibit proliferation of leukemia cells. Microvesicles released from human embryonic stem cell derived-mesenchymal stem cells (hESCMSCMVs) might play an important part in antitumor activity. Microvesicles were isolated by ultracentrifugation and identified under a scanning electron microscopy and transmission electron microscope separately. After 48-h cocultured with hESCMSCs and hESCMSCMVs, the number of K562 and HL60 was counted and tumor cell viability was measured by CCK8 assay. The expression of proteins Bcl-2 and Bax were estimated by western blotting. Transmission electron microscope and western blot analysis were adopted to evaluate the autophagy level. Results showed that both hESCMSCs and hESCMSCMVs inhibited proliferation of leukemia cells in a concentration-dependent manner. hESCMSCMVs reduced the ratio of Bcl/Bax, enhanced the protein level of Beclin-1 and LC3-II conversion, thus upregulating autophagy and apoptosis. In conclusion, microvesicles released from human embryonic stem cell derived-mesenchymal stem cells inhibited tumor growth and stimulated autophagy and excessive autophagy might induce apoptosis.

Jia, Z., et al. (2018). "Isolation and characterization of human mesenchymal stem cells derived from synovial fluid by magnetic-activated cell sorting (MACS)." Cell Biol Int **42**(3): 262-271.

Mesenchymal stem cells (MSCs) are the primary source of cells used for cell-based therapy in tissue engineering. MSCs are found in synovial fluid, a source that could be conveniently used for cartilage tissue engineering. However, the purification and characterization of SF-MSCs has been poorly documented in the literature. Here, we outline an easy-to-perform approach for the isolation and culture of MSCs derived from human synovial fluid (hSF-MSCs). We have successfully purified hSF-MSCs using magnetic-activated cell sorting (MACS) using the MSC surface marker, CD90. Purified SF-MSCs demonstrate significant renewal capacity following several passages in culture. Furthermore, we demonstrated that MACS-sorted CD90(+) cells could differentiated into osteoblasts, adipocytes, and chondrocytes in vitro. In addition, we show that these cells can generate cartilage tissue in micromass culture as well. This study demonstrates that MACS is a useful tool that can be used for the purification of hSF-MSCs from synovial fluid. The proliferation properties and ability to differentiate into chondrocytes make these hSF-MSCs a promising source of stem cells for applications in cartilage repair.

Jiang, R., et al. (2006). "Histological type of oncogenity and expression of cell cycle genes in tumor cells from human mesenchymal stem cells." Oncol Rep **16**(5): 1021-1028.

In previous experiments, a novel tumor cell line, which was characterized by dominated F6 mutated from human mesenchymal stem cells (hMSCs), was developed. The mechanism and biological characteristics of this mutation are still unclear. In this study, the histological type of F6 cells was investigated by immunohistochemistry with specific markers: vimentin, CD117, desmin, NSE and vWF. The characteristics of proliferation and metastasis were shown by PCNA (proliferating cell nuclear antigen), and nm23 and cell cycle-related genes, such as p16, p21, p53 and pRb, were analyzed by RT-PCR and immunohistochemistry. The expression of hTRAP and BMI-1 were detected by real-time PCR and Western blotting. The activity of telomerase was analyzed by TRAP (telomerase repeat amplification protocol) assay. The results showed that multi-directional differentiation occurred in F6 cells, i.e., special markers of muscle, endothelial cell and nerve system were co-expressed in F6 cells, while hardly expressed in hMSCs. F6 cells maintained the same properties as of MSCs, such as negativity for both CD117 and vimentin. F6 cells exhibited strong positivity for PCNA and negativity for nm23. The cell cycle-related genes, such as p16, p21, p53 and pRb, were not detected in F6 cells, while the expression of hTRAP and BMI-1 was significantly higher. The activity of telomerase was also significantly higher in F6 cells than that in hMSCs. These findings indicated that multi-directional differentiation occurred during the transformation of hMSCs into F6 cells, and that the genes of cell cycle and cell senescence may also be associated with the neoplasia of adult stem cells.

Jin, H. J., et al. (2013). "Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy." Int J Mol Sci **14**(9): 17986-18001.

Various source-derived mesenchymal stem cells (MSCs) have been considered for cell therapeutics in incurable diseases. To characterize MSCs from different sources, we compared human bone marrow (BM), adipose tissue (AT), and umbilical cord blood-derived MSCs (UCB-MSCs) for surface antigen expression, differentiation ability, proliferation

capacity, clonality, tolerance for aging, and paracrine activity. Although MSCs from different tissues have similar levels of surface antigen expression, immunosuppressive activity, and differentiation ability, UCB-MSCs had the highest rate of cell proliferation and clonality, and significantly lower expression of p53, p21, and p16, well known markers of senescence. Since paracrine action is the main action of MSCs, we examined the anti-inflammatory activity of each MSC under lipopolysaccharide (LPS)-induced inflammation. Co-culture of UCB-MSCs with LPS-treated rat alveolar macrophage, reduced expression of inflammatory cytokines including interleukin-1alpha (IL-1alpha), IL-6, and IL-8 via angiopoietin-1 (Ang-1). Using recombinant Ang-1 as potential soluble paracrine factor or its small interference RNA (siRNA), we found that Ang-1 secretion was responsible for this beneficial effect in part by preventing inflammation. Our results demonstrate that primitive UCB-MSCs have biological advantages in comparison to adult sources, making UCB-MSCs a useful model for clinical applications of cell therapy.

Jin, H. J., et al. (2016). "Downregulation of Melanoma Cell Adhesion Molecule (MCAM/CD146) Accelerates Cellular Senescence in Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells." Stem Cells Transl Med **5**(4): 427-439.

UNLABELLED: Therapeutic applications of mesenchymal stem cells (MSCs) for treating various diseases have increased in recent years. To ensure that treatment is effective, an adequate MSC dosage should be determined before these cells are used for therapeutic purposes. To obtain a sufficient number of cells for therapeutic applications, MSCs must be expanded in long-term cell culture, which inevitably triggers cellular senescence. In this study, we investigated the surface markers of human umbilical cord blood-derived MSCs (hUCB-MSCs) associated with cellular senescence using fluorescence-activated cell sorting analysis and 242 cell surface-marker antibodies. Among these surface proteins, we selected the melanoma cell adhesion molecule (MCAM/CD146) for further study with the aim of validating observed expression differences and investigating the associated implications in hUCB-MSCs during cellular senescence. We observed that CD146 expression markedly decreased in hUCB-MSCs following prolonged in vitro expansion. Using preparative sorting, we found that hUCB-MSCs with high CD146 expression displayed high growth rates, multilineage differentiation, expression of stemness markers, and telomerase activity, as well as significantly lower expression of the senescence markers p16, p21, p53, and senescence-associated beta-galactosidase, compared with that observed in hUCB-MSCs with low-level CD146 expression. In contrast, CD146 downregulation with small interfering RNAs enhanced the senescence phenotype. In addition, CD146 suppression in hUCB-MSCs caused downregulation of other cellular senescence regulators, including Bmi-1, Id1, and Twist1. Collectively, our results suggest that CD146 regulates cellular senescence; thus, it could be used as a therapeutic marker to identify senescent hUCB-MSCs. SIGNIFICANCE: One of the fundamental requirements for mesenchymal stem cell (MSC)-based therapies is the expansion of MSCs during long-term culture because a sufficient number of functional cells is required. However, long-term growth inevitably induces cellular senescence, which potentially causes poor clinical outcomes by inducing growth arrest and the loss of stem cell properties. Thus, the identification of markers for evaluating the status of MSC senescence during long-term culture may enhance the success of MSC-based therapy. This study provides strong evidence that CD146 is a novel and useful marker for predicting senescence in human umbilical cord blood-derived MSCs (hUCB-MSCs), and CD146 can potentially be applied in quality-control assessments of hUCB-MSC-based therapy.

Jinfeng, L., et al. (2016). "Therapeutic Effects of CUR-Activated Human Umbilical Cord Mesenchymal Stem Cells on 1-Methyl-4-phenylpyridine-Induced Parkinson's Disease Cell Model." Biomed Res Int **2016**: 9140541.

The purpose of this study is to evaluate the therapeutic effects of human umbilical cord-derived mesenchymal stem cells (hUC-MSC) activated by curcumin (CUR) on PC12 cells induced by 1-methyl-4-phenylpyridinium ion (MPP+), a cell model of Parkinson's disease (PD). The supernatant of hUC-MSC and hUC-MSC activated by 5 micromol/L CUR (hUC-MSC-CUR) were collected in accordance with the same concentration. The cell proliferation and differentiation potential to dopaminergic neuronal cells and antioxidation were observed in PC12 cells after being treated with the above two supernatants and 5 micromol/L CUR. The results showed that the hUC-MSC-CUR could more obviously promote the proliferation and the expression of tyrosine hydroxylase (TH) and microtubule associated protein-2 (MAP2) and significantly decreased the expression of nitric oxide (NO) and inducible nitric oxide synthase (iNOS) in PC12 cells. Furtherly, cytokines detection gave a clue that the expression of IL-6, IL-10, and NGF was significantly higher in the group treated with the hUC-MSC-CUR compared to those of other two groups. Therefore, the hUC-MSC-CUR may be a potential strategy to promote the proliferation and differentiation of PD cell model, therefore providing new insights into a novel therapeutic approach in PD.

Jo, J., et al. (2012). "Regulation of differentiation potential of human mesenchymal stem cells by intracytoplasmic delivery of coactivator-associated arginine methyltransferase 1 protein using cell-penetrating peptide." Stem Cells **30**(8): 1703-1713.

Recent studies suggest that epigenetic modifications, such as DNA methylation and histone modification, can alter the differentiation potential of stem cells or progenitor cells. Specifically, coactivator-associated arginine methyltransferase 1 (CARM1) is known to act as a coactivator for various transcription factors and to regulate gene expression by chromatin remodeling through histone methylation. Here, for the first time, we have used direct protein delivery of CARM1 using cell-penetrating peptide (CPP) to regulate the differentiation potential of human mesenchymal stem cells (hMSCs). Immunofluorescence showed that the CPP-CARM1 protein is successfully delivered into the nuclei of hMSCs. Further experiments using immunofluorescence and Western blotting showed that the delivered CARM1 protein can effectively methylate the arginine 17 residue of histone H3 in both bone marrow (BM)- and adipose-derived (AD)-hMSCs, thus suggesting that the CARM1 protein delivered by the CPP system is biologically active in hMSCs. Chromatin immunoprecipitation (ChIP) assay and genome-wide gene expression profiling supported the result that delivered CARM1 protein can cause chromatin remodeling through histone methylation. Finally, the CPP-CARM1 protein efficiently elevated the differentiation efficiency of BM-hMSCs and AD-hMSCs into adipogenic, osteogenic, and myogenic cell lineages in vitro. Altered expression of critical genes after hMSC differentiation was reconfirmed by real-time reverse transcription polymerase chain reaction (qRT-PCR). Collectively, our results suggest that CPP-CARM1 can elevate the differentiation potential of hMSCs into various cell types, and that this system using CPP is a useful tool for exogenous protein delivery in clinical applications of cell-based therapy.

Joddar, B., et al. (2018). "A Contact-Based Method for Differentiation of Human Mesenchymal Stem Cells into an Endothelial Cell-Phenotype." Cell Biochem Biophys **76**(1-2): 187-195.

Adult stem cells such as mesenchymal stem cells (MSC) are known to possess the ability to augment neovascularization processes and are thus widely popular as an autologous source of progenitor cells. However there is a huge gap in our current knowledge of mechanisms involved in differentiating MSC into endothelial cells (EC), essential for lining engineered blood vessels. To fill up this gap, we attempted to differentiate human MSC into EC, by culturing the former onto chemically fixed layers of EC or its ECM, respectively. We expected direct contact of MSC when cultured atop fixed EC or its ECM, would coax the former to differentiate into EC. Results showed that human MSC cultured atop chemically fixed EC or its ECM using EC-medium showed enhanced expression of CD31, a marker for EC, compared to other cases. Further in all human MSC cultured using EC-medium, typically characteristic cobble stone shaped morphologies were noted in comparison to cells cultured using MSC medium, implying that the differentiated cells were sensitive to soluble VEGF supplementation present in the EC-medium. Results will enhance and affect therapies utilizing autologous MSC as a cell source for generating vascular cells to be used in a variety of tissue engineering applications.

Joensuu, K., et al. (2011). "Interaction between marrow-derived human mesenchymal stem cells and peripheral blood mononuclear cells in endothelial cell differentiation." Scand J Surg **100**(3): 216-222.

BACKGROUND AND AIMS: In adult connective tissues, mesenchymal stem cells (MSCs) play a key role in normal tissue turnover and repair. MSCs can participate in these processes not only through proliferation and differentiation but also through paracrine/autocrine functions. These characteristics make MSCs the optimal target in the development of cell-based therapies. This study describes a novel interaction between human MSC and blood mononuclear cells (MNCs), resulting in formation of blood vessel-like structures. MATERIALS AND METHODS: Human marrow-derived MSCs and peripheral blood MNCs were co-cultured in monolayer cultures as well as in bovine collagen sponge up to 20 days. No exogenously supplied growth factors were applied. Morphological changes and formations of three dimensional structures were detected by light microscopy. The process was further stu-died for the expression of different endothelial cell markers. The expression of PECAM-1 and endoglin was studied by immunohistochemistry and the expression of vascular endothelial growth factor receptors 1 and 2 using quantitative real time PCR. RESULTS: In co-cultures of human MSCs and MNCs, the previously nonadherent cells attached and started to elongate and formed tube-like structures within one week. At day 10, elongated PECAM-1 and endoglin expressing cells were detected in co-cultures. At day 20, PECAM-1 and endoglin-positive vessel-like structures were observed. VEGFR1 was up-regulated in co-cultures after 10 days, and expression levels increased with time. No PECAM-1, endoglin or VEGFR1 expressing cells were discovered in MSC-cultures without MNCs at any time point. CONCLUSIONS: This study

demonstrates induction of endothelial differentiation in co-cultures of human MSCs and MNCs, indicating a mechanism by which local application of MSCs could induce angiogenesis in vivo.

Ju, G. Q., et al. (2015). "Microvesicles derived from human umbilical cord mesenchymal stem cells facilitate tubular epithelial cell dedifferentiation and growth via hepatocyte growth factor induction." PLoS One **10**(3): e0121534.

During acute kidney injury (AKI), tubular cell dedifferentiation initiates cell regeneration; hepatocyte growth factor (HGF) is involved in modulating cell dedifferentiation. Mesenchymal stem cell (MSC)-derived microvesicles (MVs) deliver RNA into injured tubular cells and alter their gene expression, thus regenerating these cells. We boldly speculated that MVs might induce HGF synthesis via RNA transfer, thereby facilitating tubular cell dedifferentiation and regeneration. In a rat model of unilateral AKI, the administration of MVs promoted kidney recovery. One of the mechanisms of action is the acceleration of tubular cell dedifferentiation and growth. Both in vivo and in vitro, rat HGF expression in damaged rat tubular cells was greatly enhanced by MV treatment. In addition, human HGF mRNA present in MVs was delivered into rat tubular cells and translated into the HGF protein as another mechanism of HGF induction. RNase treatment abrogated all MV effects. In the in vitro experimental setting, the conditioned medium of MV-treated injured tubular cells, which contains a higher concentration of HGF, strongly stimulated cell dedifferentiation and growth, as well as Erk1/2 signaling activation. Intriguingly, these effects were completely abrogated by either c-Met inhibitor or MEK inhibitor, suggesting that HGF induction is a crucial contributor to the acceleration of cell dedifferentiation and growth. All these findings indicate that MV-induced HGF synthesis in damaged tubular cells via RNA transfer facilitates cell dedifferentiation and growth, which are important regenerative mechanisms.

Kadam, S., et al. (2010). "Human placenta-derived mesenchymal stem cells and islet-like cell clusters generated from these cells as a novel source for stem cell therapy in diabetes." Rev Diabet Stud **7**(2): 168-182.

Placental tissue holds great promise as a source of cells for regenerative medicine due to its plasticity, and easy availability. Human placenta-derived mesenchymal stem cells (hPDMSCs) have the potential to differentiate into insulin-producing cells. Upon transplantation, they can reverse experimental diabetes in mice. However, it is not known whether culture-expanded undifferentiated hPDMSCs are capable of restoring normoglycemia upon transplantation in streptozotocin (STZ)-induced diabetic mice. Hence we prepared long-term cultures of hPDMSCs from the chorionic villi of full-term human placenta. Flow cytometry analyses and immunocytochemistry study revealed bonafide mesenchymal nature of the isolated hPDMSCs. These cultures could differentiate into adipogenic, oesteogenic, chondrogenic, and neuronal lineages on exposure to lineage-specific cocktails. Furthermore, we showed that hPDMSCs can form islet-like cell clusters (ILCs) on stepwise exposure to serum-free defined media containing specific growth factors and differentiating agents. qRT-PCR showed the expression of insulin, glucagon, and somatostatin in undifferentiated hPDMSCs and in ILCs. Differentiated ILCs were found to express human insulin, glucagon, and somatostatin by immunocytochemistry. Additionally, ILCs also showed abundance of pancreatic transcription factors ngn3 and isl1. Both undifferentiated hPDMSCs and ILCs exihibited insulin secretion in response to glucose. Transplantation of hPDMSCs or ILCs derived from hPDMSCs in STZ-induced diabetic mice led to restoration of normoglycemia. Our results demonstrate, for the first time, reversal of hyperglycemia by undifferentiated hPDMSCs and ILCs derived from hPDMSCs. These results suggest human placenta-derived MSCs as an alternative source for cell replacement therapy in diabetes.

Kanemura, Y. (2010). "Development of cell-processing systems for human stem cells (neural stem cells, mesenchymal stem cells, and iPS cells) for regenerative medicine." Keio J Med **59**(2): 35-45.

Regenerative medicine using human stem cells is one of the newest and most promising fields for treating various intractable diseases and damaged organs. For clinical applications, choosing which human stem cells to use, i.e. according to tissue of origin and progenitor type, is a critical issue. Neural stem/progenitor cells (NSPCs) hold promise for treating various neurological diseases. We have shown that the transporter protein ABCB1 is predominantly expressed in immature human fetal NSPCs, and thus could be used as a phenotypic marker to investigate and monitor NSPCs in culture. We describe our proposed model for the in vitro proliferative process of aggregated human NSPCs and show that neurosphere enlargement and NSPC proliferation are mutually reinforcing. We have established that human neurospheres contain a heterogeneous cell population, knowledge that will contribute to the development of human neurospheres with desirable characteristics for clinical applications. Furthermore, decidua-derived mesenchymal cells (DMCs), which we isolated from

human placenta, have unique properties as mesenchymal stem cells. They also generate a pericellular matrix (PCM-DM) that supports the growth and pluripotency of human embryonic stem cells and induced pluripotent stem cells (hiPS) cells. The newly developed re-programming techniques for generating hiPS cells should greatly contribute to cell therapies using human pluripotent stem cells, including those derived from DMCs. Our DMC-derived hiPS cells are a promising candidate source of allogeneic hiPS cells for clinical applications. We hope our findings will contribute to the development of cell-culture systems for generating human allogeneic stem cells for clinical use in regenerative medicine.

Kanzawa, M., et al. (2013). "WNT5A is a key regulator of the epithelial-mesenchymal transition and cancer stem cell properties in human gastric carcinoma cells." Pathobiology **80**(5): 235-244.

OBJECTIVE: Direct interaction with cancer-associated fibroblasts triggers WNT5A expression in human gastric carcinoma (GC) cells. In this study, we performed gene transduction experiments to investigate the significance of WNT5A in the GC tumor microenvironment. METHODS: Gene transduction (pWNT5A and shWNT5A) was performed in human GC-derived MKN-7 cells. Altered gene expression was examined by RT-PCR and cDNA microarray analysis. Immunohistochemical examination was carried out in human GC tissues. RESULTS: Transduction of exogenous WNT5A expression into MKN-7 cells upregulated genes related to the epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs), and the pWNT5A transfectant showed high tumorigenicity in vivo. These results were confirmed by knockdown experiments using a lentivirus expressing shWNT5A. A cDNA microarray analysis suggested that depletion of endogenous WNT5A downregulated genes involved in intracellular signaling, chemokine-cytokine interaction and focal adhesion. High levels of WNT5A expression were observed in 66% of GC cases, with significant correlation with histological type. Interestingly, in intestinal-type GCs, WNT5A expression was detected in the periphery of tumor nests. CONCLUSIONS: WNT5A regulates the induction of EMT and the maintenance of CSC properties in MKN-7 cells. WNT5A may play an important role in constructing an advantageous tumor microenvironment for the progression and development of human GC.

Karaoz, E., et al. (2011). "Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells." Histochem Cell Biol **136**(4): 455-473.

Dental pulp stem cells (hDP-SCs) were primarily derived from pulp tissues of primary incisors, exfoliated deciduous and permanent third molar teeth. To understand the characteristics of hDP-SCs from impacted third molar, proliferation capacities, gene expression profiles, phenotypic, ultrastructural, and differentiation characteristics were analyzed in comparison with human bone marrow-derived mesenchymal stem cells (hBM-MSCs), extensively. hDP-SCs showed more developed and metabolically active cells. Contrary to hBM-MSCs, hDP-SCs strongly expressed both cytokeratin (CK)-18 and -19, which could involve in odontoblast differentiation and dentine repair. The intrinsic neuro-glia characteristics of hDP-MSCs were demonstrated by the expression of several specific transcripts and proteins of neural stem cell and neurons. These cells not only differentiate into adipogenic, osteogenic, and chondrogenic lineage, but also share some special characteristics of expressing some neural stem cell and epithelial markers. Under defined conditions, hDP-SCs are able to differentiate into both neural and vascular endothelial cells in vitro. Dental pulp might provide an alternative source for human MSCs. hDP-SCs with a promising differentiation capacity could be easily isolated, and possible clinical use could be developed for neurodegenerative and oral diseases in the future.

Kasten, P., et al. (2008). "Instant stem cell therapy: characterization and concentration of human mesenchymal stem cells in vitro." Eur Cell Mater **16**: 47-55.

In regenerative medicine, there is an approach to avoid expansion of the mesenchymal stem cell (MSC) before implantation. The aim of this study was to compare methods for instant MSC therapy by use of a portable, automatic and closed system centrifuge that allows for the concentration of MSCs. The main outcome measures were the amount of MSCs per millilitre of bone marrow (BM), clusters of differentiation (CD), proliferation and differentiation capacities of the MSC. A volume reduction protocol was compared to the traditional laboratory methods of isolation using a Ficoll gradient and native BM. Fifty millilitres of BM were obtained from haematologically healthy male Caucasians (n=10, age 8 to 49 years). The number of colony forming units-fibroblast (CFU-F)/ml BM was highest in the centrifuge volume reduction protocol, followed by the native BM (not significant), the centrifuge Ficoll (p=0.042) and the manual Ficoll procedure (p=0.001). The MSC of all groups could differentiate into the mesenchymal lineages without significant differences between the groups. The CD pattern was identical for all groups:

CD13+; CD 44+; CD73 +; CD90+; CD105+; HLA-A,B,C+; CD14-; CD34-; CD45-; CD271-; HLA-DR-. In a further clinical pilot study (n=5) with 297 ml BM (SD 18.6), the volume reduction protocol concentrated the MSC by a factor of 14: there were 1.08 x 10(2) MSC/ml BM (standard deviation (SD) 1.02 x 10(2)) before concentration, 14.8 x 10(2) MSC/ ml BM (SD 12.4 x 10(2)) after concentration, and on average 296 x 10(2) MSC (SD 248.9 x 10(2), range 86.4-691.5 x 10(2)) were available for MSC therapy. The volume reduction protocol of the closed centrifuge allows for the highest concentration of the MSC, and therefore, is a promising candidate for instant stem cell therapy.

Katayama, N., et al. (2014). "The effects of synthetic oligopeptide derived from enamel matrix derivative on cell proliferation and osteoblastic differentiation of human mesenchymal stem cells." Int J Mol Sci **15**(8): 14026-14043.

Enamel matrix derivative (EMD) is widely used in periodontal tissue regeneration therapy. However, because the bioactivity of EMD varies from batch to batch, and the use of a synthetic peptide could avoid use from an animal source, a completely synthetic peptide (SP) containing the active component of EMD would be useful. In this study an oligopeptide synthesized derived from EMD was evaluated for whether it contributes to periodontal tissue regeneration. We investigated the effects of the SP on cell proliferation and osteoblast differentiation of human mesenchymal stem cells (MSCs), which are involved in tissue regeneration. MSCs were treated with SP (0 to 1000 ng/mL), to determine the optimal concentration. We examined the effects of SP on cell proliferation and osteoblastic differentiation indicators such as alkaline phosphatase activity, the production of procollagen type 1 C-peptide and osteocalcin, and on mineralization. Additionally, we investigated the role of extracellular signal-related kinases (ERK) in cell proliferation and osteoblastic differentiation induced by SP. Our results suggest that SP promotes these processes in human MSCs, and that ERK inhibitors suppress these effects. In conclusion, SP promotes cell proliferation and osteoblastic differentiation of human MSCs, probably through the ERK pathway.

Kelly, A. M., et al. (2016). "Glucocorticoid Cell Priming Enhances Transfection Outcomes in Adult Human Mesenchymal Stem Cells." Mol Ther **24**(2): 331-341.

Human mesenchymal stem cells (hMSCs) are one of the most widely researched stem cell types with broad applications from basic research to therapeutics, the majority of which require introduction of exogenous DNA. However, safety and scalability issues hinder viral delivery, while poor efficiency hinders nonviral gene delivery, particularly to hMSCs. Here, we present the use of a pharmacologic agent (glucocorticoid) to overcome barriers to hMSC DNA transfer to enhance transfection using three common nonviral vectors. Glucocorticoid priming significantly enhances transfection in hMSCs, demonstrated by a 3-fold increase in efficiency, 4-15-fold increase in transgene expression, and prolonged transgene expression when compared to transfection without glucocorticoids. These effects are dependent on glucocorticoid receptor binding and caused in part by maintenance of normal metabolic function and increased cellular (5-fold) and nuclear (6-10-fold) DNA uptake over hMSCs transfected without glucocorticoids. Results were consistent across five human donors and in cells up to passage five. Glucocorticoid cell priming is a simple and effective technique to significantly enhance nonviral transfection of hMSCs that should enhance their clinical use, accelerate new research, and decrease reliance on early passage cells.

Kemp, K., et al. (2017). "Mesenchymal Stem Cell-Derived Factors Restore Function to Human Frataxin-Deficient Cells." Cerebellum **16**(4): 840-851.

Friedreich's ataxia is an inherited neurological disorder characterised by mitochondrial dysfunction and increased susceptibility to oxidative stress. At present, no therapy has been shown to reduce disease progression. Strategies being trialled to treat Friedreich's ataxia include drugs that improve mitochondrial function and reduce oxidative injury. In addition, stem cells have been investigated as a potential therapeutic approach. We have used siRNA-induced knockdown of frataxin in SH-SY5Y cells as an in vitro cellular model for Friedreich's ataxia. Knockdown of frataxin protein expression to levels detected in patients with the disorder was achieved, leading to decreased cellular viability, increased susceptibility to hydrogen peroxide-induced oxidative stress, dysregulation of key anti-oxidant molecules and deficiencies in both cell proliferation and differentiation. Bone marrow stem cells are being investigated extensively as potential treatments for a wide range of neurological disorders, including Friedreich's ataxia. The potential neuroprotective effects of bone marrow-derived mesenchymal stem cells were therefore studied using our frataxin-deficient cell model. Soluble factors secreted by mesenchymal stem cells protected against cellular changes induced by frataxin deficiency, leading to restoration in frataxin levels and anti-oxidant defences, improved survival against oxidative stress and stimulated both cell proliferation and differentiation down the Schwann cell lineage. The demonstration

that mesenchymal stem cell-derived factors can restore cellular homeostasis and function to frataxin-deficient cells further suggests that they may have potential therapeutic benefits for patients with Friedreich's ataxia.

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

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

Khodabandeh, Z., et al. (2017). "Hepatogenic Differentiation Capacity of Human Wharton's Jelly Mesenchymal Stem Cell in a Co-culturing System with Endothelial Cells in Matrigel/collagen Scaffold in the Presence of Fetal Liver Extract." Int J Stem Cells **10**(2): 218-226.

Background: Human Wharton's jelly mesenchymal stem cells (HWJMSCs) isolated from medical waste product can be considered as an accessible source of cells in regenerative medicine. Stem cell-derived hepatocytes have poor function and need appropriate niche to reconstruct the liver structure. Therefore, we attempted to find a novel approach in differentiating HWJMSCs into functional hepatic cells using 3D culture conditions and liver extract that recapitulates vital stage in liver development. Materials and Methods: HWJMSCs were extracted from human Wharton's jelly, characterized by flow cytometry, and differentiated towards osteogenic and adipogenic lineages. HWJMSCs were co-cultured with HUVECs in 3D matrigel/ collagen scaffolds in the presence of fetal liver extract for 14 days. The expression of specific liver genes were evaluated by lectins, PAS and immunocytochemistry. Results: According to flow cytometry data, isolated cells from HWJMSCs were shown to express MSC markers. HWJMSCs co-cultured with HUVECs in matrigel/collagen scaffold with extract expressed albumin, lectins UEA and PNA. Immunohistochemistry of the cells in matrigel/collagen scaffold with or without extract exhibited a positive reaction for CK19. Conclusions: Co-culturing of the HWJMSC/HUVEC in 3D matrigel/collagen scaffold is bimimicary of in vivo cell condition. The results showed that administration of the liver extract in 3D matrigel/collagen culture of HWJMSC/HUVEC can induce hepatocyte marker expression.

Kim, D. S., et al. (2014). "Gene expression profiles of human adipose tissue-derived mesenchymal stem cells are modified by cell culture density." PLoS One **9**(1): e83363.

Previous studies conducted cell expansion ex vivo using low initial plating densities for optimal expansion and subsequent differentiation of mesenchymal stem cells (MSCs). However, MSC populations are heterogeneous and culture conditions can affect the characteristics of MSCs. In this study, differences in gene expression profiles of adipose tissue (AT)-derived MSCs were examined after harvesting cells cultured at different densities. AT-MSCs from three different donors were plated at a density of 200 or 5,000 cells/cm (2). After 7 days in culture, detailed gene expression profiles were investigated using a DNA chip microarray, and subsequently validated using a reverse transcription polymerase chain reaction (RT-PCR) analysis. Gene expression profiles were influenced primarily by the level of cell confluence at harvest. In MSCs harvested at approximately 90% confluence, 177 genes were up-regulated and 102 genes down-regulated relative to cells harvested at approximately 50% confluence ($P<0.05$, $FC>2$). Proliferation-related genes were highly expressed in MSCs harvested at low density, while genes that were highly expressed in MSCs harvested at high density (approximately 90% confluent) were linked to immunity and defense, cell communication, signal transduction and cell motility. Several cytokine, chemokine and growth factor genes

involved in immunosuppression, migration, and reconstitution of damaged tissues were up-regulated in MSCs harvested at high density compared with MSCs harvested at low density. These results imply that cell density at harvest is a critical factor for modulating the specific gene-expression patterns of heterogeneous MSCs.

Kim, H. J. and J. S. Park (2017). "Usage of Human Mesenchymal Stem Cells in Cell-based Therapy: Advantages and Disadvantages." Dev Reprod **21**(1): 1-10.

The use of human mesenchymal stem cells (hMSCs) in cell-based therapy has attracted extensive interest in the field of regenerative medicine, and it shows applications to numerous incurable diseases. hMSCs show several superior properties for therapeutic use compared to other types of stem cells. Different cell types are discussed in terms of their advantages and disadvantages, with focus on the characteristics of hMSCs. hMSCs can proliferate readily and produce differentiated cells that can substitute for the targeted affected tissue. To maximize the therapeutic effects of hMSCs, a substantial number of these cells are essential, requiring extensive ex vivo cell expansion. However, hMSCs have a limited lifespan in an in vitro culture condition. The senescence of hMSCs is a double-edged sword from the viewpoint of clinical applications. Although their limited cell proliferation potency protects them from malignant transformation after transplantation, senescence can alter various cell functions including proliferation, differentiation, and migration, that are essential for their therapeutic efficacy. Numerous trials to overcome the limited lifespan of mesenchymal stem cells are discussed.

Koh, S. H., et al. (2005). "Co-culture of human CD34+ cells with mesenchymal stem cells increases the survival of CD34+ cells against the 5-aza-deoxycytidine- or trichostatin A-induced cell death." Biochem Biophys Res Commun **329**(3): 1039-1045.

It has been suggested that epigenetic regulation plays an important role in maintaining the stemness and lineage differentiation of hematopoietic stem cells (HSCs), 5-aza-deoxycytidine (aza-D) and Trichostatin A (TSA) being candidate additives for HSC ex vivo expansion. Although they have potent activity to maintain the stemness, they can also cause serious cell death. This study examined the effects of mesenchymal stem cells (MSCs) on the maintenance of CD34+ cells driven by aza-D and TSA in culture with the combined cytokines of thrombopoietin, flt-3 ligand, stem cell factor, interleukin-3, and interleukin-6. In cultures without MSCs, although aza-D and TSA retained the CD34 frequency 4 to 8 times more than in the cytokines alone, a large portion of cells underwent apoptotic cell death. Consequently, CD34+ cell expansion could not be achieved in any condition without MSCs. In cultures with MSCs, the total cell number was higher in aza-D or TSA than in any conditions in the cultures without MSCs. The CD34 frequency was also similar to the level in the cultures in aza-D or TSA without the MSCs. These results suggest that a co-culture of CD34+ cells with the MSCs might not simply deliver the proliferation signals but also stemness and survival signals, and overlap the action of epigenetic regulators.

Kohli, N., et al. (2015). "An In Vitro Comparison of the Incorporation, Growth, and Chondrogenic Potential of Human Bone Marrow versus Adipose Tissue Mesenchymal Stem Cells in Clinically Relevant Cell Scaffolds Used for Cartilage Repair." Cartilage **6**(4): 252-263.

AIM: To compare the incorporation, growth, and chondrogenic potential of bone marrow (BM) and adipose tissue (AT) mesenchymal stem cells (MSCs) in scaffolds used for cartilage repair. METHODS: Human BM and AT MSCs were isolated, culture expanded, and characterised using standard protocols, then seeded into 2 different scaffolds, Chondro-Gide or Alpha Chondro Shield. Cell adhesion, incorporation, and viable cell growth were assessed microscopically and following calcein AM/ethidium homodimer (Live/Dead) staining. Cell-seeded scaffolds were treated with chondrogenic inducers for 28 days. Extracellular matrix deposition and soluble glycosaminoglycan (GAG) release into the culture medium was measured at day 28 by histology/immunohistochemistry and dimethylmethylene blue assay, respectively. RESULTS: A greater number of viable MSCs from either source adhered and incorporated into Chondro-Gide than into Alpha Chondro Shield. In both cell scaffolds, this incorporation represented less than 2% of the cells that were seeded. There was a marked proliferation of BM MSCs, but not AT MSCs, in Chondro-Gide. MSCs from both sources underwent chondrogenic differentiation following induction. However, cartilaginous extracellular matrix deposition was most marked in Chondro-Gide seeded with BM MSCs. Soluble GAG secretion increased in chondrogenic versus control conditions. There was no marked difference in GAG secretion by MSCs from either cell source. CONCLUSION: Chondro-Gide and Alpha Chondro Shield were permissive to the incorporation and chondrogenic differentiation of human BM and AT MSCs. Chondro-Gide seeded with BM MSCs demonstrated the greatest increase in MSC number and deposition of a cartilaginous tissue.

Koltsova, A. M., et al. (2015). "[Characteristics of New Mesenchymal Stem Cell Line Derived from Human Embryonic Stem Cells]." Tsitologiia **57**(11): 761-770.

New nonimmortalized fibroblast-like cell line SC6-MSC has been obtained from a line of human embryonic stem cells (ESC)--SC6. Numerical and structural karyotypic analysis has shown hypodiploidy karyotypic: 45, X0 in this line. The average cell population doublings time, for SC6-MSC is 26.0 +/- 0.4 h at the 8th passage and 82.0 +/- 9.2 h at the 18th passage. The growth curves showed active proliferation for 8-10 passages with a consequent gradual decrease of proliferative activity, which ended to 20th passage. To determine the line's status, the analysis of the surface markers by flow cytometry was carried out. We have revealed the expression of surface antigens CD44, CD73, CD90, CD105 and HLA-ABC characteristic for human MSC, and the absence of CD34 and HLA-DR expression. However, the level of expression of surface markers CD90 and CD105 was significantly lower in comparison with other MSC lines including the line SC5-MSC derived from the line human ESC-SC5. Immunofluorescence analysis of the expression of the surface markers and transcription factor Oct-4 characteristic for human embryonic stem cells showed the absence of Oct-4 expression and the presence of SSEA-4 and TRA-1-60 expression, which is characteristic for a number of MSC lines with normal karyotype. Immunofluorescence analysis has shown the presence of the markers of early differentiation in the derivates of three germ layers, characteristic for human ESC, which in corresponding microenvironments may allow MSC to be useful for reparation of tissue injures. The directed osteogenic and chondrogenic differentiation of line SC6-MSC has shown. However, no directed adipogenic differentiation of this line has been found. The obtained results with high probability may indicate what alteration of chromosomal and, accordingly, gene balance, in line SC6-MSC with karyotype 45, X0 resulted in decrease in differential potential, in expression CD90, associated in particular with the processes of differentiation and aging of cells.

Komoda, H., et al. (2010). "Reduction of N-glycolylneuraminic acid xenoantigen on human adipose tissue-derived stromal cells/mesenchymal stem cells leads to safer and more useful cell sources for various stem cell therapies." Tissue Eng Part A **16**(4): 1143-1155.

Adipose tissue is an attractive source for somatic stem cell therapy. Currently, human adipose tissue-derived stromal cells/mesenchymal stem cells (hADSCs/MSCs) are cultured with fetal bovine serum (FBS). Recently, however, not only human embryonic stem cell lines cultured on mouse feeder cells but also bone marrow-derived human MSCs cultured with FBS were reported to express N-glycolylneuraminic acid (Neu5Gc) xenoantigen. Human serum contains high titers of natural preformed antibodies against Neu5Gc. We studied the presence of Neu5Gc on hADSCs/MSCs cultured with FBS and human immune response mediated by Neu5Gc. Our data indicated that hADSCs/MSCs cultured with FBS expressed Neu5Gc and that human natural preformed antibodies could bind to hADSCs/MSCs. However, hADSCs/MSCs express complement regulatory proteins such as CD46, CD55, and CD59 and are largely resistant to complement-mediated cytotoxicity. hADSCs/MSCs cultured with FBS could be injured by antibody-dependent cell-mediated cytotoxicity mechanism. Further, human monocyte-derived macrophages could phagocytose hADSCs/MSCs cultured with FBS and this phagocytic activity was increased in the presence of human serum. Culturing hADSCs/MSCs with heat-inactivated human serum for a week could markedly reduce Neu5Gc on hADSCs/MSCs and prevent immune responses mediated by Neu5Gc, such as binding of human natural preformed antibodies, antibody-dependent cell-mediated cytotoxicity, and phagocytosis. Adipogenic and osteogenic differentiation potentials of hADSCs/MSCs cultured with heat-inactivated human serum were not less than that of those cultured with FBS. For stem cell therapies based on hADSCs/MSCs, hADSCs/MSCs that presented Neu5Gc on their cell surfaces after exposure to FBS should be cleaned up to be rescued from xenogeneic rejection.

Kong, Y., et al. (2014). "Sphingosine-1-phosphate/S1P receptors signaling modulates cell migration in human bone marrow-derived mesenchymal stem cells." Mediators Inflamm **2014**: 565369.

The recruitment of bone marrow-derived mesenchymal stem cells (BMSCs) to damaged tissues and sites of inflammation is an essential step for clinical therapy. However, the signals regulating the motility of these cells are still not fully understood. Sphingosine-1-phosphate (S1P), a bioactive sphingolipid metabolite, is known to have a variety of biological effects on various cells. Here, we investigated the roles of S1P and S1P receptors (S1PRs) in migration of human BMSCs. We found that S1P exerted a powerful migratory action on human BMSCs. Moreover, by employing RNA interference technology and pharmacological tools, we demonstrated that S1PR1 and S1PR3 are responsible for S1P-induced migration of human BMSCs. In contrast, S1PR2 mediates the inhibition of migration. Additionally, we explored the downstream signaling

pathway of the S1P/S1PRs axis and found that activation of S1PR1 or S1PR3 increased migration of human BMSCs through a G i /extracellular regulated protein kinases 1/2- (ERK1/2-) dependent pathway, whereas activation of S1PR2 decreased migration through the Rho/Rho-associated protein kinase (ROCK) pathway. In conclusion, we reveal that the S1P/S1PRs signaling axis regulates the migration of human BMSCs via a dual-directional mechanism. Thus, selective modulation of S1PR's activity on human BMSCs may provide an effective approach to immunotherapy or tissue regeneration.

Kono, K., et al. (2015). "Characterization of the cell growth analysis for detection of immortal cellular impurities in human mesenchymal stem cells." Biologicals **43**(2): 146-149.

The analysis of in vitro cell senescence/growth after serial passaging can be one of ways to show the absence of immortalized cells, which are frequently tumorigenic, in human cell-processed therapeutic products (hCTPs). However, the performance of the cell growth analysis for detection of the immortalized cellular impurities has never been evaluated. In the present study, we examined the growth rates of human mesenchymal stem cells (hMSCs, passage 5 (P = 5)) contaminated with various doses of HeLa cells, and compared with that of hMSCs alone. The growth rates of the contaminated hMSCs were comparable to that of hMSCs alone at P = 5, but significantly increased at P = 6 (0.1% and 0.01% HeLa) or P = 7 (0.001% HeLa) within 30 days. These findings suggest that the cell growth analysis is a simple and sensitive method to detect immortalized cellular impurities in hCTPs derived from human somatic cells.

Kostiuk, S. V., et al. (2012). "[Cell-free DNA fragments increase transcription in human mesenchymal stem cells, activate TLR-dependent signal pathway and supress apoptosis]." Biomed Khim **58**(6): 673-683.

Human mesenchymal stem cells (MSCs) are now widely adopted in regenerative medicine. However, many questions on the role of different signaling pathways in the regulation of stem cell (SC) functional activity within the organism remain unaswered. In damaged regions the level of cell death increases and DNA fragments from dead cells (cell-free DNA, cfDNA) are accumulated in blood. We showed that in adipose-derived MSCs exposed in vitro to cfDNA fragments the transcription level increased (the total amount of cellular RNA and the rRNA amount rose). GC-rich CfDNA fragments (GC-DNA) activated the TLR9-dependent signal pathway: the expression of TLR9 and of TLR9-signaling pathway adapter--MyD88--was up-regulated. AT-rich DNA fragments did not increase the TLR9 expression, though, the MyD88 expression level rose. So we suggest that AT-DNA acts via some other receptors that nevertheless activate MyD88-dependent signalling in MSCs. We also showed that cfDNA fragments decreased the activity of caspase, an apoptotic enzyme. So, ctDNA can significantly influence the functional activity ofMSC by activating TLR9- and MyD88-dependent signal pathways and lowering the apoptosis level.

Kouroupis, D., et al. (2014). "The assessment of CD146-based cell sorting and telomere length analysis for establishing the identity of mesenchymal stem cells in human umbilical cord." F1000Res **3**: 126.

Adult stem cells are characterised by longer telomeres compared to mature cells from the same tissue. In this study, candidate CD146 (+) umbilical cord (UC) mesenchymal stem cells (MSCs) were purified by cell sorting from UC tissue digests and their telomere lengths were measured in comparison to donor-matched CD146-negative fraction. UC tissue fragments were enzymatically treated with collagenase and the cells were used for cell sorting, colony-forming fibroblast (CFU-F) assay or for long-term MSC cultivation. Telomere lengths were measured by qPCR in both culture-expanded MSCs and candidate native UC MSCs. Immunohistochemistry was undertaken to study the topography of CD146 (+) cells. Culture-expanded UC MSCs had a stable expression of CD73, CD90 and CD105, whereas CD146 declined in later passages which correlated with the shortening of telomeres in the same cultures. In five out of seven donors, telomeres in candidate native UC MSCs (CD45 (-)CD235alpha (-)CD31 (-)CD146 (+)) were longer compared to donor-matched CD146 (-) population (CD45 (-)CD235alpha (-)CD31 (-)CD146 (-)). The frequency of CD45 (-)CD235alpha (-)CD31 (-)CD146 (+) cells measured by flow cytometry was ~1000-fold above that of CFU-Fs (means 10.4% and 0.01%, respectively). CD146 (+) cells were also abundant in situ having a broad topography including high levels of positivity in muscle areas in addition to vessels. Although qPCR-based telomere length analysis in sorted populations could be limited in its sensitivity, very high frequency of CD146 (+) cells in UC tissue suggests that CD146 expression alone is unlikely to be sufficient to identify and purify native MSCs from the UC tissue.

Kronsteiner, B., et al. (2011). "Human mesenchymal stem cells and renal tubular epithelial cells differentially influence monocyte-derived dendritic cell differentiation and maturation." Cell Immunol **267**(1): 30-38.

Mesenchymal stem cells (MSCs) possess immunosuppressive properties. But also fully

differentiated human renal tubular epithelial cells (RTECs) are able to modulate T-cell proliferation in vitro. In this study we compared two MSC populations, human adipose derived stem cells (ASCs) and human amniotic mesenchymal stromal cells (hAMSCs), and RTECs regarding their potential to inhibit monocyte-derived dendritic cell (DC) differentiation and maturation in indirect co-culture. In the presence of hAMSCs and RTECs, monocytes stimulated to undergo DC differentiation were inhibited to acquire surface phenotype of immature and mature DCs. In contrast, ASCs showed only limited suppressive capacity. Secretion of IL-12p70 was suppressed in hAMSC co-cultures and high IL-10 levels were detected in all co-cultures. Prostaglandin E (2) was found in ASC and hAMSC co-cultures, whereas soluble human leukocyte antigen-G was highly elevated only in RTEC co-cultures. Thus, inhibition of DC generation by MSCs and RTECs might be mediated by different soluble factors.

Krylova, T. A., et al. (2012). "[Comparative characteristics of new mesenchymal stem cell lines derived from human embryonic stem cells, bone marrow and foreskin]." Tsitologiia **54**(1): 5-16.

New nonimmortalized fibroblast-like cell lines SC5-MSC and SC3a-MSC, FetMSC, FRSN were obtained from human embryonic stem cells (ESC), bone marrow of a 5-6-days embryo and foreskin of a 3-years-old boy, respectively. All the lines are successfully used as the feeder at human ESC cultivation. It is determined that the average cell population doublings time varies from 25.5 h for ISC5-MSC to 38.8 h for SC3a-MSC. Active proliferation of all the lines is also shown by the corresponding growth curves. Numerical and structural karyotypic analysis showed that these lines had normal karyotype: 46,XX (SC5-MSC and SC3a-MSC) and 46,XY (FetMSC and FRSN). To determine the status of the lines, their cell surface markers were analyzed by flow cytometry. This analysis revealed the presence of surface antigens CD44, CD73, CD90, CD105 and HLA-ABC, characteristic of human MSC, and the absence of CD34 and HLA-DR. Different lines were found to express CD117(c-kit) to a different level. Immunofluorescence and flow cytometry analysis did not detect TRA-1-60 and Oct-4, characteristic of human embryonic stem cells, and revealed interlinear variations in the level of SSEA, which did not depend on the cell origin. It is not clear yet whether these interlinear variations affect functional MSC status. In all the lines, immunofluorescence analysis showed the presence of the markers of early differentiation in the derivates of three germ layers which may allow MSC to be useful, in corresponding microenvironments, for reparation of tissue injures. Adipogenic and osteogenic differentiatiation of all cell lines has been shown.

Kuhn, L. T., et al. (2014). "Developmental-like bone regeneration by human embryonic stem cell-derived mesenchymal cells." Tissue Eng Part A **20**(1-2): 365-377.

The in vivo osteogenesis potential of mesenchymal-like cells derived from human embryonic stem cells (hESC-MCs) was evaluated in vivo by implantation on collagen/hydroxyapatite scaffolds into calvarial defects in immunodeficient mice. This study is novel because no osteogenic or chondrogenic differentiation protocols were applied to the cells prior to implantation. After 6 weeks, X-ray, microCT, and histological analysis showed that the hESC-MCs had consistently formed a highly vascularized new bone that bridged the bone defect and seamlessly integrated with host bone. The implanted hESC-MCs differentiated in situ to functional hypertrophic chondrocytes, osteoblasts, and osteocytes forming new bone tissue via an endochondral ossification pathway. Evidence for the direct participation of the human cells in bone morphogenesis was verified by two separate assays: with Alu and by human mitochondrial antigen positive staining in conjunction with co-localized expression of human bone sialoprotein in histologically verified regions of new bone. The large volume of new bone in a calvarial defect and the direct participation of the hESC-MCs far exceeds that of previous studies and that of the control adult hMSCs. This study represents a key step forward for bone tissue engineering because of the large volume, vascularity, and reproducibility of new bone formation and the discovery that it is advantageous to not over-commit these progenitor cells to a particular lineage prior to implantation. The hESC-MCs were able to recapitulate the mesenchymal developmental pathway and were able to repair the bone defect semi-autonomously without preimplantation differentiation to osteo- or chondroprogenitors.

Kuo, C. H., et al. (2014). "17beta-estradiol inhibits mesenchymal stem cells-induced human AGS gastric cancer cell mobility via suppression of CCL5-Src/Cas/Paxillin signaling pathway." Int J Med Sci **11**(1): 7-16.

Gender differences in terms of mortality among many solid organ malignancies have been proved by epidemiological data. Estrogen has been suspected to cast a protective effect against cancer because of the lower mortality of gastric cancer in females and the benefits of hormone replacement therapy (HRT) in gastric cancer. Hence, it suggests that 17beta-estradiol (E2) may affect the behavior of cancer cells. One of

the key features of cancer-related mortality is metastasis. Accumulating evidences suggest that human bone marrow mesenchymal stem cells (HBMMSCs) and its secreted CCL-5 have a role in enhancing the metastatic potential of breast cancer cells. However, it is not clear whether E2 would affect HBMMSCs-induced mobility in gastric cancer cells. In this report, we show that CCL-5 secreted by HBMMSCs enhanced mobility in human AGS gastric cancer cells via activation of Src/Cas/Paxillin signaling pathway. Treatment with specific neutralizing antibody of CCL-5 significantly inhibited HBMMSCs-enhanced mobility in human AGS gastric cancer cells. We further observe that 17beta-estradiol suppressed HBMMSCs-enhanced mobility by down-regulating CCL5-Src/Cas/paxillin signaling pathway in AGS cells. Collectively, these results suggest that 17beta-estradiol treatment significantly inhibits HBMMSCS-induced mobility in human AGS gastric cancer cells.

Kupcsik, L., et al. (2009). "Statin-induced calcification in human mesenchymal stem cells is cell death related." J Cell Mol Med **13**(11-12): 4465-4473.

Statins are widely used in clinics to lower cholesterol levels. Recently, they have been shown to positively affect bone formation and bone mass in a rat model. The aim of this study was to investigate the effect of pravastatin, simvastatin and lovastatin on the osteoblastic differentiation of human mesenchymal stem cells (MSCs) in vitro. Cell number, alkaline phosphatase (ALP) activity, matrix mineralization and gene expression pattern were determined. Pravastatin did not affect cell differentiation. Simvastatin and lovastatin enhanced bone morphogenetic protein 2 (BMP-2) mRNA levels. In contrast, ALP activity and mRNA levels were suppressed by statins, as well as the DNA content and cell activity (MTT). An increase in apoptotic events was observed at high concentrations of statins, along with high Ca-45 incorporation. Lower concentrations of statins did not increase apoptotic staining, but also failed to induce calcification. When statin-induced calcification did occur, the morphology of the deposits was very different from the conventional nodule formation; the calcium was laid down along the membranes of the rounded cells suggesting it was as a result of cell death. Our results indicate that statins are not able to differentiate human MSCs into osteoblasts and that high concentrations of statins (>1 microM) have a cytotoxic effect.

Kurogi, R., et al. (2018). "Inhibition of glioblastoma cell invasion by hsa-miR-145-5p and hsa-miR-31-5p co-overexpression in human mesenchymal stem cells." J Neurosurg: 1-12.

OBJECTIVE Human bone marrow-derived mesenchymal stem cells (hMSCs) show tropism for brain tumors and may be a useful vehicle for drug or gene delivery to malignant gliomas. Recently, some microRNAs (miRNAs) have been shown to suppress the invasiveness of malignant gliomas. METHODS To test their potential to become vehicles for the delivery of miRNA to malignant gliomas, hMSCs were engineered so that hMSC secretion of miRNAs that inhibit glioma cell invasion was enabled without altering the hMSC tropism for glioma cells. RESULTS In coculture, hMSCs cotransfected with hsa-miR-145-5p and -31-5p miRNAs showed markedly reduced invasion by U87 glioma cells in a contact-dependent manner both in vitro and ex vivo, with invasion of hMSCs cotransfected with these 2 miRNAs by the U87 cells reduced to 60.7% compared with control cells. According to a Matrigel invasion assay, the tropism of the hMSCs for U87 cells was not affected. In glioma cell lines U251 and LN229, hMSCs exhibited tropism in vivo, and invasion of hMSCs cotransfected with hsa-miR-145-5p and -31-5p was also significantly less than that of control cells. When U87 cells were coimplanted into the striatum of organotypic rat brain slices with hMSCs cotransfected with hsa-miR-145 and -31-5p, the relative invasive area decreased by 37.1%; interestingly, these U87 cells showed a change to a rounded morphology that was apparent at the invasion front. Whole-genome microarray analysis of the expression levels of 58,341 genes revealed that the co-overexpression of hsa-miR-145-5p and -31-5p downregulated FSCN1 expression in U87 cells. CONCLUSIONS This study demonstrates that miRNA overexpression in hMSCs can alter the function of glioma cells via contact-dependent transfer. Co-overexpression of multiple miRNAs may be a useful and novel therapeutic strategy. The study results suggest that hMSCs can be applied as a delivery vehicle for miRNAs.

Kwon, S. H., et al. (2013). "Modulation of BMP-2-induced chondrogenic versus osteogenic differentiation of human mesenchymal stem cells by cell-specific extracellular matrices." Tissue Eng Part A **19**(1-2): 49-58.

Bone morphogenetic protein-2 (BMP-2) is known to induce both osteogenic and chondrogenic commitment of human mesenchymal stem cells (hMSCs). However, factors influencing BMP-2-dependent chondrogenic and osteogenic differentiation have not been investigated. In this study, we demonstrated that extracellular microenvironments, in the form of cell-derived matrices, play important roles in determining the specific lineage commitment of hMSCs in the presence of BMP-2. Extracellular matrices (ECMs) derived from osteoblasts and

chondrocytes were utilized to regulate cell differentiation. Osteogenic and chondrogenic differentiation of hMSCs cultured on the two different cell-derived ECMs were assessed by quantitative real-time-polymerase chain reaction, immunocytochemistry, and western blot analysis. To minimize the effects of the cell-adhesion proteins contained in serum on the ECMs, hMSCs were cultured in serum-free osteogenic or chondrogenic differentiation medium. Fibronectin-, collagen type I-, or collagen type II-coated substrates were utilized as ECM controls. The ECM specific to each cell type promoted lineage-specific commitment of hMSCs in the presence of BMP-2, that is, osteoblast- and chondrocyte-derived ECM promoted osteogenic and chondrogenic commitment, respectively. Therefore, cell-specific ECMs are capable of modulating the BMP-2-induced osteogenic and chondrogenic differentiation of hMSCs.

Lavery, K., et al. (2008). "BMP-2/4 and BMP-6/7 differentially utilize cell surface receptors to induce osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells." J Biol Chem **283**(30): 20948-20958.

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-beta superfamily of growth factors and are used clinically to induce new bone formation. The purpose of this study was to evaluate receptor utilization by BMP-2, BMP-4, BMP-6, and BMP-7 in primary human mesenchymal stem cells (hMSC), a physiologically relevant cell type that probably mediates the in vivo effects of BMPs. RNA interference-mediated gene knockdown revealed that osteoinductive BMP activities in hMSC are elicited through the type I receptors ACVR1A and BMPR1A and the type II receptors ACVR2A and BMPR2. BMPR1B and ACVR2B were expressed at low levels and were not found to play a significant role in signaling by any of the BMPs evaluated in this study. Type II receptor utilization differed significantly between BMP-2/4 and BMP-6/7. A greater reliance on BMPR2 was observed for BMP-2/4 relative to BMP-6/7, whereas ACVR2A was more critical to signaling by BMP-6/7 than BMP-2/4. Significant differences were also observed for the type I receptors. Although BMP-2/4 used predominantly BMPR1A for signaling, ACVR1A was the preferred type I receptor for BMP-6/7. Signaling by both BMP-2/4 and BMP-6/7 was mediated by homodimers of ACVR1A or BMPR1A. A portion of BMP-2/4 signaling also required concurrent BMPR1A and ACVR1A expression, suggesting that BMP-2/4 signal in part through ACVR1A/BMPR1A heterodimers. The capacity of ACVR1A and BMPR1A to form homodimers and heterodimers was confirmed by bioluminescence resonance energy transfer analyses. These results suggest different mechanisms for BMP-2/4- and BMP-6/7-induced osteoblastic differentiation in primary hMSC.

Le Blanc, K. and O. Ringden (2005). "Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation." Biol Blood Marrow Transplant **11**(5): 321-334.

Mesenchymal stem cells (MSCs) may be derived from adult bone marrow, fat, and several fetal tissues. In vitro, MSCs can be expanded and have the capacity to differentiate into several mesenchymal tissues, such as bone, cartilage, and fat. They escape the immune system in vitro, and this may make them candidates for cellular therapy in an allogeneic setting. They also have immunomodulatory effects, inhibit T-cell proliferation in mixed lymphocyte cultures, prolong skin allograft survival, and may decrease graft-versus-host disease (GVHD) when cotransplanted with hematopoietic stem cells. MSCs induce their immunosuppressive effect via a soluble factor. Some candidates have been suggested, and various mechanisms have also been suggested, although contradictory data exist; this may be due to differences in the cells and systems tested. A major problem has been that it has been difficult to identify and isolate MSCs after transplantation in vivo. However, MSCs seem to enhance hematopoietic engraftment in recipients of autologous and allogeneic grafts. Recently, they were found to reverse grade IV acute GVHD of the gut and liver. No tolerance was induced, however. Controlled studies are warranted. Thus, in allogeneic stem cell transplantation, MSCs may be used for hematopoiesis enhancement, as GVHD prophylaxis, and for the treatment of severe acute GVHD. They are also of potential use in the treatment of organ transplant rejection and in autoimmune inflammatory bowel disorders where immunomodulation and tissue repair are needed.

LeBlon, C. E., et al. (2015). "Correlation between in vitro expansion-related cell stiffening and differentiation potential of human mesenchymal stem cells." Differentiation **90**(1-3): 1-15.

Human mesenchymal stem cells (hMSCs) are an attractive cell source for tissue regeneration, given their self-renewal and multilineage potential. However, they are present in only small percentages in human bone marrow, and are generally propagated in vitro prior to downstream use. Previous work has shown that hMSC propagation can lead to alterations in cell behavior and differentiation potency, yet optimization of differentiation based on starting cell elastic modulus is an area still under investigation. To further advance

the knowledge in this field, hMSCs were cultured and routinely passaged on tissue-culture polystyrene to investigate the correlation between cell stiffening and differentiation potency during in vitro aging. Local cell elastic modulus was measured at every passage using atomic force microscopy indentation. At each passage, cells were induced to differentiate down myogenic and osteogenic paths. Cells induced to differentiate, as well as undifferentiated cells were assessed for gene and protein expression using quantitative polymerase chain reaction and immunofluorescent staining, respectively, for osteogenic and myogenic markers. Myogenic and osteogenic cell potential are highly reliant on the elastic modulus of the starting cell population (of undifferentiated cells), and this potential appears to peak when the innate cell elastic modulus is close to that of differentiated tissue. However, the latent expression of the same markers in undifferentiated cells also appears to undergo a correlative relationship with cell elastic modulus, indicating some endogenous effects of cell elastic modulus and gene/protein expression. Overall, this study correlates age-related changes with regards to innate cell stiffening and gene/protein expression in commercial hMSCs, providing some guidance as to maintenance and future use of hMSCs in future tissue engineering applications.

Lee, C. W., et al. (2018). "Improvement of Cell Cycle Lifespan and Genetic Damage Susceptibility of Human Mesenchymal Stem Cells by Hypoxic Priming." Int J Stem Cells **11**(1): 61-67.

Hypoxic culture is widely recognized as a method to efficiently expand human mesenchymal stem cells (MSCs) without loss of stem cell properties. However, the molecular basis of how hypoxia priming benefits MSC expansion remains unclear. We report that hypoxic priming markedly extends the cell cycle lifespan rather than augmenting the multipotency of MSC differentiation lineage. Hypoxic priming does not affect to chromosome damage but significantly attenuates the susceptibility of chromosome damage. Our results provide important evidence that multipotency of human MSCs by hypoxic priming is determined by cell cycle lifespan.

Lee, D. H., et al. (2006). "Chemotactic migration of human mesenchymal stem cells and MC3T3-E1 osteoblast-like cells induced by COS-7 cell line expressing rhBMP-7." Tissue Eng **12**(6): 1577-1586.

During bone development, remodeling, and repair, bone morphogenetic proteins (BMPs) induce the differentiation of mesenchymal progenitor cells (MPCs) that enter into the osteoblastic lineage, and enhance the recruitment of MPCs and osteogenic cells. The process of migration is believed to be regulated, in part, by growth factors stored within the bone matrix, which are released by bone resorption. In this study, primary human mesenchymal stem cells (hMSCs) and MC3T3-E1 osteoblasts were examined for chemotaxis in response to recombinant human BMP-7 (rhBMP-7) produced in COS-7 cells (co-culture system). In order to produce BMP-7 transfected cells (BTCs), which serve as suppliers of rhBMP-7 under in vitro culture conditions, the encoding DNA was transferred into the pTARGET expression vector and introduced into COS-7 cells by conventional genetic engineering techniques. In cell culture studies, the rhBMP-7 produced in BTCs stimulated the specific activity of ALP, the production of cAMP in response to PTH, and the synthesis of osteocalcin. Migration assays were conducted with a computer-aided time-lapse video-microscopy system, to allow the rapid and precise analysis of cell migration and for the dynamic measurement of cell position and morphology. The migration distance and speed of the MC3T3-E1 cells, or hMSCs, co-cultured with BTCs, using a band-type seeding method, were significantly increased ($p < 0.001$), compared to those of the MC3T3-E1 cells (or hMSCs) only. In conclusion, these studies revealed that rhBMP-7 plays a role in the migration of bone-forming cells, and that the co-culture model (co-culture of bone-forming cells with BMP-7-producing cells) using a computer-aided, time-lapse video-microscopy system, is useful for the chemotactic migration assay of other chemotactic growth factors.

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

Human embryonic stem (hES) cells have been successfully maintained using human-cell feeder systems or feeder-free systems. However, despite advances in culture techniques, hES cells require supplementation with fibroblast growth factor 2 (FGF-2), an exogenous stemness factor, which is needed to sustain the authentic undifferentiated status. We developed a new culture system for hES cells; this system does not require supplementation with FGF-2 to obtain hES cells that are suitable for tissue engineering and regenerative medicine. This culture system employed mesenchymal stem cells derived from hES cells (hESC-MSCs) as autologous human feeder cells in the absence of FGF-2. The hES cell line SNUhES3 cultured in this new autologous feeder culture system maintained the typical morphology of hES cells and expression of pluripotency-related proteins, SSEA-4, TRA-1-60, OCT4, and alkaline phosphatase, without development of abnormal karyotypes after more than 30 passages. RNA

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

Lee, H. R., et al. (2014). "Effects of octylphenol on the expression of cell cycle-related genes and the growth of mesenchymal stem cells derived from human umbilical cord blood." Int J Mol Med **33**(1): 221-226.

Umbilical cord blood (UCB) is defined as blood that exists in the placenta and in the attached umbilical cord following childbirth. Cord blood is now used for research purposes as it contains mesenchymal stem cells (MSCs), multipotent stromal cells which have the ability to differentiate into a variety of cell types. Among endocrine disrupting chemicals (EDCs), octylphenol (OP) is one of the alkylphenols, which are widely used industrial chemicals; these chemicals cause a number of serious side-effects, such as reproductive abnormalities. In this study, we isolated human MSCs from UCB and demonstrate that cultured MSCs express the surface marker, CD34, but not CD105. We further examined the effects of OP on human UCB-derived MSCs following exposure to OP by cell proliferation assay, semi-quantitative RT-PCR and western blot analysis. The results revealed that the transcriptional and translational levels of cyclin D1 were increased, while the levels of p21 were suppressed in the MSCs treated with OP compared with the negative controls. This collapse of the regulation of the cell cycle may directly stimulate the growth of the MSCs under culture conditions. The results from the present study provide further insight into the effects of common EDCs on MSCs derived from human UCB. However, further studies are required to identify the signaling pathways which mediate the effects of EDCs on MSCs.

Lee, J., et al. (2014). "Controlling cell geometry on substrates of variable stiffness can tune the degree of osteogenesis in human mesenchymal stem cells." J Mech Behav Biomed Mater **38**: 209-218.

The physical properties of the extracellular matrix (ECM) play an important role in regulating tissue-specific human mesenchymal stem cell (MSC) differentiation. Protein-coated hydrogels with tunable stiffness have been shown to influence lineage specific gene expression in MSCs. In addition, the control of cell shape - either through changing substrate stiffness or restricting spreading with micropatterning - has proved to be important in guiding the differentiation of MSCs. However, few studies have explored the interplay between these physical cues during MSC lineage specification. Here, we demonstrate geometric control of osteogenesis in MSCs cultured on micropatterned polyacrylamide gels. Cells cultured on fibronectin-coated gels express markers associated with osteogenesis in a stiffness dependent fashion with a maximum at ~30kPa. Controlling the geometry of single cells across the substrate demonstrates elevated osteogenesis when cells are confined to shapes that promote increased cytoskeletal tension. Patterning MSCs across hydrogels of variable stiffness will enable the exploration of the interplay between these physical cues and their relationship with the mechanochemical signals that guide stem cell fate decisions.

Lee, J. H., et al. (2011). "Schwann cell-like remyelination following transplantation of human umbilical cord blood (hUCB)-derived mesenchymal stem cells in dogs with acute spinal cord injury." J Neurol Sci **300**(1-2): 86-96.

Human umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs) have significant therapeutic potential in cell-based therapies following spinal cord injury (SCI). To evaluate this potential, we conducted our preliminary investigations on the remyelination of injured spinal cords with hUCB-MSC transplantations and we observed its long term effects on dogs with SCI. Of the ten injured dogs, seven were transplanted with hUCB-MSCs 1 week after SCI, whereas the remaining three dogs were not transplanted. Two transplanted dogs died over the first month after transplantation because of urinary tract infection, bedsores and sepsis. The SCI dogs showed no improvement in motor and sensory functions and their urinary dysfunction persisted until they were euthanized (from 3 months to 1 year) while hind-limb recovery in 4 dogs among the five transplanted dogs was significantly improved. In the recovered dogs, functional recovery was sustained for three years following transplantation. Histological results from five transplanted dogs showed that many axons were remyelinated by P0-positive myelin sheaths after transplantation. Our results suggest that transplantation of hUCB-derived MSCs may have beneficial therapeutic effects. Furthermore, histological results

provided the first in vivo evidence that hUCB-MSCs are able to enhance the remyelination of peripheral-type myelin sheaths following SCI.

Lotfinia, M., et al. (2016). "Effect of Secreted Molecules of Human Embryonic Stem Cell-Derived Mesenchymal Stem Cells on Acute Hepatic Failure Model." Stem Cells Dev **25**(24): 1898-1908.

Adult tissue-derived mesenchymal stem cells (MSCs) show tremendous promise for a wide array of therapeutic applications predominantly through paracrine activity. Recent reports showed that human embryonic stem cell (ESC)-derived MSCs are an alternative for regenerative cellular therapy due to manufacturing large quantities of MSCs from a single donor. However, no study has been reported to uncover the secretome of human ESC-MSCs as treatment of an acute liver failure (ALF) mouse model. We demonstrated that human ESC-MSCs showed similar morphology and cell surface markers compared with bone marrow-derived MSCs. ESC-MSCs exhibited a higher growth rate during early in vitro expansion, along with adipogenic and osteogenic differentiation potential. Treatment with ESC-MSC-conditioned medium (CM) led to statistically significant enhancement of primary hepatocyte viability and increased immunomodulatory interleukin-10 secretion from lipopolysaccharide-induced human blood mononuclear cells. Analysis of the MSCs secretome by a protein array screen showed an association between higher frequencies of secretory proteins such as vascular endothelial growth factor (VEGF) and regulation of cell proliferation, cell migration, the development process, immune system process, and apoptosis. In this thioacetamide-induced mouse model of acute liver injury, we observed that systemic infusion of VEGF led to significant survival. These data have provided the first experimental evidence of the therapeutic potential of human ESC-MSC-derived molecules. These molecules show trophic support to hepatocytes, which potentially creates new avenues for the treatment of ALF, as an inflammatory condition.

Lozito, T. P., et al. (2009). "Human mesenchymal stem cells express vascular cell phenotypes upon interaction with endothelial cell matrix." J Cell Biochem **107**(4): 714-722.

Mesenchymal stem cells (MSCs) are thought to occupy a perivascular niche where they are exposed to signals originating from vascular cells. This study focused on the effects of endothelial cell (EC)-derived signals on MSC differentiation toward vascular cell lineages. Upon co-culture with two types of ECs, macrovascular (macro) ECs and microvascular (micro) ECs, the former caused MSCs to increase expression of both EC and smooth muscle cell (SMC) markers, while the latter induced expression of EC markers only. These marker changes in MSCs were linked to the extracellular matrixes secreted by the ECs (EC-matrix) rather than soluble EC-secreted factors. Beyond enhanced marker expression, EC-matrix also induced functional changes in MSCs indicative of development of a genuine vascular cell phenotype. These included enhanced incorporation into vessels and cytoskeletal localization of vascular SMC-specific contractile elements. The bioactivity of EC-matrix was sensitive to EDTA washes and required sulfated glycosaminoglycans. However, neither soluble VEGF nor substrate surfaces coated with fibronectin, collagen type IV, or laminin recreated the effects of EC-matrix on MSC vascular differentiation. In conclusion, these results identified EC-matrix as a critical regulator of vascular cell differentiation of MSCs. Elucidating these MSC-EC-matrix interactions and identifying the specific EC-matrix components involved will shed light on the perivascular signals seen by MSCs in vivo.

Lu, Z. Y., et al. (2016). "TNF-alpha enhances vascular cell adhesion molecule-1 expression in human bone marrow mesenchymal stem cells via the NF-kappaB, ERK and JNK signaling pathways." Mol Med Rep **14**(1): 643-648.

The migration of circulating mesenchymal stem cells (MSCs) to injured tissue is an important step in tissue regeneration and requires adhesion to the microvascular endothelium. The current study investigated the underlying mechanism of MSC adhesion to endothelial cells during inflammation. In in vitro MSC culture, tumor necrosis factoralpha (TNFalpha) increased the level of vascular cell adhesion molecule1 (VCAM1) expression in a dosedependent manner. The nuclear factor-kappaB (NF-kappaB), extracellular signalregulated kinase (ERK) and cJun Nterminal kinase (JNK) signaling pathway inhibitors, pyrrolidine dithiocarbamate (PDTC), U0126 and SP600125, respectively, suppressed VCAM1 expression induced by TNFalpha at the mRNA and protein levels ($P<0.05$). TNFalpha augmented the activation of NFkappaB, ERK and JNK, and promoted MSC adhesion to human umbilical vein endothelial cells; however, the inhibitors of NFkappaB, ERK and JNK did not affect this process in these cells. The results of the current study indicate that adhesion of circulating MSCs to the endothelium is regulated by TNF-alpha-induced VCAM-1 expression, which is potentially mediated by the NFkappaB, ERK and JNK signaling pathways.

Luan, X., et al. (2013). "Human placenta-derived mesenchymal stem cells suppress T cell proliferation and support the culture expansion of cord blood

CD34(+) cells: a comparison with human bone marrow-derived mesenchymal stem cells." Tissue Cell **45**(1): 32-38.

Human placenta-derived mesenchymal stem cells (hPMSCs) have been shown to possess immunosuppressive effects against T cells and support the expansion of hematopoietic stem/progenitor cells (HSPCs) from umbilical cord blood (UCB). However, the characteristics of hPMSCs compared with human bone marrow-derived mesenchymal stem cells (hBMSCs) are not fully understood. Here, we show that hPMSCs have similar regulatory effects on T cell activation, proliferation and cytokine secretion as hBMSCs and demonstrate that PDL1 and B7H4, negative co-stimulatory molecules, are involved in the T cell immunosuppressive activities of hPMSCs and hBMSCs, respectively. hPMSCs efficiently enhanced the expansion of CD34(+) cells from UCB compared with hBMSCs. Furthermore, hPMSCs maintained the expression of adhesion molecules (CD11a, CD44 and CD49e) in CD34(+) cells. Similar effects were observed for both hPMSCs and hBMSCs on CD34(+) cell chemotaxis and cytokine production, such as SDF-1alpha, IL-6 and SCF. Therefore, hPMSCs may be an ideal alternative source of hBMSCs for basic research and clinical applications, which may be significant in future efforts to explore the potential clinical utility of hPMSCs.

Luan, X. Y. and X. B. Liu (2010). "[Comparison the inhibitory effects of human bone marrow mesenchymal stem cells and human placenta mesenchymal stem cells on T cell proliferation]." Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi **26**(9): 849-851.

AIM: To compare and study the inhibitory effects of human bone marrow mesenchymal stem cells (HBMSCs) and human palacenta mesenchymal stem cells (HPMSCs) on T cell proliferation, and the underlying mechanism. METHODS: The expression of B7H4 on HBMSCs or the expression of PDL1 on HPMSCs were detected by FCM. Blocking experiment was used to analyze the effects of B7H4 or PDL1 on HBMSCs or HPMSCs mediating suppression on T cell proliferation and cell cycle. RESULTS: FCM detection showed that HBMSCs highly expressed B7H4, while HPMSCs highly expressed PDL1, the negative immune molecules. Blockade B7H4 on HBMSCs with B7H4mAb significantly attenuated the inhibitory effects of HBMSCs on T cell proliferation. Likewise, blocking the expression of PDL1 on HPMSCs obviously weakened the suppressive effects of HPMSCs on T cell proliferation activated by PHA. Moreover, Blockade B7H4 on HBMSCs with B7H4mAb or PDL1 on HPMSCs with PDL1mAb significantly weakened the inhibitory effects of HBMSCs or HPMSCs on T cell cycle through down-regulating the cell number in G (0);/G (1); phase and up-regulating the cell number in S phase. CONCLUSION: HBMSCs and HPMSCs could mediate the suppressive effects on T cell proliferation through expressing different negative immune molecules.

Luan, X. Y., et al. (2009). "[The effects of B7H4 on human bone marrow mesenchymal stem cell inhibiting proliferation of PHA activated T cells]." Zhonghua Xue Ye Xue Za Zhi **30**(10): 689-693.

OBJECTIVE: To investigate the effects of B7H4 on human bone marrow mesenchymal stem cells (HBMSC) mediating immune suppression. METHODS: The expression of the negative immunoregulatory factor B7H4 on HBMSC were analyzed by RT-PCR and flow cytometry (FCM), respectively. The blocking experiment was used to detect the effects of B7H4 on HBMSC mediating suppression on PHA induced T cell activation, proliferation and cell cycle. HBMSC inhibiting T cell proliferation was examined by transwell cell culture system. RESULTS: B7H4 was highly expressed on HBMSC. Blocking the B7H4 expression by B7H4mAb significantly attenuated the inhibitory effects of HBMSC on T cell proliferation. Compared with that of the unblocking group, T cell stimulator index (SI) of the B7H4 blocked group was significantly increased (53 +/- 5 vs 15 +/- 8, $P < 0.01$) and the inhibitory effects of HBMSC on T cell cycle were weakened significantly through down-regulating the cell number in G (0)/G (1) phase \ [(85.6 +/- 9.9)% vs (95.8 +/- 9.9)%\] and up-regulating those in S phase\ [(5.8 +/- 3.2)% vs (2.3 +/- 2.2)%, $P < 0.05$\]. The suppressive effects of HBMSC on T cell proliferation were significantly weakened after separating HBMSC from T cells by transwell cell culture system. Compared with the cell to cell contact group, T cell SI was significantly increased (27 +/- 17 vs 15 +/- 3, $P < 0.01$). CONCLUSION: HBMSC highly express B7H4, which plays an important role in the suppressive effects of HBMSC on T cell proliferation.

Ma, J., et al. (2017). "Multiphoton Fabrication of Fibronectin-Functionalized Protein Micropatterns: Stiffness-Induced Maturation of Cell-Matrix Adhesions in Human Mesenchymal Stem Cells." ACS Appl Mater Interfaces **9**(35): 29469-29480.

Cell-matrix adhesions are important structures governing the interactions between cells and their microenvironment at the cell-matrix interface. The focal complex (FC) and focal adhesion (FA) have been substantially investigated in conventional planar culture systems using fibroblasts as an in vitro model. However, the formation of more mature types of cell-

matrix adhesion in human mesenchymal stem cells (hMSCs), including fibrillar adhesion (FBA) and 3D matrix adhesion (3DMA), have not been fully elucidated. Here we investigate the niche factor (s) that influence (s) the maturation of FBA and 3DMA by using multiphoton fabrication-based micropatterning. First, the bovine serum albumin (BSA)-made protein micropatterns were functionalized by incorporating various concentrations of fibronectin (FN) in fabrication solution. The amount of cross-linked FN is positively correlated with the initial concentration of FN in the reaction liquid, as verified by immunofluorescence staining. On the other hand, the anisotropic FN-functionalized micropatterns were fabricated by varying the length (i.e., in-plane stiffness) and height (i.e., bending stiffness) of micropatterns, respectively. Finally, hMSCs were cultured on these micropatterns for 2 h and 1 day to determine the formation of FBA and 3DMA, respectively, using immunofluorescence staining. Results demonstrated that FN-functionalized micropatterns with high anisotropy in x-y dimension benefit FBA maturation. Furthermore, niche factors such as higher bending and in-plane stiffness and the presence of abundant fibronectin have a positive effect on the maturation of FN-based cell-matrix adhesion. These findings could provide some new perspectives on designing platforms for further cell niche study and rationalizing scaffold design for tissue engineering.

Ma, K., et al. (2011). "Generation of neural stem cell-like cells from bone marrow-derived human mesenchymal stem cells." Neurol Res **33**(10): 1083-1093.

Under appropriate culture conditions, bone marrow (BM)-derived mesenchymal stem cells are capable of differentiating into diverse cell types unrelated to their phenotypical embryonic origin, including neural cells. Here, we report the successful generation of neural stem cell (NSC)-like cells from BM-derived human mesenchymal stem cells (hMSCs). Initially, hMSCs were cultivated in a conditioned medium of human neural stem cells. In this culture system, hMSCs were induced to become NSC-like cells, which proliferate in neurosphere-like structures and express early NSC markers. Like central nervous system-derived NSCs, these BM-derived NSC-like cells were able to differentiate into cells expressing neural markers for neurons, astrocytes, and oligodendrocytes. Whole-cell patch clamp recording revealed that neuron-like cells, differentiated from NSC-like cells, exhibited electrophysiological properties of neurons, including action potentials. Transplantation of NSC-like cells into mouse brain confirmed that these NSC-like cells retained their capability to differentiate into neuronal and glial cells in vivo. Our data show that multipotent NSC-like cells can be efficiently produced from BM-derived hMSCs in culture and that these cells may serve as a useful alternative to human neural stem cells for potential clinical applications such as autologous neuroreplacement therapies.

Ma, L. J., et al. (2006). "[Effects of human mesenchymal stem cells and fibroblastoid cell line as feeder layers on expansion of umbilical cord blood CD34(+) cells in vitro]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **14**(5): 949-954.

To investigate the effects of human mesenchymal stem cells (MSC) and human fibroblastoid cell line (HFCL) as feeder layer on expansion of umbilical cord blood CD34(+) cells in vitro, (60)Co gamma-ray irradiated MSC and HFCL were used as feeder layer to expand cord blood CD34(+) cells in culture. The efficiencies of MSC and HFCL on expansion of CD34(+) cells in culture with or without cytokines were compared. The results showed that no matter whether cytokines (rhFL, rhSCF, rhTPO) were added, the proliferation of nucleated cells after expansion for 12 days in HFCL group was statistically higher than that in MSC group, i.e. with cytokines (9797 +/- 361)% vs (7061 +/- 418)%; without cytokines (5305 +/- 354)% vs (1992 +/- 247)%, when the cell numbers at day 0 was accounted as 100%), $P < 0.01$. The proliferation of propagated CD34(+) cells between MSC group and HFCL without addition of cytokines was not statistically different (820 +/- 191)% vs (825 +/- 305)%, $P > 0.05$. However, in the presence of cytokines, the propagating rate of MSC group was lower than that of HFCL group (939 +/- 212)% vs (1617 +/- 222)%, $P < 0.01$. MSC was better than HFCL in maintaining the LTC-IC of UCB CD34(+) cells, i.e. the number of CFU-GM colonies in the fifth week was (129.95 +/- 8.73) /10(5) seeded cells vs (89.81 +/- 10.29) colonies/10(5) cells, $P < 0.05$; with addition of cytokines, the effect was more obvious, i.e. the number of CFU-GM colonies in the fifth week (192.93 +/- 4.95)/10(5) seeded cells vs (90.47 +/- 14.28) colonies/10(5) seeded cells, $P < 0.01$. MSC mixed with a certain proportion of HFCL facilitated maintaining the LTC-IC of UCB CD34(+) cells. When the proportion was 4:1, the number of CFU-GM colonies was the highest (186.89 +/- 11.11)/10(5) seeded cells, which was higher than that of both 3:2 group [(138.92 +/- 14.84) colonies/10(5) seeded cells] and MSC only group, i.e. (64.63 +/- 6.11) colonies/10(5) seeded cells, both $P < 0.01$. It is concluded that HFCL is better than MSC in maintaining the expansion of CD34(+) cells and cytokines can enhance this effect, while MSC are stronger than HFCL in maintaining the LTC-IC of UCB CD34(+) cells in vitro. MSC with addition of a

certain proportion of HFCL can significantly enhance the efficiency of CD34(+) cell expansion.

Maccario, R., et al. (2005). "Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype." Haematologica **90**(4): 516-525.

BACKGROUND AND OBJECTIVES: Experimental evidence and preliminary clinical studies have demonstrated that human mesenchymal stem cells (MSC) have an important immune modulatory function in the setting of allogeneic hematopoietic stem cell (HSC) transplantation. We extended the evaluation of mechanisms responsible for the immune regulatory effect derived from the interaction of human MSC with cells involved in alloantigen-specific immune response in mixed lymphocyte culture (MLC). DESIGN AND METHODS: Dendritic cell (DC) differentiation, T- and natural killer (NK)-lymphocyte expansion, alloantigen-specific cytotoxic activity and differentiation of CD4+ T-cell subsets co-expressing CD25 and/or CTLA4 molecules were assessed, comparing the effect observed using third-party MSC with that obtained employing MSC autologous to the MLC responder. RESULTS: We found that human MSC strongly inhibit alloantigen-induced DC1 differentiation, down-regulate alloantigen-induced lymphocyte expansion, especially that of CD8+ T cells and of NK lymphocytes, decrease alloantigen-specific cytotoxic capacity mediated by either cytotoxic T lymphocytes or NK cells and favor the differentiation of CD4+ T-cell subsets co-expressing CD25 and/or CTLA4. More effective suppressive activity on MLC-induced T-cell activation was observed when MSC were third-party, rather than autologous, with respect to MLC-responder cells. INTERPRETATION AND CONCLUSIONS: Our results strongly suggest that MSC-mediated inhibition of alloantigen-induced DC1 differentiation and preferential activation of CD4+ CD25+ T-cell subsets with presumed regulatory activity represent important mechanisms contributing to the immunosuppressive activity of MSC. Collectively, these data provide immunological support for the use of MSC to prevent immune complications related to both HSC and solid organ transplantation and to the theory that MSC are universal suppressors of immune reactivity.

Madsen, S. D., et al. (2017). "Decoy TRAIL receptor CD264: a cell surface marker of cellular aging for human bone marrow-derived mesenchymal stem cells." Stem Cell Res Ther **8**(1): 201.

BACKGROUND: Mesenchymal stem cells (MSCs) are a mixture of progenitors that are heterogeneous in their regenerative potential. Development of MSC therapies with consistent efficacy is hindered by the absence of an immunophenotype of MSC heterogeneity. This study evaluates decoy TRAIL receptor CD264 as potentially the first surface marker to detect cellular aging in heterogeneous MSC cultures. METHODS: CD264 surface expression, regenerative potential, and metrics of cellular aging were assessed in vitro for marrow MSCs from 12 donors ages 20-60 years old. Male and female donors were age matched. Expression of CD264 was compared with that of p16, p21, and p53 during serial passage of MSCs. RESULTS: When CD264(+) cell content was 20% to 35%, MSC cultures from young (ages 20-40 years) and older (ages 45-60 years) donors proliferated rapidly and differentiated extensively. Older donor MSCs containing < 35% CD264(+) cells had a small size and negligible senescence despite the donor's advanced chronological age. Above the 35% threshold, CD264 expression inversely correlated with proliferation and differentiation potential. When CD264(+) cell content was 75%, MSCs were enlarged and mostly senescent with severely compromised regenerative potential. There was no correlation of the older donors' chronological age to either CD264(+) cell content or the regenerative potential of the donor MSCs. CD264 was upregulated after p53 and had a similar expression profile to that of p21 during serial passage of MSCs. No sex-linked differences were detected in this study. CONCLUSIONS: These results suggest that CD264 is a surface marker of cellular age for MSCs, not the chronological age of the MSC donor. CD264 is first upregulated in MSCs at an intermediate stage of cellular aging and remains upregulated as aging progresses towards senescence. The strong inverse correlation of CD264(+) cell content to the regenerative potential of MSCs has possible application to assess the therapeutic potential of patient MSCs, standardize the composition and efficacy of MSC therapies, and facilitate aging research on MSCs.

Maeda, S., et al. (2011). "Shrinkage-free preparation of scaffold-free cartilage-like disk-shaped cell sheet using human bone marrow mesenchymal stem cells." J Biosci Bioeng **111**(4): 489-492.

Aiming for the clinical application of cartilage regeneration, a culture method for mesenchymal stem cells (MSCs) derived from human bone marrow to obtain scaffold-free cartilage-like disk-shaped sheet of uniform sizes without the shrinkage was investigated. A disk-shaped cell sheet having the same diameter as that of the membrane without the shrinkage was formed after the cultivation of MSCs (18.6 x 10(5)cells/well) for 3 weeks in a cell culture insert

(CCI) containing a flat membrane whose porosity was 12%, while 6.2 and 31.0 x 10(5)MSCs/well, respectively, resulted in the shrinkage of the aggregate and the hole formation in the center part of the sheet. Cell aggregates shrunk also in a 96-well plate and CCIs having lower porosity. The disk-shaped cell sheet showed the comparable thickness (1.2mm) and sulfated glycosaminoglycan (sGAG) density to those of the pellet formed in a pellet culture. The gene expression levels of aggrecan and type II collagen in the disk-shaped cell sheet were not lower than those in the pellet. In conclusion, the usage of CCI having 12% porosity and 18.6 x 10(5)MSCs/well could avoid the shrinkage from the formation of the scaffold-free cartilage-like disk-shaped cell sheet.

Mahalingaiah, P. K., et al. (2015). "Chronic oxidative stress leads to malignant transformation along with acquisition of stem cell characteristics, and epithelial to mesenchymal transition in human renal epithelial cells." J Cell Physiol **230**(8): 1916-1928.

Oxidative injury to cellular macromolecules has been suggested as a common pathway shared by multiple etiological factors for kidney cancer. Whether the chronic oxidative stress alone is sufficient to induce malignant transformation in human kidney cells is not clear. Therefore, the objective of this study was to evaluate the effect of H2O2-induced chronic oxidative stress on growth, and malignant transformation of HK-2 normal kidney epithelial cells. This study revealed that chronic oxidative stress causes increased growth and neoplastic transformation in normal kidney epithelial cells at non-cytotoxic dose and increased adaptation to cytotoxic level. This was confirmed by gene expression changes, cell cycle analysis, anchorage independent growth assay and in vivo tumorigenicity in nude mice. Stem cells characteristics as revealed by up-regulation of stem cell marker genes, and morphological changes indicative of EMT with up regulation of mesenchymal markers were also observed in cells exposed to chronic oxidative stress. Antioxidant NAC did not reverse the chronic oxidative stress-induced growth, and adaptation suggesting that perturbed biological function in these cells are permanent. Partial reversal of oxidative stress-induced growth, and adaptation by silencing of Oct 4 and Snail1, respectively, suggest that these changes are mediated by acquisition of stem cell and EMT characteristics. In summary, this study for the first time suggests that chronic exposure to elevated levels of oxidative stress is sufficient to induce malignant transformation in kidney epithelial cells through acquisition of stem cell characteristics. Additionally, the EMT plays an important role in increased adaptive response of renal cells to oxidative stress.

Maitra, B., et al. (2004). "Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation." Bone Marrow Transplant **33**(6): 597-604.

Bone marrow-derived mesenchymal stem cells (MSCs) are known to interact with hematopoietic stem cells (HSCs) and immune cells, and represent potential cellular therapy to enhance allogeneic hematopoietic engraftment and prevent graft-versus-host disease (GVHD). We investigated the role of human MSCs in NOD-SCID mice repopulation by unrelated human hematopoietic cells and studied the immune interactions between human MSCs and unrelated donor blood cells in vitro. When hematopoietic stem cell numbers were limited, human engraftment of NOD-SCID mice was observed only after coinfusion of unrelated human MSCs, but not with coinfusion of mouse mesenchymal cell line. Unrelated human MSCs did not elicit T-cell activation in vitro and suppressed T-cell activation by Tuberculin and unrelated allogeneic lymphocytes in a dose-dependent manner. Cell-free MSC culture supernatant, mouse stromal cells and human dermal fibroblasts did not elicit this effect. These preclinical data suggest that unrelated, human bone marrow-derived, culture-expanded MSCs may improve the outcome of allogeneic transplantation by promoting hematopoietic engraftment and limiting GVHD and their therapeutic potential should be tested in clinic.

Majumdar, M. K., et al. (2003). "Characterization and functionality of cell surface molecules on human mesenchymal stem cells." J Biomed Sci **10**(2): 228-241.

We have characterized adhesion molecules on the surface of multipotential human mesenchymal stem cells (hMSCs) and identified molecules whose ligands are present on mature hematopoietic cells. Flow cytometric analysis of hMSCs identified the expression of integrins: alpha1, alpha2, alpha3, alpha5, alpha6, alphav, beta1, beta3, and beta4, in addition to ICAM-1, ICAM-2, VCAM-1, CD72, and LFA-3. Exposure of hMSCs to IL-1alpha, TNFalpha or IFNgamma up-modulated ICAM-1 surface expression, whereas only IFNgamma increased both HLA-class I and -class II molecules on the cell surface. Whole cell-binding assays between the hMSCs and hematopoietic cell lines showed that T lymphocytic lines bound hMSCs with higher affinity than lines of either B lymphocytes or those of myeloid lineage. Experiments using autologous T lymphocytes isolated from peripheral blood mononuclear cells showed that hMSCs exhibited increased affinity for activated T-lymphocytes compared to resting T cells by quantitative whole cell binding and rosetting assays.

Flow cytometric analysis of rosetted cells demonstrated that both CD4+ and CD8+ cells bound to hMSCs. To determine the functional significance of these findings, we tested the ability of hMSCs to present antigen to T lymphocytes. hMSCs pulsed with tetanus toxoid stimulated proliferation and cytokine production (IL-4, IL-10, and IFNgamma) in a tetanus-toxoid-specific T cell line. Maximal cytokine production correlated with maximal antigen-dependent proliferation. These data demonstrate physiological outcome as a consequence of interactions between hMSCs and human hematopoietic lineage cells, suggesting a role for hMSCs in vivo to influence both hematopoietic and immune function (s).

Maleki, M., et al. (2014). "Comparison of mesenchymal stem cell markers in multiple human adult stem cells." Int J Stem Cells **7**(2): 118-126.

OBJECTIVES: Mesenchymal stem cells (MSCs) are adult stem cells which identified by adherence to plastic, expression of cell surface markers including CD44, CD90, CD105, CD106, CD166, and Stro-1, lack of the expression of hematopoietic markers, no immunogenic effect and replacement of damaged tissues. These properties led to development of progressive methods to isolation and characterization of MSCs from various sources for therapeutic applications in regenerative medicine. METHODS: We isolated MSC-like cells from testis biopsies, ovary, hair follicle and umbilical cord Wharton's jelly and investigated the expression of specific cell surface antigens using flow cytometry in order to verify stemness properties of these cells. RESULTS: All four cell types adhered to plastic culture flask a few days after primary culture. All our cells positively expressed common MSC- specific cell surface markers. Moreover, our results revealed the expression of CD19and CD45 antigens in these cells. CONCLUSION: According to our results, high expression of CD44 in spermatogonial stem cells (SSCs), hair follicle stem cells (HFSCs),granulosa cells (GCs)and Wharton's jelly- MSCs (WJ-MSCs)may help them to maintain stemness properties. Furthermore, we suggest that CD105+SSCs, HFSCs and WJ-MSCs revealed the osteogenic potential of these cells. Moreover, high expression of CD90 in SSCs and HFSCs may associate to higher growth and differentiation potential of these cells. Further, the presence of CD19 on SSCs and GCs may help them to efficiency in response to trans-membrane signals. Thus, these four types of MSCs may be useful in clinical applications and cell therapy.

Mao, Y., et al. (2017). "Cell type-specific extracellular matrix guided the differentiation of human mesenchymal stem cells in 3D polymeric scaffolds." J Mater Sci Mater Med **28**(7): 100.

The tissue microenvironment has profound effects on tissue-specific regeneration. The 3-dimensional extracellular matrix (ECM) niche influences the linage-specific differentiation of stem cells in tissue. To understand how ECM guides tissue-specific regeneration, we established a series of 3D composite scaffolds containing ECMs derived from different primary cells isolated from a single animal species and assessed their impact on the differentiation of human mesenchymal stem cells (hMSCs). Synthetic microfiber scaffolds (fiber mats) were fabricated by electrospinning tyrosine-derived polycarbonates (pDTEC). The bovine primary fibroblasts, chondrocytes and osteoblasts cultured on the fiber mats produced and assembled their ECMs, infiltrating the pores of the fibrous scaffold. The composite scaffolds were decellularized to remove cellular components, preserve ECM and minimally affect polymer integrity. Characterization of the ECMs derived from different primary cells in the composite scaffolds showed overlapping but distinct compositions. The chondrogenic and osteogenic differentiation of hMSCs on the different composite scaffolds were compared. Our results showed that ECM derived from chondrocytes cultured in synthetic fiber mats promoted the chondrogenic differentiation of hMSC in the presence or absence of soluble inducing factors. ECM derived from co-culture of osteoblasts and chondrocytes promoted osteogenic differentiation in hMSCs better than ECM derived from chondrocytes. This study demonstrated that decellularized ECMs derived from different cell types formed within synthetic fiber scaffolds guide the tissue-specific differentiation of hMSCs. These composite scaffolds may be developed into models to study the mechanisms of ECM-induced tissue regeneration.

Maqbool, M., et al. (2011). "Human mesenchymal stem cells protect neutrophils from serum-deprived cell death." Cell Biol Int **35**(12): 1247-1251.

We have previously shown that human MSC (mesenchymal stem cells) inhibit the proliferation of most of the immune cells. However, there are innate immune cells such as neutrophils and other PMN (polymorphonuclear) cells that do not require an extensive proliferation prior to their effector function. In this study, the effect of MSC on neutrophils in the presence of complete and serum-deprived culture media was investigated. In the presence of MSC, the viability of neutrophils increase as measured in 24 h of incubation at various supplementation of serum concentration. We have utilized Annexin V and PI

(propidium iodide) staining to confirm whether the enhancement of neutrophil's viability is due to a reduction in PCD (programmed cell death). MSC significantly rescue neutrophils from apoptosis at 1, 5 and 10% of FBS (fetal bovine serum) supplementation. The fractions of viable and dead cells were increased and decreased respectively in the presence of MSC. Our results indicate MSC rescue neutrophils from nutrient- or serum-deprived cell death. However, whether this effect is exerted through a specific signalling pathway or confining neutrophils in resting state by MSC requires further investigation.

Marrelli, M., et al. (2013). "Cells isolated from human periapical cysts express mesenchymal stem cell-like properties." Int J Biol Sci **9**(10): 1070-1078.

We provide a detailed description of mesenchymal stem cells (MSCs) isolated from human periapical cysts, which we have termed hPCy-MSCs. These cells have a fibroblast-like shape and adhere to tissue culture plastic surfaces. hPCy-MSCs possess high proliferative potential and self-renewal capacity properties. We characterised the immunophenotype of hPCy-MSCs (CD73(+), CD90(+), CD105(+), CD13(+), CD29(+), CD44(+), CD45(-), STRO-1(+), CD146(+)) by flow cytometry and immunofluorescence. hPCy-MSCs possess the potential to differentiate into osteoblast- and adipocyte-like cells in vitro. Multi-potentiality was evaluated with culture-specific staining and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis for osteo/odontogenic and adipogenic markers. This is the first report to indicate that human periapical cysts contain cells with MSC-like properties. Taken together, our findings indicate that human periapical cysts could be a rich source of MSCs.

McFadden, T. M., et al. (2013). "The delayed addition of human mesenchymal stem cells to pre-formed endothelial cell networks results in functional vascularization of a collagen-glycosaminoglycan scaffold in vivo." Acta Biomater **9**(12): 9303-9316.

This paper demonstrates a method to engineer, in vitro, a nascent microvasculature within a collagen-glycosaminoglycan scaffold with a view to overcoming the major issue of graft failure due to avascular necrosis of tissue-engineered constructs. Human umbilical vein endothelial cells (ECs) were cultured alone and in various co-culture combinations with human mesenchymal stem cells (MSCs) to determine their vasculogenic abilities in vitro. Results demonstrated that the delayed addition of MSCs to pre-formed EC networks, whereby MSCs act as pericytes to the nascent vessels, resulted in the best developed vasculature. The results also demonstrate that the crosstalk between ECs and MSCs during microvessel formation occurs in a highly regulated, spatio-temporal fashion, whereby the initial seeding of ECs results in platelet derived growth factor (PDGF) release; the subsequent addition of MSCs 3 days later leads to a cessation in PDGF production, coinciding with increased vascular endothelial cell growth factor expression and enhanced vessel formation. Functional assessment of these pre-engineered constructs in a subcutaneous rat implant model demonstrated anastomosis between the in vitro engineered vessels and the host vasculature, with significantly increased vascularization occurring in the co-culture group. This study has thus provided new information on the process of in vitro vasculogenesis within a three-dimensional porous scaffold for tissue engineering and demonstrates the potential for using these vascularized scaffolds in the repair of critical sized bone defects.

Medda, R., et al. (2014). "Investigation of early cell-surface interactions of human mesenchymal stem cells on nanopatterned beta-type titanium-niobium alloy surfaces." Interface Focus **4**(1): 20130046.

Multi-potent adult mesenchymal stem cells (MSCs) derived from bone marrow have therapeutic potential for bone diseases and regenerative medicine. However, an intrinsic heterogeneity in their phenotype, which in turn results in various differentiation potentials, makes it difficult to predict the response of these cells. The aim of this study is to investigate initial cell-surface interactions of human MSCs on modified titanium alloys. Gold nanoparticles deposited on beta-type Ti-40Nb alloys by block copolymer micelle nanolithography served as nanotopographical cues as well as specific binding sites for the immobilization of thiolated peptides present in several extracellular matrix proteins. MSC heterogeneity persists on polished and nanopatterned Ti-40Nb samples. However, cell heterogeneity and donor variability decreased upon functionalization of the gold nanoparticles with cyclic RGD peptides. In particular, the number of large cells significantly decreased after 24 h owing to the arrangement of cell anchorage sites, rather than peptide specificity. However, the size and number of integrin-mediated adhesion clusters increased in the presence of the integrin-binding peptide (cRGDfK) compared with the control peptide (cRADfK). These results suggest that the use of integrin ligands in defined patterns could improve MSC-material interactions, not only by regulating cell adhesion locally, but also by reducing population heterogeneity.

Menge, T., et al. (2013). "Human mesenchymal stem cells inhibit endothelial proliferation and angiogenesis via cell-cell contact through modulation

of the VE-Cadherin/beta-catenin signaling pathway." Stem Cells Dev **22**(1): 148-157.

Over the past 10 years, a great deal has been learned about the fundamental biology and therapeutic application of bone marrow-derived human mesenchymal stem cells (MSCs). Intravenous administration of these cells is the preferred route for therapeutic delivery of MSCs. Vascular endothelial cells (ECs) are the first cell type that MSCs encounter following IV administration. However, little is known about the biological consequences of interactions between MSCs and ECs, and if any therapeutic benefit results from this interaction. We show that MSCs exert potent stabilizing effects on ECs using an in vitro coculture system. Such effects include decreased EC proliferation and the reduction of EC vascular network formation in matrigel. Interestingly, these effects appear to require EC-MSC contact and result in enhanced colocalization of VE-Cadherin and beta-catenin at the cell membrane. Disruption of the VE-Cadherin/beta-catenin interaction abrogates the observed effects. As a functional in vivo correlate, we show that intravenously administered MSCs strongly inhibit angiogenesis in a matrigel plug assay. Taken together, these results identify a novel mechanism of action of MSCs that involves a contact-dependent EC interaction. These findings are relevant to intravenous use of MSCs and provide insight into further optimizing therapeutic strategies involving MSCs.

Merceron, C., et al. (2011). "The effect of two- and three-dimensional cell culture on the chondrogenic potential of human adipose-derived mesenchymal stem cells after subcutaneous transplantation with an injectable hydrogel." Cell Transplant **20**(10): 1575-1588.

Articular cartilage is an avascular tissue composed of chondrocytes, a unique cell type responsible for abundant matrix synthesis and maintenance. When damaged, it never heals spontaneously under physiological circumstances. Therefore, the delivery of mesenchymal stem cells using hydrogel has been considered for cartilage repair. This study aims at investigating the influence of in vitro chondrogenic differentiation of human adipose tissue-derived stem cells (hATSCs) on in vivo cartilage formation when associated with a cellulose-based self-setting hydrogel (Si-HPMC). hATSCs were characterized for their proliferation, surface marker expression, and multipotency. The in vitro chondrogenic potential of hATSCs cultured within Si-HPMC in control or chondrogenic medium was evaluated by measuring COL2A1, ACAN, SOX9, and COMP expression by real-time PCR. Alcian blue and type II collagen staining were also performed. To determine whether in vitro chondrogenically differentiated hATSCs may give rise to cartilage in vivo, cells differentiated as a monolayer or in pellets were finally associated with Si-HPMC and implanted subcutaneously into nude mice. Cartilage formation was assessed histologically by alcian blue and type II collagen staining. Our data demonstrate that hATSCs exhibited proliferation and self-renewal. hATSCs also expressed typical stem cell surface markers and were able to differentiate towards the adipogenic, osteogenic, and chondrogenic lineages. Real-time PCR and histological analysis indicated that Si-HPMC enabled chondrogenic differentiation of hATSCs in inductive medium, as demonstrated by increased expression of chondrogenic markers. In addition, histological analysis of implants showed that chondrogenically differentiated hATSCs (monolayers or pellets) have the ability to form cartilaginous tissue, as indicated by the presence of sulphated glycosaminoglycans and type II collagen. This study therefore suggests that an in vitro induction of hATSCs in 2D was sufficient to obtain cartilaginous tissue formation in vivo. Si-HPMC associated with autologous hATSCs could thus be a significant tool for regenerative medicine in the context of cartilage damage.

Milanesi, A., et al. (2012). "beta-Cell regeneration mediated by human bone marrow mesenchymal stem cells." PLoS One **7**(8): e42177.

Bone marrow mesenchymal stem cells (BMSCs) have been shown to ameliorate diabetes in animal models. The mechanism, however, remains largely unknown. An unanswered question is whether BMSCs are able to differentiate into beta-cells in vivo, or whether BMSCs are able to mediate recovery and/or regeneration of endogenous beta-cells. Here we examined these questions by testing the ability of hBMSCs genetically modified to transiently express vascular endothelial growth factor (VEGF) or pancreatic-duodenal homeobox 1 (PDX1) to reverse diabetes and whether these cells were differentiated into beta-cells or mediated recovery through alternative mechanisms. Human BMSCs expressing VEGF and PDX1 reversed hyperglycemia in more than half of the diabetic mice and induced overall improved survival and weight maintenance in all mice. Recovery was sustained only in the mice treated with hBMSCs-VEGF. However, de novo beta-cell differentiation from human cells was observed in mice in both cases, treated with either hBMSCs-VEGF or hBMSCs- PDX1, confirmed by detectable level of serum human insulin. Sustained reversion of diabetes mediated by hBMSCs-VEGF was secondary to endogenous beta-cell regeneration and correlated with activation of the insulin/IGF receptor signaling pathway involved in maintaining beta-cell mass and

function. Our study demonstrated the possible benefit of hBMSCs for the treatment of insulin-dependent diabetes and gives new insight into the mechanism of beta-cell recovery after injury mediated by hBMSC therapy.

Minguell, J. J., et al. (2005). "Nonstimulated human uncommitted mesenchymal stem cells express cell markers of mesenchymal and neural lineages." Stem Cells Dev **14**(4): 408-414.

Ex vivo cultures of human bone marrow-derived mesenchymal stem cells (MSCs) contain subsets of progenitors exhibiting dissimilar properties. One of these subsets comprises uncommitted progenitors displaying distinctive features, such as morphology, a quiescent condition, growth factor production, and restricted tissue biodistribution after transplantation. In this study, we assessed the competence of these cells to express, in the absence of differentiation stimuli, markers of mesoderm and ectodermic (neural) cell lineages. Fluorescence microscopy analysis showed a unique pattern of expression of osteogenic, chondrogenic, muscle, and neural markers. The depicted "molecular signature" of these early uncommitted progenitors, in the absence of differentiation stimuli, is consistent with their multipotentiality and plasticity as suggested by several in vitro and in vivo studies.

Moghadasali, R., et al. (2013). "Mesenchymal stem cell-conditioned medium accelerates regeneration of human renal proximal tubule epithelial cells after gentamicin toxicity." Exp Toxicol Pathol **65**(5): 595-600.

Bone marrow-derived mesenchymal stem cells (MSCs) have the capacity to regenerate renal tubule epithelia and repair renal function without fusing with resident tubular cells. The goal of the present project was to investigate the role of MSCs secreted cytokines on tubule cell viability and regeneration after a toxic insult, using a conditionally immortalized human proximal tubule epithelial cell (ciPTEC) line. Gentamicin was used to induce nephrotoxicity, and cell viability and migration were studied in absence and presence of human MSC-conditioned medium (hMSC-CM) i.e. medium containing soluble factors produced and secreted by MSCs. Exposure of ciPTEC to 0-3000 mug/ml gentamicin for 24 h caused a significant dose-dependent increase in cell death. We further demonstrated that the nephrotoxic effect of 2000 mug/ml gentamicin was recovered partially by exposing cells to hMSC-CM. Moreover, exposure of ciPTEC to gentamicin (1500-3000 mug/ml) for 7 days completely attenuated the migratory capacity of the cells. In addition, following scrape-wounding, cell migration of both untreated and gentamicin-exposed cells was increased in the presence of hMSC-CM, as compared to exposures to normal medium, indicating improved cell recovery. Our data suggest that cytokines secreted by MSCs stimulate renal tubule cell regeneration after nephrotoxicity.

Montanucci, P., et al. (2016). "Restoration of t cell substes of patients with type 1 diabetes mellitus by microencapsulated human umbilical cord Wharton jelly-derived mesenchymal stem cells: An in vitro study." Clin Immunol **163**: 34-41.

Human umbilical cord Wharton jelly-derived mesenchymal stem cells (hUCMS) might apply to treating chronic autoimmune disorders, as already shown for Sjogren's syndrome, including type 1 diabetes mellitus (T1D). Since naked hUCMS grafts encountered restraints, we enveloped hUCMS, within immunoisolatory microcapsules (CpS-hUCMS), made of our endotoxin-free, clinical grade alginate. We then examined the vitro effects of interferon (IFN)-gamma-pretreated CpS-hUCMS on Th17 and Treg of T1D patients (n=15) and healthy controls (n=10). Peripheral blood mononuclear cells (PBMCs) were co-cultured with PBMC/CpS-hUCMS: lymphocyte proliferation was assessed by carboxyfluorescein succinimidyl esther (CFSE) dilution assay, and phenotypic analysis of regulatory and effector Tc was also performed. Cytokine expression was performed by bead array and qPCR on IFN-gamma-pretreated hUCMS before PBMCs co-culture. CpS-hUCMS restored a correct Treg/Th17 ratio, relevant to the T1D disease process. In summary, we have preliminarily developed a new biohybrid system, associated with immunoregulatory properties, that is ready for in vivo application.

Morandi, F., et al. (2008). "Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T-cell responses against viral or tumor-associated antigens." Stem Cells **26**(5): 1275-1287.

Human mesenchymal stem cells (MSC) are immunosuppressive and poorly immunogenic but may act as antigen-presenting cells (APC) for CD4(+) T-cell responses; here we have investigated their ability to serve as APC for in vitro CD8(+) T-cell responses. MSC pulsed with peptides from viral antigens evoked interferon (IFN)-gamma and Granzyme B secretion in specific cytotoxic T lymphocytes (CTL) and were lysed, although with low efficiency. MSC transfected with tumor mRNA or infected with a viral vector carrying the Hepatitis C virus NS3Ag gene induced cytokine release but were not killed by specific CTL, even following pretreatment with IFN-gamma. To investigate the mechanisms involved in MSC resistance to CTL-mediated lysis, we analyzed expression of human leukocyte antigen (HLA) class I-related antigen-processing machinery (APM)

components and of immunosuppressive HLA-G molecules in MSC. The LMP7, LMP10, and ERp57 components were not expressed and the MB-1 and zeta molecules were downregulated in MSC either unmanipulated or pretreated with IFN-gamma. Surface HLA-G was constitutively expressed on MSC but was not involved in their protection from CTL-mediated lysis. MSC supernatants containing soluble HLA-G (sHLA-G) inhibited CTL-mediated lysis, whereas those lacking sHLA-G did not. The role of sHLA-G in such inhibition was unambiguously demonstrated by partial restoration of lysis following sHLA-G depletion from MSC supernatants. In conclusion, human MSC can process and present HLA class I-restricted viral or tumor antigens to specific CTL with a limited efficiency, likely because of some defects in APM components. However, they are protected from CTL-mediated lysis through a mechanism that is partly sHLA-G-dependent.

Moreno, R., et al. (2017). "Human Menstrual Blood-Derived Mesenchymal Stem Cells as Potential Cell Carriers for Oncolytic Adenovirus." Stem Cells Int **2017**: 3615729.

Antitumor efficacy of systemically administered oncolytic adenoviruses (OAdv) is limited due to diverse factors such as liver sequestration, neutralizing interactions in blood, elimination by the immune system, and physical barriers in tumors. It is therefore of clinical relevance to improve OAdv bioavailability and tumor delivery. Among the variety of tumor-targeting strategies, the use of stem cells and specifically bone marrow-derived mesenchymal stem cells (BM-MSCs) is of particular interest due to their tumor tropism and immunomodulatory properties. Nonetheless, the invasive methods to obtain these cells, the low number of MSCs present in the bone marrow, and their restricted in vitro expansion represent major obstacles for their use in cancer treatments, pointing out the necessity to identify an alternative source of MSCs. Here, we have evaluated the use of menstrual blood-derived mesenchymal stem cells (MenSCs) as cell carriers for regional delivery of an OAdv in the tumor. Our results indicate that MenSCs can be isolated without invasive methods, they have an increased proliferation rate compared to BM-MSCs, and they can be efficiently infected with different serotype 5-based capsid-modified adenoviruses, leading to viral replication and release. In addition, our in vivo studies confirmed the tumor-homing properties of MenSCs after regional administration.

Moshtagh, P. R., et al. (2013). "Differentiation of human adipose-derived mesenchymal stem cell into insulin-producing cells: an in vitro study." J Physiol Biochem **69**(3): 451-458.

Stem cells with the ability to differentiate into insulin-producing cells (IPCs) are becoming the most promising therapy for diabetes mellitus and reduce the major limitations of availability and allogeneic rejection of beta cell transplantations. Mesenchymal stem cells (MSCs) are pluripotent stromal cells with the ability to proliferate and differentiate into a variety of cell types including endocrine cells of the pancreas. This study sought to inspect the in vitro differentiation of human adipose-derived tissue stem cells into IPCs which could provide an abundant source of cells for the purpose of diabetic cell therapy in addition to avoid immunological rejection. Adipose-derived MSCs were obtained from liposuction aspirates and induced to differentiate into insulin-secreting cells under a three-stage protocol based on a combination of low-glucose DMEM medium, beta-mercaptoethanol, and nicotinamide for pre-induction and high-glucose DMEM, beta-mercaptoethanol, nicotinamide, and exendin-4 for induction stages of differentiation. Differentiation was evaluated by the analysis of morphology, dithizone staining, RT-PCR, and immunocytochemistry. Morphological changes including typical islet-like cell clusters were observed by phase-contrast microscope at the end of differentiation protocol. Based on dithizone staining, differentiated cells were positive and undifferentiated cells were not stained. Furthermore, RT-PCR results confirmed the expression of insulin, PDX1, Ngn3, PAX4, and GLUT2 in differentiated cells. Moreover, insulin production by the IPCs was confirmed by immunocytochemistry analysis. It is concluded that adipose-derived MSCs could differentiate into insulin-producing cells in vitro.

Moslem, M., et al. (2013). "Therapeutic potential of human induced pluripotent stem cell-derived mesenchymal stem cells in mice with lethal fulminant hepatic failure." Cell Transplant **22**(10): 1785-1799.

Large-scale production and noninvasive methods for harvesting mesenchymal stem cells (MSCs), particularly in elderly individuals, has prompted researchers to find new patient-specific sources for MSCs in regenerative medicine. This study aims to produce MSCs from human induced pluripotent stem cells (hiPSCs) and to evaluate their therapeutic effects in a CCl4-induced mouse model of fulminant hepatic failure (FHF). hiPSC-MSCs have shown MSC morphology, antigen profile and differentiation capabilities, and improved hepatic function in our model. hiPSC-MSC-transplanted animals provide significant benefit in terms of survival, serum LDH, total bilirubin, and lipid peroxidation. hiPSC-MSC therapy resulted in a one-third reduction of histologic activity index and a threefold increase in the number of proliferating hepatocytes. This was accompanied by

a significant decrease in the expression levels of collagen type I, Mmp13, Mmp2, and Mmp9 genes and increase in Timp1 and Timp2 genes in transplanted groups. hiPSC-MSCs secreted hepatocyte growth factor (HGF) in vitro and also expressed HGF in evaluated liver sections. Similar results were observed with human bone marrow (hBM)-derived MSCs. In conclusion, our results have demonstrated that hiPSC-MSCs might be valuable appropriate alternatives for hBM-MSCs in FHF liver repair and support liver function by cell therapy with a large-scale production capacity, patient-specific nature, and no invasive MSC harvesting.

Motaln, H., et al. (2010). "Human mesenchymal stem cells and their use in cell-based therapies." Cancer **116**(11): 2519-2530.

The human population is increasingly facing various diseases, including types of cancer, that cannot be cured with conventional drugs. Advanced drug targeting of tumor cells is also often impossible when treating highly invasive and infiltrative tumors such as glioblastoma or pulmonary cancer, because of tumor cells' high migration and invasiveness. Pluripotent human mesenchymal stem cells (hMSCs) have been extensively studied, and strategies are being proposed for treating "incurable" cancers and injury/disease-affected organs. Because of their own intrinsic properties, involving homing and immunomodulatory potency, hMSCs could be used as an excellent cell/drug delivery vehicle in those cell-based therapies. Their unprecedented use has been shadowed, however, by their spontaneous transformation, which links them to cancer-initiating cells during tumor development. How malignant initiation proceeds in vivo, and what are the exact characteristics of the cancer-initiating cells, still remain to be investigated. In the present review, the authors summed up the most recent knowledge about hMSC characteristics, their malignant transformation, and outlined the possibilities of their safe use in novel cell-based therapies.

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.

Mullick, M. and D. Sen (2018). "The Delta Opioid Peptide DADLE Represses Hypoxia-Reperfusion Mimicked Stress Mediated Apoptotic Cell Death in Human Mesenchymal Stem Cells in Part by Downregulating the Unfolded Protein Response and ROS along with Enhanced Anti-Inflammatory Effect." Stem Cell Rev **14**(4): 558-573.

Hypoxia-reperfusion (H/R) emblems a plethora of pathological conditions which is potent in contributing to the adversities encountered by human mesenchymal stem cells (hMSCs) in post-transplant microenvironment, resulting in transplant failure. D-Alanine 2, Leucine 5 Enkephaline (DADLE)-mediated delta opioid receptor (DOR) activation is well-known for its recuperative properties in different cell types like neuronal and cardiomyocytes. In the current study its effectiveness in assuaging hMSC mortality under H/R-like insult has been delineated. The CoCl2 mimicked H/R conditions in vitro was investigated upon DOR activation, mediated via DADLE. hMSCs loss of viability, reactive oxygen species (ROS) production, inflammatory responses and disconcerted unfolded protein response (UPR) were assessed using AnnexinV/PI flow cytometry, fluorescence imaging, mitochondrial complex 1 assay, quantitative PCR, immunoblot analysis and ELISA. H/R like stress induced apoptosis of hMSCs was significantly mitigated by DADLE via modulation of the apoptotic regulators (Bcl-2/Bax) along with significant curtailment of ROS and mitochondrial complex 1 activity. DADLE concomitantly repressed the misfolded protein aggregation, alongside the major UPR sensors: PERK/BiP/IRE-1alpha /ATF-6, evoked due to the H/R mimicked endoplasmic reticulum stress. Undermined phosphorylation of the Akt signalling pathway was observed, which concerted its effect onto regulating both the pro and anti-inflammatory cytokines, actuated as a response to the H/R-like insult. The effects of DADLE were subdued by naltrindole (specific DOR antagonist) reaffirming the involvement of DOR in the process. Taken together these results promulgate the role of DADLE-induced DOR

activation on improved hMSC survival, which signifies the plausible implications of DOR-activation in cell-transplantation therapies and tissue engineering aspect.

Najimi, M., et al. (2017). "Human liver mesenchymal stem/progenitor cells inhibit hepatic stellate cell activation: in vitro and in vivo evaluation." Stem Cell Res Ther **8**(1): 131.

BACKGROUND: Progressive liver fibrosis leads to cirrhosis and end-stage liver disease. This disease is a consequence of strong interactions between matrix-producing hepatic stellate cells (HSCs) and resident and infiltrating immune cell populations. Accumulated experimental evidence supports the involvement of adult-derived human liver mesenchymal stem/progenitor cells (ADHLSCs) in liver regeneration. The aim of the present study was to evaluate the influence of ADHLSCs on HSCs, both in vitro and in vivo. METHODS: Activated human HSCs were co-cultured with ADHLSCs or ADHLSC-conditioned culture medium. The characteristics of the activated human HSCs were assessed by microscopy and biochemical assays, whereas proliferation was analyzed using flow cytometry and immunocytochemistry. The secretion profile of activated HSCs was evaluated by ELISA and Luminex. ADHLSCs were transplanted into a juvenile rat model of fibrosis established after co-administration of phenobarbital and CCl4. RESULTS: When co-cultured with ADHLSCs or conditioned medium, the proliferation of HSCs was inhibited, beginning at 24 h and for up to 7 days. The HSCs were blocked in G0/G1 phase, and showed decreased Ki-67 positivity. Pro-collagen I production was reduced, while secretion of HGF, IL-6, MMP1, and MMP2 was enhanced. Neutralization of HGF partially blocked the inhibitory effect of ADHLSCs on the proliferation and secretion profile of HSCs. Repeated intrahepatic transplantation of cryopreserved/thawed ADHLSCs without immunosuppression inhibited the expression of markers of liver fibrosis in 6 out of 11 rats, as compared to their expression in the vehicle-transplanted group. CONCLUSIONS: These data provide evidence for a direct inhibitory effect of ADHLSCs on activated HSCs, which supports their development for the treatment of liver fibrosis.

Nakahata, A. M., et al. (2010). "Human glioblastoma cells display mesenchymal stem cell features and form intracranial tumors in immunocompetent rats." J Stem Cells **5**(3): 103-111.

Isolation of highly tumorigenic stem-like cells from human glioblastoma specimens and cell lines has been focusing on their neural stem cells properties or capacity to efflux fluorescent dyes. Here, we report that, under standard culture conditions, human glioblastoma cells of the U87MG cell line display a predominant mesenchymal phenotype and share some of the in vitro properties of mesenchymal stem cells. Moreover, these cells were capable of forming tumors in immunocompetent rats. Infiltrative intracranial tumors could be detected 15 to 30 days post-stereotaxic cell injection within the motor cortex. Tumors were comprised by pleomorphic and mitotically active cells and displayed necrotic and hemorrhagic foci, which are common features of human glioblastomas. This rather unexpected in vivo tumorigenesis in the absence of immune suppression more closely mimics the physiological milieu encountered by tumor cells and could be explored as a xenograft orthotopic model of human glioblastomas to address new therapeutic approaches, particularly those involving immune effector mechanisms.

Nakamura, K., et al. (2018). "Initial cell plating density affects properties of human primary synovial mesenchymal stem cells." J Orthop Res.

Synovial mesenchymal stem cells (MSCs) appear to be an attractive cell source in cartilage and meniscus regeneration because of their high proliferative and chondrogenic potentials. Two methods are used to culture synovial nucleated cells in the preparation of primary synovial MSCs. In one method, the cells are plated at low density to make cell colonies. In the other method, the cells are plated at high density. We investigated the effects of initial cell density on proliferation, surface markers, and multipotentiality, including chondrogenesis in primary synovial MSCs. Human synovium was obtained from the knee joints of patients with osteoarthritis after total knee arthroplasty. Immediately after enzyme digestion, the synovial nucleated cells were plated in densities of 10(3), 10(4), or 10(5) cells/60-cm (2) dish and cultured for 14 days. Proliferation, surface markers, chondrogenesis, adipogenesis, and calcification were examined in three populations. The cell colonies were distinct in the 10(3) cells/dish group, faint in the 10(4) cells/dish group, and obscure in the 10(5) cells/dish group. The total number of cells/dish was positively related to plating density, whereas the fold increase was negatively related to plating density (n = 13). Among 12 surface markers, a negative relation to plating density was distinct in CD105. The cartilage pellet weight was negatively related to the initial plating density. The oil red-o positive area and alizarin red positive area were positively related to the initial plating density. The initial cell plating density affected the properties of primary synovial MSCs. Synovial nucleated cells proliferated better when plated at low density, and the synovial MSCs obtained by this method contained a high chondrogenic potential. (c) 2018 The Authors.

Journal of Orthopaedic Research (R) Published by Wiley Periodicals, Inc. J Orthop Res.

Nakayama, C., et al. (2018). "The development of induced pluripotent stem cell-derived mesenchymal stem/stromal cells from normal human and RDEB epidermal keratinocytes." J Dermatol Sci **91**(3): 301-310.

BACKGROUND: Epidermolysis bullosa (EB) is a group of hereditary disorders caused by mutations in the genes encoding structural molecules of the dermal-epidermal junction (DEJ). Cell-based therapies such as allogeneic mesenchymal stem/stromal cell (MSC) transplantation have recently been explored for severe EB types, such as recessive dystrophic EB (RDEB). However, hurdles exist in current MSC-based therapies, such as limited proliferation from a single cell source and limited cell survival due to potential allogenic rejection. OBJECTIVES: We aimed to develop MSCs from keratinocyte-derived induced pluripotent stem cells (iPSCs). METHODS: Keratinocyte-derived iPSCs (KC-iPSCs) of a healthy human and an RDEB patient were cultured with activin A, 6-bromoindirubin-3'-oxime and bone morphogenetic protein 4 to induce mesodermal lineage formation. These induced cells were subjected to immunohistochemical analysis, flow cytometric analysis and RNA microarray analysis in vitro, and were injected subcutaneously and intravenously to wounded immunodeficient mice to assess their wound-healing efficacy. RESULTS: After their induction, KC-iPSC-induced cells were found to be compatible with MSCs. Furthermore, with the subcutaneous and intravenous injection of the KC-iPSC-induced cells into wounded immunodeficient mice, human type VII collagen was detected at the DEJ of epithelized areas. CONCLUSIONS: We successfully established iPSC-derived MSCs from keratinocytes (KC-iPSC-MSCs) of a normal human and an RDEB patient. KC-iPSC-MSCs may have potential in therapies for RDEB.

Narang, H., et al. (2015). "Effect of proton and gamma irradiation on human lung carcinoma cells: Gene expression, cell cycle, cell death, epithelial-mesenchymal transition and cancer-stem cell trait as biological end points." Mutat Res **780**: 35-46.

Proton beam therapy is a cutting edge modality over conventional gamma radiotherapy because of its physical dose deposition advantage. However, not much is known about its biological effects vis-a-vis gamma irradiation. Here we investigated the effect of proton- and gamma- irradiation on cell cycle, death, epithelial-mesenchymal transition (EMT) and "stemness" in human non-small cell lung carcinoma cells (A549). Proton beam (3MeV) was two times more cytotoxic than gamma radiation and induced higher and longer cell cycle arrest. At equivalent doses, numbers of genes responsive to proton irradiation were ten times higher than those responsive to gamma irradiation. At equitoxic doses, the proton-irradiated cells had reduced cell adhesion and migration ability as compared to the gamma-irradiated cells. It was also more effective in reducing population of Cancer Stem Cell (CSC) like cells as revealed by aldehyde dehydrogenase activity and surface phenotyping by CD44(+), a CSC marker. These results can have significant implications for proton therapy in the context of suppression of molecular and cellular processes that are fundamental to tumor expansion.

Narita, Y., et al. (2008). "Effects of transforming growth factor-beta 1 and ascorbic acid on differentiation of human bone-marrow-derived mesenchymal stem cells into smooth muscle cell lineage." Cell Tissue Res **333**(3): 449-459.

Bone-marrow-derived mesenchymal stem cells (MSCs) can differentiate into a variety of cell types including smooth muscle cells (SMCs). We have attempted to demonstrate that, following treatment with transforming growth factor-beta 1 (TGF-beta1) and ascorbic acid (AA), human bone-marrow-derived MSCs differentiate into the SMC lineage for use in tissue engineering. Quantitative polymerase chain reaction for SMC-specific gene (alpha smooth muscle actin, h1-calponin, and SM22alpha) expression was performed on MSCs, which were cultured with various concentrations of TGF-beta1 or AA. TGF-beta1 had a tendency to up-regulate the expression of SMC-specific genes in a dose-dependent manner. The expression of SM22alpha was significantly up-regulated by 30 microM AA. We also investigated the additive effect of TGF-beta1 and AA for differentiation into SMCs and compared this effect with that of other factors including platelet-derived growth factor BB (PDGF-BB). In addition to SMC-specific gene expression, SMC-specific proteins increased by two to four times when TGF-beta1 and AA were used together compared with their administration alone. PDGF did not increase the expression of SMC-specific markers. MSCs cultured with TGF-beta1 and AA did not differentiate into osteoblasts and adipocytes. These results suggest that a combination of TGF-beta1 and AA is useful for the differentiation of MSCs into SMCs for use in tissue engineering.

Nasef, A., et al. (2007). "Identification of IL-10 and TGF-beta transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells." Gene Expr **13**(4-5): 217-226.

Mesenchymal stem cells (MSC) inhibit the response of allogeneic T lymphocytes in culture. Because the mechanisms of this effect may differ according to the existence of cell contact, we investigated the differences in gene expression of inhibitory molecules during MSC-T lymphocyte coculture when cell contact does and does not occur. Human MSC and T lymphocytes were cultured together in standard and transwell cultures. MSC gene expression was analyzed by semiquantitative real-time RT-PCR. MSC elicited a high dose-dependent inhibition of T lymphocytes in cultures with cell contact, but inhibition occurred even without cell contact. In both cases, we observed significant upregulation of IDO, LIF, and HLA-G, along with downregulation of HGF and SDF1. In cultures with cell contact, IL-10 and TGF-beta transcripts were expressed in a significantly higher level than in cultures without this contact. Furthermore, in the latter, the increased inhibition of T-cell proliferation was positively correlated with IDO gene expression and negatively correlated with SDF1 gene expression. MSC appear to induce T-cell tolerance by two distinct mechanisms. The first of these, which does not require cell contact, induces expression of the tolerogenic genes IDO, LIF, and HLA-G. The second mechanism, which is contact dependent, modulates IL-10 and TGF-beta gene expression. These two mechanisms probably play separate roles in MSC-induced tolerance in allogeneic hematopoietic stem cell transplantation.

Nedopil, A., et al. (2010). "MR signal characteristics of viable and apoptotic human mesenchymal stem cells in matrix-associated stem cell implants for treatment of osteoarthritis." Invest Radiol **45**(10): 634-640.

OBJECTIVE: To compare magnetic resonance (MR) signal characteristics of contrast agent-labeled apoptotic and viable human mesenchymal stem cells (hMSCs) in matrix-associated stem cell implants. METHODS: hMSCs were labeled with Food and Drug Administration-approved ferumoxides nanoparticles. One group (A) remained untreated whereas a second group (B) underwent mitomycin C-induced apoptosis induction. Viability of group A and apoptosis of group B was confirmed by caspase-assays and terminal dUTP nick-end labeling (TUNEL) stains. Labeled viable hMSCs, unlabeled viable hMSCs, labeled apoptotic hMSCs, and unlabeled apoptotic hMSCs (n = 7 samples each) in an agarose scaffold were implanted into cartilage defects of porcine patellae specimens and underwent MR imaging at 7 T, using T1-weighted spin-echo sequences, T2-weighted spin-echo sequences, and T2*-weighted gradient-echo sequences. Signal-to-noise ratios (SNR) of the implants were calculated and compared between different experimental groups using linear mixed regression models. RESULTS: Ferumoxides-labeled hMSCs provided a strong negative T2 and T2*-enhancement. Corresponding SNR data of labeled hMSCs were significantly lower compared with unlabeled controls ($P < 0.05$). Apoptosis induction resulted in a significant signal decline of ferumoxides-labeled hMSC transplants on short echo time T2-weighted spinecho sequences. SNR data of labeled apoptotic hMSCs were significantly lower compared with labeled viable hMSCs ($P < 0.05$). CONCLUSION: Apoptosis of transplanted ferumoxides-labeled stem cells in cartilage defects can be visualized noninvasively by a significant signal decline on T2-weighted MR images. The described MR signal characteristics may serve as a noninvasive outcome measure for the assessment of matrix-associated stem cell implants in clinical practice. Additional studies are needed to further enhance the observed differences between viable and apoptotic cells, for example, by further optimizing the applied MR pulse sequence parameters or intracellular contrast agent concentration.

Nejad, N. A., et al. (2015). "Male germ-like cell differentiation potential of human umbilical cord Wharton's jelly-derived mesenchymal stem cells in co-culture with human placenta cells in presence of BMP4 and retinoic acid." Iran J Basic Med Sci **18**(4): 325-333.

OBJECTIVES: Mesenchymal stem cells (MSCs) derived from Wharton's jelly (WJ-MSCs) are now much more appealing for cell-based infertility therapy. Hence, WJ-MSCs differentiation toward germ layer cells for cell therapy purposes is currently under intensive study. MATERIALS AND METHODS: MSCs were isolated from human Wharton's jelly and treated with BMP4, retinoic acid (RA) or co-cultured on human amniotic epithelial (HAE) and chorionic plate (HCP) placenta feeder cells. profile of POU5F1, Fragilis, Plzf, DDX4, Piwil2, Stra8, Dazl, beta1- and alpha6-integrins (ITBeta1, ITA6) genes expression as germ cell markers were analyzed using RT-PCR and real-time PCR. Immunocytochemistry of surface markers were conducted. RESULTS: After 3 weeks treatment with different reagents and co-culture system, morphology of WJ-MSCs changed to shiny clusters and germ cell specific markers in mRNA were up-regulated in both placental feeder + RA and BMP4 + RA. Induction of hWJ-MSCs with BMP4 in presence of RA resulted in significant up-regulation ($P</=0.05$) of all germ cell specific genes (c-Kit; 2.84+/-0.59, DDX4; 1.69+/-0.39, Piwil2; 1.14+/-0.21, Dazl; 0.65+/-0.25, alpha6 integrin; 1.26+/-0.53, beta1 integrins; 1.18+/-0.65) compared to control and placental feeder cells + RA. Our results indicated that

HAE and HCP followed by RA treatment were involved in human germ cell development. CONCLUSION: We demonstrated that under the right conditions, hWJ-MSCs have the ability to differentiate to germ cells and this provides an excellent pattern to study infertility cause and treatment.

Neuss, S., et al. (2004). "Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing." Stem Cells **22**(3): 405-414.

Human mesenchymal stem cells (hMSC) are adult stem cells with multipotent capacities. The ability of mesenchymal stem cells to differentiate into many cell types, as well as their high ex vivo expansion potential, makes these cells an attractive therapeutic tool for cell transplantation and tissue engineering. hMSC are thought to contribute to tissue regeneration, but the signals governing their mobilization, diapedesis into the bloodstream, and migration into the target tissue are largely unknown. Here we report that hepatocyte growth factor (HGF) and the cognate receptor HGFR/c-met are expressed in hMSC, on both the RNA and the protein levels. The expression of HGF was downregulated by transforming growth factor beta. HGF stimulated chemotactic migration but not proliferation of hMSC. Therefore the HGF/c-met signaling system may have an important role in hMSC recruitment sites of tissue regeneration. The controlled regulation of HGF/c-met expression may be beneficial in tissue engineering and cell therapy employing hMSC.

Nguyen, T. M., et al. (2013). "EphB and Ephrin-B interactions mediate human mesenchymal stem cell suppression of activated T-cells." Stem Cells Dev **22**(20): 2751-2764.

Mesenchymal stromal/stem cells (MSC) express the contact-dependent erythropoietin-producing hepatocellular (Eph) receptor tyrosine kinase family and their cognate ephrin ligands, which are known to regulate thymocyte maturation and selection, T-cell transendothelial migration, activation, co-stimulation, and proliferation. However, the contribution of Eph/ephrin molecules in mediating human MSC suppression of activated T-cells remains to be determined. In the present study, we showed that EphB2 and ephrin-B2 are expressed by ex vivo expanded MSC, while the corresponding ligands, ephrin-B1 and EphB4, respectively, are highly expressed by T-cells. Initial studies demonstrated that EphB2-Fc and ephrin-B2-Fc molecules suppressed T-cell proliferation in allogeneic mixed lymphocyte reaction (MLR) assays compared with human IgG-treated controls. While the addition of a third-party MSC population demonstrated dramatic suppression of T-cell proliferation responses in the MLR, blocking the function of EphB2 or EphB4 receptors using inhibitor binding peptides significantly increased T-cell proliferation. Consistent with these observations, shRNA EphB2 or ephrin-B2 knockdown expression in MSC reduced their ability to inhibit T-cell proliferation. Importantly, the expression of immunosuppressive factors, indoleamine 2, 3-dioxygenase, transforming growth factor-beta1, and inducible nitric oxide synthase expressed by MSC, was up-regulated after stimulation with EphB4 and ephrin-B1 in the presence of interferon (IFN)-gamma, compared with untreated controls. Conversely, key factors involved in T-cell activation and proliferation, such as interleukin (IL)-2, IFN-gamma, tumor necrosis factor-alpha, and IL-17, were down-regulated by T-cells treated with EphB2 or ephrin-B2 compared with untreated controls. Studies utilizing signaling inhibitors revealed that inhibition of T-cell proliferation is partly mediated through EphB2-induced ephrin-B1 reverse signaling or ephrin-B2-mediated EphB4 forward signaling by activating Src, PI3Kinase, Abl, and JNK kinase pathways, activated by tyrosine phosphorylation. Taken together, these observations suggest that EphB/ephrin-B interactions play an important role in mediating human MSC inhibition of activated T cells.

Nieto-Miguel, T., et al. (2013). "In vitro simulation of corneal epithelium microenvironment induces a corneal epithelial-like cell phenotype from human adipose tissue mesenchymal stem cells." Curr Eye Res **38**(9): 933-944.

PURPOSE: Transplantation of autologous corneal stem cells in not possible in cases of bilateral limbal stem cell deficiency (LSCD). To restore the ocular surface in these patients, an autologous extraocular source of stem cells is desirable to avoid dependence on deceased donor tissue and host immunosuppression of allogenic transplants. While bone marrow-derived mesenchymal stem cells (MSCs) can acquire certain characteristics of corneal epithelial cells, subcutaneous adipose tissue (AT) is more readily available and accessible. The aim of this study was to determine if extraocular human AT-derived MSCs (hAT-MSCs) can acquire in vitro some features of corneal epithelial-like cells. METHODS: hAT-MSCs were isolated from human lipoaspirates and expanded up to 3-4 passages. We studied the immunophenotype of MSCs and demonstrated its multipotent capacity to differentiate toward osteoblasts, adipocytes and chondrocytes. To test the capacity of differentiation of hAT-MSCs toward corneal epithelial-like cells, hAT-MSCs were cultured on substrata of plastic or collagen IV. We used basal culture medium (BM), BM

conditioned with human corneal epithelial cells (HCEcBM) and BM conditioned with limbal fibroblasts (LFcBM). RESULTS: The hAT-MSCs incubated for 15 days with HCEcBM acquired more polygonal and complex morphology as evaluated by phase-contrast microscopy and flow cytometry. Additionally, the expression of transforming growth factor-beta receptor CD105 and corneal epithelial marker CK12 got increased as evaluated by flow cytometry, real-time reverse-transcription polymerase chain reaction, western blot and immunostaining. These changes were absent in hAT-MSCs incubated with unconditioned BM or with LFcBM. CONCLUSIONS: Corneal epithelial-like cells can be induced from extraocular hAT-MSCs by subjecting them to an in vitro microenvironment containing conditioning signals derived from differentiated human corneal epithelial cells. Our results suggest that hAT-MSCs could provide a novel source of stem cells that hold the potential to restore sight lost in patients suffering from bilateral ocular surface failure due to LSCD.

Nikolaev, N. I., et al. (2014). "Changes in the stiffness of human mesenchymal stem cells with the progress of cell death as measured by atomic force microscopy." J Biomech **47**(3): 625-630.

This note reports observations of the change of stiffness of human mesenchymal stem cells (hMSCs) with the progress of cell death as measured by AFM. hMSC with impaired membrane, dead and viable cells were labelled with Annexin V and Propidium Iodide after 24h cold storage, followed by AFM measurement and Young's modulus of cells was derived. Viable hMSCs have a Young's modulus (E) in the range of 0.81-1.13kPa and consistent measurement was observed when different measurement locations were chosen. E of cells with partially impaired membrane was 0.69+/-0.17kPa or in the range of 2.04-4.74kPa, depending upon the measurement locations. With the loss of membrane integrity, though there was no variation on measured E between different locations, a mixed picture of cell stiffness was observed as indicated by cells with E as low as 0.09+/-0.03kPa, in a mid-range of 4.62+/-0.67kPa, and the highest of up to 48.98+/-19.80kPa. With the progress of cell death, the highest stiffness was noticed for cells showing a more granular appearance; also the lowest stiffness for cells with vacuole appearance. Findings from this study indicate that cell stiffness is significantly altered with the progress of cell death.

Noh, Y. K., et al. (2016). "Polymer mesh scaffold combined with cell-derived ECM for osteogenesis of human mesenchymal stem cells." Biomater Res **20**: 6.

BACKGROUND: Tissue-engineered scaffold should mimic the structure and biological function of the extracellular matrix and have mechanically supportive properties for tissue regeneration. In this study, we utilized a PLGA/PLA mesh scaffold, coated with cell-derived extracellular matrix (CDM) and assessed its potential as an osteogenic microenvironment for human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs). CDM was obtained by decellularization of in vitro-cultured type I collagen overexpressing (Col I -293 T-DK) cells. Test groups are mesh itself (control), fibronectin-coated (FN-mesh), and CDM-coated mesh scaffold (CDM-mesh). CDM was then solubilized and used for scaffold coating. RESULTS: CDM was successfully collected and applied to mesh scaffolds. The presence of CDM was confirmed via SEM and FN immunofluorescence. After then, UCB-MSCs were seeded into the scaffolds and subjected to the induction of osteogenic differentiation for 21 days in vitro. We found that the seeded cells were viable and have better proliferation activity on CDM-mesh scaffold. In addition, when osteogenic differentiation of UCB-MSCs was examined for up to 21 days, alkaline phosphatase (ALP) activity and osteogenic marker (COL I, ALP, osteocalcin, bone sialoprotein) expression were significantly improved with UCB-MSCs when cultured in the CDM-mesh scaffold compared to the control and FN-mesh. CONCLUSION: Polymer mesh scaffold incorporated with CDM can provide UCB-MSCs with a better microenvironment for osteogenesis in vitro.

Nong, K., et al. (2016). "Hepatoprotective effect of exosomes from human-induced pluripotent stem cell-derived mesenchymal stromal cells against hepatic ischemia-reperfusion injury in rats." Cytotherapy **18**(12): 1548-1559.

BACKGROUND: This study aimed to evaluate the effect of exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) on hepatic ischemia-reperfusion (I/R) injury. METHODS: Exosomes were isolated and concentrated from conditioned medium using ultracentrifugation and ultrafiltration. hiPSC-MSCs-Exo were injected systemically via the inferior vena cava in a rat model of 70% warm hepatic I/R injury, and the therapeutic effect was evaluated. The serum levels of transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were measured using an automatic analyzer. The expression of inflammatory factors was measured using enzyme-linked immunosorbent assay (ELISA). Histological changes indicated changes in pathology and inflammatory infiltration in liver tissue. Apoptosis of hepatic cells in liver tissue was measured using

terminal-deoxynucleoitidyl transferase mediated nick end labeling (TUNEL) staining along with apoptotic markers. RESULTS: hiPSCs were efficiently induced into hiPSC-MSCs with typical MSC characteristics. hiPSC-MSCs-Exo had diameters ranging from 50 to 60 nm and expressed exosomal markers (CD9, CD63 and CD81). Hepatocyte necrosis and sinusoidal congestion were markedly suppressed with a lower Suzuki score after hiPSC-MSCs-Exo administration. The levels of the hepatocyte injury markers AST and ALT were significantly lower in the treated group than in the control group. Inflammatory markers, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and high mobility group box 1 (HMGB1), were significantly reduced after administration of hiPSC-MSCs-Exo, which suggests that the exosomes have a role in suppressing the inflammatory response. Additionally, in liver tissues from the experimental group, the levels of apoptotic markers, such as caspase-3 and bax, were significantly lower and the levels of oxidative markers, such as glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), were significantly higher than in the control group. These data point to an anti-apoptotic, anti-oxidative stress response role for hiPSC-MSCs-Exo. CONCLUSIONS: Our results demonstrated that hiPSC-MSCs-Exo alleviate hepatic I/R injury, possibly via suppression of inflammatory responses, attenuation of the oxidative stress response and inhibition of apoptosis.

Ogura, N., et al. (2004). "Differentiation of the human mesenchymal stem cells derived from bone marrow and enhancement of cell attachment by fibronectin." J Oral Sci **46**(4): 207-213.

The ability of human mesenchymal stem cells (hMSC) to differentiate into osteoblasts was examined through the use of osteogenic induction medium (MSCOIM) cultures. hMSC first attached to the dish surface and exhibited fibroblast-like spindle shapes, and after proliferation, formed cuboidal shapes. Calcium assays and the use of von Kossa and alizarin red S staining showed that hMSC were capable of mineralization when cultured in MSCOIM. Gene expressions of Cbfa-1 and BMP-4, which are markers for osteogenic differentiation, were also increased during the hMSC differentiation into osteoblasts. When compared to albumin (Alb)-coated dishes, microscopic observation documented enhanced cell attachment and spreading when hMSC were cultured on fibronectin (FN)-coated dishes. Adherent cell numbers also exhibited a greater increase on the FN-coated dishes during earlier culture stages than that seen for the Alb-coated dishes. These findings suggest that hMSC have the capability to differentiate into osteoblasts and that FN can stimulate the attachment and spreading of the hMSC.

Okura, H., et al. (2010). "Properties of hepatocyte-like cell clusters from human adipose tissue-derived mesenchymal stem cells." Tissue Eng Part C Methods **16**(4): 761-770.

There are only a few reports that describe the hepatocytic differentiation potential of human adipose tissue-derived mesenchymal stem cells (hADMSCs) and no reports that describe the in vivo functions of hepatocyte-like cells differentiated from somatic stem cells including hADMSCs. In this study, we established a new method for generation of functional hepatocyte-like cell clusters using floating culture method and induced functional hepatocyte-like cell clusters, which functioned effectively not only in vitro but also in vivo. The generated hepatocyte-like cell clusters were characterized by gene expression analysis, functional assays, and transplantation into non-obese diabetic severe combined immunodeficiency (NOD-SCID) mouse with chronic liver injury. The generated hepatocyte-like cell clusters expressed various genes normally found on mature hepatocytes. The cell clusters exhibited functional characteristics of hepatocytes: they expressed albumin, secreted urea, had cytochrome P450 activity, could take up low-density lipoprotein, and stored glycogen. Transplantation of these cell clusters into NOD-SCID mouse with chronic liver injury resulted in a significant improvement of serum albumin and total bilirubin levels. In summary, we established a new protocol for efficient induction of hADMSCs into functional hepatocyte-like cell clusters.

Omoto, M., et al. (2009). "The use of human mesenchymal stem cell-derived feeder cells for the cultivation of transplantable epithelial sheets." Invest Ophthalmol Vis Sci **50**(5): 2109-2115.

PURPOSE: To report the efficacy of human bone marrow-derived mesenchymal stem cells as a source of feeder cells for the cultivation of transplantable corneal epithelial cell sheets. METHODS: Human mesenchymal stem cells (marrow adherent stem cells; MASCs) were cultured in alpha-modified Eagle's medium with 10% serum and were treated with mitomycin C. Expression of cytokines in MASCs was confirmed by reverse transcription-polymerase chain reaction. Human limbal epithelial cells were cocultured with MASCs or 3T3 feeder cells to compare colony-forming efficiency (CFE). Limbal epithelial cells were cultured on MASCs or 3T3 feeder cells at the air-liquid interface to allow stratification, and stratified epithelial sheets were analyzed by immunohistochemistry against cytokeratin 3 (K3), K15, p63alpha, and ABCG2. Rabbit limbal epithelial

cell sheets were cultivated with MASC feeder cells and transplanted to the ocular surface of the limbal-deficient rabbits. Epithelial grafts were observed by slit lamp microscopy for 4 weeks and then evaluated by histology and immunohistochemistry against K3 and K4. RESULTS: MASC feeder cells expressed keratinocyte growth factor, hepatocyte growth factor, and N-cadherin. The CFE of human limbal epithelial cells was similar in MASC and 3T3 feeder groups. Stratified cell sheets were successfully cultivated with MASC feeder cells expressing K3, K15, p63alpha, and ABCG2. Transplanted epithelial sheets regenerated the corneal phenotype in limbal-deficient rabbits. CONCLUSIONS: MASC-derived feeder cells are suitable for the engineering of epithelial sheets, avoiding the use of potentially hazardous xenologic feeder cells.

Oodi, A., et al. (2012). "Expression of P16 cell cycle inhibitor in human cord blood CD34+ expanded cells following co-culture with bone marrow-derived mesenchymal stem cells." Hematology **17**(6): 334-340.

BACKGROUND: Because of insufficient number of cord blood hematopoietic stem cells (CB-HSC), expansion of these cells seems to be important for clinical application in adults. Cell cycle inhibitors are important regulators in normal hematopoietic regeneration. In this study, mRNA expression and promoter methylation status of p16 were evaluated during CB-HSC ex vivo expansion using cytokines and a co-culture system with mesenchymal stem cells (MSCs) feeder layer. METHODS: ex vivo cultures of CB-HSCs were performed in three culture conditions for 14 days: cytokines with MSCs feeder layer, cytokines without MSCs feeder layer, and co-culture with MSCs without cytokine. After expansion, measuring total number of cells, CD34+ cells and colony-forming unit (CFU) assay was performed. Methylation status of the p16(INK4a) gene promoter was analyzed using methylation-specific polymerase chain reaction (PCR), and p16 mRNA expression was evaluated by real-time reverse transcriptase-PCR. RESULTS: Maximum CB-HSC expansion was observed in day 10 of expansion. The data showed that after 10 days, p16 mRNA expression in the expanded cells at the co-culture system without cytokine was higher than in CD34+ fresh cells ($P < 0.01$); however, p16 mRNA expression in the expanded cells at both cytokine cultures with and without MSCs feeder layer was decreased. p16 gene promoter of expanded CD34+ cells remained in unmethylated form just like fresh CD34+ cells in all the three culture conditions at days 5, 10, and 14 of culture. CONCLUSION: Expression in HSCs of p16(INK4a), an important cell cycle regulator in normal hematopoietic regeneration disruption of which is involved in leukemic cell development, was increased during 10 days of expansion in co-culture with MSCs feeder layers. Also, no methylation of p16 promoter was observed, which is capable of initiating some leukemic cell progression or disruption in hematopoietic regeneration.

Orciani, M., et al. (2010). "Oxidative stress defense in human-skin-derived mesenchymal stem cells versus human keratinocytes: Different mechanisms of protection and cell selection." Free Radic Biol Med **49**(5): 830-838.

Stem cells are undifferentiated cells with the capacity for self-renewal and differentiation. Here we have determined the susceptibility to oxidative stress of isolated mesenchymal stem cells from human skin (S-MSCs) in comparison with keratinocytes, which are differentiated cells of the same lineage. To induce pro-oxidant conditions, S-MSCs and keratinocytes were exposed to 0.5mM H (2)O (2) for 2 h, with oxidative effects analyzed after 4, 12, 24, and 48 h of recovery, in terms of cell growth, vitality, apoptosis, DNA damage, variations in individual antioxidant defense and total oxyradical scavenging capacity toward peroxyl and hydroxyl radicals. The data indicate different abilities across these two cell types to counteract this oxidative stress, which reflects stress that would normally be experienced by these cells under basal conditions. Human keratinocytes seem to have much greater antioxidant defense to counteract the oxidative injury to which they are continuously exposed in the skin. The S-MSCs are surrounded by a complex microenvironment that protects them from external insults, and so they do not have a particularly efficient defense system, and they were generally less responsive to enhanced pro-oxidant challenge. S-MSCs seem particularly prone to apoptotic events, which might thus represent their primary defense mechanism against stress.

Ouchi, T., et al. (2016). "LNGFR (+)THY-1(+) human pluripotent stem cell-derived neural crest-like cells have the potential to develop into mesenchymal stem cells." Differentiation **92**(5): 270-280.

Mesenchymal stem cells (MSCs) are defined as non-hematopoietic, plastic-adherent, self-renewing cells that are capable of tri-lineage differentiation into bone, cartilage or fat in vitro. Thus, MSCs are promising candidates for cell-based medicine. However, classifications of MSCs have been defined retrospectively; moreover, this conventional criterion may be inaccurate due to contamination with other hematopoietic lineage cells. Human MSCs can be enriched by selection for LNGFR and THY-1, and this population may be analogous to murine PDGFRalpha (+)Sca-1(+) cells, which are developmentally derived from neural crest cells (NCCs). Murine NCCs were

labeled by fluorescence, which provided definitive proof of neural crest lineage, however, technical considerations prevent the use of a similar approach to determine the origin of human LNGFR (+)THY-1(+) MSCs. To further clarify the origin of human MSCs, human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) were used in this study. Under culture conditions required for the induction of neural crest cells, human ESCs and iPSCs-derived cells highly expressed LNGFR and THY-1. These LNGFR (+)THY-1(+) neural crest-like cells, designated as LT-NCLCs, showed a strong potential to differentiate into both mesenchymal and neural crest lineages. LT-NCLCs proliferated to form colonies and actively migrated in response to serum concentration. Furthermore, we transplanted LT-NCLCs into chick embryos, and traced their potential for survival, migration and differentiation in the host environment. These results suggest that LNGFR (+)THY-1(+) cells identified following NCLC induction from ESCs/iPSCs shared similar potentials with multipotent MSCs.

Ozdemir, A. T., et al. (2016). "The paracrine immunomodulatory interactions between the human dental pulp derived mesenchymal stem cells and CD4 T cell subsets." Cell Immunol **310**: 108-115.

Mesenchymal stem cells (MSCs) have strong immunomodulatory properties, however these properties may show some differences according to the tissue type of their isolate. In this study we investigated the paracrine interactions between human DP derived MSCs (hDP-MSCs) and the CD4(+) T helper cell subsets to establish their immunomodulatory mechanisms. We found that the CD4(+)-Tbet (+) (Th1) and CD4(+)-Gata3(+) (Th2) cells were suppressed by the hDP-MSCs, but the CD4(+)-Stat3(+) (Th17) and CD4(+)-CD25(+)-FoxP3(+) (Treg) cells were stimulated. The expressions of T cell specific cytokines interferon gamma (IFN-g), interleukin (IL)-4 and IL-17a decreased, but IL-10 and transforming growth factor beta-1 (TGF-b1) increased with the hDP-MSCs. The expressions of indoleamine-pyrrole 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), soluble human leukocyte antigen G (sHLA-G) derived from hDP-MSCs slightly increased, but hepatocyte growth factor (HGF) significantly increased in the co-culture groups. According to our findings, the hDP-MSCs can suppress the Th1 and Th2 subsets but stimulate the Th17 and Treg subsets. The Stat3 expression of Th17 cells may have been stimulated by the HGF, and thus the pro-inflammatory Th17 cells may have altered into the immunosuppressive regulatory Th17 cells. Further prospective studies are needed to confirm our findings.

Park, H. E., et al. (2011). "Real-time monitoring of neural differentiation of human mesenchymal stem cells by electric cell-substrate impedance sensing." J Biomed Biotechnol **2011**: 485173.

Stem cells are useful for cell replacement therapy. Stem cell differentiation must be monitored thoroughly and precisely prior to transplantation. In this study we evaluated the usefulness of electric cell-substrate impedance sensing (ECIS) for in vitro real-time monitoring of neural differentiation of human mesenchymal stem cells (hMSCs). We cultured hMSCs in neural differentiation media (NDM) for 6 days and examined the time-course of impedance changes with an ECIS array. We also monitored the expression of markers for neural differentiation, total cell count, and cell cycle profiles. Cellular expression of neuron and oligodendrocyte markers increased. The resistance value of cells cultured in NDM was automatically measured in real-time and found to increase much more slowly over time compared to cells cultured in non-differentiation media. The relatively slow resistance changes observed in differentiating MSCs were determined to be due to their lower growth capacity achieved by induction of cell cycle arrest in G0/G1. Overall results suggest that the relatively slow change in resistance values measured by ECIS method can be used as a parameter for slowly growing neural-differentiating cells. However, to enhance the competence of ECIS for in vitro real-time monitoring of neural differentiation of MSCs, more elaborate studies are needed.

Park, H. W., et al. (2010). "Human mesenchymal stem cell-derived Schwann cell-like cells exhibit neurotrophic effects, via distinct growth factor production, in a model of spinal cord injury." Glia **58**(9): 1118-1132.

Human bone marrow-derived mesenchymal stem cells (hMSCs) are considered a desirable cell source for autologous cell transplantation therapy to treat nervous system injury due to their ability to differentiate into specific cell types and render the tissue microenvironment more favorable for tissue repair by secreting various growth factors. To potentiate their possible trophic effect, hMSCs were induced without genetic modification to adopt characteristics of Schwann cells (SCs), which provide trophic support for regenerating axons. The induced hMSCs (shMSCs) adopted a SC-like morphology and expressed SC-specific proteins including the p75 neurotrophin receptor, which correlated with cell-cycle exit. In addition, shMSCs secreted higher amounts of several growth factors, such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) when compared with uninduced hMSCs. Coculture of shMSCs with Neuro2A cells significantly

increased neurite outgrowth and cell proliferation but decreased cell death. Transplantation of shMSCs in an ex vivo model of spinal cord injury dramatically enhanced axonal outgrowth, which was mediated by HGF and VEGF secretion and also decreased cell death. These results demonstrate that shMSCs could serve as an endogenous source of neurotrophic growth factors to facilitate axonal regeneration while at the same time protecting the resident cells at the site of tissue injury. We propose that these induced hMSCs without genetic modification are useful for autologous cell therapy to treat nervous system injury.

Park, J. R., et al. (2010). "DNER modulates adipogenesis of human adipose tissue-derived mesenchymal stem cells via regulation of cell proliferation." Cell Prolif **43**(1): 19-28.

OBJECTIVES: In recent years, obesity has become a global epidemic, highlighting the necessity for basic research into mechanisms underlying growth of adipose tissue and differentiation of stem cells into adipocytes, in humans. For better understanding of cell signalling in adipogenesis, the role of DNER (delta/Notch-like EGF-related receptor) in adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells (hAMSC) was investigated. MATERIALS AND METHODS: To assess the role of DNER in hAMSC adipogenesis, hAMSCs were transfected with DNER small interfering RNA (siDNER). Real-time quantitative reverse transcriptase polymerase chain reactions to assess expression levels of adipogenesis-related genes regulated by siDNER, cell cycle and immunoblot analyses were performed. RESULTS: First, it was determined that DNER mRNA was profoundly expressed in hAMSCs and reduced during adipogenic differentiation. Knockdown of DNER altered cell morphology, inhibited proliferation and increased frequency and efficiency of adipogenesis in hAMSC. Expression of CCAAT/enhancer-binding protein delta increased and proportion of cells in S phase decreased by knockdown of DNER, using specific siRNA. Moreover, adipocyte-specific genes including peroxisome proliferator-activated receptor gamma, fatty acid binding protein 4 and perilipin were up-regulated in siDNER compared to the siControl group during adipogenesis in hAMSC. CONCLUSIONS: These results indicate that DNER knockdown in hAMSC accelerated onset of adipogenic differentiation by bypassing mitotic clonal expansion during the early stages of adipogenesis.

Park, J. S., et al. (2016). "Regulation of Cell Signaling Factors Using PLGA Nanoparticles Coated/Loaded with Genes and Proteins for Osteogenesis of Human Mesenchymal Stem Cells." ACS Appl Mater Interfaces **8**(44): 30387-30397.

Transfection of specific genes and transportation of proteins into cells have been a focus of stem cell differentiation research. However, it is not easy to regulate codelivery of a gene and a protein into cells. For codelivery into undifferentiated cells (human mesenchymal stem cells (hMSCs)), we used biodegradable carriers loaded with Runt-related transcription factor 2 (RUNX2) protein and coated with bone morphogenetic protein 2 (BMP2) plasmid DNA (pDNA) to induce osteogenesis. The released gene and protein were first localized in the cytosol of transfected hMSCs, and the gene then moved into the nucleus. The levels of internalized PLGA nanoparticles were tested using different doses and incubation durations. Then, transfection of BMP2 pDNA was confirmed by determining mRNA and protein levels and acquiring cell images. The same techniques were used to assess osteogenesis of hMSCs both in vitro and in vivo upon internalization of PLGA NPs carrying the BMP2 gene and RUNX2 protein. Detection of specific genes and proteins demonstrated that cells transfected with PLGA NPs carrying both the BMP2 gene and RUNX2 protein were highly differentiated compared with other samples. Histological and immunofluorescence analyses demonstrated that transfection of PLGA nanoparticles carrying both the BMP2 gene and RUNX2 protein dramatically enhanced osteogenesis of hMSCs.

Park, J. Y., et al. (2013). "Comparative analysis of mesenchymal stem cell surface marker expression for human dental mesenchymal stem cells." Regen Med **8**(4): 453-466.

AIM: Human dental mesenchymal stem cells (hDMSCs) have been isolated from extracted human teeth and proven to have different proliferation and differentiation abilities among the subtypes. Despite increasing interest in the clinical use of hDMSCs, a well-defined specific marker has been absent for these stem cells. In this study, a comparative analysis with known mesenchymal stem cell surface markers such as STRO-1, CD90, CD146, CD34 and TfR (CD71) was performed. MATERIALS & METHODS: Four subtypes of the hDMSCs were obtained and cultured. The hDMSCs were processed by flow cytometric analysis, fluorescence immunocytostaining for in vitro study and in situ immunohistochemical staining for in vivo study. RESULTS & CONCLUSION: The previously known positive and negative MSC markers, such as STRO-1, CD90, CD146 and CD34 showed comparative expression profiles of hDMSC subtypes. TfR was highly positive in hDMSCs compared with the control cells; therefore, TfR was suggested as a new marker for hDMSCs in this study.

Park, Y. B., et al. (2017). "Single-stage cell-based cartilage repair in a rabbit model: cell tracking and in vivo chondrogenesis of human umbilical cord blood-derived mesenchymal stem cells and hyaluronic acid hydrogel composite." Osteoarthritis Cartilage **25**(4): 570-580.

OBJECTIVE: Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have gained popularity as a promising cell source for regenerative medicine, but limited in vivo studies have reported cartilage repair. In addition, the roles of MSCs in cartilage repair are not well-understood. The purpose of this study was to investigate the feasibility of transplanting hUCB-MSCs and hyaluronic acid (HA) hydrogel composite to repair articular cartilage defects in a rabbit model and determine whether the transplanted cells persisted or disappeared from the defect site. DESIGN: Osteochondral defects were created in the trochlear grooves of the knees. The hUCB-MSCs and HA composite was transplanted into the defect of experimental knees. Control knees were transplanted by HA or left untreated. Animals were sacrificed at 8 and 16 weeks post-transplantation and additionally at 2 and 4 weeks to evaluate the fate of transplanted cells. The repair tissues were evaluated by gross, histological and immunohistochemical analysis. RESULTS: Transplanting hUCB-MSCs and HA composite resulted in overall superior cartilage repair tissue with better quality than HA alone or no treatment. Cellular architecture and collagen arrangement at 16 weeks were similar to those of surrounding normal articular cartilage tissue. Histological scores also revealed that cartilage repair in experimental knees was better than that in control knees. Immunohistochemical analysis with anti-human nuclear antibody confirmed that the transplanted MSCs disappeared gradually over time. CONCLUSION: Transplanting hUCB-MSCs and HA composite promote cartilage repair and interactions between hUCB-MSCs and host cells initiated by paracrine action may play an important role in cartilage repair.

Peng, J., et al. (2011). "Human umbilical cord Wharton's jelly-derived mesenchymal stem cells differentiate into a Schwann-cell phenotype and promote neurite outgrowth in vitro." Brain Res Bull **84**(3): 235-243.

Cell-based therapy has achieved promising functional recovery for peripheral nerve repair. Although Schwann cells (SCs) and bone marrow derived mesenchymal stromal cells (BM-MSCs) are the main cell source for nerve tissue engineering, the clinical application is limited because of donor site morbidity, the invasive procedure, and the decreased number of SCs and BM-MSCs. Wharton's jelly-derived mesenchymal stem cells (WJMSCs) could be a promising cell source for nerve tissue engineering because they are easily accessible and their use has no ethical issues. We investigated the phenotypic, molecular and functional characteristics of WJMSCs differentiated along a Schwann-cell lineage. Cultured WJMSCs were isolated from human umbilical cord, and the undifferentiated WJMSCs were confirmed by the detection of MSC-specific cell-surface markers. WJMSCs treated with a mixture of glial growth factors (basic fibroblast growth factor, platelet-derived growth factor and forskolin) adopted a spindle-like morphology similar to SCs. Immunocytochemical staining, RT-PCR analysis, and Western blot analysis revealed that the treated cells expressed the glial markers glial fibrillary acidic protein, p75, S100 and P0 and indicative of differentiation. On co-culture with dorsal root ganglia neurons, the differentiated WJMSCs enhanced the number of sprouting neurites and neurite length in dorsal root ganglia neurons. Furthermore, using enzyme-linked immunosorbent assay and RT-PCR methodology, we found differentiated WJMSCs secrete and express neurotrophic factors, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin-3 (NT-3). Quantification of neurite outgrowth from PC12 cells grown in differentiated WJMSCs-conditioned media demonstrates that the neurite length is significantly more than control medium and undifferentiated WJMSCs group. WJMSCs can be differentiated into cells that are Schwann-like in terms of morphologic features, phenotype, and function and could be suitable Schwann-cell substitutes for nerve repair in clinical applications.

Peng, K. Y., et al. (2016). "Human pluripotent stem cell (PSC)-derived mesenchymal stem cells (MSCs) show potent neurogenic capacity which is enhanced with cytoskeletal rearrangement." Oncotarget **7**(28): 43949-43959.

Mesenchymal stem cells (MSCs) are paraxial mesodermal progenitors with potent immunomodulatory properties. Reports also indicate that MSCs can undergo neural-like differentiation, offering hope for use in neurodegenerative diseases. However, ex vivo expansion of these rare somatic stem cells for clinical use leads to cellular senescence. A newer source of MSCs derived from human pluripotent stem cells (PSC) can offer the 'best-of-both-worlds' scenario, abrogating the concern of teratoma formation while preserving PSC proliferative capacity. PSC-derived MSCs (PSC-MSCs) also represent MSCs at the earliest developmental stage, and we found that these MSCs harbor stronger neuro-

differentiation capacity than post-natal MSCs. PSC-MSCs express higher levels of neural stem cell (NSC)-related genes and transcription factors than adult bone marrow MSCs at baseline, and rapidly differentiate into neural-like cells when cultured in either standard neurogenic differentiation medium (NDM) or when the cytoskeletal modulator RhoA kinase (ROCK) is inhibited. Interestingly, when NDM is combined with ROCK inhibition, PSC-MSCs undergo further commitment, acquiring characteristics of post-mitotic neurons including nuclear condensation, extensive dendritic growth, and neuron-restricted marker expression including NeuN, beta-III-tubulin and Doublecortin. Our data demonstrates that PSC-MSCs have potent capacity to undergo neural differentiation and also implicate the important role of the cytoskeleton in neural lineage commitment.

Peng, X., et al. (2011). "[Cloning, expression and characterization of gene encoding human stem cell growth factor-alpha and its synergetic effect with rhGM-CSF on proliferation of human umbilical cord mesenchymal stem cells]." Sheng Wu Gong Cheng Xue Bao **27**(11): 1667-1676.

To investigate the effect of hSCGF-alpha on human Umbilical Cord Mesenchymal Stem Cells (hUCMSCs), we obtained hSCGF-alpha using genetic engineering, hSCGF-alpha gene was amplified from hUCMSCs cDNA using two-step PCR and was inserted into pET-28a (+) plasmid vector. Induced by IPTG at 20 degrees Celsius for 24 h, the fusion protein expressed in E. coli BL21 (DE3) was mainly existing in soluble form. The recombinant hSCGF-a was purified using NI-NTA affinity chromatography and the purity was up to 90%. The colony forming test revealed that combined use hSCGF-alpha and rmGM-CSF (recombinant murine GM-colony stimulating factor, rmGM-CSF) had granulocyte/macrophage (GM) promoting effects on murine bone marrow GM progenitor. In addition, the results indicated that hSCGF-alpha and rhGM-CSF had stimulatory effect on hUCMSCs and their synergetic effect was the strongest.

Perez-Ilzarbe, M., et al. (2009). "Comparison of ex vivo expansion culture conditions of mesenchymal stem cells for human cell therapy." Transfusion **49**(9): 1901-1910.

BACKGROUND: Mesenchymal stem cells (MSCs) are multipotent stem cells. Based on their properties, several clinical trials have been designed to explore their potential therapeutic effect. Fetal calf serum (FCS, commonly used for in vitro expansion) is an undesirable source of xenogeneic antigens and bears the risk of transmitting contaminations. As an alternative for FCS, platelet lysate (PL) and both autologous and allogeneic human serum have been proposed. The aim of this study is to compare the culture of bone marrow (BM)-derived MSCs in the presence of different serum supplements to determine the effect on cell growth, differentiation potential, and immunologic function. STUDY DESIGN AND METHODS: MSCs from BM of healthy volunteer donors were grown in the presence of 10% FCS supplemented with 1 ng/mL basic fibroblast growth factor (bFGF), 10% human serum supplemented with 1 ng/mL bFGF, 5% PL, and PL 5% supplemented with 1 ng/mL bFGF (PL plus bFGF). RESULTS: MSCs that expanded in either medium showed a comparable morphology, phenotype, and proliferative and differentiation capacity. While the presence of MSCs in vitro significantly decreased CD3/CD28-mediated T-cell activation, this effect was significantly higher in MSCs cultured with human serum. Production of interferon-gamma was inhibited by cocultured media with MSCs while MSCs also induced a significant inhibition of cell cycle in T cells. DISCUSSION: In conclusion, PL or autologous serum could offer an alternative to the use of FCS in MSC expansion for clinical use maintaining the same growing potential, phenotype, immunomodulatory properties, and differentiation potential.

Periasamy, V. S., et al. (2016). "Fe3 O4 nanoparticle redox system modulation via cell-cycle progression and gene expression in human mesenchymal stem cells." Environ Toxicol **31**(8): 901-912.

The use of engineered nanoparticles (NPs) across multiple fields and applications has rapidly increased over the last decade owing to their unusual properties. However, there is an increased need in understanding their toxicological effect on human health. Particularly, iron oxide (Fe3 O4) have been used in various sectors, including biomedical, food, and agriculture, but the current understanding of their impact on human health is inadequate. In this investigation, we assessed the toxic effect of Fe3 O4 NPs on human mesenchymal stem cells (hMSCs) adopting cell viability, cellular morphological changes, mitochondrial transmembrane potential, and cell-cycle progression assessment methodologies. Furthermore, the expression of oxidative stress, cell death, and cell-cycle regulatory genes was assessed using quantitative polymerase chain reaction. The Fe3 O4 NPs induced cytotoxicity and nuclear morphological changes in hMSCs by dose and time exposure. Cell-cycle analysis indicated that Fe3 O4 NPs altered the cell-cycle progression through a decrease in the proportion of cells in the G0 -G1 phase. The hMSC mitochondrial membrane potential loss increased with an increase in the concentration of Fe3 O4 NPs exposure. The observed expression levels

of the CYP1A, TNF3, TNFSF10, E2F1, and CCNC genes were significantly upregulated in hMSCs in response to Fe3 O4 NPs exposure. Our findings suggest that Fe3 O4 NPs caused metabolic stress through altered cell cycle, oxidative stress, and cell death regulatory gene expression in hMSCs. The results of this investigation revealed that Fe3 O4 NPs exhibited moderate toxicity on hMSCs and that Fe3 O4 NPs may have biomedical applications at low concentrations. (c) 2014 Wiley Periodicals, Inc. Environ Toxicol 31: 901-912, 2016.

Periasamy, V. S., et al. (2016). "Aluminum oxide nanoparticles alter cell cycle progression through CCND1 and EGR1 gene expression in human mesenchymal stem cells." Biotechnol Appl Biochem **63**(3): 320-327.

Aluminum oxide nanoparticles (Al2 O3 -NPs) are important ceramic materials that have been used in a variety of commercial and industrial applications. However, the impact of acute and chronic exposure to Al2 O3 -NPs on the environment and on human health has not been well studied. In this investigation, we evaluated the cytotoxic effects of Al2 O3 -NPs on human mesenchymal stem cells (hMSCs) by using a cell viability assay and observing cellular morphological changes, analyzing cell cycle progression, and monitoring the expression of cell cycle response genes (PCNA, EGR1, E2F1, CCND1, CCNC, CCNG1, and CYCD3). The Al2 O3 -NPs reduced hMSC viability in a dose- and time-dependent manner. Nuclear condensation and fragmentation, chromosomal DNA fragmentation, and cytoplasmic vacuolization were observed in Al2 O3 -NP-exposed cells. The nuclear morphological changes indicated that Al2 O3 -NPs alter cell cycle progression and gene expression. The cell cycle distribution revealed that Al2 O3 -NPs cause cell cycle arrest in the sub-G0-G1 phase, and this is associated with a reduction in the cell population in the G2/M and G0/G1 phases. Moreover, Al2 O3 -NPs induced the upregulation of cell cycle response genes, including EGR1, E2F1, and CCND1. Our results suggested that exposure to Al2 O3 -NPs could cause acute cytotoxic effects in hMSCs through cell cycle regulatory genes.

Pinho, S., et al. (2013). "PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion." J Exp Med **210**(7): 1351-1367.

The intermediate filament protein Nestin labels populations of stem/progenitor cells, including self-renewing mesenchymal stem cells (MSCs), a major constituent of the hematopoietic stem cell (HSC) niche. However, the intracellular location of Nestin prevents its use for prospective live cell isolation. Hence it is important to find surface markers specific for Nestin (+) cells. In this study, we show that the expression of PDGFRalpha and CD51 among CD45(-) Ter119(-) CD31(-) mouse bone marrow (BM) stromal cells characterizes a large fraction of Nestin (+) cells, containing most fibroblastic CFUs, mesenspheres, and self-renewal capacity after transplantation. The PDGFRalpha (+) CD51 (+)subset of Nestin (+) cells is also enriched in major HSC maintenance genes, supporting the notion that niche activity co-segregates with MSC activity. Furthermore, we show that PDGFRalpha (+) CD51(+) cells in the human fetal BM represent a small subset of CD146(+) cells expressing Nestin and enriched for MSC and HSC niche activities. Importantly, cultured human PDGFRalpha (+) CD51(+) nonadherent mesenspheres can significantly expand multipotent hematopoietic progenitors able to engraft immunodeficient mice. These results thus indicate that the HSC niche is conserved between the murine and human species and suggest that highly purified nonadherent cultures of niche cells may represent a useful novel technology to culture human hematopoietic stem and progenitor cells.

Pisati, F., et al. (2007). "Induction of neurotrophin expression via human adult mesenchymal stem cells: implication for cell therapy in neurodegenerative diseases." Cell Transplant **16**(1): 41-55.

In animal models of neurological disorders for cerebral ischemia, Parkinson's disease, and spinal cord lesions, transplantation of mesenchymal stem cells (MSCs) has been reported to improve functional outcome. Three mechanisms have been suggested for the effects of the MSCs: transdifferentiation of the grafted cells with replacement of degenerating neural cells, cell fusion, and neuroprotection of the dying cells. Here we demonstrate that a restricted number of cells with differentiated astroglial features can be obtained from human adult MSCs (hMSCs) both in vitro using different induction protocols and in vivo after transplantation into the developing mouse brain. We then examined the in vitro differentiation capacity of the hMSCs in coculture with slices of neonatal brain cortex. In this condition the hMSCs did not show any neuronal transdifferentiation but expressed neurotrophin low-affinity (NGFR (p75)) and high-affinity (trkC) receptors and released nerve growth factor (NGF) and neurotrophin-3 (NT-3). The same neurotrophin's expression was demonstrated 45 days after the intracerebral transplantation of hMSCs into nude mice with surviving astroglial cells. These data further confirm the limited capability of adult hMSC to differentiate into neurons whereas they differentiated in astroglial cells. Moreover, the secretion of neurotrophic factors combined with

activation of the specific receptors of transplanted hMSCs demonstrated an alternative mechanism for neuroprotection of degenerating neurons. hMSCs are further defined in their transplantation potential for treating neurological disorders.

Polacek, M., et al. (2011). "The secretory profiles of cultured human articular chondrocytes and mesenchymal stem cells: implications for autologous cell transplantation strategies." Cell Transplant **20**(9): 1381-1393.

This study was undertaken to compare the phenotype of human articular chondrocytes (ACs) and bone marrow-derived mesenchymal stem cells (MSCs) after cell expansion by studying the spectrum of proteins secreted by cells into the culture medium. ACs and MSCs were expanded in monolayer cultures for some weeks, as done in standard cell transplantation procedures. Initially, the expression of cartilage signature genes was compared by real-time PCR. Metabolic labeling of proteins (SILAC) in combination with mass spectrometry (LC/MS-MS) was applied to investigate differences in released proteins. In addition, multiplex assays were carried out to quantify the amounts of several matrix metalloproteases (MMPs) and their natural inhibitors (TIMPs). Expanded chondrocytes showed a slightly higher expression of cartilage-specific genes than MSCs, whereas the overall spectra of released proteins were very similar for the two cell types. In qualitative terms MSCs seemed to secrete similar number of extracellular matrix proteins (43% vs. 45% of total proteins found) and catabolic agents (9% vs. 10%), and higher number of anabolic agents (12 % vs. 7%) compared to ACs. Some matrix-regulatory agents such as serpins, BMP-1, and galectins were detected only in MSC supernatants. Quantitative analyses of MMPs and TIMPs revealed significantly higher levels of MMP-1, MMP-2, MMP-3, and MMP-7 in the medium of ACs. Our data show that after the expansion phase, both ACs and MSCs express a dedifferentiated phenotype, resembling each other. ACs hold a phenotype closer to native cartilage at the gene expression level, whereas MSCs show a more anabolic profile by looking at the released proteins pattern. Our data together with the inherent capability of MSCs to maintain their differentiation potential for longer cultivation periods would favor the use of these cells for cartilage reconstruction.

Polzer, H., et al. (2010). "Quantification of fluorescence intensity of labeled human mesenchymal stem cells and cell counting of unlabeled cells in phase-contrast imaging: an open-source-based algorithm." Tissue Eng Part C Methods **16**(6): 1277-1285.

Assessment of cell fate is indispensable to evaluate cell-based therapies in regenerative medicine. Therefore, a widely used technique is fluorescence labeling. A major problem still is the standardized, noninvasive, and reliable quantification of fluorescence intensity of adherent cell populations on single-cell level, since total fluorescence intensity must be correlated to the cell number. Consequently, the aim of the present study was to produce and validate an open-source-based algorithm, capable of measuring the total fluorescence intensity of cell populations and assessing the total cell number in phase-contrast images. To verify the algorithms' capacity to assess fluorescence intensity, human mesenchymal stem cells were transduced to stably express enhanced green fluorescent protein and results produced by the algorithm were compared to flow cytometry analysis. No significant differences could be observed at any time ($p >/= 0.443$). For validation of the algorithm for cell counting in phase-contrast images, adherent human mesenchymal stem cells were manually counted and compared to results produced by the algorithm (correlation coefficient [CC] $r = 0.975$), nuclei staining (CC $r = 0.997$), and hemocytometer (CC $r = 0.629$). We conclude that applying the developed algorithm in routine practice allows robust, fast, and reproducible assessment of fluorescence intensity and cell numbers in simple large-scale microscopy. The method is easy to perform and open source based.

Portas, M., et al. (2016). "Use of Human Cadaveric Mesenchymal Stem Cells for Cell Therapy of a Chronic Radiation-Induced Skin Lesion: A Case Report." Radiat Prot Dosimetry **171**(1): 99-106.

Acute and late radiation-induced injury on skin and subcutaneous tissues are associated with substantial morbidity in radiation therapy, interventional procedures and also are of concern in the context of nuclear or radiological accidents. Pathogenesis is initiated by depletion of acutely responding epithelial tissues and damage to vascular endothelial microvessels. Efforts for medical management of severe radiation-induced lesions have been made. Nevertheless, the development of strategies to promote wound healing, including stem cell therapy, is required. From 1997 to 2014, over 248 patients were referred to the Radiopathology Committee of Hospital de Quemados del Gobierno de la Ciudad de Buenos Aires (Burns Hospital) for the diagnosis and therapy of radiation-induced localized lesions. As part of the strategies for the management of severe cases, there is an ongoing research and development protocol on 'Translational Clinical Trial phases I/II to evaluate the safety and efficacy of adult mesenchymal stem cells from bone marrow for the

treatment of large burns and radiological lesions'. The object of this work was to describe the actions carried out by the Radiopathology Committee of the Burns Hospital in a chronic case with more than 30 years of evolution without positive response to conventional treatments. The approach involved the evaluation of the tissular compromise of the lesion, the prognosis and the personalized treatment, including regenerative therapy.

Potapova, I. A., et al. (2010). "Von willebrand factor increases endothelial cell adhesiveness for human mesenchymal stem cells by activating p38 mitogen-activated protein kinase." Stem Cell Res Ther **1**(5): 35.

INTRODUCTION: Delivered systemically or natively circulating mesenchymal stem cells accumulate in injured tissues. During homing mesenchymal stem cells adhere to endothelial cells and infiltrate underlying tissue. Previously we have shown that adhesiveness of endothelial cells for mesenchymal stem cells correlates with the inhibition of mitochondrial function of endothelial cells and secretion of von Willebrand factor. We hypothesized that von Willebrand factor is an auto/paracrine regulator of endothelial cell adhesiveness and studied the effect of von Willebrand factor on adhesion of mesenchymal stem cells to endothelial cells. METHODS: We used Affymetrix DNA microarrays, human protein phospho-MAPK array, Western blot, cell-based ELISA and flow cytometry analysis to study the activation of endothelial cells by von Willebrand factor. Cell adhesion assay and protein kinase inhibitors were used to evaluate the role of mitogen-activated protein kinases in the regulation of endothelial cell adhesiveness for mesenchymal stem cell. RESULTS: Treatment of endothelial cells with von Willebrand factor stimulated the mesenchymal stem cell adhesion in a time- and concentration-dependent manner. Mesenchymal stem cells did not adhere to immobilized von Willebrand factor and did not express receptors for von Willebrand factor suggesting that the stimulation of the mesenchymal stem cell adhesion is a result of endothelial cell activation with von Willebrand factor. Treatment of endothelial cells with von Willebrand factor activated ERK-1,2 and p38 MAPK without an effect on gene or cell surface expression of E-selectin, P-selectin, VCAM1 and ICAM1. Inhibition of p38 MAPK, but not ERK-1,2, in endothelial cells completely abrogated the stimulation of the mesenchymal stem cell adhesion by von Willebrand factor. CONCLUSIONS: Von Willebrand factor is an auto/paracrine regulator of endothelial cells. Activation of p38 MAPK in endothelial cells by von Willebrand factor is responsible for the regulation of endothelial cell adhesiveness for mesenchymal stem cells.

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.

Prieto, P., et al. (2016). "Cell Expansion-Dependent Inflammatory and Metabolic Profile of Human Bone Marrow Mesenchymal Stem Cells." Front Physiol **7**: 548.

Stem cell therapy has emerged as a promising new area in regenerative medicine allowing the recovery of viable tissues. Among the many sources of adult stem cells, bone marrow-derived are easy to expand in culture via plastic adherence and their multipotentiality for differentiation make them ideal for clinical applications. Interestingly, several studies have indicated that MSCs expansion in vitro may be limited mainly due to "cell aging" related to the number of cell divisions in culture. We have determined that MSCs exhibit a progressive decline

across successive passages in the expression of stem cell markers, in plasticity and in the inflammatory response, presenting low immunogenicity. We have exposed human MSCs after several passages to TLRs ligands and analyzed their inflammatory response. These cells responded to pro-inflammatory stimuli (i.e., NOS-2 expression) and to anti-inflammatory cytokines (i.e., HO1 and Arg1) until two expansions, rapidly declining upon subculture. Moreover, in the first passages, MSCs were capable to release IL1beta, IL6, and IL8, as well as to produce active MMPs allowing them to migrate. Interestingly enough, after two passages, anaerobic glycolysis was enhanced releasing high levels of lactate to the extracellular medium. All these results may have important implications for the safety and efficacy of MSCs-based cell therapies.

Pustlauk, W., et al. (2017). "Modulation of chondrogenic differentiation of human mesenchymal stem cells in jellyfish collagen scaffolds by cell density and culture medium." J Tissue Eng Regen Med **11**(6): 1710-1722.

Studies on tissue-engineering approaches for the regeneration of traumatized cartilage focus increasingly on multipotent human mesenchymal stem cells (hMSCs) as an alternative to autologous chondrocytes. The present study applied porous scaffolds made of collagen from the jellyfish Rhopilema esculentum for the in vitro chondrogenic differentiation of hMSCs. Culture conditions in those scaffolds differ from conditions in high-density pellet cultures, making a re-examination of these data necessary. We systematically investigated the influence of seeding density, basic culture media [Dulbecco's modified Eagle's medium (DMEM), alpha-minimum essential medium (alpha-MEM)] with varying glucose content and supplementation with fetal calf serum (FCS) or bovine serum albumin (BSA) on the chondrogenic differentiation of hMSCs. Gene expression analyses of selected markers for chondrogenic differentiation and hypertrophic development were conducted. Furthermore, the production of cartilage extracellular matrix (ECM) was analysed by quantification of sulphated glycosaminoglycan and collagen type II contents. The strongest upregulation of chondrogenic markers, along with the highest ECM deposition was observed in scaffolds seeded with 2.4 x 10(6) cells/cm (3) after cultivation in high-glucose DMEM and 0.125% BSA. Lower seeding densities compared to high-density pellet cultures were sufficient to induce in vitro chondrogenic differentiation of hMSCs in collagen scaffolds, which reduces the amount of cells required for the seeding of scaffolds and thus the monolayer expansion period. Furthermore, examination of the impact of FCS and alpha-MEM on chondrogenic MSC differentiation is an important prerequisite for the development of an osteochondral medium for simultaneous osteogenic and chondrogenic differentiation in biphasic scaffolds for osteochondral tissue regeneration. Copyright (c) 2015 John Wiley & Sons, Ltd.

Rajan, T. S., et al. (2017). "Conditioned medium from human gingival mesenchymal stem cells protects motor-neuron-like NSC-34 cells against scratch-injury-induced cell death." Int J Immunopathol Pharmacol **30**(4): 383-394.

Neuronal cell death is a normal process during central nervous system (CNS) development and is also involved in the death of motor neurons in diverse spinal motor neuron degenerative diseases. Here, we investigated the neuroprotective effect of secretory factors released from human gingival mesenchymal stem cells (hGMSCs) in mechanically injured murine motor-neuron-like NSC-34 cells. The cells were exposed to scratch injury and the markers for apoptosis and oxidative stress were examined. Immunocytochemistry results showed that proapoptotic markers cleaved caspase-3 and Bax were elevated while anti-apoptotic protein Bcl-2 was suppressed in scratch-injured NSC-34 cells. Oxidative stress markers SOD-1, inducible nitric oxide synthase (iNOS), Cox-2, and proinflammatory cytokine tumor necrosis factor alpha (TNF-alpha) were activated. Conditioned medium (CM) derived from hGMSCs (hGMSC-CM) significantly blocked the cell death by suppressing SOD-1, iNOS, TNF-alpha, cleaved caspase-3, and Bax. Bcl-2 and anti-inflammatory cytokine anti-interleukin 10 (IL-10) were increased in hGMSC-CM-treated injured cells. Moreover, hGMSC-CM treatment upregulated neurotrophins anti-brain-derived neurotrophic factor (BDNF) and NT3. Western blot data of hGMSC-CM revealed the presence of neurotrophins nerve growth factor (NGF), NT3, anti-inflammatory cytokines IL-10, and transforming growth factor beta (TGF-beta), suggesting their positive role to elicit neuroprotection. Our results propose that hGMSC-CM may serve as a simple and potential autologous therapeutic tool to treat motor neuron injury.

Ramasamy, R., et al. (2008). "The immunosuppressive effects of human bone marrow-derived mesenchymal stem cells target T cell proliferation but not its effector function." Cell Immunol **251**(2): 131-136.

Mesenchymal stem cells (MSC) are non-haematopoietic stem cells that are capable of differentiating into tissues of mesodermal origin. MSC play an important role in supporting the development of fetal and adult haematopoiesis. More recently, MSC

have also been found to exhibit inhibitory effect on T cell responses. However, there is little information on the mechanism of this immunosuppression and our study addresses this issue by targeting T cell functions at various level of immune responses. We have generated MSC from human adult bone marrow (BM) and investigated their immunoregulatory function at different phases of T cell responses. MSC showed the ability to inhibit mitogen (CD3/CD28 microbeads)-activated T cell proliferation in a dose-dependent manner. In order to evaluate the specificity of this immunosuppression, the proliferation of CD4(+) and CD8(+) cells were measured. MSC equally inhibit CD4(+) and CD8(+) subpopulations of T cells in response to PHA stimulation. However, the antiproliferative effect of MSC is not due to the inhibition of T cell activation. The expression of early activation markers of T cells, namely CD25 and CD69 were not significantly altered by MSC at 24, 48 and 72h. Furthermore, the immunosuppressive effect of MSC mainly targets T cell proliferation rather than their effector function since cytotoxicity of T cells is not affected. This work demonstrates that the immunosuppressive effect of MSC is exclusively a consequence of an anti-proliferative activity, which targets T cells of different subpopulations. For this reason, they have the potential to be exploited in the control of unwanted immune responses such as graft versus host disease (GVHD) and autoimmunity.

Ramasamy, R., et al. (2012). "Basic fibroblast growth factor modulates cell cycle of human umbilical cord-derived mesenchymal stem cells." Cell Prolif **45**(2): 132-139.

BACKGROUND: Mesenchymal stem cells (MSC) have great potential in regenerative medicine, immunotherapy and gene therapy due to their unique properties of self-renewal, high plasticity, immune modulation and ease for genetic modification. However, production of MSC at sufficient clinical scale remains an issue as in vitro generation of MSC inadequately fulfils the demand with respect to patients. OBJECTIVES: This study has aimed to establish optimum conditions to generate and characterize MSC from human umbilical cord (UC-MSC). MATERIALS AND METHODS: To optimize MSC population growth, basic fibroblast growth factor (bFGF) was utilized in culture media. Effects of bFGF on expansion kinetics, cell cycle, survival of UC-MSC, cytokine secretion, expression of early stem-cell markers and immunomodulation were investigated. RESULTS: bFGF supplementation profoundly enhanced UC-MSC proliferation by reducing population doubling time without altering immunophenotype and immunomodulatory function of UC-MSC. However, cell cycle studies revealed that bFGF drove the cells into the cell cycle, as a higher proportion of cells resided in S phase and progressed into M phase. Consistent with this, bFGF was shown to promote expression of cyclin D proteins and their relevant kinases to drive UC-MSC to transverse cell cycle check points, thus, committing the cells to DNA synthesis. Furthermore, supplementation with bFGF changed the cytokine profiles of the cells and reduced their apoptotic level. CONCLUSION: Our study showed that bFGF supplementation of UC-MSC culture enhanced the cells' growth kinetics without compromising their nature.

Ramos-Mejia, V., et al. (2012). "Maintenance of human embryonic stem cells in mesenchymal stem cell-conditioned media augments hematopoietic specification." Stem Cells Dev **21**(9): 1549-1558.

The realization of human embryonic stem cells (hESC) as a model for human developmental hematopoiesis and in potential cell replacement strategies relies on an improved understanding of the extrinsic and intrinsic factors regulating hematopoietic-specific hESC differentiation. Human mesenchymal stem cells (hMSCs) are multipotent cells of mesodermal origin that form a part of hematopoietic stem cell niches and have an important role in the regulation of hematopoiesis through production of secreted factors and/or cell-to-cell interactions. We have previously shown that hESCs may be successfully maintained feeder free using hMSC-conditioned media (MSC-CM). Here, we hypothesized that hESCs maintained in MSC-CM may be more prone to differentiation toward hematopoietic lineage than hESCs grown in standard human foreskin fibroblast-conditioned media. We report that specification into hemogenic progenitors and subsequent hematopoietic differentiation and clonogenic progenitor capacity is robustly enhanced in hESC lines maintained in MSC-CM. Interestingly, co-culture of hESCs on hMSCs fully abrogates hematopoietic specification of hESCs, thus suggesting that the improved hematopoietic differentiation is mediated by MSC-secreted factors rather than by MSC-hESC physical interactions. To investigate the molecular mechanism involved in this process, we analyzed global (LINE-1) methylation and genome-wide promoter DNA methylation. hESCs grown in MSC-CM showed a decrease of 17% in global DNA methylation and a promoter DNA methylation signature consisting of 45 genes commonly hypomethylated and 102 genes frequently hypermethylated. Our data indicate that maintenance of hESCs in MSC-CM robustly augments hematopoietic specification and that the process seems mediated by MSC-secreted factors conferring a DNA methylation signature to undifferentiated hESCs which

may influence further predisposition toward hematopoietic specification.

Reitinger, S., et al. (2012). "Electric impedance sensing in cell-substrates for rapid and selective multipotential differentiation capacity monitoring of human mesenchymal stem cells." Biosens Bioelectron **34**(1): 63-69.

Biosensor systems which enable impedance measurements on adherent cell layers under label-free conditions are considered powerful tools for monitoring specific biological characteristics. A radio frequency identification-based sensor platform was adopted to characterize cultivation and differentiation of human bone marrow-derived multipotent stem cells (bmMSC) over periods of up to several days and weeks. Electric cell-substrate impedance sensing was achieved through fabrication of sensitive elements onto glass substrates which comprised two comb-shaped interdigitated gold electrodes covering an area of 1.8 mmx2 mm. The sensing systems were placed into the wells of a 6-well tissue culture plate, stacked onto a reader unit and could thus be handled and operated under sterile conditions. Continuous measurements were carried out with a sinusoidal voltage of 35 mV at a frequency of 10 kHz. After seeding of human bmMSC, this sensor was able to trace significant impedance changes contingent upon cell spreading and adhesion. The re-usable system was further proven suitable for live examination of cell-substrate attachment or continuous cell monitoring up to several weeks. Induction of either osteogenic or adipogenic differentiation could be validated in bmMSC cultures within a few days, in contrast to state-of-the-art protocols, which require several weeks of cultivation time. In the context of medical cell production in a GMP-compliant process, the here presented interdigitated electric microsensor technology allows the documentation of MSC quality in a fast, efficient and reliable fashion.

Ren, H., et al. (2016). "Comparative Analysis of Human Mesenchymal Stem Cells from Umbilical Cord, Dental Pulp, and Menstrual Blood as Sources for Cell Therapy." Stem Cells Int **2016**: 3516574.

Although mesenchymal stem cells (MSCs) based therapy has been considered as a promising tool for tissue repair and regeneration, the optimal cell source remains unknown. Umbilical cord (UC), dental pulp (DP), and menstrual blood (MB) are easily accessible sources, which make them attractive candidates for MSCs. The goal of this study was to compare the biological characteristics, including morphology, proliferation, antiapoptosis, multilineage differentiation capacity, and immunophenotype of UC-, DP-, and MB-MSCs in order to provide a theoretical basis for clinical selection and application of these cells. As a result, all UC-, DP-, and MB-MSCs have self-renewal capacity and multipotentiality. However, the UC-MSCs seemed to have higher cell proliferation ability, while DP-MSCs may have significant advantages for osteogenic differentiation, lower cell apoptosis, and senescence. These differences may be associated with the different expression level of cytokines, including vascular endothelial growth factor, fibroblast growth factor, keratinocyte growth factor, and hepatocyte growth factor in each of the MSCs. Comprehensively, our results suggest DP-MSCs may be a desired source for clinical applications of cell therapy.

Ren, X., et al. (2005). "A novel human artificial chromosome vector provides effective cell lineage-specific transgene expression in human mesenchymal stem cells." Stem Cells **23**(10): 1608-1616.

Mesenchymal stem cells (MSCs) hold promise for use in adult stem cell-mediated gene therapy. One of the major aims of stem cell-mediated gene therapy is to develop vectors that will allow appropriate levels of expression of therapeutic genes along differentiation under physiological regulation of the specialized cells. Human artificial chromosomes (HACs) are stably maintained as independent chromosomes in host cells and should be free from potential insertional mutagenesis problems of conventional transgenes. Therefore, HACs have been proposed as alternative implements to cell-mediated gene therapy. Previously, we constructed a novel HAC, termed 21 Deltapq HAC, with a loxP site in which circular DNA can be reproducibly inserted by the Cre/loxP system. We here assessed the feasibility of lineage-specific transgene expression by the 21Deltapq HAC vector using an in vitro differentiation system with an MSC cell line, hiMSCs, which has potential for osteogenic, chondrogenic, and adipogenic differentiation. An enhanced green fluorescent protein (EGFP) gene driven by a promoter for osteogenic lineage-specific osteopontin (OPN) gene was inserted onto the 21 Deltapq HAC and then transferred into hiMSC. The expression cassette was flanked by the chicken HS4 insulators to block promoter interference from adjacent drug-resistant genes. The EGFP gene was specifically expressed in the hiMSC that differentiated into osteocytes in coordination with the transcription of endogenous OPN gene but was not expressed after adipogenic differentiation induction or in noninduction culture. These results suggest that use of the HAC vector is suitable for regulated expression of transgenes in stem cell-mediated gene therapy.

Reza, A. M., et al. (2016). "Human adipose mesenchymal stem cell-derived exosomal-miRNAs

are critical factors for inducing anti-proliferation signalling to A2780 and SKOV-3 ovarian cancer cells." Sci Rep **6**: 38498.

An enigmatic question exists concerning the pro- or anti-cancer status of mesenchymal stem cells (MSCs). Despite growing interest, this question remains unanswered, and the debate became intensified with new evidences backing each side. Here, we showed that human adipose MSC (hAMSC)-derived conditioned medium (CM) exhibited inhibitory effects on A2780 human ovarian cancer cells by blocking the cell cycle, and activating mitochondria-mediated apoptosis signalling. Explicitly, we demonstrated that exosomes, an important biological component of hAMSC-CM, could restrain proliferation, wound-repair and colony formation ability of A2780 and SKOV-3 cancer cells. Furthermore, hAMSC-CM-derived exosomes induced apoptosis signalling by upregulating different pro-apoptotic signalling molecules, such as BAX, CASP9, and CASP3, as well as downregulating the anti-apoptotic protein BCL2. More specifically, cancer cells exhibited reduced viability following fresh or protease-digested exosome treatment; however, treatment with RNase-digested exosomes could not inhibit the proliferation of cancer cells. Additionally, sequencing of exosomal RNAs revealed a rich population of microRNAs (miRNAs), which exhibit anti-cancer activities by targeting different molecules associated with cancer survival. Our findings indicated that exosomal miRNAs are important players involved in the inhibitory influence of hAMSC-CM towards ovarian cancer cells. Therefore, we believe that these comprehensive results will provide advances concerning ovarian cancer research and treatment.

Richardson, S. M., et al. (2008). "Human mesenchymal stem cell differentiation to NP-like cells in chitosan-glycerophosphate hydrogels." Biomaterials **29**(1): 85-93.

Intervertebral disc (IVD) degeneration is one of the major causes of low back pain. As current clinical treatments are aimed at restoring biomechanical function and providing symptomatic relief, interest in methods focused on biological repair has increased. Several tissue engineering approaches using different cell types and hydrogels/scaffolds have been proposed. Owing to the unsuitable nature of degenerate cells for tissue engineering attention has focused on the use of mesenchymal stem cells (MSCs). Additionally, while rigid scaffolds have been demonstrated to allow MSC differentiation to the chondrocyte-like cells of the IVD, hydrogels are being increasingly studied as they allow minimally invasive implantation without extensive damage to the IVD. Here, we have studied the temperature-sensitive hydrogel chitosan-glycerophosphate (C/Gp), seeded with human MSCs and cultured for 4 weeks in standard medium. We have analysed the gene and protein expression profile of the MSCs and compared it to that of both nucleus pulposus (NP) cells and articular chondrocytes cultured in C/Gp. Gene expression analysis for chondrocytic-cell marker genes demonstrated differentiation of MSCs to a phenotype which showed similarities to both articular chondrocytes and NP cells. Conventional PCR demonstrated a lack of expression of osteogenic marker genes and the hypertrophic marker gene type X collagen. MSCs also secreted both proteoglycans and collagens in a ratio, which more closely resembled that of NP cells than articular chondrocytes. These results therefore suggest that MSC-seeded C/Gp gels could be used clinically for the regeneration of the degenerate human IVD.

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.

Rizvanov, A. A., et al. (2010). "Interaction and self-organization of human mesenchymal stem cells and neuro-blastoma SH-SY5Y cells under co-culture conditions: A novel system for modeling cancer cell micro-environment." Eur J Pharm Biopharm **76**(2): 253-259.

The common drawback of many in vitro cell culture systems is the absence of appropriate micro-environment, which is formed by the combination of factors such as cell-cell contacts, extracellular matrix and paracrine regulation. Micro-environmental factors in a tumor tissue can influence physiological status of the cancer cells and their susceptibility to anticancer therapies. Interaction of cancer cells with their micro-environment and regional stem cells, therefore, is of particular interest. Development of in vitro systems which allow more accurate modeling of complex relations occurring in real tumor environments can increase efficiency of preclinical assays for screening anticancer drugs. The aim of this work was to study interactions between human mesenchymal stem cells (MSCs) and neuro-blastoma cancer SH-SY5Y cells under co-culture conditions on different coated surfaces to determine the effect of co-existence of cancer and stem cells on each cellular population under various stress conditions. We developed an efficient in vitro system for studying individual cancer and stem cell populations during co-culture using differential live fluorescent membrane labeling, and demonstrated self-organization of cancer and stem cells during co-culture on various coated surfaces. Our findings support the evidence that cancer and stem cell interactions play important roles in cellular behavior of cancer cells. These properties can be used in different fields of cancer research, tissue engineering and biotechnology.

Roche, S., et al. (2009). "Comparative proteomic analysis of human mesenchymal and embryonic stem cells: towards the definition of a mesenchymal stem cell proteomic signature." Proteomics **9**(2): 223-232.

Mesenchymal stem cells (MSC) are adult multipotential progenitors which have a high potential in regenerative medicine. They can be isolated from different tissues throughout the body and their homogeneity in terms of phenotype and differentiation capacities is a real concern. To address this issue, we conducted a 2-DE gel analysis of mesenchymal stem cells isolated from bone marrow (BM), adipose tissue, synovial membrane and umbilical vein wall. We confirmed that BM and adipose tissue derived cells were very similar, which argue for their interchangeable use for cell therapy. We also compared human mesenchymal to embryonic stem cells and showed that umbilical vein wall stem cells, a neo-natal cell type, were closer to BM cells than to embryonic stem cells. Based on these proteomic data, we could propose a panel of proteins which were the basis for the definition of a mesenchymal stem cell proteomic signature.

Rodini, C. O., et al. (2018). "Mesenchymal stem cells enhance tumorigenic properties of human glioblastoma through independent cell-cell communication mechanisms." Oncotarget **9**(37): 24766-24777.

Mesenchymal stem cells (MSC) display tumor tropism and have been addressed as vehicles for delivery of anti-cancer agents. As cellular components of the tumor microenvironment, MSC also influence tumor progression. However, the contribution of MSC in brain cancer is not well understood since either oncogenic or tumor suppressor effects have been reported for these cells. Here, MSC were found capable of stimulating human Glioblastoma (GBM) cell proliferation through a paracrine effect mediated by TGFB1. Moreover, when in direct cell-cell contact with GBM cells, MSC elicited an increased proliferative and invasive tumor cell behavior under 3D conditions, as well as accelerated tumor development in nude mice, independently of paracrine TGFB1. A secretome profiling of MSC-GBM co-cultures identified 126 differentially expressed proteins and 10 proteins exclusively detected under direct cell-cell contact conditions. Most of these proteins are exosome cargos and are involved in cell motility and tissue development. These results indicate a dynamic interaction between MSC and GBM cells, favoring aggressive tumor cell traits through alternative and independent mechanisms. Overall, these findings indicate that MSC may exert pro-tumorigenic effects when in close contact with tumor cells, which must be carefully considered when employing MSC in targeted cell therapy protocols against cancer.

Rohaina, C. M., et al. (2014). "Reconstruction of limbal stem cell deficient corneal surface with induced human bone marrow mesenchymal stem cells on amniotic membrane." Transl Res **163**(3): 200-210.

The cornea can be damaged by a variety of clinical disorders or chemical, mechanical, and thermal injuries. The objectives of this study were to induce bone marrow mesenchymal stem cells (BMSCs) to corneal lineage, to form a tissue engineered corneal substitute (TEC) using BMSCs, and to treat corneal surface defects in a limbal stem cell deficiency model. BMSCs were induced to corneal lineage using limbal

medium for 10 days. Induced BMSCs demonstrated upregulation of corneal stem cell markers; beta1-integrin, C/EBPdelta, ABCG2, and p63, increased protein expression of CK3 and p63 significantly compared with the uninduced ones. For TEC formation, passage 1 BMSCs were trypsinized and seeded on amniotic membrane in a transwell co-culture system and were grown in limbal medium. Limbal stem cell deficiency models were induced by alkaline injury, and the TEC was implanted for 8 weeks. Serial slit lamp evaluation revealed remarkable improvement in corneal regeneration in terms of corneal clarity and reduced vascularization. Histologic and optical coherence tomography analyses demonstrated comparable corneal thickness and achieved stratified epithelium with a compact stromal layer resembling that of normal cornea. CK3 and p63 were expressed in the newly regenerated cornea. In conclusion, BMSCs can be induced into corneal epithelial lineage, and these cells are viable for the formation of TEC, to be used for the reconstruction of the corneal surface in the limbal stem cell deficient model.

Sa, Y. L., et al. (2010). "[Effects of human bone marrow mesenchymal stem cells on cytokines secretion from allogeneic dendritic cell activated cytokine-induced killer cells]." Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi **26**(10): 988-991.

AIM: to study the effect of human bone marrow derived mesenchymal stem cells (hMSCs) on cytokines secretion (IFN-gamma, TNF-alpha, IL-10, IL-6, IL-4 and IL-2) of allogeneic DC-CIK cells (in co-culture of CIK cells with DC), which investigate the mechanism of immunoregulation induced by hMSCs. METHODS: the hMSCs from bone marrow were isolated, expanded and identified by cell morphology, differentiation into neuron-like cells with NSE, fat-like cells with red-oil stain, and expression of CD29, CD44. The DC and CIK cells from peripheral blood were isolated, expanded and identified by CD1alpha, HLA-DR or CD3(+);CD56(+);. The hMSCs were co-cultured with DC-CIK cells according to ratio 1:10. The expression of the six cytokines in supernatant was evaluated by flow cytometry after 4 days of DC-activated CIK cells in co-culture with hMSCs. RESULTS: the hMSCs displayed a fibroblast-like morphology and the positive cells of CD29 and CD44 were 96.6%, 94.6%, which have the capacity of differentiation into neuron-like cells with expressed NSE as well as fat-like cells with red-oil stain positive. The expression of CD1alpha, HLA-DR in DC was (91.9 +/- 10.04)% and (88.8 +/- 8.92)%. The CD3(+);CD56(+); double positive cells in DC-CIK cells was (29.23 +/- 12.23)% compared to CIK cells with (15.98 +/- 2.49)%. The cytokines secretion of DC-CIK cells in co-culture with hMSCs was IFN-gamma (135.05 +/- 48.19) ng/L; TNF-alpha (11.33 +/- 1.42) ng/L; IL-10 (10.15 +/- 2.25) ng/L; IL-6 (494.63 +/- 235.222) ng/L; IL-4 (7.07 +/- 2.30) ng/L and IL-2 (1074.6 3 +/- 303.74) ng/L. In control group (DC-CIK cells) the secretion of IFN-gamma, TNF-alpha, IL-10, IL-6, IL-4 and IL-2 was (717.6 +/- 248.15) ng/L; (17.78 +/- 7.52) ng/L; (29.95 +/- 12.76) ng/L; (8.03 +/- 0.21) ng/L, (9.08 +/- 3.07) ng/L as well as IL-2 1 250 ng/L. CONCLUSION: the secretion of IFN-gamma and IL-10 were down-regulated. It probably implied that hMSCs had the effect of immunoregulation on DC-CIK cells.

Sabri, A., et al. (2011). "Crosstalk of EGF-directed MAPK signalling pathways and its potential role on EGF-induced cell proliferation and COX-2 expression in human mesenchymal stem cells." Cell Biochem Funct **29**(1): 64-70.

Epidermal growth factor (EGF) promotes proliferation in human mesenchymal stem cells (hMSCs) during in vitro propagation. In this study, we investigated the effects of PI3K/AKT, ERK1/2, P38 and JNK on EGF signalling in hMSCs. The effects of EGF on MAPKs and PI3K/AKT crosstalk were investigated by immunoblotting; cyclooxygenase-2 (COX-2) expression was studied by real-time RT-PCR; and cell proliferation was evaluated by methylthiazolyl tetrazolium bromide assay. Our results showed that EGF immediately activated all four pathways, induced proliferation and increased COX-2 expression. Interestingly, inhibition of PI3K/AKT-enhanced EGF-stimulated ERK1/2 activity, and inhibition of ERK1/2 and JNK reduced AKT phosphorylation. Furthermore, EGF-induced proliferation as well as EGF-augmented COX2 expression was hindered by ERK1/2 and p38 inhibitors. The results of this study provide evidences to be used in extended proliferation of hMSCs for cell therapy.

Saito, S., et al. (2011). "Use of BAC array CGH for evaluation of chromosomal stability of clinically used human mesenchymal stem cells and of cancer cell lines." Hum Cell **24**(1): 2-8.

Array-based comparative genomic hybridization (aCGH) using bacterial artificial chromosomes (BAC) is a powerful method to analyze DNA copy number aberrations of the entire human genome. In fact, CGH and aCGH have revealed various DNA copy number aberrations in numerous cancer cells and cancer cell lines examined so far. In this report, BAC aCGH was applied to evaluate the stability or instability of cell lines. Established cell lines have greatly contributed to advancements in not only biology but also medical science. However, cell lines have serious problems, such as alteration of biological properties during long-

term cultivation. Firstly, we investigated two cancer cell lines, HeLa and Caco-2. HeLa cells, established from a cervical cancer, showed significantly increased DNA copy number alterations with passage time. Caco-2 cells, established from a colon cancer, showed no remarkable differences under various culture conditions. These results indicate that BAC aCGH can be used for the evaluation and validation of genomic stability of cultured cells. Secondly, BAC aCGH was applied to evaluate and validate the genomic stabilities of three patient's mesenchymal stem cells (MSCs), which were already used for their treatments. These three MSCs showed no significant differences in DNA copy number aberrations over their entire chromosomal regions. Therefore, BAC aCGH is highly recommended for use for a quality check of various cells before using them for any kind of biological investigation or clinical application.

Sakaguchi, Y., et al. (2005). "Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source." Arthritis Rheum **52**(8): 2521-2529.

OBJECTIVE: To compare the properties of human mesenchymal stem cells (MSCs) isolated from bone marrow, synovium, periosteum, skeletal muscle, and adipose tissue. METHODS: Human mesenchymal tissues were obtained from 8 donors during knee surgery for ligament injury. After collagenase digestion or gradient-density separation, nucleated cells were plated at an appropriate density for expansion at the maximum rate without colony-to-colony contact. Yield, expandability, differentiation potential, and epitope profile were compared among MSCs from the 5 different tissue sources. RESULTS: Colony number per 10(3) nucleated cells was lower, and cell number per colony was higher, in bone marrow than in other mesenchymal tissues. When the cells were replated at low density every 14 days, bone marrow-, synovium-, and periosteum-derived cells retained their proliferation ability even at passage 10. In chondrogenesis studies in which the cells were pelleted and cultured in vitro, pellets from bone marrow-, synovium-, and periosteum-derived cells were shown to be larger and stained more extensively for cartilage matrix. Synovium-derived cells, in particular, had the greatest ability for chondrogenesis. In adipogenesis experiments, the frequency of oil red O-positive colonies was highest in synovium- and adipose tissue-derived cells. In studies of osteogenesis, the rate of alizarin red-positive colonies was highest in bone marrow-, synovium-, and periosteum-derived cells. For epitope profiling, 15 surface antigens were measured. Most appeared to have similar epitope profiles irrespective of cell source. CONCLUSION: Our findings indicate that there are significant differences in MSC properties according to tissue source, beyond donor and experimental variation. Superiority of synovium as a potential source of MSCs for clinical applications was demonstrated.

Saleh, F. A., et al. (2011). "Effects of endothelial cells on human mesenchymal stem cell activity in a three-dimensional in vitro model." Eur Cell Mater **22**: 242-257; discussion 257.

An increasing body of data suggest that mesenchymal stem cells (MSCs) reside in a perivascular niche. To more closely mimic this in vivo microenvironment and for better understanding of its complexity, and the factors that regulate the MSC activity, human umbilical vein endothelial cells (HUVECs) were co-cultured with human bone marrow MSCs--using a novel three-dimensional (3D) spheroid co-culture system. Using confocal microscopy of fluorescently labelled cells, we observed HUVECs and MSCs to self-assemble and form organised structures with segregated cell-type partitioning. Under osteogenic conditions, the rate and extent of differentiation in MSC/HUVEC spheroids was significantly elevated compared to 3D co-cultures of MSCs and human dermal fibroblast controls as shown by alkaline phosphatase staining. Conversely, HUVECs inhibited adipogenic differentiation and the proliferation of MSCs in 3D co-cultures indicating that HUVECs suppressed MSC cycling and selectively promoted osteogenic differentiation in 3D. We have also shown that HUVECs enhanced activation of endogenous Wnt signalling and bone morphogenetic protein (BMP) signalling as shown by increased levels of active nuclear beta-catenin and pSmad 1/5/8 immunopositivity respectively. These data suggest strongly that endothelial cells regulate the MSC activity in simulated in vivo conditions, by maintaining quiescence and facilitating niche exit via osteogenic differentiation following appropriate cues. Our findings also underline the importance of 3D heterotypic cell-cell interactions in the regulation of MSC behaviour, suggesting that multicellular cocktails and/or 3D-based delivery strategies may be beneficial for bone repair.

Sardesai, V. S., et al. (2017). "Avoidance of Maternal Cell Contamination and Overgrowth in Isolating Fetal Chorionic Villi Mesenchymal Stem Cells from Human Term Placenta." Stem Cells Transl Med **6**(4): 1070-1084.

Human placenta is rich in mesenchymal stem/stromal cells (MSC), with their origin widely presumed fetal. Cultured placental MSCs are confounded by a high frequency of maternal cell contamination. Our recent systematic review concluded that only a small minority of placental MSC

publications report fetal/maternal origin, and failed to discern a specific methodology for isolation of fetal MSC from term villi. We determined isolation conditions to yield fetal and separately maternal MSC during ex vivo expansion from human term placenta. MSCs were isolated via a range of methods in combination; selection from various chorionic regions, different commercial media, mononuclear cell digest and/or explant culture. Fetal and maternal cell identities were quantitated in gender-discordant pregnancies by XY chromosome fluorescence in situ hybridization. We first demonstrated reproducible maternal cell contamination in MSC cultures from all chorionic anatomical locations tested. Cultures in standard media rapidly became composed entirely of maternal cells despite isolation from fetal villi. To isolate pure fetal cells, we validated a novel isolation procedure comprising focal dissection from the cotyledonary core, collagenase/dispase digestion and explant culture in endothelial growth media that selected, and provided a proliferative environment, for fetal MSC. Comparison of MSC populations within the same placenta confirmed fetal to be smaller, more osteogenic and proliferative than maternal MSC. We conclude that in standard media, fetal chorionic villi-derived MSC (CV-MSC) do not grow readily, whereas maternal MSC proliferate to result in maternal overgrowth during culture. Instead, fetal CV-MSCs require isolation under specific conditions, which has implications for clinical trials using placental MSC. Stem Cells Translational Medicine 2017;6:1070-1084.

Sarmadi, V. H., et al. (2008). "The effect of human mesenchymal stem cells on tumour cell proliferation." Med J Malaysia **63 Suppl A**: 63-64.

The therapeutic effect of mesenchymal stem cells (MSC) has been extensively investigated in recent decades, however this therapeutic effect has not been fully characterised. The aim of this study is to elucidate the inhibitory effect of MSC on haematopoietic tumour cells proliferation such as BV173 cell line. To this end, MSC generated from bone marrow, after immunophenotyping, they were co-cultured with tumour cell. The result shows that MSC profoundly inhibit the tumour cell proliferation via arresting the tumour cells at G0 and G1 phase of cell cycle.

Sato, Y., et al. (2013). "Xeno-free and shrinkage-free preparation of scaffold-free cartilage-like disc-shaped cell sheet using human bone marrow mesenchymal stem cells." J Biosci Bioeng **116**(6): 734-739.

Aiming for the clinical application of cartilage regeneration, the xeno-free cultivation method to obtain a scaffold-free cartilage-like disc-shaped cell sheet using mesenchymal stem cells (MSCs) derived from human bone marrow without the shrinkage of the sheet was investigated. MSCs were inoculated into Cell Culture Insert (0.3 cm (2), pore size; 0.4 mum, pore density; 1.0 x 10(8)/cm (2)) using serum-free chondrogenic differentiation medium containing TGF-beta3, IGF-1 and dexamethasone or other modified media, and cultured at 37 degrees C in 5% CO2 for 3 weeks. Sheet thickness, cartilage specific genes expression, ECM accumulation were determined, and the sections of sheets were stained with alcian blue. A novel mixed medium consisting of a growth medium (10% FCS) with a serum-free chondrogenic differentiation medium could prevent the shrinkage of the sheet and produced a disc-shaped cell sheet. The depth of the sheet was approximately 0.7 mm and the gene expression levels were higher than those in cells in normal human cartilage. The use of human serum instead of FCS did not cause shrinkage and did not decrease the accumulation levels of sGAG and type 2 collagen in the sheet. The cultivation of MSCs grown with completely xeno-free materials using the mixed medium containing human serum in a cell culture insert showed a sheet depth of 1.0 mm and gene expression levels higher than those in normal cartilage. The scaffold-free and xeno-free cartilage-like cell sheet was successfully formed without shrinkage using human bone marrow MSCs and the chondrogenic differentiation medium containing human serum.

Sawada, R., et al. (2006). "Changes in expression of genes related to cell proliferation in human mesenchymal stem cells during in vitro culture in comparison with cancer cells." J Artif Organs **9**(3): 179-184.

We investigated the expression levels of several genes related to cell proliferation in human mesenchymal stem cells (hMSCs) during in vitro culture for use in clinical applications. In this study, we focused on the relationship between hMSC proliferation and transforming growth factor beta (TGFbeta) signaling during in vitro culture. The proliferation rate of hMSCs gradually decreased and marked changes in hMSC morphology were not observed in 3 months of in vitro culture. The mRNA expressions of TGFbeta1, TGFbeta2, and TGFbeta receptor type I (TGFbetaRI) in hMSCs increased with the length of cell culture. There had been no change in the TGFbeta3, TGFbetaRII, and TGFbetaRIII mRNA expressions by the 12th passage from the primary culture (at about 3 months). The mRNA expression of Smad3 increased, but those of c-myc and nucleostemin decreased with the length of hMSC in vitro culture. In addition, the expression profiles of the genes that regulate cellular proliferation in hMSCs were significantly different from those of cancer cells. In

conclusion, hMSCs derived from bone marrow seldom underwent spontaneous transformation during 1-2 months of in vitro culture for use in clinical applications. In hMSCs as well as in epithelial cells, growth might be controlled by the TGFbeta family signaling.

Scavo, L. M., et al. (2004). "Insulin-like growth factor-I stimulates both cell growth and lipogenesis during differentiation of human mesenchymal stem cells into adipocytes." J Clin Endocrinol Metab **89**(7): 3543-3553.

Insulin is known to regulate adipocyte differentiation and lipid accumulation, but the specific mechanism by which precursor cells differentiate into adipocytes is not clearly understood. This study evaluated the role of the IGF-I receptor in the process of adipocyte differentiation in bone marrow-derived human mesenchymal stem cells (HMSCs). The results demonstrated that nanomolar concentrations of IGF-I adequately replaced micromolar concentrations of insulin in supporting differentiation and lipid accumulation in HMSCs. The addition of IGF-I specifically increased cell proliferation and lipid accumulation in HMSCs, but a mixture of differentiation factors including dexamethasone, indomethacin, and 3-isobutyl-1-methylxanthine did not. These effects were blocked by the alphaIR-3 antibody, which inhibits IGF-I receptor activity. We also describe the pattern of differentiation with regard to cell growth, lipid accumulation, and morphologic changes and define the changes in these parameters that are influenced by IGF-I. Finally, peroxisome proliferator activating receptor-gamma immunoreactivity was also increased in response to IGF-I, and this effect was blocked in cells treated with the alphaIR-3 antibody. Taken together, these findings suggest that IGF-I plays a critical role in adipocyte differentiation and lipid accumulation.

Scherzad, A., et al. (2015). "Human mesenchymal stem cells enhance cancer cell proliferation via IL-6 secretion and activation of ERK1/2." Int J Oncol **47**(1): 391-397.

Human mesenchymal stem cells (hMSC) are frequently used in tissue engineering. Due to their strong tumor tropism, hMSC seem to be a promising vehicle for anticancer drugs. However, interactions between hMSC and cancer are ambiguous. Particularly the cytokines and growth factors seem to play an important role in cancer progression and metastasis. The present study evaluated the effects of hMSC on head and neck squamous cell carcinoma (HNSCC) cell lines (FaDu and HLaC78) in vitro. hMSC released several cytokines and growth factors. FaDu and HLaC78 showed a significant enhancement of cell proliferation after cultivation with hMSC-conditioned medium as compared to control. This proliferation improvement was inhibited by the addition of anti-IL-6. The western blot showed an activation of Erk1/2 in FaDu and HLaC78 by hMSC-conditioned medium. HNSCC cell lines expressed EGFR. The current study confirms the importance of cytokines secreted by hMSC in cancer biology. Especially IL-6 seems to play a key role in cancer progression. Thus, the use of hMSC as a carrier for cancer therapy must be discussed critically. Future studies should evaluate the possibility of generating genetically engineered hMSC with, for example, the absence of IL-6 secretion.

Schieker, M., et al. (2007). "Human mesenchymal stem cells at the single-cell level: simultaneous seven-colour immunofluorescence." J Anat **210**(5): 592-599.

Extracellular, intracellular or surface proteins can be used as putative markers to characterize human mesenchymal stem cells (hMSC). However, these markers are also expressed by other cell types and primary cell pools reveal considerable heterogeneity. Therefore, the simultaneous detection of several markers on a single cell appears to be an attractive approach to identify hMSC. Here we demonstrate the specific distinction of human MSC from human osteoblasts via seven-colour fluorescence on the single cell level with simultaneous marker detection of CD44, CD105/endoglin, CD106/VCAM-1, collagen-IV, fibronectin, actin and DAPI nuclear staining. We performed spectral image acquisition using a Sagnac-type interferometer. Subsequent linear unmixing allowed for decomposition of each pixel in its spectral components. Our approach reveals a typical expression profile of the adherent singular cells, allowing the specific distinction between hMSC and osteoblasts on the single cell level.

Schulze, J., et al. (2017). "Effect of hyperbaric oxygen on BDNF-release and neuroprotection: Investigations with human mesenchymal stem cells and genetically modified NIH3T3 fibroblasts as putative cell therapeutics." PLoS One **12**(5): e0178182.

Hyperbaric oxygen therapy (HBOT) is a noninvasive widely applied treatment that increases the oxygen pressure in tissues. In cochlear implant (CI) research, intracochlear application of neurotrophic factors (NTFs) is able to improve survival of spiral ganglion neurons (SGN) after deafness. Cell-based delivery of NTFs such as brain-derived neurotrophic factor (BDNF) may be realized by cell-coating of the surface of the CI electrode. Human mesenchymal stem cells (MSC) secrete a variety of different neurotrophic factors and may be used for the development of a biohybrid electrode in order to release endogenously-

derived neuroprotective factors for the protection of residual SGN and for a guided outgrowth of dendrites in the direction of the CI electrode. HBOT could be used to influence cell behaviour after transplantation to the inner ear. The aim of this study was to investigate the effect of HBOT on the proliferation, BDNF-release and secretion of neuroprotective factors. Thus, model cells (an immortalized fibroblast cell line (NIH3T3)-native and genetically modified) and MSCs were repeatedly (3 x - 10 x) exposed to 100% oxygen at different pressures. The effects of HBO on cell proliferation were investigated in relation to normoxic and normobaric conditions (NOR). Moreover, the neuroprotective and neuroregenerative effects of HBO-treated cells were analysed by cultivation of SGN in conditioned medium. Both, the genetically modified NIH3T3/BDNF and native NIH3T3 fibroblasts, showed a highly significant increased proliferation after five days of HBOT in comparison to normoxic controls. By contrast, the number of MSCs was decreased in MSCs treated with 2.0 bar of HBO. Treating SGN cultures with supernatants of fibroblasts and MSCs significantly increased the survival rate of SGN. HBO treatment did not influence (increase / reduce) this effect. Secretome analysis showed that HBO treatment altered the protein expression pattern in MSCs.

Schwab, K. E. and C. E. Gargett (2007). "Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium." Hum Reprod **22**(11): 2903-2911.

BACKGROUND: Human endometrium has immense regenerative capacity, growing ~5 mm in 7 days every month. We have previously identified a small population of colony-forming endometrial stromal cells which we hypothesize are mesenchymal stem cells (MSC). The aim of this study was to determine if the co-expression of two perivascular cell markers, CD146 and platelet-derived growth factor-receptor beta (PDGF-Rbeta), will prospectively isolate endometrial stromal cells which exhibit MSC properties, and determine their location in human endometrium. METHODS: Single cell suspensions of human endometrial stromal cells were fluorescence activated cell sorting (FACS) sorted into CD146(+)PDGF-Rbeta (+) and CD146(-)PDGF-Rbeta (-) populations and analysed for colony-forming ability, in vitro differentiation and expression of typical MSC markers. Full thickness human endometrial sections were co-stained for CD146 and PDGF-Rbeta. RESULTS: FACS stromal CD146(+)PDGF-Rbeta (+) stromal cells (1.5% of sorted population) were enriched for colony-forming cells compared with CD146(-)PDGF-Rbeta (-) cells (7.7 +/- 1.7 versus 0.7 +/- 0.2% P <0.0001), and also underwent differentiation into adipogenic, osteogenic, myogenic and chondrogenic lineages. They expressed MSC phenotypic surface markers and were located near blood vessels. CONCLUSION: This study shows that human endometrium contains a small population of MSC-like cells that may be responsible for its cyclical growth, and may provide a readily available source of MSC for tissue engineering applications.

Seebach, C., et al. (2010). "Comparison of six bone-graft substitutes regarding to cell seeding efficiency, metabolism and growth behaviour of human mesenchymal stem cells (MSC) in vitro." Injury **41**(7): 731-738.

INTRODUCTION: Various synthetic bone-graft substitutes are used commercially as osteoconductive scaffolds in the treatment of bone defects and fractures. The role of bone-graft substitutes is changing from osteoconductive conduits for growth to an delivery system for biologic fracture treatments. Achieving optimal bone regeneration requires biologics (e.g. MSC) and using the correct scaffold incorporated into a local environment for bone regeneration. The need for an unlimited supply with high quality bone-graft substitutes continue to find alternatives for bone replacement surgery. MATERIALS AND METHODS: This in vitro study investigates cell seeding efficiency, metabolism, gene expression and growth behaviour of MSC sown on six commercially clinical available bone-graft substitutes in order to define their biological properties: synthetic silicate-substituted porous hydroxyapatite (Actifuse ABX), synthetic alpha-TCP (Biobase), synthetic beta-TCP (Vitoss), synthetic beta-TCP (Chronos), processed human cancellous allograft (Tutoplast) and processed bovines hydroxyapatite ceramic (Cerabone). 250,000 MSC derived from human bone marrow (n=4) were seeded onto the scaffolds, respectively. On days 2, 6 and 10 the adherence of MSC (fluorescence microscopy) and cellular activity (MTT assay) were analysed. Osteogenic gene expression (cbfa-1) was analysed by RT-PCR and scanning electron microscopy was performed. RESULTS: The highest number of adhering cells was found on Tutoplast (e.g. day 6: 110.0+/-24.0 cells/microscopic field; $p<0.05$) followed by Chronos (47.5+/-19.5, $p<0.05$), Actifuse ABX (19.1+/-4.4), Biobase (15.7+/-9.9), Vitoss (8.8+/-8.7) and Cerabone (8.1+/-2.2). MSC seeded onto Tutoplast showed highest metabolic activity and gene expression of cbfa-1. These data are confirmed by scanning electron microscopy. The cell shapes varied from round-shaped cells to wide spread cells and cell clusters, depending on the bone-graft substitutes. Processed human cancellous allograft is a well-structured and biocompatible scaffold for ingrowing MSC in vitro. Of all other synthetical scaffolds, beta-

tricalcium phosphate (Chronos) have shown the best growth behaviour for MSC. DISCUSSION: Our results indicate that various bone-graft substitutes influence cell seeding efficiency, metabolic activity and growth behaviour of MSC in different manners. We detected a high variety of cellular integration of MSC in vitro, which may be important for bony integration in the clinical setting.

Seiler, C., et al. (2014). "Time-lapse microscopy and classification of 2D human mesenchymal stem cells based on cell shape picks up myogenic from osteogenic and adipogenic differentiation." J Tissue Eng Regen Med **8**(9): 737-746.

Current methods to characterize mesenchymal stem cells (MSCs) are limited to CD marker expression, plastic adherence and their ability to differentiate into adipogenic, osteogenic and chondrogenic precursors. It seems evident that stem cells undergoing differentiation should differ in many aspects, such as morphology and possibly also behaviour; however, such a correlation has not yet been exploited for fate prediction of MSCs. Primary human MSCs from bone marrow were expanded and pelleted to form high-density cultures and were then randomly divided into four groups to differentiate into adipogenic, osteogenic chondrogenic and myogenic progenitor cells. The cells were expanded as heterogeneous and tracked with time-lapse microscopy to record cell shape, using phase-contrast microscopy. The cells were segmented using a custom-made image-processing pipeline. Seven morphological features were extracted for each of the segmented cells. Statistical analysis was performed on the seven-dimensional feature vectors, using a tree-like classification method. Differentiation of cells was monitored with key marker genes and histology. Cells in differentiation media were expressing the key genes for each of the three pathways after 21 days, i.e. adipogenic, osteogenic and chondrogenic, which was also confirmed by histological staining. Time-lapse microscopy data were obtained and contained new evidence that two cell shape features, eccentricity and filopodia (= 'fingers') are highly informative to classify myogenic differentiation from all others. However, no robust classifiers could be identified for the other cell differentiation paths. The results suggest that non-invasive automated time-lapse microscopy could potentially be used to predict the stem cell fate of hMSCs for clinical application, based on morphology for earlier time-points. The classification is challenged by cell density, proliferation and possible unknown donor-specific factors, which affect the performance of morphology-based approaches.

Seo, S. K., et al. (2013). "Overexpression of human arginine decarboxylase rescues human mesenchymal stem cells against H (2)O (2) toxicity through cell survival protein activation." J Korean Med Sci **28**(3): 366-373.

In this study, we explored the potentiality of human arginine decarboxylase (ADC) to enhance the survival of mesenchymal stem cells (MSCs) against unfavorable milieu of host tissues as the low survival of MSCs is the issue in cell transplantation therapy. To address this, human MSCs overexpressing human ADC were treated with H2O2 and the resultant intracellular events were examined. First, we examined whether human ADC is overexpressed in human MSCs. Then, we investigated cell survival or death related events. We found that the overexpression of human ADC increases formazan production and reduces caspase 3 activation and the numbers of FITC, hoechst, or propidium iodide positive cells in human MSCs exposed to H2O2. To elucidate the factors underlying these phenomena, AKT, CREB, and BDNF were examined. We found that the overexpression of human ADC phosphorylates AKT and CREB and increases BDNF level in human MSCs exposed to H2O2. The changes of these proteins are possibly relevant to the elevation of agmatine. Collectively, our data demonstrate that the overexpression of human ADC stimulates pro-survival factors to protect human MSCs against H2O2 toxicity. In conclusion, the present findings support that ADC can enhance the survival of MSCs against hostile environment of host tissues.

Seo, Y., et al. (2011). "Human umbilical cord blood-derived mesenchymal stem cells protect against neuronal cell death and ameliorate motor deficits in Niemann Pick type C1 mice." Cell Transplant **20**(7): 1033-1047.

Niemann Pick disease type C1 (NPC) is an autosomal recessive disease characterized by progressive neurological deterioration leading to premature death. In this study, we hypothesized that human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have the multifunctional abilities to ameliorate NPC symptoms in the brain. To test this hypothesis, hUCB-MSCs were transplanted into the hippocampus of NPC mice in the early asymptomatic stage. This transplantation resulted in the recovery of motor function in the Rota Rod test and impaired cholesterol homeostasis leading to increased levels of cholesterol efflux-related genes such as LXRalpha, ABCA1, and ABCG5 while decreased levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase were observed in NPC mice. In the cerebrum, hUCB-MSCs enhanced neuronal cell survival and proliferation, where they directly

differentiated into electrically active MAP2-positive neurons as demonstrated by whole-cell patch clamping. In addition, we observed that hUCB-MSCs reduced Purkinje neuronal loss by suppression of inflammatory and apoptotic signaling in the cerebellum as shown by immunohistochemistry. We further investigated how hUCB-MSCs enhance cellular survival and inhibit apoptosis in NPC mice. Neuronal cell survival was associated with increased PI3K/AKT and JAK2/STAT3 signaling; moreover, hUCB-MSCs modulated the levels of GABA/glutamate transporters such as GAT1, EAAT2, EAAT3, and GAD6 in NPC mice as assessed by Western blot analysis. Taken together, our findings suggest that hUCB-MSCs might play multifunctional roles in neuronal cell survival and ameliorating motor deficits of NPC mice.

Sepulveda, J. C., et al. (2014). "Cell senescence abrogates the therapeutic potential of human mesenchymal stem cells in the lethal endotoxemia model." Stem Cells **32**(7): 1865-1877.

Mesenchymal stem cells (MSCs) possess unique paracrine and immunosuppressive properties, which make them useful candidates for cellular therapy. Here, we address how cellular senescence influences the therapeutic potential of human MSCs (hMSCs). Senescence was induced in bone marrow-derived hMSC cultures with gamma irradiation. Control and senescent cells were tested for their immunoregulatory activity in vitro and in vivo, and an extensive molecular characterization of the phenotypic changes induced by senescence was performed. We also compared the gene expression profiles of senescent hMSCs with a collection of hMSCs used in an ongoing clinical study of Graft Versus Host disease (GVHD). Our results show that senescence induces extensive phenotypic changes in hMSCs and abrogates their protective activity in a murine model of LPS-induced lethal endotoxemia. Although senescent hMSCs retain an ability to regulate the inflammatory response on macrophages in vitro, and, in part retain their capacity to significantly inhibit lymphocyte proliferation, they have a severely impaired migratory capacity in response to proinflammatory signals, which is associated with an inhibition of the AP-1 pathway. Additionally, expression analysis identified PLEC, C8orf48, TRPC4, and ZNF14, as differentially regulated genes in senescent hMSCs that were similarly regulated in those hMSCs which failed to produce a therapeutic effect in a GVHD trial. All the observed phenotypic alterations were confirmed in replicative-senescent hMSCs. In conclusion, this study highlights important changes in the immunomodulatory phenotype of senescent hMSCs and provides candidate gene signatures which may be useful to evaluate the therapeutic potential of hMSCs used in future clinical studies.

Sequiera, G. L., et al. (2017). "Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells as an Individual-Specific and Renewable Source of Adult Stem Cells." Methods Mol Biol **1553**: 183-190.

This chapter deals with the employment of human-induced pluripotent stem cells (hiPSCs) as a candidate to differentiate into mesenchymal stem cells (MSCs). This would enable to help establish a regular source of human MSCs with the aim of avoiding the problems associated with procuring the MSCs either from different healthy individuals or patients, limited extraction potentials, batch-to-batch variations or from diverse sources such as bone marrow or adipose tissue. The procedures described herein allow for a guided and ensured approach for the regular maintenance of hiPSCs and their subsequent differentiation into MSCs using the prescribed medium. Subsequently, an easy protocol for the successive isolation and purification of the hiPSC-differentiated MSCs is outlined, which is carried out through passaging and can be further sorted through flow cytometry. Further, the maintenance and expansion of the resultant hiPSC-differentiated MSCs using appropriate characterization techniques, i.e., Reverse-transcription PCR and immunostaining is also elaborated. The course of action has been deliberated keeping in mind the awareness and the requisites available to even beginner researchers who mostly have access to regular consumables and medium components found in the general laboratory.

Shahdadfar, A., et al. (2005). "In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability." Stem Cells **23**(9): 1357-1366.

Human bone marrow mesenchymal stem cells (hMSCs) represent an appealing source of adult stem cells for cell therapy and tissue engineering, as they are easily obtained and expanded while maintaining their multilineage differentiation potential. All current protocols for in vitro culture of hMSCs include fetal bovine serum (FBS) as nutritional supplement. FBS is an undesirable additive to cells that are expanded for therapeutic purposes in humans because the use of FBS carries the risk of transmitting viral and prion diseases and proteins that may initiate xenogeneic immune responses. In the present study, we have therefore investigated if autologous serum (AS) or allogeneic human serum (alloHS) could replace FBS for the expansion of hMSCs in vitro. We discovered that the choice of serum affected hMSCs at several different levels. First, hMSCs in AS proliferated

markedly faster than hMSCs in FBS, whereas use of alloHS resulted in hMSC growth arrest and death. Second, hMSCs in FBS differentiated more rapidly toward mesenchymal lineages compared with hMSCs in AS. Interestingly, genome-wide microarray analysis identified several transcripts involved in cell cycle and differentiation that were differentially regulated between hMSCs in FBS and AS. Finally, several transcripts, including some involved in cell cycle inhibition, were upregulated in hMSCs in FBS at a late passage, whereas the hMSC transcriptome in AS was remarkably stable. Thus, hMSCs may be expanded rapidly and with stable gene expression in AS in the absence of growth factors, whereas FBS induces a more differentiated and less stable transcriptional profile.

Simpson, D. L., et al. (2012). "Use of human embryonic stem cell derived-mesenchymal cells for cardiac repair." Biotechnol Bioeng **109**(1): 274-283.

Human mesenchymal stem cells (hMSC) have proven beneficial in the repair and preservation of infarcted myocardium. Unfortunately, MSCs represent a small portion of the bone marrow and require ex vivo expansion. To further advance the clinical usefulness of cellular cardiomyoplasty, derivation of "MSC-like" cells that can be made available "off-the-shelf" are desirable. Recently, human embryonic stem cell-derived mesenchymal cells (hESC-MC) were described. We investigated the efficacy of hESC-MC for cardiac repair after myocardial infarction (MI) compared to hMSC. Because of increased efficacy of cell delivery, cells were embedded into collagen patches and delivered to infarcted myocardium. Culture of hMSC and hESC-MCs in collagen patches did not induce differentiation or significant loss in viability. Transplantation of hMSC and hES-MC patches onto infarcted myocardium of athymic nude rats prevented adverse changes in infarct wall thickness and fractional area change compared to a non-viable patch control. Hemodynamic assessment showed that hMSCs and hES-MC patch application improved end diastolic pressure equivalently. There were no changes in systolic function. hES-MC and hMSC construct application enhanced neovessel formation compared to a non-viable control, and each cell type had similar efficacy in stimulating endothelial cell growth in vitro. In summary, the use of hES-MC provides similar efficacy for cellular cardiomyoplasty as compared to hMSC and may be considered a suitable alternative for cell therapy.

Sogo, Y., et al. (2007). "Fibronectin-calcium phosphate composite layer on hydroxyapatite to enhance adhesion, cell spread and osteogenic differentiation of human mesenchymal stem cells in vitro." Biomed Mater **2**(2): 116-123.

Fibronectin (Fn) and type I collagen (Col) were immobilized on a surface of a hydroxyapatite (HAP) ceramic by coprecipitation with calcium phosphate in a supersaturated calcium phosphate solution prepared by mixing clinically approved infusion fluids. These proteins and the calcium phosphate precipitate formed a composite surface layer. As a result, the proteins were immobilized firmly as not to be released completely for 3 d in a physiological salt solution. When human mesenchymal stem cells (hMSCs) were cultured on a HAP ceramic in a differentiation medium supplemented with dexamethasone, beta-glycerophosphate and ascorbic acid, hMSCs spread well within 1 h. The alkaline phosphatase (ALP) activity of hMSCs cultured on the Fn-calcium phosphate composite layer significantly increased compared with that of hMSCs cultured on the untreated HAP ceramic. On the other hand, Col did not increase the ALP activity of hMSCs and no synergy between Fn and Col was observed. Therefore, the Fn-calcium phosphate composite layer formed on the HAP is useful for the enhancement of the spreading and osteogenic differentiation of hMSCs in vitro.

Sohn, H. S., et al. (2013). "Duration of in vitro storage affects the key stem cell features of human bone marrow-derived mesenchymal stromal cells for clinical transplantation." Cytotherapy **15**(4): 460-466.

BACKGROUND AIMS: Mesenchymal stromal cells (MSCs) have the ability to self-renew and differentiate into various cell types. Their plasticity and easy availability make them promising candidates for regenerative medicine. However, for successful clinical application, MSCs need to be expanded under a Good Manufacturing Practices-compliant system to obtain a large quantity of these cells. Although the viability and potency of these in vitro-expanded MSCs need to be maintained during preparation and transportation before transplantation, these characteristics have not thoroughly been examined. Our goal in this study was to standardize MSC preparation and storage before their clinical application to ensure reproducible quality and potency for their clinically intended purpose. METHODS: We examined the viability, self-renewal capacity and differentiation capability of MSCs on short-term in vitro storage in saline or dextrose solution at 4 degrees C and room temperature. RESULTS: MSCs harvested and suspended in saline for 1-2 h showed >90% viability regardless of storage temperature. However, when cells were stored for >2 h in saline, their viability decreased gradually over time. The viability of cells in dextrose deteriorated rapidly. MSCs lost colony-forming unit and differentiation capacities

rapidly as storage time increased. Collectively, we found that a storage period >2 h resulted in a significant decrease in cell viability, cell proliferation capacity and differentiation potency. CONCLUSIONS: Storage of culture-harvested MSCs for >2 h is likely to result in suboptimal MSC-mediated tissue regeneration because of decreased cell viability and differentiation capacity.

Somoza, R., et al. (2010). "Intranigral transplantation of epigenetically induced BDNF-secreting human mesenchymal stem cells: implications for cell-based therapies in Parkinson's disease." Biol Blood Marrow Transplant **16**(11): 1530-1540.

It is thought that the ability of human mesenchymal stem cells (hMSC) to deliver neurotrophic factors might be potentially useful for the treatment of neurodegenerative disorders. The aim of the present study was to characterize signals and/or molecules that regulate brain-derived neurotrophic factor (BDNF) protein expression/delivery in hMSC cultures and evaluate the effect of epigenetically generated BDNF-secreting hMSC on the intact and lesioned substantia nigra (SN). We tested 4 different culture media and found that the presence of fetal bovine serum (FBS) decreased the expression of BDNF, whereas exogenous addition of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) to serum-free medium was required to induce BDNF release (125 +/- 12 pg/day/10(6) cells). These cells were called hM (N)SC. Although the induction medium inhibited the expression of alpha smooth muscle actin (ASMA), an hMSC marker, and increased the nestin-positive subpopulation of hMSC cultures, the ability to express BDNF was restricted to the nestin-negative subpopulation. One week after transplantation into the SN, the human cells integrated into the surrounding tissue, and some showed a dopaminergic phenotype. We also observed the activation of Trk receptors for neurotrophic factors around the implant site, including the BDNF receptor TrkB. When we transplanted these cells into the unilateral lesioned SN induced by striatal injection of 6-hydroxydopamine (6-OHDA), a significant hypertrophy of nigral tyrosine hydroxylase (TH) (+) cells, an increase of striatal TH-staining and stabilization of amphetamine-induced motor symptoms were observed. Therefore, hMSC cultures exposed to the described induction medium might be highly useful as a vehicle for neurotrophic delivery to the brain and specifically are strong candidates for future therapeutic application in Parkinson's disease.

Son, M., et al. (2017). "Protection against RAGE-mediated neuronal cell death by sRAGE-secreting human mesenchymal stem cells in 5xFAD transgenic mouse model." Brain Behav Immun **66**: 347-358.

Alzheimer's disease (AD), which is the most commonly encountered neurodegenerative disease, causes synaptic dysfunction and neuronal loss due to various pathological processes that include tau abnormality and amyloid beta (Abeta) accumulation. Abeta stimulates the secretion and the synthesis of Receptor for Advanced Glycation End products (RAGE) ligand by activating microglial cells, and has been reported to cause neuronal cell death in Abeta1-42 treated rats and in mice with neurotoxin-induced Parkinson's disease. The soluble form of RAGE (sRAGE) is known to reduce inflammation, and to decrease microglial cell activation and Abeta deposition, and thus, it protects from neuronal cell death in AD. However, sRAGE protein has too a short half-life for therapeutic purposes. We developed sRAGE-secreting umbilical cord derived mesenchymal stem cells (sRAGE-MSCs) to enhance the inhibitory effects of sRAGE on Abeta deposition and to reduce the secretion and synthesis of RAGE ligands in 5xFAD mice. In addition, these cells improved the viability of injected MSCs, and enhanced the protective effects of sRAGE by inhibiting the binding of RAGE and RAGE ligands in 5xFAD mice. These findings suggest sRAGE protein from sRAGE-MSCs has better protection against neuronal cell death than sRAGE protein or single MSC treatment by inhibiting the RAGE cell death cascade and RAGE-induce inflammation.

Song, C., et al. (2015). "Use of Ferritin Expression, Regulated by Neural Cell-Specific Promoters in Human Adipose Tissue-Derived Mesenchymal Stem Cells, to Monitor Differentiation with Magnetic Resonance Imaging In Vitro." PLoS One **10**(7): e0132480.

The purpose of this study was to establish a method for monitoring the neural differentiation of stem cells using ferritin transgene expression, under the control of a neural-differentiation-inducible promoter, and magnetic resonance imaging (MRI). Human adipose tissue-derived mesenchymal stem cells (hADMSCs) were transduced with a lentivirus containing the human ferritin heavy chain 1 (FTH1) gene coupled to one of three neural cell-specific promoters: human synapsin 1 promoter (SYN1p, for neurons), human glial fibrillary acidic protein promoter (GFAPp, for astrocytes), and human myelin basic protein promoter (MBPp, for oligodendrocytes). Three groups of neural-differentiation-inducible ferritin-expressing (NDIFE) hADMSCs were established: SYN1p-FTH1, GFAPp-FTH1, and MBPp-FTH1. The proliferation rate of the NDIFE hADMSCs was evaluated using a Cell Counting Kit-8

assay. Ferritin expression was assessed with western blotting and immunofluorescent staining before and after the induction of differentiation in NDIFE hADMSCs. The intracellular iron content was measured with Prussian blue iron staining and inductively coupled plasma mass spectrometry. R2 relaxation rates were measured with MRI in vitro. The proliferation rates of control and NDIFE hADMSCs did not differ significantly ($P > 0.05$). SYN1p-FTH1, GFAPp-FTH1, and MBPp-FTH1 hADMSCs expressed specific markers of neurons, astrocytes, and oligodendrocytes, respectively, after neural differentiation. Neural differentiation increased ferritin expression twofold, the intracellular iron content threefold, and the R2 relaxation rate two- to threefold in NDIFE hADMSCs, resulting in notable hypointensity in T2-weighted images ($P < 0.05$). These results were cross-validated. Thus, a link between neural differentiation and MRI signals (R2 relaxation rate) was established in hADMSCs. The use of MRI and neural-differentiation-inducible ferritin expression is a viable method for monitoring the neural differentiation of hADMSCs.

Song, N., et al. (2014). "Multipotent mesenchymal stem cells from human subacromial bursa: potential for cell based tendon tissue engineering." Tissue Eng Part A **20**(1-2): 239-249.

Rotator cuff injuries are a common clinical problem either as a result of overuse or aging. Biological approaches to tendon repair that involve use of scaffolding materials or cell-based approaches are currently being investigated. The cell-based approaches are focused on applying multipotent mesenchymal stem cells (MSCs) mostly harvested from bone marrow. In the present study, we focused on characterizing cells harvested from tissues associated with rotator cuff tendons based on an assumption that these cells would be more appropriate for tendon repair. We isolated MSCs from bursa tissue associated with rotator cuff tendons and characterized them for multilineage differentiation in vitro and in vivo. Human bursa was obtained from patients undergoing rotator cuff surgery and cells within were isolated using collagenase and dispase digestion. The cells isolated from the tissues were characterized for osteoblastic, adipogenic, chondrogenic, and tenogenic differentiation in vitro and in vivo. The results showed that the cells isolated from bursa tissue exhibited MSCs characteristics as evidenced by the expression of putative cell surface markers attributed to MSCs. The cells exhibited high proliferative capacity and differentiated toward cells of mesenchymal lineages with high efficiency. Bursa-derived cells expressed markers of tenocytes when treated with bone morphogenetic protein-12 (BMP-12) and assumed aligned morphology in culture. Bursa cells pretreated with BMP-12 and seeded in ceramic scaffolds formed extensive bone, as well as tendon-like tissue in vivo. Bone formation was demonstrated by histological analysis and immunofluorescence for DMP-1 in tissue sections made from the scaffolds seeded with the cells. Tendon-like tissue formed in vivo consisted of parallel collagen fibres typical of tendon tissues. Bursa-derived cells also formed a fibrocartilagenous tissue in the ceramic scaffolds. Taken together, the results demonstrate a new source of MSCs with a high potential for application in tendon repair.

Song, Y. S., et al. (2007). "Potential differentiation of human mesenchymal stem cell transplanted in rat corpus cavernosum toward endothelial or smooth muscle cells." Int J Impot Res **19**(4): 378-385.

One of the causes of erectile dysfunction (ED) is the damaged penile cavernous smooth muscle cells (SMCs) and sinus endothelial cells (ECs). To investigate the feasibility of applying immortalized human mesenchymal stem cells (MSCs) to penile cavernous ECs or SMCs repair in the treatment of ED, the in vivo potential differentiation of the immortalized human MSCs toward penile cavernous endothelial or smooth muscle was investigated. One clone of immortalized human bone marrow mesenchymal stem cell line B10 cells via retroviral vector encoding v-myc were transplanted into the cavernosum of the Sprague-Dawley rats and harvested 2 weeks later. The expression of CD31, von Willebrand factor (vWF), smooth muscle cell actin (SMA), calponin and desmin was determined immunohistochemically in rat penile cavernosum. Multipotency of B10 to adipogenic, osteogenic or chondrogenic differentiation was found. Expression of EC specific markers (CD31 or vWF protein) and expression of SMC specific markers (calponin, SMA or desmin protein) were demonstrated in grafted B10 cells. When human MSCs were transplanted into the penile cavernosum, they have the potential to differentiate toward ECs or SMCs. Human MSCs may be a good candidate in the treatment of penile cavernosum injury.

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

Human embryonic stem cells (hESCs) are pluripotent cells that have the potential to differentiate into the three germ layers and possibly all tissues of the human body. To fulfil the clinical potentials for cell-based therapy, banks of hESC lines that express different combinations of the major histocompatibility

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

Sousa, M. F., et al. (2015). "Production of oncolytic adenovirus and human mesenchymal stem cells in a single-use, Vertical-Wheel bioreactor system: Impact of bioreactor design on performance of microcarrier-based cell culture processes." Biotechnol Prog **31**(6): 1600-1612.

Anchorage-dependent cell cultures are used for the production of viruses, viral vectors, and vaccines, as well as for various cell therapies and tissue engineering applications. Most of these applications currently rely on planar technologies for the generation of biological products. However, as new cell therapy product candidates move from clinical trials towards potential commercialization, planar platforms have proven to be inadequate to meet large-scale manufacturing demand. Therefore, a new scalable platform for culturing anchorage-dependent cells at high cell volumetric concentrations is urgently needed. One promising solution is to grow cells on microcarriers suspended in single-use bioreactors. Toward this goal, a novel bioreactor system utilizing an innovative Vertical-Wheel technology was evaluated for its potential to support scalable cell culture process development. Two anchorage-dependent human cell types were used: human lung carcinoma cells (A549 cell line) and human bone marrow-derived mesenchymal stem cells (hMSC). Key hydrodynamic parameters such as power input, mixing time, Kolmogorov length scale, and shear stress were estimated. The performance of Vertical-Wheel bioreactors (PBS-VW) was then evaluated for A549 cell growth and oncolytic adenovirus type 5 production as well as for hMSC expansion. Regarding the first cell model, higher cell growth and number of infectious viruses per cell were achieved when compared with stirred tank (ST) bioreactors. For the hMSC model, although higher percentages of proliferative cells could be reached in the PBS-VW compared with ST bioreactors, no significant differences in the cell volumetric concentration and expansion factor were observed. Noteworthy, the hMSC population generated in the PBS-VW showed a significantly lower percentage of apoptotic cells as well as reduced levels of HLA-DR positive cells. Overall, these results showed that process transfer from ST bioreactor to PBS-VW, and scale-up was successfully carried out for two different microcarrier-based cell cultures. Ultimately, the data herein generated demonstrate the potential of Vertical-Wheel bioreactors as a new scalable biomanufacturing platform for microcarrier-based cell cultures of complex biopharmaceuticals.

Spitzhorn, L. S., et al. (2018). "Transplanted human pluripotent stem cell-derived mesenchymal stem cells support liver regeneration in Gunn rats." Stem Cells Dev.

Gunn rats bear a mutation within the uridine diphosphate glucuronosyltransferase-1A1 (Ugt1a1) gene resulting in high serum bilirubin levels as seen in Crigler-Najjar syndrome. In the present study, the Gunn rat was used as an animal model for heritable liver dysfunction. Human mesenchymal stem cells (iMSC) derived from embryonic stem cells (H1) and induced pluripotent stem cells were transplanted into Gunn rats after partial hepatectomy. The iMSCs engrafted and survived in the liver for up to 2 months. The transplanted iMSCs differentiated into functional hepatocytes as evidenced by partially suppressed hyperbilirubinemia and expression of multiple human-specific hepatocyte markers such as Albumin, hepatocyte nuclear factor 4alpha, UGT1A1, Cytokeratin 18, bile salt export pump, multidrug resistance protein 2, Na/taurocholate-cotransporting polypeptide and alpha-Fetoprotein. These findings imply that transplanted human iMSCs can contribute to liver regeneration in vivo and thus represent a promising tool for the treatment of inherited liver diseases.

Sriram, G., et al. (2016). "Innate Immune Response of Human Embryonic Stem Cell-Derived Fibroblasts and Mesenchymal Stem Cells to Periodontopathogens." Stem Cells Int **2016**: 8905365.

Periodontitis involves complex interplay of bacteria and host immune response resulting in destruction of supporting tissues of the tooth. Toll-like receptors (TLRs) play a role in recognizing microbial

pathogens and eliciting an innate immune response. Recently, the potential application of multipotent stem cells and pluripotent stem cells including human embryonic stem cells (hESCs) in periodontal regenerative therapy has been proposed. However, little is known about the impact of periodontopathogens on hESC-derived progenies. This study investigates the effects of heat-killed periodontopathogens, namely, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans, on TLR and cytokine expression profile of hESC-derived progenies, namely, fibroblasts (hESC-Fib) and mesenchymal stem cells (hESC-MSCs). Additionally, the serotype-dependent effect of A. actinomycetemcomitans on hESC-derived progenies was explored. Both hESC-Fib and hESC-MSCs constitutively expressed TLR-2 and TLR-4. hESC-Fib upon exposure to periodontopathogens displayed upregulation of TLRs and release of cytokines (IL-1beta, IL-6, and IL-8). In contrast, hESC-MSCs were largely nonresponsive to bacterial challenge, especially in terms of cytokine production. Further, exposure of hESC-Fib to A. actinomycetemcomitans serotype c was associated with higher IL-8 production than serotype b. In contrast, the hESC-MSCs displayed no serotype-dependent response. Differential response of the two hESC progenies implies a phenotype-dependent response to periodontopathogens and supports the concept of immunomodulatory properties of MSCs.

Stanco, D., et al. (2015). "Multidifferentiation potential of human mesenchymal stem cells from adipose tissue and hamstring tendons for musculoskeletal cell-based therapy." Regen Med **10**(6): 729-743.

AIM: Adipose-derived stem cells (ASCs) have been deeply characterized for their usefulness in musculoskeletal tissue regeneration; recently, other mesenchymal stem cell (MSC) sources have also been proposed. This study compares for the first time human tendon stem/progenitor cells isolated from hamstring tendons with human ASCs. MATERIALS & METHODS: Human TSPCs and ASCs were isolated from hamstring tendon portions and adipose tissue of healthy donors undergoing ACL reconstruction or liposuction, respectively (n = 7). Clonogenic ability, immunophenotype and multi-differentiation potential were assessed and compared. RESULTS: Both populations showed similar proliferation and clonogenic ability and expressed embryonic stem cell genes and MSC surface markers. Tendon stem/progenitor cells showed lower adipogenic and osteogenic ability, but after the chondrogenic differentiation, they produced more abundant glycosaminoglycans and expressed higher levels of aggrecan with regards to ASCs. The tenogenic induction with BMP-12 upregulated SCX and DCN gene expression in both populations. CONCLUSION: Our results demonstrate that waste hamstring tendon fragments could represent a convenient MSC source for musculoskeletal regenerative medicine.

Steinert, A. F., et al. (2011). "Mesenchymal stem cell characteristics of human anterior cruciate ligament outgrowth cells." Tissue Eng Part A **17**(9-10): 1375-1388.

When ruptured, the anterior cruciate ligament (ACL) of the human knee has limited regenerative potential. However, the goal of this report was to show that the cells that migrate out of the human ACL constitute a rich population of progenitor cells and we hypothesize that they display mesenchymal stem cell (MSC) characteristics when compared with adherent cells derived from bone marrow or collagenase digests from ACL. We show that ACL outgrowth cells are adherent, fibroblastic cells with a surface immunophenotype strongly positive for cluster of differentiation (CD)29, CD44, CD49c, CD73, CD90, CD97, CD105, CD146, and CD166, weakly positive for CD106 and CD14, but negative for CD11c, CD31, CD34, CD40, CD45, CD53, CD74, CD133, CD144, and CD163. Staining for STRO-1 was seen by immunohistochemistry but not flow cytometry. Under suitable culture conditions, the ACL outgrowth-derived MSCs differentiated into chondrocytes, osteoblasts, and adipocytes and showed capacity to self-renew in an in vitro assay of ligamentogenesis. MSCs derived from collagenase digests of ACL tissue and human bone marrow were analyzed in parallel and displayed similar, but not identical, properties. In situ staining of the ACL suggests that the MSCs reside both aligned with the collagenous matrix of the ligament and adjacent to small blood vessels. We conclude that the cells that emigrate from damaged ACLs are MSCs and that they have the potential to provide the basis for a superior, biological repair of this ligament.

Stoddart, M. J. (2011). "WST-8 analysis of cell viability during osteogenesis of human mesenchymal stem cells." Methods Mol Biol **740**: 21-25.

WST-8 is one of the newer generation formazan-based dyes, which release the converted product into the medium in a soluble form. This allows for a non-destructive determination of viability enabling the cells to be subject to further investigations. This is a major advantage in cases where cell phenotype is being investigated and data such as matrix synthesis is correlated to cell number. This chapter describes the

use of WST-8 to normalise alkaline phosphatase activity.

Stolzing, A., et al. (2008). "Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies." Mech Ageing Dev **129**(3): 163-173.

Human mesenchymal stem cells (hMSC) represent a promising cell-based therapy for a number of degenerative conditions. Understanding the effect of aging on hMSCs is crucial for autologous therapy development in older subject whom degenerative diseases typically afflict. Previous investigations into the effects of aging on hMSC have proved contradictory due to the relative narrow age ranges of subjects assessed and the exclusive reliance of in vitro assays. This study seeks to address this controversy by using a wider range of donor ages and by measuring indices of cellular aging as well as hMSC numbers ex vivo and proliferation rates. CFU-f analysis and flow cytometry analysis using a CD45(low)/D7fib (+ve)/LNGF (+ve) gating strategy were employed. In addition a variety of markers of cellular aging, oxidative damage and senescence measured. A reduction in CFU-f and CD45(low)/D7fib (+ve)/LNGF (+ve) cell numbers were noted in adulthood relative to childhood. Indices of aging including oxidative damage, ROS levels and p21 and p53 all increased suggesting a loss of MSC fitness with age. These data suggest that hMSC numbers obtained by marrow aspiration decline with age. Furthermore, there is an age-related decline in overall BM MSC "fitness" which might lead to problems when using autologous aged MSC for cell-based therapies.

Stopp, S., et al. (2013). "Expression of the melanoma cell adhesion molecule in human mesenchymal stromal cells regulates proliferation, differentiation, and maintenance of hematopoietic stem and progenitor cells." Haematologica **98**(4): 505-513.

The melanoma cell adhesion molecule defines mesenchymal stromal cells in the human bone marrow that regenerate bone and establish a hematopoietic microenvironment in vivo. The role of the melanoma cell adhesion molecule in primary human mesenchymal stromal cells and the maintenance of hematopoietic stem and progenitor cells during ex vivo culture has not yet been demonstrated. We applied RNA interference or ectopic overexpression of the melanoma cell adhesion molecule in human mesenchymal stromal cells to evaluate the effect of the melanoma cell adhesion molecule on their proliferation and differentiation as well as its influence on co-cultivated hematopoietic stem and progenitor cells. Knockdown and overexpression of the melanoma cell adhesion molecule affected several characteristics of human mesenchymal stromal cells related to osteogenic differentiation, proliferation, and migration. Furthermore, knockdown of the melanoma cell adhesion molecule in human mesenchymal stromal cells stimulated the proliferation of hematopoietic stem and progenitor cells, and strongly reduced the formation of long-term culture-initiating cells. In contrast, melanoma cell adhesion molecule-overexpressing human mesenchymal stromal cells provided a supportive microenvironment for hematopoietic stem and progenitor cells. Expression of the melanoma cell adhesion molecule increased the adhesion of hematopoietic stem and progenitor cells to human mesenchymal stromal cells and their migration beneath the monolayer of human mesenchymal stromal cells. Our results demonstrate that the expression of the melanoma cell adhesion molecule in human mesenchymal stromal cells determines their fate and regulates the maintenance of hematopoietic stem and progenitor cells through direct cell-cell contact.

Strojny, C., et al. (2015). "Interferon Gamma-treated Dental Pulp Stem Cells Promote Human Mesenchymal Stem Cell Migration In Vitro." J Endod **41**(8): 1259-1264.

INTRODUCTION: Chronic inflammation disrupts dental pulp regeneration by disintegrating the recruitment process of progenitors for repair. Bone marrow-derived mesenchymal stem cells (BM-MSCs) share the common features with dental pulp stem cells (DPSCs). The aim of the study was to investigate the migration of BM-MSCs toward DPSCs in response to inflammatory chemoattractants. Additionally, our studies also delineated the signaling mechanisms from BM-MSCs in mediating the proliferation and differentiation of DPSCs in vitro. METHODS: Human DPSCs and BM-MSCs between passages 2 and 4 were used and were grown in odontogenic differentiation medium. Mineralization was determined by alizarin red staining analysis. Migration was assessed using crystal violet staining in cells grown in Boyden chamber Transwell inserts (Corning Inc Foundation, Tewksbury, MA). The mineralization potential of DPSCs was evaluated using alkaline phosphatase activity assay. Real-time polymerase chain reaction analysis was performed to assess the gene expression profile of chemokine (C-X-C motif) ligand (Cxcl) 3, 5, 6, 10, 11, 12, 14, and 16; stromal cell-derived factor (SDF) alpha; vascular endothelial growth factor; and fibroblast growth factor. RESULTS: Interferon gamma (FN-gamma) treatment significantly abrogated the differentiation potential of DPSCs as shown by using alizarin red and alkaline phosphatase activity analysis.

An increase in the migration of BM-MSCs was documented when cocultured with IFN-gamma-treated DPSCs. RNA expression studies showed an increase in the levels of Cxcl6 and Cxcl12 in BM-MSCs when cocultured with IFN-gamma-treated DPSCs. Additionally, an up-regulation of proangiogenic factors vascular endothelial growth factor and fibroblast growth factor were observed in DPSCs exposed to IFN-gamma. CONCLUSIONS: Our findings indicate that inflamed IFN-gamma-treated DPSCs release factors (presumably Cxcl6 and 12) that contribute to the homing of MSCs. This model might provide a potential research tool for studying MSC-DPSC cross talk and for future studies involving the recruitment and sustainability of progenitor stem cells sustaining the inflammatory cascade to treat pulp inflammation.

Suzuki, S., et al. (2010). "Effects of extracellular matrix on differentiation of human bone marrow-derived mesenchymal stem cells into smooth muscle cell lineage: utility for cardiovascular tissue engineering." Cells Tissues Organs **191**(4): 269-280.

BACKGROUND: Bone marrow-derived mesenchymal stem cells (MSCs) can differentiate into various types of cell, and the extracellular matrix (ECM) is acknowledged to be important for the regulation of cell functions. In this study, we demonstrated the effects of ECMs on the differentiation of human bone marrow-derived MSCs into a smooth muscle cell (SMC) lineage. METHODS: Human MSCs (hMSCs) were cultured on dishes coated with 3 types of ECM including laminin (LM), collagen type IV (Col-IV) and fibronectin for 7 days, and simultaneously cultured on a noncoated dish as a control. Cell numbers of these cultured hMSCs were counted, and their expression of SMC-specific genes and proteins was evaluated. hMSCs were then seeded on LM-coated biodegradable sheets and implanted into rat subcutaneous space. After 2 weeks of implantation, these tissues were evaluated. RESULTS: The number of hMSCs was significantly increased by culture on Col-IV-coated dishes. The expression of SMC-specific genes and proteins (alpha-smooth muscle actin, ASMA; h1-calponin, CALP) in hMSC was significantly upregulated from culture on LM-coated dishes. LM-coated sheets showed a significantly increased expression of ASMA and CALP protein in vivo. Moreover, a fully differentiated marker (SM2) was expressed in the in vivo implanted hMSCs in the course of 2 weeks on the LM-coated sheet. CONCLUSION: These results suggest that the signal transduction of the cell-matrix interaction for the differentiation of hMSCs into SMCs was activated when cultured with LM. LM-coated materials may thus be useful for cardiovascular tissue engineering.

Sze, S. K., et al. (2007). "Elucidating the secretion proteome of human embryonic stem cell-derived mesenchymal stem cells." Mol Cell Proteomics **6**(10): 1680-1689.

Transplantation of mesenchymal stem cells (MSCs) has been used to treat a wide range of diseases, and the mechanism of action is postulated to be mediated by either differentiation into functional reparative cells that replace injured tissues or secretion of paracrine factors that promote tissue repair. To complement earlier studies that identified some of the paracrine factors, we profiled the paracrine proteome to better assess the relevance of MSC paracrine factors to the wide spectrum of MSC-mediated therapeutic effects. To evaluate the therapeutic potential of the MSC paracrine proteome, a chemically defined serum-free culture medium was conditioned by MSCs derived from human embryonic stem cells using a clinically compliant protocol. The conditioned medium was analyzed by multidimensional protein identification technology and cytokine antibody array analysis and revealed the presence of 201 unique gene products. 86-88% of these gene products had detectable transcript levels by microarray or quantitative RT-PCR assays. Computational analysis predicted that these gene products will significantly drive three major groups of biological processes: metabolism, defense response, and tissue differentiation including vascularization, hematopoiesis, and skeletal development. It also predicted that the 201 gene products activate important signaling pathways in cardiovascular biology, bone development, and hematopoiesis such as Jak-STAT, MAPK, Toll-like receptor, transforming growth factor-beta, and mTOR (mammalian target of rapamycin) signaling pathways. This study identified a large number of MSC secretory products that have the potential to act as paracrine modulators of tissue repair and replacement in diseases of the cardiovascular, hematopoietic, and skeletal tissues. Moreover our results suggest that human embryonic stem cell-derived MSC-conditioned medium has the potency to treat a variety of diseases in humans without cell transplantation.

Takagi, M., et al. (2007). "High inoculation cell density could accelerate the differentiation of human bone marrow mesenchymal stem cells to chondrocyte cells." J Biosci Bioeng **103**(1): 98-100.

The effects of the density of human mesenchymal stem cells (MSCs) on their differentiation to chondrocytes in a differentiation medium supplemented with dexamethasone, TGFbeta3, and IGF-1 were investigated for the regenerative therapy of cartilage. The increase in the initial density

of MSCs from 0.05 x 10(4) to 0.9 x 10(4) cells/cm (2) accelerated the increase in the expression level of aggrecan mRNA during the differentiation culture for 7 d. The conditioned medium harvested at 7 d from the differentiation culture with an initial MSC density of 0.3 x 10(4) cells/cm (2) accelerated the initial increase in the expression level for 3 d in the differentiation culture with an initial MSC density of 0.3 x 10(4) cells/cm (2), whereas the conditioned medium harvested at 7 d in the differentiation culture with an initial MSC density of 0.05 x 10(4) cells/cm (2) did not. The differentiation culture after 14 d with an initial MSC concentration of 0.3 x 10(4) cells/cm (2) showed an expression level 1.7-fold that in the case of the culture with an initial MSC concentration of 0.05 x 10(4) cells/cm (2). Thus, a high MSC inoculum density might be appropriate for the rapid differentiation of MSCs to chondrocytes.

Tan, S. L., et al. (2011). "Human amnion as a novel cell delivery vehicle for chondrogenic mesenchymal stem cells." Cell Tissue Bank **12**(1): 59-70.

This study investigates the feasibility of processed human amnion (HAM) as a substrate for chondrogenic differentiation of mesenchymal stem cells (MSCs). HAM preparations processed by air drying (AD) and freeze drying (FD) underwent histological examination and MSC seeding in chondrogenic medium for 15 days. Monolayer cultures were used as control for chondrogenic differentiation and HAMs without cell seeding were used as negative control. Qualitative observations were made using scanning electron microscopy analysis and quantitative analyses were based on the sulfated glycosaminoglycans (GAG) assays performed on day 1 and day 15. Histological examination of HAM substrates before seeding revealed a smooth surface in AD substrates, while the FD substrates exhibited a porous surface. Cell attachment to AD and FD substrates on day 15 was qualitatively comparable. GAG were significantly highly expressed in cells seeded on FD HAM substrates. This study indicates that processed HAM is a potentially valuable material as a cell-carrier for MSC differentiation.

Tanabe, S., et al. (2008). "Gene expression profiling of human mesenchymal stem cells for identification of novel markers in early- and late-stage cell culture." J Biochem **144**(3): 399-408.

Human mesenchymal stem cells (hMSCs) are multipotent cells that differentiate into several cell types, and are expected to be a useful tool for cellular therapy. Although the hMSCs differentiate into osteogenic cells during early to middle stages, this differentiation capacity decreases during the late stages of cell culture. To test a hypothesis that there are biomarkers indicating the differentiation potential of hMSCs, we performed microarray analyses and profiled the gene expression in six batches of hMSCs (passages 4-28). At least four genes [necdin homolog (mouse) (NDN), EPH receptor A5 (EPHA5), nephroblastoma overexpressed gene (NOV) and runt-related transcription factor 2 (RUNX2)] were identified correlating with the passage numbers in all six batches. The results showed that the osteogenic differentiation capacity of hMSCs is down-regulated in the late stages of cell culture. It seemed that adipogenic differentiation capacity was also down-regulated in late stage of the culture. The cells in late stage are oligopotent and the genes identified in this study have the potential to act as quality-control markers of the osteogenic differentiation capacity of hMSCs.

Tang, K., et al. (2015). "Brief Report: Human Mesenchymal Stem-Like Cells Facilitate Floating Tumorigenic Cell Growth via Glutamine-Ammonium Cycle." Stem Cells **33**(9): 2877-2884.

How mesenchymal stem cells (MSCs) promote tumor growth remains incompletely understood. Here, we show that mesenchymal stem-like cells (MSLCs) are commonly present in malignant pleural effusion or ascites of cancer patients, where they directly interact with tumor cells. Chemokines and chemokine receptors, especially the CCL2/CCR2 pathway, are involved in this interaction. As a result, MSLCs exert tumor-promoting effects by enhancing the proliferation and colony formation of tumor-repopulating cells. The underlying molecular basis involves MSLC release of glutamine to tumorigenic cells. Inhibition of glutamine uptake impedes MSC-mediated tumor-promoting effects. More intriguingly, MSLCs take up tumor cell-released ammonium that, in turn, favors MSLC growth. Thus, glutamine and ammonium form a vicious cycle between MSLCs and tumorigenic cells. These findings suggest a potential clinical application by targeting MSLCs in patients with malignant pleural effusions or ascites.

Tang, Y. J., et al. (2011). "[Prostate cancer cell line PC-3 conditioned medium promotes proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells]." Zhonghua Nan Ke Xue **17**(3): 229-236.

OBJECTIVE: To investigate the effects of prostate cancer cell line PC-3 conditioned medium (PC- 3-CM) on the proliferation and osteogenic differentiation of human bone marrow human basalis mesenchymal stem cells (hBMSCs). METHODS: hBMSCs were isolated and culture-expanded by density gradient centrifugation from normal volunteers.

PC-3 cells were cultured till the time of logarithmic growth and then transferred to a fresh medium, which, after 24 hours of incubation, was collected as PC-3-CM. Passage 3 hBMSCs were cultured in the fresh medium alone (the control group) or that with 50% PC-3-CM (the experimental group), and the effect of PC-3-CM on the proliferation activity of the hBMSCs was detected by WST-8 assay. Based on the types of medium used, the hBMSCs were divided into Groups I (control), II (50% PC-3-CM), III (osteoblast inducer) and IV (osteoblast inducer containing 50% PC-3 CM). The effects of PC-3-CM on the osteoblastic differentiation of the hBMSCs were determined by ALP staining, ALP activity detection, Von Kossa staining, and calcium quantitation. RESULTS: At 1, 3, 5 and 7 days of incubation, the absorbance values of the cells in the experimental group were 0.4370 +/- 0.0285, 0.7980 +/- 0.0213, 1.9090 +/- 0.0612 and 2.3023 +/- 0.0610, and those in the control group were 0.4060 +/- 0.0223, 0.6643 +/- 0.0075, 1.3727 +/- 0.0176 and 1.7947 +/- 0.0115, respectively, with significant differences between the two groups ($P < 0.01$) except on day 1 ($P > 0.05$). The positive rate and intensity of ALP staining were gradually increased in the four groups, with the ALP activities of 0.29 +/- 0.03, 1.30 +/- 0.03, 2.13 +/- 0.08, and 3.80 +/- 0.03, respectively ($P < 0.01$), and so was the intensity of Von Kossa staining, with the calcium depositions of 0.04 +/- 0.01, 0.44 +/- 0.05, 0.98 +/- 0.03, and 1.27 +/- 0.04, respectively ($P < 0.01$). CONCLUSION: PC-3-CM can promote the proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells.

Tao, H., et al. (2005). "Cytokine-induced stable neuronal differentiation of human bone marrow mesenchymal stem cells in a serum/feeder cell-free condition." Dev Growth Differ **47**(6): 423-433.

The characteristics and multilineage differentiation potential of bone marrow mesenchymal stem cells (BM MSC) remain controversial. This study aimed to characterize human BM MSC isolated by plastic adherent or antibody selection and their neuronal differentiation potential using growth factors or chemical inducing agents. MSC were found to express low levels of neuronal markers: neurofilament-M, beta tubulin III, and neuron specific enolase. Under a serum- and feeder cell-free condition, basic fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor induced neuronal morphology in MSC. In addition to the above markers, these cells expressed neurotransmitters or associated proteins: gamma-aminobutyric acid, tyrosine hydroxylase and serotonin. These changes were maintained for up to 3 months in all bone marrow specimens (N = 6). In contrast, butylated hydroxyanisole and dimethylsulfoxide were unable to induce sustained neuronal differentiation. Our results show that MSC isolated by two different procedures produced identical lineage differentiation with defined growth factors in a serum- and feeder cell-free condition.

Tao, R., et al. (2014). "Optimization of in vitro cell labeling methods for human umbilical cord-derived mesenchymal stem cells." Eur Rev Med Pharmacol Sci **18**(8): 1127-1134.

BACKGROUND AND OBJECTIVES: Human umbilical cord-derived mesenchymal stem cells (hUCMSCs) are a novel source of seed cells for cell therapy and tissue engineering. However, in vitro labeling methods for hUCMSCs need to be optimized for better detection of transplanted cells. AIM OF THE STUDY: To identify the most stable and efficient method for labeling hUCMSCs in vitro. MATERIALS AND METHODS: hUCMSCs were isolated using a modified enzymatic digestion procedure and cultured. hUCMSCs of passage three (P3) were then labeled with BrdU, PKH26, or lentivirus-GFP and passaged further. Cells from the first labeled passage (LP1), the fourth labeled passage (LP4) and later passages were observed using a fluorescence microscope. The differentiation potential of LP4 cells was assessed by induction with adipogenic and osteogenic medium. Flow cytometry was used to measure the percentage of labeled cells and the percentage of apoptotic or dead cells. The labeling efficiencies of the three hUCMSC-labeling methods were compared in vitro. RESULTS: BrdU, PKH26, and lentivirus-GFP all labeled LP1 cells with high intensity and clarity. However, the BrdU labeling of the LP4 cells was vague and not localized to the cell nuclei; LP9 cells were not detected under a fluorescence microscope. There was also a significant decrease in the fluorescence intensity of PKH26-labeled LP4 cells, and LP11 cells were not detected under a fluorescence microscope. However, the fluorescence of LP4 cells labeled with lentivirus-GFP remained strong, and cells labeled with lentivirus-GFP were detected up to LP14 under a fluorescence microscope. Statistical analyses indicated that percentages of LP1 cells labeled with PKH26 and lentivirus-GFP were significantly higher than that of cells labeled with BrdU ($p < 0.05$), and that the LP4 cells were more efficiently labeled with lentivirus-GFP than with PKH26 or BrdU ($p < 0.05$). BrdU-, PKH26-, and lentivirus-GFP labeled LP4 cells were all differentiated to adipocytes or osteoblasts with adipogenic and osteogenic medium. No statistical significance ($p > 0.05$) was observed between the death rates of labeled and unlabeled cells. CONCLUSIONS: Lentivirus-GFP is a valid method for long-term in vitro labeling, and it may be used as a

long-term hUCMSC tracker following transplantation in vivo.

Tatullo, M., et al. (2017). "Potential Use of Human Periapical Cyst-Mesenchymal Stem Cells (hPCy-MSCs) as a Novel Stem Cell Source for Regenerative Medicine Applications." Front Cell Dev Biol **5**: 103.

Mesenchymal stem cells (MSCs) are attracting growing interest by the scientific community due to their huge regenerative potential. Thus, the plasticity of MSCs strongly suggests the utilization of these cells for regenerative medicine applications. The main issue about the clinical use of MSCs is related to the complex way to obtain them from healthy tissues; this topic has encouraged scientists to search for novel and more advantageous sources of these cells in easily accessible tissues. The oral cavity hosts several cell populations expressing mesenchymal stem cell like-features, furthermore, the access to oral and dental tissues is simple and isolation of cells is very efficient. Thus, oral-derived stem cells are highly attractive for clinical purposes. In this context, human periapical cyst mesenchymal stem cells (hPCy-MSCs) exhibit characteristics similar to other dental-derived MSCs, including their extensive proliferative potential, cell surface marker profile and the ability to differentiate into various cell types such as osteoblasts, adipocytes and neurons. Importantly, hPCy-MSCs are easily collected from the surgically removed periapical cysts; this reusing of biological waste guarantees a smart source of stem cells without any impact on the surrounding healthy tissues. In this review, we report the most interesting research topics related to hPCy-MSCs with a newsworthy discussion about the future insights. This newly discovered cell population exhibits interesting and valuable potentialities that could be of high impact in the future regenerative medicine applications.

Tatullo, M., et al. (2015). "Dental Pulp Stem Cells and Human Periapical Cyst Mesenchymal Stem Cells in Bone Tissue Regeneration: Comparison of Basal and Osteogenic Differentiated Gene Expression of a Newly Discovered Mesenchymal Stem Cell Lineage." J Biol Regul Homeost Agents **29**(3): 713-718.

Bone regeneration is an interesting field of biomedicine. The most recent studies are aimed to achieve a bone regeneration using mesenchymal stem cells (MSCs) taken from more accessible sites: oral and dental tissues have been widely investigated as a rich accessible source of MSCs. Dental Pulp Stem Cells (DPSCs) and human Periapical Cysts Mesenchymal Stem Cells (hPCy-MSCs) represent the new generation MSCs. The aim of this study is to compare the gene expression of these two innovative cell types to highlight the advantages of their use in bone regeneration. The harvesting, culturing and differentiating of cells isolated from dental pulp as well as from periapical cystic tissue were carried out as described in previously published reports. qRT-PCR analyses were performed on osteogenic genes in undifferentiated and osteogenic differentiated cells of DPSC and hPCy-MSC lineage. Real-time RT-PCR data suggested that both DPSCs and hPCy-MSCs cultured in osteogenic media are able to differentiate into osteoblast/odontoblast-like cells: however, some differences indicated that DPSCs seem to be directed more towards dentinogenesis, while hPCy-MSCs seem to be directed more towards osteogenesis.

Thankamony, S. P. and R. Sackstein (2011). "Enforced hematopoietic cell E- and L-selectin ligand (HCELL) expression primes transendothelial migration of human mesenchymal stem cells." Proc Natl Acad Sci U S A **108**(6): 2258-2263.

According to the multistep model of cell migration, chemokine receptor engagement (step 2) triggers conversion of rolling interactions (step 1) into firm adhesion (step 3), yielding transendothelial migration. We recently reported that glycosyltransferase-programmed stereosubstitution (GPS) of CD44 on human mesenchymal stem cells (hMSCs) creates the E-selectin ligand HCELL (hematopoietic cell E-selectin/L-selectin ligand) and, despite absence of CXCR4, systemically administered HCELL (+)hMSCs display robust osteotropism visualized by intravital microscopy. Here we performed studies to define the molecular effectors of this process. We observed that engagement of hMSC HCELL with E-selectin triggers VLA-4 adhesiveness, resulting in shear-resistant adhesion to ligand VCAM-1. This VLA-4 activation is mediated via a Rac1/Rap1 GTPase signaling pathway, resulting in transendothelial migration on stimulated human umbilical vein endothelial cells without chemokine input. These findings indicate that hMSCs coordinately integrate CD44 ligation and integrin activation, circumventing chemokine-mediated signaling, yielding a step 2-bypass pathway of the canonical multistep paradigm of cell migration.

Thapsukhon, B., et al. (2014). "Effect of topology of poly (L-lactide-co-epsilon-caprolactone) scaffolds on the response of cultured human umbilical cord Wharton's jelly-derived mesenchymal stem cells and neuroblastoma cell lines." J Biomater Sci Polym Ed **25**(10): 1028-1044.

In this study, for the first time, a biodegradable poly (L-lactide-co-epsilon-caprolactone), PLC 67:33 copolymer was developed for use as temporary

scaffolds in reconstructive nerve surgery. The effect of the surface topology and pore architecture were studied on the biocompatibility for supporting the growth of human umbilical cord Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs) and human neuroblastoma cells (hNBCs) as cell models. Porous PLC membranes were prepared by electrospinning and phase immersion precipitation with particulate leaching and nonporous PLC membranes were prepared by solvent casting. From the results, the porous PLC membranes can support hWJ-MSCs and hNBCs cells better than the nonporous PLC membrane, and the interconnected pore scaffold prepared by electrospinning exhibited a more significant supporting attachment of the cells than the open pore and nonporous membranes. We can consider that these electrospun PLC membranes with 3-D interconnecting fiber networks and a high porosity warrant a potential use as nerve guides in reconstructive nerve surgery.

Thiel, A., et al. (2015). "Human embryonic stem cell-derived mesenchymal cells preserve kidney function and extend lifespan in NZB/W F1 mouse model of lupus nephritis." Sci Rep **5**: 17685.

Adult tissue-derived mesenchymal stromal cells (MSCs) are showing promise in clinical trials for systemic lupus erythematosus (SLE). However, the inability to manufacture large quantities of functional cells from a single donor as well as donor-dependent variability in quality limits their clinical utility. Human embryonic stem cell (hESC)-derived MSCs are an alternative to adult MSCs that can circumvent issues regarding scalability and consistent quality due to their derivation from a renewable starting material. Here, we show that hESC-MSCs prevent the progression of fatal lupus nephritis (LN) in NZB/W F1 (BWF1) mice. Treatment led to statistically significant reductions in proteinuria and serum creatinine and preserved renal architecture. Specifically, hESC-MSC treatment prevented disease-associated interstitial inflammation, protein cast deposition, and infiltration of CD3(+) lymphocytes in the kidneys. This therapy also led to significant reductions in serum levels of tumor necrosis factor alpha (TNFalpha) and interleukin 6 (IL-6), two inflammatory cytokines associated with SLE. Mechanistically, in vitro data support these findings, as co-culture of hESC-MSCs with lipopolysaccharide (LPS)-stimulated BWF1 lymphocytes decreased lymphocyte secretion of TNFalpha and IL-6, and enhanced the percentage of putative regulatory T cells. This study represents an important step in the development of a commercially scalable and efficacious cell therapy for SLE/LN.

Thimm, B. W., et al. (2011). "Initial cell pre-cultivation can maximize ECM mineralization by human mesenchymal stem cells on silk fibroin scaffolds." Acta Biomater **7**(5): 2218-2228.

Fast remineralization of bone defects by means of tissue engineering is one of many targets in orthopedic regeneration. This study investigated the influence of a range of pre-culture durations for human bone marrow derived mesenchymal stem cells (hMSC) before inducing differentiation into osteoblast-like cells. The aim was to find the conditions that lead to maximal extracellular matrix (ECM) mineralization, in terms of both amount and best distribution. Additionally, the influence of silk fibroin scaffold pore size on mineralization was assessed. The formation of mineralized ECM by hMSCs cultured in osteogenic medium on silk fibroin scaffolds was monitored and quantified for up to 72 days in culture using non-invasive time-lapse micro-computed tomography (micro-CT). ECM mineralization increased linearly 3 weeks after the beginning of the experiment with addition of differentiation medium. Biochemical end-point assays measured the amount of DNA, calcium deposits, alkaline phosphatase activity and cell metabolic activity to corroborate the hypothesis that an initial pre-culture period of hMSCs on silk fibroin scaffolds can accelerate mineralized ECM formation. According to the micro-CT analysis mineralization on silk fibroin scaffolds with pores of 112-224 mum diameter was most efficient with an initial cell pre-culture period of 9 days, showing 6.87+/-0.81x higher mineralization values during the whole cultivation period than without an initial cell pre-culture period.

Thomas, R. and E. Ratcliffe (2012). "Automated adherent human cell culture (mesenchymal stem cells)." Methods Mol Biol **806**: 393-406.

Human cell culture processes developed at research laboratory scale need to be translated to large-scale production processes to achieve commercial application to a large market. To allow this transition of scale with consistent process performance and control of costs, it will be necessary to reduce manual processing and increase automation. There are a number of commercially available platforms that will reduce manual process intervention and improve process control for different culture formats. However, in many human cell-based applications, there is currently a need to remain close to the development format, usually adherent culture on cell culture plastic or matrix-coated wells or flasks due to deterioration of cell quality in other environments, such as suspension. This chapter presents an example method for adherent automated human stem cell culture using a specific automated flask handling platform, the CompacT SelecT.

Thrivikraman, G., et al. (2014). "Intermittent electrical stimuli for guidance of human mesenchymal stem cell lineage commitment towards neural-like cells on electroconductive substrates." Biomaterials **35**(24): 6219-6235.

In the context of the role of multiple physical factors in dictating stem cell fate, the present paper demonstrates the effectiveness of the intermittently delivered external electric field stimulation towards switching the stem cell fate to specific lineage, when cultured in the absence of biochemical growth factors. In particular, our findings present the ability of human mesenchymal stem cells (hMSCs) to respond to the electric stimuli by adopting extended neural-like morphology on conducting polymeric substrates. Polyaniline (PANI) is selected as the model system to demonstrate this effect, as the electrical conductivity of the polymeric substrates can be systematically tailored over a broad range (10(-9) to 10 S/cm) from highly insulating to conducting by doping with varying concentrations (10(-5) to 1 m) of HCl. On the basis of the culture protocol involving the systematic delivery of intermittent electric field (dc) stimulation, the parametric window of substrate conductivity and electric field strength was established to promote significant morphological extensions, with minimal cellular damage. A time dependent morphological change in hMSCs with significant filopodial elongation was observed after 7 days of electrically stimulated culture. Concomitant with morphological changes, a commensurate increase in the expression of neural lineage commitment markers such as nestin and betaIII tubulin was recorded from hMSCs grown on highly conducting substrates, as revealed from the mRNA expression analysis using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) as well as by immune-fluorescence imaging. Therefore, the present work establishes the key role of intermittent and systematic delivery of electric stimuli as guidance cues in promoting neural-like differentiation of hMSCs, when grown on electroconductive substrates.

Tian, L. L., et al. (2011). "Human mesenchymal stem cells play a dual role on tumor cell growth in vitro and in vivo." J Cell Physiol **226**(7): 1860-1867.

Nowadays, some evidences demonstrate that human mesenchymal stem cells (hMSCs) favor tumor growth; however, others show that hMSCs can suppress tumorigenesis and tumor growth. With the indeterminateness of the effect of hMSCs on tumors, we investigated the effect of hMSCs on lung cancer cell line A549 and esophageal cancer cell line Eca-109 in vitro and in vivo. Our results revealed that hMSCs inhibited the proliferation and invasion of A549 and Eca-109 cells, arrested tumor cells in the G1 phase of the cell cycle and induced the apoptosis of tumor cells in vitro by using a co-culture system and the hMSCs-conditioned medium. However, animal study showed that hMSCs enhanced tumor formation and growth in vivo. Western blotting and immunoprecipitation data showed that the expressions of proliferating cell nuclear antigen (PCNA), Cyclin E, phospho-retinoblastoma protein (pRb), B-cell lymphoma/leukemia-2 (Bcl-2), Bcl-xL, and matrix metalloproteinase 2 (MMP-2) were downregulated and the formation of Cyclin E-cyclin-dependent kinase 2 (CDK2) complexes was inhibited in the tumor cells treated with the hMSCs-conditioned medium. According to the observation of tumor mass and the result of microvessel density (MVD), we found that the promoting role of hMSCs on tumor growth was related with the increase of tumor vessel formation. Our present study suggests that hMSCs have a contradictory effect on tumor cell growth between in vitro and in vivo, and therefore, the exploitation of hMSCs in new therapeutic strategies should be cautious under the malignant conditions.

Tierney, E. G., et al. (2013). "High levels of ephrinB2 over-expression increases the osteogenic differentiation of human mesenchymal stem cells and promotes enhanced cell mediated mineralisation in a polyethyleneimine-ephrinB2 gene-activated matrix." J Control Release **165**(3): 173-182.

Gene therapy can be combined with tissue engineering constructs to produce gene-activated matrices (GAMs) with enhanced capacity for repair. Polyethyleneimine (PEI), a non-viral vector, has previously been optimised for high efficiency gene transfer in rat mesenchymal stem cells (rMSCs). The use of PEI to transfect human MSCs (hMSCs) with ephrinB2 is assessed here. Recently a role for the ephrinB2 ligand and EphB4 receptor duo has been proposed in bone remodelling. Herein, over-expression of the ephrinB2 ligand resulted in increased osteogenic differentiation in hMSCs. As ephrinB2 is a cell surface anchored ligand which only interacts with cells expressing the cognate EphB4 receptor through direct contact, we have shown that direct cell-cell contact between two neighbouring cells is responsible for enhanced osteogenesis. In an effort to begin to elucidate the molecular mechanisms at play downstream of ephrinB2 over-expression, RT-PCR was performed on the GAMs which revealed no significant changes in runx2 or BMP2 expression but an upregulation of osterix (Osx) and Dlx5 expression prompting the belief that the mode of osteogenesis is independent of the BMP2 pathway. This select interaction, coupled with the transient gene expression

profile of PEI, makes the PEI-ephrinB2 GAM an ideal candidate matrix for a bone targeted GAM.

Tobin, L. M., et al. (2013). "Human mesenchymal stem cells suppress donor CD4(+) T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease." Clin Exp Immunol **172**(2): 333-348.

Acute graft-versus-host disease (aGVHD) is a life-threatening complication following allogeneic haematopoietic stem cell transplantation (HSCT), occurring in up to 30-50% of patients who receive human leucocyte antigen (HLA)-matched sibling transplants. Current therapies for steroid refractory aGVHD are limited, with the prognosis of patients suboptimal. Mesenchymal stem or stromal cells (MSC), a heterogeneous cell population present in many tissues, display potent immunomodulatory abilities. Autologous and allogeneic ex-vivo expanded human MSC have been utilized to treat aGVHD with promising results, but the mechanisms of therapeutic action remain unclear. Here a robust humanized mouse model of aGVHD based on delivery of human peripheral blood mononuclear cells (PBMC) to non-obese diabetic (NOD)-severe combined immunodeficient (SCID) interleukin (IL)-2rgamma (null) (NSG) mice was developed that allowed the exploration of the role of MSC in cell therapy. MSC therapy resulted in the reduction of liver and gut pathology and significantly increased survival. Protection was dependent upon the timing of MSC therapy, with conventional MSC proving effective only after delayed administration. In contrast, interferon (IFN)-gamma-stimulated MSC were effective when delivered with PBMC. The beneficial effect of MSC therapy in this model was not due to the inhibition of donor PBMC chimerism, as CD45(+) and T cells engrafted successfully in this model. MSC therapy did not induce donor T cell anergy, FoxP3(+) T regulatory cells or cause PBMC apoptosis in this model; however, it was associated with the direct inhibition of donor CD4(+) T cell proliferation and reduction of human tumour necrosis factor-alpha in serum.

Tohamy, K. M., et al. (2018). "Novel alginate/hydroxyethyl cellulose/hydroxyapatite composite scaffold for bone regeneration: In vitro cell viability and proliferation of human mesenchymal stem cells." Int J Biol Macromol **112**: 448-460.

Sodium alginate (SA)/hydroxyethylcellulose (HEC)/hydroxyapatite (HA) composite scaffolds were explored for enhanced in vitro bone regeneration. The SA/HEC/HA composites were synthesized using the lyophilization technique and further cross-linked in the presence of calcium ions to form composite hydrogel networks. The physicochemical, thermal behavior and morphology properties of the prepared scaffolds were characterized through XRD, DSC/TGA, FTIR and SEM. Furthermore, the mechanical behavior of the under investigated scaffolds was determined using texture analyzer. The in vitro bioactivity in SBF and adsorption of bovine serum albumin as well as cell viability for all the prepared scaffolds were also tested. The results indicated that the higher HA concentration (40wt%) enhanced the mechanical properties (23.9MPa), bioactivity and protein adsorption. Cell viability of the tested scaffolds confirmed the non-toxicity of the fabricated systems on the human mesenchymal stem cells (hMSCs). Proliferation capability was also confirmed for the tested scaffolds after 3 and 7days, but the higher HA-containing scaffold showed increased cell populations specially after 7days compared to HA-free scaffolds. This novel composite material could be used in bone tissue engineering as a scaffold material to deliver cells and biologically active molecules.

Tokumitsu, A., et al. (2009). "Noninvasive estimation of cell cycle phase and proliferation rate of human mesenchymal stem cells by phase-shifting laser microscopy." Cytotechnology **59**(3): 161-167.

The simultaneous determination of the cell cycle phase of individual adherent mesenchymal stem cells (MSCs) using a fluorescence microscope after staining with 4',6-diamidine-2'-phenylindole dihydrochloride and bromodeoxyuridine and the laser phase shift by phase-shifting laser microscopy (PLM) revealed that the laser phase shift of cells in the G (2)/M phase was markedly higher than that of cells in the G (0)/G (1) phase. Even in the synchronous cultures to G (0)/G (1) and G (2)/M cell cycle phases, the laser phase shift of the cells in the G (2)/M phase was markedly higher than that of the cells in the G (0)/G (1) phase. The analysis of the cultures of MSCs from different donors with the addition of FGF2 at different concentrations revealed that there was a marked negative correlation between the average phase shift and mean generation time. In conclusion, it is possible to estimate noninvasively the proliferation activity of MSCs population by measuring the phase shift using PLM.

Tomar, G. B., et al. (2010). "Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine." Biochem Biophys Res Commun **393**(3): 377-383.

Mesenchymal stem cells (MSCs) are capable of self-renewal and differentiation into multiple cell lineages. Presently, bone marrow is considered as a prime source of MSCs; however, there are some drawbacks and limitations in use of these MSCs for

cell therapy. In this study, we demonstrate that human gingival tissue-derived MSCs have several advantages over bone marrow-derived MSCs. Gingival MSCs are easy to isolate, homogenous and proliferate faster than bone marrow MSCs without any growth factor. Importantly, gingival MSCs display stable morphology and do not loose MSC characteristic at higher passages. In addition, gingival MSCs maintain normal karyotype and telomerase activity in long-term cultures, and are not tumorigenic. Thus, we reveal that human gingiva is a better source of MSCs than bone marrow, and large number of functionally competent clinical grade MSCs can be generated in short duration for cell therapy in regenerative medicine and tissue engineering.

Tome, M., et al. (2011). "miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells." Cell Death Differ **18**(6): 985-995.

In spite of the extensive potential of human mesenchymal stem cells (hMSCs) in cell therapy, little is known about the molecular mechanisms that regulate their therapeutic properties. We aimed to identify microRNAs (miRNAs) involved in controlling the transition between the resting and reparative phenotypes of hMSCs, hypothesizing that these miRNAs must be present in the undifferentiated cells and downregulated to allow initiation of distinct activation/differentiation programs. Differential miRNA expression analyses revealed that miR-335 is significantly downregulated upon hMSC differentiation. In addition, hMSCs derived from a variety of tissues express miR-335 at a higher level than human skin fibroblasts, and overexpression of miR-335 in hMSCs inhibited their proliferation and migration, as well as their osteogenic and adipogenic potential. Expression of miR-335 in hMSCs was upregulated by the canonical Wnt signaling pathway, a positive regulator of MSC self-renewal, and downregulated by interferon-gamma (IFN-gamma), a pro-inflammatory cytokine that has an important role in activating the immunomodulatory properties of hMSCs. Differential gene expression analyses, in combination with computational searches, defined a cluster of 62 putative target genes for miR-335 in hMSCs. Western blot and 3'UTR reporter assays confirmed RUNX2 as a direct target of miR-335 in hMSCs. These results strongly suggest that miR-335 downregulation is critical for the acquisition of reparative MSC phenotypes.

Tremain, N., et al. (2001). "MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages." Stem Cells **19**(5): 408-418.

Mesenchymal stem cells (MSCs) isolated from the bone marrow of adult organisms are capable of differentiating into adipocytes, chondrocytes, myoblasts, osteoblasts, and hematopoiesis-supporting stroma. We recently demonstrated that MSCs also adopt glial cell fates when transplanted into the developing central nervous system and hence can produce tissue elements derived from a separate embryonic layer. Despite these remarkable properties, it has been difficult to establish specific criteria to characterize MSCs. Using a modified protocol for micro-serial analysis of gene expression, we cataloged 2,353 unique genes expressed by a single cell-derived colony of undifferentiated human MSCs. This analysis revealed that the MSC colony simultaneously expressed transcripts characteristic of various mesenchymal cell lineages including chondrocytes, myoblasts, osteoblasts, and hematopoiesis-supporting stroma. Therefore, the profile of expressed transcripts reflects the developmental potential of the cells. Additionally, the MSC colony expressed mRNAs characteristic of endothelial, epithelial and neuronal cell lineages, a combination that provides a unique molecular signature for the cells. Other expressed transcripts included various products involved in wound repair as well as several neurotrophic factors. A total of 268 novel transcripts were also identified, one of which is the most abundantly expressed mRNA in MSCs. This study represents the first extensive gene expression analysis of MSCs and as such reveals new insight into the biology, ontogeny, and in vivo function of the cells.

Trivanovic, D., et al. (2014). "Characteristics of human adipose mesenchymal stem cells isolated from healthy and cancer affected people and their interactions with human breast cancer cell line MCF-7 in vitro." Cell Biol Int **38**(2): 254-265.

Adipose tissue is an attractive source of mesenchymal stem/stromal cells (MSCs) with potential applications in reconstructive plastic surgery and regenerative medicine. The aim of this study was to characterise human adipose tissue MSCs (ASCs) derived from healthy individuals and cancer patients and to compare their interactions with tumour cells. ASCs were isolated from adipose tissue of healthy donors, breast cancer-adjacent adipose tissue of breast cancer patients and tumour-adjacent adipose tissue of non-breast cancer patients. Their proliferation, differentiation, immunophenotype and gene expression were assessed and effects on the proliferation of human breast cancer cell line MCF-7 compared. ASCs from all sources exhibited similar morphology, proliferative and differentiation potential, showing the

characteristic pattern of mesenchymal surface markers expression (CD90, CD105, CD44H, CD73) and the lack of HLA-DR and hematopoietic markers (CD11a, CD33, CD45, Glycophorin-CD235a), but uneven expression of CD34. ASCs also shared a common positive gene expression of HLA-DR, HLA-A, IL-6, TGF-beta and HIF-1, but were negative for HLA-G, while the expression levels of Cox-2 and IDO-1 varied. All ASCs significantly stimulated the proliferation of MCF-7 tumour cells in direct mixed co-cultures and transwell system, although their conditioned media displayed antiproliferative activity. Data obtained showed that ASCs with similar characteristics are easily isolated from various donors and sites of origin, although ASCs could both suppress and favour tumour cells growth, emphasising the importance of cellular context within the microenvironment and pointing to the significance of safety studies to exclude any potential clinical risk of their application in regenerative medicine.

Trivedi, H. L., et al. (2008). "Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin." Transplant Proc **40**(4): 1135-1139.

BACKGROUND: Type 1 diabetes mellitus (DM) is an autoimmune disorder with disturbed glucose/insulin metabolism, which has no medical treatment other than life-long insulin therapy, despite which 30% of subjects develop organ failure. Herein we have reported the use of human adipose-tissue-derived, insulin-making mesenchymal stem cells (h-AD-MSC) transfused with unfractionated cultured bone marrow (CBM) in 5 insulinopenic DM patients. PATIENTS AND METHODS: Five (M:F, 2:3) insulinopenic DM patients of 0.6 to 10 years' duration, ages 14 to 28 years under treatment insulin (Human with 14-70 U/d) showed postprandial blood sugars between 156 to 470 mg%, glycosylated hemoglobin 6.8% to 9.9% and c-peptide levels of 0.02 to 0.2 ng/mL. They underwent intraportal administration of xenogeneic-free h-AD-MSC (mean dose = 1.5 mL; cell counts, 2.1 x 10(3)/muL). The CD45-/90+/73(+) cells (29.8/16.8%) showed c-peptide levels of 3.08 ng/mL, insulin level of 1578 micro IU/mL. The aliquot was supplemented with CBM (mean dose 94 mL with cell counts: 18.7 x 10(3)/microL) containing CD45-/34+ elements of 0.93%. The Institutional Review Board approved the study protocol and consent forms. RESULTS: All patients were successfully infused CBM plus h-AD-MSC without any untoward effects and showed 30% to 50% decreased insulin requirements with 4- to 26-fold increased serum c-peptide levels, with a mean follow-up of 2.9 months. CONCLUSION: This report describes safe and effective treatment of insulinopenic diabetics using insulin-producing h-AD-MSC plus CBM without xenogeneic materials.

Trubiani, O., et al. (2012). "Overexpression of interleukin-6 and -8, cell growth inhibition and morphological changes in 2-hydroxyethyl methacrylate-treated human dental pulp mesenchymal stem cells." Int Endod J **45**(1): 19-25.

AIM: To evaluate morphological features, cell growth and interleukin-6 (IL-6) and interleukin-8 (IL-8) secretion in expanded ex vivo human dental pulp mesenchymal stem cells (DP-MSCs) after exposure to 2-hydroxyethyl methacrylate (HEMA). METHODOLOGY: Dental pulp mesenchymal stem cells were derived from the dental pulps of 10 young donors. After in vitro isolation, DP-MSCs were treated with 3 and 5 mmol L (-1) HEMA, and after 24, 48 and 72 h of incubation, their morphological features, cell growth, IL-6 and IL-8 secretion were analysed. Differences in the cell growth and in the interleukin secretion were analysed for statistical significance with two-way anova tests and the Holm-Sidak method for multiple comparisons. RESULTS: Dental pulp mesenchymal stem cells revealed a decrease in cell growth with both treatments ($P < 0.05$), more evident at 5 mmol L (-1). Microscopic analysis displayed extensive cytotoxic effects in treated cells, which lost their fibroblastoid features and became retracted, even roundish, with a large number of granules. An up-regulation of IL-6 and IL-8 in treated cells cytokines was evident ($P < 0.05$). CONCLUSIONS: 2-Hydroxyethyl methacrylate exhibited cytotoxicity, inhibited cell growth and induced morphological changes in cultured DP-MSCs. Moreover, in treated samples, an up-regulation of soluble mediators of inflammation such as IL-6 and IL-8 cytokines was found. The direct application of HEMA potentially induces an inflammation process that could be the starting point for toxic response and cell damage in DP-MSCs.

van Zoelen, E. J., et al. (2016). "TGFbeta-induced switch from adipogenic to osteogenic differentiation of human mesenchymal stem cells: identification of drug targets for prevention of fat cell differentiation." Stem Cell Res Ther **7**(1): 123.

BACKGROUND: Patients suffering from osteoporosis show an increased number of adipocytes in their bone marrow, concomitant with a reduction in the pool of human mesenchymal stem cells (hMSCs) that are able to differentiate into osteoblasts, thus leading to suppressed osteogenesis. METHODS: In order to be able to interfere with this process, we have investigated in-vitro culture conditions whereby adipogenic differentiation of hMSCs is impaired and osteogenic differentiation is promoted. By means of

gene expression microarray analysis, we have investigated genes which are potential targets for prevention of fat cell differentiation. RESULTS: Our data show that BMP2 promotes both adipogenic and osteogenic differentiation of hMSCs, while transforming growth factor beta (TGFbeta) inhibits differentiation into both lineages. However, when cells are cultured under adipogenic differentiation conditions, which contain cAMP-enhancing agents such as IBMX of PGE2, TGFbeta promotes osteogenic differentiation, while at the same time inhibiting adipogenic differentiation. Gene expression and immunoblot analysis indicated that IBMX-induced suppression of HDAC5 levels plays an important role in the inhibitory effect of TGFbeta on osteogenic differentiation. By means of gene expression microarray analysis, we have investigated genes which are downregulated by TGFbeta under adipogenic differentiation conditions and may therefore be potential targets for prevention of fat cell differentiation. We thus identified nine genes for which FDA-approved drugs are available. Our results show that drugs directed against the nuclear hormone receptor PPARG, the metalloproteinase ADAMTS5, and the aldo-keto reductase AKR1B10 inhibit adipogenic differentiation in a dose-dependent manner, although in contrast to TGFbeta they do not appear to promote osteogenic differentiation. CONCLUSIONS: The approach chosen in this study has resulted in the identification of new targets for inhibition of fat cell differentiation, which may not only be relevant for prevention of osteoporosis, but also of obesity.

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.

Vellasamy, S., et al. (2013). "Mesenchymal stem cells of human placenta and umbilical cord suppress T-cell proliferation at G0 phase of cell cycle." Cell Biol Int **37**(3): 250-256.

Mesenchymal stem cells (MSC) generated from human umbilical cord (UC-MSC) and placenta (PLC-MSC) were assessed and compared for their immunomodulatory function on T cells proliferation by analysis of the cell cycle. Mitogen stimulated or resting T cells were co-cultured in the presence or absence of MSC. T-cell proliferation was assessed by tritiated thymidine ((3) H-TdR) assay and the mechanism of inhibition was examined bycell cycle and apoptosis assay. Both UC-MSC and PLC-MSC profoundly inhibited the proliferation of T-cell, mainly via cell-to-cell contact. MSC-mediated anti-proliferation does not lead to apoptosis,but prevented T cells from entering S phase and they therefore accumulated in the G (0) /G (1) phases. The anti-proliferative activity of MSC was related to this cell cycle arrest of T-cell. UC-MSC produced a greater inhibition than PLC-MSC in all measured parameters.

Venugopal, B., et al. (2018). "Cell density overrides the effect of substrate stiffness on human mesenchymal stem cells' morphology and proliferation." Biomater Sci **6**(5): 1109-1119.

The effect of substrate stiffness on the cellular morphology, proliferation, and differentiation of human mesenchymal stem cells (hMSCs) has been extensively researched and well established. However, the majority of these studies are done with a low seeding density where cell to cell interactions do not play a significant role. While these conditions permit an analysis of cell-substratum interactions at the single cell level, such a model system fails to capture a critical aspect of the cellular micro-environment in vivo, i.e. the cell-cell interaction via matrix deformation (i.e., strain). To address this question, we seeded hMSCs on soft poly-acrylamide (PAA) gels, at a seeding density that permits cells to be mechanically interacting via the underlying substrate. We found that as the intercellular distance decreases with the increasing seeding density, cellular sensitivity towards the substrate rigidity becomes significantly diminished. With the increasing seeding density, the cell spread area increased on a soft substrate (500 Pa) but reduced on an even slightly stiffer substrate (2 kPa) as well as on glass making them indistinguishable at a high seeding density. Not only in terms of cell spread area but also at a high seeding density, cells formed mature focal adhesions and prominent stress fibres on a soft

substrate similar to that of the cells being cultured on a stiff substrate. The decreased intercellular distance also influenced the proliferation rate of the cells: higher seeding density on the soft substrate showed cell cycle progression similar to that of the cells on glass substrates. In summary, this paper demonstrates how the effect of substrate rigidity on the cell morphology and fate is a function of inter-cellular distance when seeded on a soft substrate. Our AFM data suggest that such changes happen due to local strain stiffening of the soft PAA gel, an effect that has been rarely reported in the literature so far.

Veraitch, O., et al. (2017). "Induction of hair follicle dermal papilla cell properties in human induced pluripotent stem cell-derived multipotent LNGFR (+)THY-1(+) mesenchymal cells." Sci Rep **7**: 42777.

The dermal papilla (DP) is a specialised mesenchymal component of the hair follicle (HF) that plays key roles in HF morphogenesis and regeneration. Current technical difficulties in preparing trichogenic human DP cells could be overcome by the use of highly proliferative and plastic human induced pluripotent stem cells (hiPSCs). In this study, hiPSCs were differentiated into induced mesenchymal cells (iMCs) with a bone marrow stromal cell phenotype. A highly proliferative and plastic LNGFR (+)THY-1(+) subset of iMCs was subsequently programmed using retinoic acid and DP cell activating culture medium to acquire DP properties. The resultant cells (induced DP-substituting cells [iDPSCs]) exhibited up-regulated DP markers, interacted with human keratinocytes to up-regulate HF related genes, and when co-grafted with human keratinocytes in vivo gave rise to fibre structures with a hair cuticle-like coat resembling the hair shaft, as confirmed by scanning electron microscope analysis. Furthermore, iDPSCs responded to the clinically used hair growth reagent, minoxidil sulfate, to up-regulate DP genes, further supporting that they were capable of, at least in part, reproducing DP properties. Thus, LNGFR (+)THY-1(+) iMCs may provide material for HF bioengineering and drug screening for hair diseases.

Veriter, S., et al. (2015). "Human Adipose-Derived Mesenchymal Stem Cells in Cell Therapy: Safety and Feasibility in Different "Hospital Exemption" Clinical Applications." PLoS One **10**(10): e0139566.

Based on immunomodulatory, osteogenic, and pro-angiogenic properties of adipose-derived stem cells (ASCs), this study aims to assess the safety and efficacy of ASC-derived cell therapies for clinical indications. Two autologous ASC-derived products were proposed to 17 patients who had not experienced any success with conventional therapies: (1) a scaffold-free osteogenic three-dimensional graft for the treatment of bone non-union and (2) a biological dressing for dermal reconstruction of non-healing chronic wounds. Safety was studied using the quality control of the final product (genetic stability, microbiological/mycoplasma/endotoxin contamination) and the in vivo evaluation of adverse events after transplantation. Feasibility was assessed by the ability to reproducibly obtain the final ASC-based product with specific characteristics, the time necessary for graft manufacturing, the capacity to produce enough material to treat the lesion, the surgical handling of the graft, and the ability to manufacture the graft in line with hospital exemption regulations. For 16 patients (one patient did not undergo grafting because of spontaneous bone healing), in-process controls found no microbiological/mycoplasma/endotoxin contamination, no obvious deleterious genomic anomalies, and optimal ASC purity. Each type of graft was reproducibly obtained without significant delay for implantation and surgical handling was always according to the surgical procedure and the implantation site. No serious adverse events were noted for up to 54 months. We demonstrated that autologous ASC transplantation can be considered a safe and feasible therapy tool for extreme clinical indications of ASC properties and physiopathology of disease.

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.

Wagner, W., et al. (2007). "Adhesion of hematopoietic progenitor cells to human mesenchymal stem cells as a model for cell-cell interaction." Exp Hematol **35**(2): 314-325.

OBJECTIVE: The significant role of direct contact between hematopoietic progenitor cells (HPC) and the cellular microenvironment for maintaining "stemness" has been demonstrated. Human mesenchymal stem cell (MSC) feeder layers represent a surrogate model for this interaction. Specific adhesion molecules are responsible for this cell-cell contact. METHODS: To define cell-cell contact between HPC and MSC, we have studied adhesive interaction of various fractions of HPC by using a novel assay based on gravitational force upon inversion. Adherent and nonadherent cells were separated and further analyzed with regard to gene expression and long-term hematopoietic culture initiating cell (LTC-IC) frequency. RESULTS: HPC subsets with higher self-renewing capacity demonstrated significantly higher adherence to human MSC (CD34(+) vs CD34(-), CD34(+)/CD38(-) vs CD34(+)/CD38(+), slow dividing fraction vs fast dividing fraction). LTC-IC frequency was significantly higher in the adherent fraction than in the nonadherent fraction. Furthermore, genes coding for adhesion proteins and extracellular matrix were higher expressed in the adherent subsets of CD34(+) cells (fibronectin 1, cadherin 11, vascular cell adhesion molecule-1, connexin 43, integrin beta-like 1, and TGFBI). CONCLUSION: In this study we have demonstrated that primitive subsets of HPC have higher affinity to human MSC. The essential role of specific junction proteins for stabilization of cell-cell contact is indicated by their significant higher expression.

Wakao, S., et al. (2012). "Isolation of adult human pluripotent stem cells from mesenchymal cell populations and their application to liver damages." Methods Mol Biol **826**: 89-102.

We have found a novel type of pluripotent stem cells, Multilineage-differentiating stress enduring (Muse) cells that can be isolated from mesenchymal cell populations. Muse cells are characterized by stress tolerance, expression of pluripotency markers, self-renewal, and the ability to differentiate into endodermal-, mesodermal-, and ectodermal-lineage cells from a single cell, demonstrating that they are pluripotent stem cells. They can be isolated as cells positive for stage-specific embryonic antigen-3, a human pluripotent stem cell marker. Here, we introduce the isolation method for Muse cells and the effect of transplantation of these cells on chronic liver diseases.

Walter, M. N., et al. (2015). "Human mesenchymal stem cells stimulate EaHy926 endothelial cell migration: combined proteomic and in vitro analysis of the influence of donor-donor variability." J Stem Cells Regen Med **11**(1): 18-24.

Mesenchymal stem cells (MSCs) stimulate angiogenesis within a wound environment and this effect is mediated through paracrine interactions with the endothelial cells present. Here we report that human MSC-conditioned medium (n=3 donors) significantly increased EaHy-926 endothelial cell adhesion and cell migration, but that this stimulatory effect was markedly donor-dependent. MALDI-TOF/TOF mass spectrometry demonstrated that whilst collagen type I and fibronectin were secreted by all of the MSC cultures, the small leucine rich proteoglycan, decorin was secreted only by the MSC culture that was least effective upon EaHy-926 cells. These individual extracellular matrix components were then tested as culture substrata. EaHy-926 cell adherence was greatest on fibronectin-coated surfaces with least adherence on decorin-coated surfaces. Scratch wound assays were used to examine cell migration. EaHy-926 cell scratch wound closure was quickest on substrates of fibronectin and slowest on decorin. However, EaHy-926 cell migration was stimulated by the addition of MSC-conditioned medium irrespective of the types of culture substrates. These data suggest that whilst the MSC secretome may generally be considered angiogenic, the composition of the secretome is variable and this variation probably contributes to donor-donor differences in activity. Hence, screening and optimizing MSC secretomes will improve the clinical effectiveness of pro-angiogenic MSC-based therapies.

West, C. C., et al. (2016). "Prospective purification of perivascular presumptive mesenchymal stem cells from human adipose tissue: process optimization and cell population metrics across a large cohort of diverse demographics." Stem Cell Res Ther **7**: 47.

BACKGROUND: Adipose tissue is an attractive source of mesenchymal stem cells (MSC) as it is largely dispensable and readily accessible through minimally invasive procedures such as liposuction.

Until recently MSC could only be isolated in a process involving ex-vivo culture and their in-vivo identity, location and frequency remained elusive. We have documented that pericytes (CD45-, CD146+, and CD34-) and adventitial cells (CD45-, CD146-, CD34+) (collectively termed perivascular stem cells or PSC) represent native ancestors of the MSC, and can be prospectively purified using fluorescence activated cell sorting (FACS). In this study we describe an optimized protocol that aims to deliver pure, viable and consistent yields of PSC from adipose tissue. We analysed the frequency of PSC within adipose tissue, and the effect of patient and procedure based variables on this yield. METHODS: Within this twin centre study we analysed the adipose tissue of n = 131 donors using flow cytometry to determine the frequency of PSC and correlate this with demographic and processing data such as age, sex, BMI and cold storage time of the tissue. RESULTS: The mean number of stromal vascular fraction (SVF) cells from 100 ml of lipoaspirate was 34.4 million. Within the SVF, mean cell viability was 83 %, with 31.6 % of cells being haematopoietic (CD45+). Adventitial cells and pericytes represented 33.0 % and 8 % of SVF cells respectively. Therefore, a 200 ml lipoaspirate would theoretically yield 23.2 million viable prospectively purified PSC - sufficient for many reconstructive and regenerative applications. Minimal changes were observed in respect to age, sex and BMI suggesting universal potential application. CONCLUSIONS: Adipose tissue contains two anatomically and phenotypically discreet populations of MSC precursors - adventitial cells and pericytes - together referred to as perivascular stem cells (PSC). More than 9 million PSC per 100 ml of lipoaspirate can be rapidly purified to homogeneity using flow cytometry in clinically relevant numbers potentially circumventing the need for purification and expansion by culture prior to clinical use. The number and viability of PSC are minimally affected by patient age, sex, BMI or the storage time of the tissue, but the quality and consistency of yield can be significantly influenced by procedure based variables.

Williams, C., et al. (2012). "A comparison of human smooth muscle and mesenchymal stem cells as potential cell sources for tissue-engineered vascular patches." Tissue Eng Part A **18**(9-10): 986-998.

In pediatric patients requiring vascular reconstruction, the development of a cell-based tissue-engineered vascular patch (TEVP) has great potential to overcome current issues with nonliving graft materials. Determining the optimal cell source is especially critical to TEVP success. In this study, we compared the ability of human aortic smooth muscle cells (HuAoSMCs) and human mesenchymal stem cells (hMSCs) to form cell sheets on thermoresponsive poly (N-isopropylacrylamide) (PIPAAm) substrates. hMSCs treated with transforming growth factor beta 1 (TGFbeta1) and ascorbic acid (AA) had higher expression of SMC-specific proteins compared to HuAoSMCs. hMSCs also had larger cell area and grew to confluence more quickly on PIPAAm than did HuAoSMCs. hMSCs typically formed cell sheets in 2-3 weeks and had greater wet tissue weight and collagen content compared with HuAoSMC sheets, which generally required growth for up to 5 weeks. Assays for calcification and alkaline phosphatase activity revealed that the osteogenic potential of TGFbeta1+AA-treated hMSCs was low; however, Alcian Blue staining suggested high chondrogenic behavior of TGFbeta1+AA-treated hMSCs. Although hMSCs are promising for cell-based TEVPs in their ability to form robust tissue with significant extracellular matrix content, improved control over hMSC behavior will be required for long-term TEVP success.

Wuchter, P., et al. (2007). "Processus and recessus adhaerentes: giant adherens cell junction systems connect and attract human mesenchymal stem cells." Cell Tissue Res **328**(3): 499-514.

Substrate-adherent cultured cells derived from human bone marrow or umbilical cord blood ("mesenchymal stem cells") are of special interest for regenerative medicine. We report that such cells, which can display considerable heterogeneity with respect to their cytoskeletal protein complement, are often interconnected by special tentacle-like cell processes contacting one or several other cells. These processus adhaerentes, studded with many (usually small) puncta adhaerentia and varying greatly in length (up to more than 400 microm long), either contact each other in the intercellular space ("ET touches") or insert in a tight-fitting manner into deep plasma membrane invaginations (recessus adhaerentes), thus forming a novel kind of long (up to 50 microm) continuous cuff-like junction (manubria adhaerentia). The cell processes contain an actin microfilament core that is stabilized with ezrin, alpha-actinin, and myosin and accompanied by microtubules, and their adhering junctions are characterized by a molecular complement comprising the transmembrane glycoproteins N-cadherin and cadherin-11, in combination with the cytoplasmic plaque proteins alpha- and beta-catenin, together with p120(ctn), plakoglobin, and afadin. The processes are also highly dynamic and rapidly foreshorten as cell colonies approach a denser state of cell packing. These structures are obviously able to establish cell-cell connections, even over long distances, and can form deep-rooted and tight cell-cell adhesions. The possible

relationship to similar cell processes in the embryonic primary mesenchyme and their potential in cell sorting and tissue formation processes in the body are discussed.

Yamada, Y., et al. (2006). "Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy." Biomaterials **27**(20): 3766-3781.

We investigated gene expression patterns and functional classifications regarding the clusters of human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs)--which possess a multipotent ability--because little is known about the precise moleculobiological clues by which these cells activate their differentiating ability or functionality to eventually form dentin and bone, respectively. We first verified the expressions of the alkaline phosphatase (ALP) gene, dentin matrix protein 1 (DMP-1), and dentinsialophosphoprotein (DSPP) by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and consequently discovered the high expressions of these genes. Total RNA was also followed by hybridization with a human microarray system consisting of 12,814 genes. Analyses of gene expression patterns indicated several genes which encode extracellular matrix components, cell adhesion molecules, growth factors, and transcription regulators. Functional and clustering analyses of differences in gene expression levels revealed cell signaling, cell communication, or cell metabolism. In the future, information on the gene expression patterns of hDPSCs and hMSCs might be useful in determining the detailed functional roles of the relevant genes and applicable to stem cell therapies, and these cells could also be used as multipotent cell sources for gene technology and tissue engineering technology.

Yamaguchi, S., et al. (2018). "Age of donor of human mesenchymal stem cells affects structural and functional recovery after cell therapy following ischaemic stroke." J Cereb Blood Flow Metab **38**(7): 1199-1212.

Cell transplantation therapy offers great potential to improve impairments after stroke. However, the importance of donor age on therapeutic efficacy is unclear. We investigated the regenerative capacity of transplanted cells focusing on donor age (young vs. old) for ischaemic stroke. The quantities of human mesenchymal stem cell (hMSC) secreted brain-derived neurotrophic factor in vitro and of monocyte chemotactic protein-1 at day 7 in vivo were both significantly higher for young hMSC compared with old hMSC. Male Sprague-Dawley rats subjected to transient middle cerebral artery occlusion that received young hMSC (trans-arterially at 24 h after stroke) showed better behavioural recovery with prevention of brain atrophy compared with rats that received old hMSC. Histological analysis of the peri-infarct cortex showed that rats treated with young hMSC had significantly fewer microglia and more vessels covered with pericytes. Interestingly, migration of neural stem/progenitor cells expressing Musashi-1 positively correlated with astrocyte process alignment, which was more pronounced for young hMSC. Aging of hMSC may be a critical factor that affects cell therapy outcomes, and transplantation of young hMSC appears to provide better functional recovery through anti-inflammatory effects, vessel maturation, and neurogenesis potentially by the dominance of trophic factor secretion.

Yang, C., et al. (2014). "Conditioned media from human adipose tissue-derived mesenchymal stem cells and umbilical cord-derived mesenchymal stem cells efficiently induced the apoptosis and differentiation in human glioma cell lines in vitro." Biomed Res Int **2014**: 109389.

Human mesenchymal stem cells (MSCs) have an intrinsic property for homing towards tumor sites and can be used as tumor-tropic vectors for tumor therapy. But very limited studies investigated the antitumor properties of MSCs themselves. In this study we investigated the antiglioma properties of two easily accessible MSCs, namely, human adipose tissue-derived mesenchymal stem cells (ASCs) and umbilical cord-derived mesenchymal stem cells (UC-MSCs). We found (1) MSC conditioned media can significantly inhibit the growth of human U251 glioma cell line; (2) MSC conditioned media can significantly induce apoptosis in human U251 cell line; (3) real-time PCR experiments showed significant upregulation of apoptotic genes of both caspase-3 and caspase-9 and significant downregulation of antiapoptotic genes such as survivin and XIAP after MSC conditioned media induction in U 251 cells; (4) furthermore, MSCs conditioned media culture induced rapid and complete differentiation in U251 cells. These results indicate MSCs can efficiently induce both apoptosis and differentiation in U251 human glioma cell line. Whereas UC-MSCs are more efficient for apoptosis induction than ASCs, their capability of differentiation induction is not distinguishable from each other. Our findings suggest MSCs themselves have favorable antitumor characteristics and should be further explored in future glioma therapy.

Yang, D. Y., et al. (2012). "Dual regeneration of muscle and nerve by intravenous administration of human amniotic fluid-derived mesenchymal stem cells

regulated by stromal cell-derived factor-1alpha in a sciatic nerve injury model." J Neurosurg **116**(6): 1357-1367.

OBJECT: Human amniotic fluid-derived mesenchymal stem cells (AFMSCs) have been shown to promote peripheral nerve regeneration. The expression of stromal cell-derived factor-1alpha (SDF-1alpha) in the injured nerve exerts a trophic effect by recruiting progenitor cells that promote nerve regeneration. In this study, the authors investigated the feasibility of intravenous administration of AFMSCs according to SDF-1alpha expression time profiles to facilitate neural regeneration in a sciatic nerve crush injury model. METHODS: Peripheral nerve injury was induced in 63 Sprague-Dawley rats by crushing the left sciatic nerve using a vessel clamp. The animals were randomized into 1 of 3 groups: Group I, crush injury as the control; Group II, crush injury and intravenous administration of AFMSCs (5 x 10(6) cells for 3 days) immediately after injury (early administration); and Group III, crush injury and intravenous administration of AFMSCs (5 x 10(6) cells for 3 days) 7 days after injury (late administration). Evaluation of neurobehavior, electrophysiological study, and assessment of regeneration markers were conducted every week after injury. The expression of SDF-1alpha and neurotrophic factors and the distribution of AFMSCs in various time profiles were also assessed. RESULTS: Stromal cell-derived factor-1alpha increased the migration and wound healing of AFMSCs in vitro, and the migration ability was dose dependent. Crush injury induced the expression of SDF-1alpha at a peak of 10-14 days either in nerve or muscle, and this increased expression paralleled the expression of its receptor, chemokine receptor type-4 (CXCR-4). Most AFMSCs were distributed to the lung during early or late administration. Significant deposition of AFMSCs in nerve and muscle only occurred in the late administration group. Significantly enhanced neurobehavior, electrophysiological function, nerve myelination, and expression of neurotrophic factors and acetylcholine receptor were demonstrated in the late administration group. CONCLUSIONS: Amniotic fluid-derived mesenchymal stem cells can be recruited by expression of SDF-1alpha in muscle and nerve after nerve crush injury. The increased deposition of AFMSCs paralleled the expression profiles of SDF-1alpha and its receptor CXCR-4 in either muscle or nerve. Administration of AFMSCs led to improvements in neurobehavior and expression of regeneration markers. Intravenous administration of AFMSCs may be a promising alternative treatment strategy in peripheral nerve disorder.

Yang, H., et al. (2016). "Human umbilical cord-derived mesenchymal stem cells suppress proliferation of PHA-activated lymphocytes in vitro by inducing CD4(+)CD25(high)CD45RA (+) regulatory T cell production and modulating cytokine secretion." Cell Immunol **302**: 26-31.

Bone marrow-derived mesenchymal stem cells (MSCs) are promising candidate cells for therapeutic application in autoimmune diseases due to their immunomodulatory properties. Unused human umbilical cords (UC) offer an abundant and noninvasive source of MSCs without ethical issues and are emerging as a valuable alternative to bone marrow tissue for producing MSCs. We thus investigated the immunomodulation effect of umbilical cord-derived MSCs (UC-MSCs) on human peripheral blood mononuclear cells (PBMCs), T cells in particular, in a co-culture system. We found that UC-MSCs efficiently suppressed the proliferation of phytohaemagglutinin (PHA)-stimulated PBMCs ($p<0.01$). Kinetic analysis revealed that UC-MSCs primarily inhibited the division of generation 3 (G3) and 4 (G4) of PBMCs. In addition, UC-MSCs augmented the expression of CD127(+) and CD45RA (+) but reduced the expression of CD25(+) in PBMCs stimulated by PHA ($p<0.05$). Furthermore, UC-MSCs inhibited PHA-resulted increase in the frequency of CD4(+)CD25(+)CD127(low/-) Tregs significantly ($p<0.01$) but augmented PHA-resulted increase in the frequency of CD4(+)CD25(high)CD45RA (+) Tregs to about three times in PBMCs. The levels of anti-inflammatory cytokines, PEG2, TGF-beta, and IL-10 were greatly up-regulated, accompanied by a significant down-regulation of pro-inflammatory IFN-gamma in the co-culture ($p<0.01$). Our results showed that UC-MSCs are able to suppress mitogen-induced PBMC activation and proliferation in vitro by altering T lymphocyte phenotypes, increasing the frequency of CD4(+)CD25(high)CD45RA (+) Tregs, and modulating the associated cytokine production. Further studies are warranted to investigate the therapeutic potential of UC-MSCs in immunologically-diseased conditions.

Yang, H., et al. (2013). "Human umbilical cord mesenchymal stem cell-derived neuron-like cells rescue memory deficits and reduce amyloid-beta deposition in an AbetaPP/PS1 transgenic mouse model." Stem Cell Res Ther **4**(4): 76.

INTRODUCTION: Cell therapy is a potential therapeutic approach for neurodegenerative disorders, such as Alzheimer disease (AD). Neuronal differentiation of stem cells before transplantation is a promising procedure for cell therapy. However, the therapeutic impact and mechanisms of action of neuron-like cells differentiated from human umbilical

cord mesenchymal stem cells in AD have not been determined. METHODS: In this study, we used tricyclodecan-9-yl-xanthogenate (D609) to induce human mesenchymal stem cells isolated from Wharton jelly of the umbilical cord (HUMSCs) to differentiate into neuron-like cells (HUMSC-NCs), and transplanted the HUMSC-NCs into an AbetaPP/PS1 transgenic AD mouse model. The effects of HUMSC-NC transplantation on the cognitive function, synapsin I level, amyloid beta-peptides (Abeta) deposition, and microglial function of the mice were investigated. RESULTS: We found that transplantation of HUMSC-NCs into AbetaPP/PS1 mice improved the cognitive function, increased synapsin I level, and significantly reduced Abeta deposition in the mice. The beneficial effects were associated with "alternatively activated" microglia (M2-like microglia). In the mice transplanted with HUMSC-NCs, M2-like microglial activation was significantly increased, and the expression of antiinflammatory cytokine associated with M2-like microglia, interleukin-4 (IL-4), was also increased, whereas the expression of proinflammatory cytokines associated with classic microglia (M1-like microglia), including interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha), was significantly reduced. Moreover, the expression of Abeta-degrading factors, insulin-degrading enzyme (IDE) and neprilysin (NEP), was increased substantially in the mice treated with HUMSC-NCs. CONCLUSIONS: HUMSC-NC transplantation decreased Abeta deposition and improved memory in AbetaPP/PS1 mice by a mechanism associated with activating M2-like microglia and modulating neuroinflammation. Transplantation of neuron-like cells differentiated from mesenchymal stem cells might be a promising cell therapy for Alzheimer disease.

Yang, H. M., et al. (2011). "The effect of human fetal liver-derived mesenchymal stem cells on CD34+ hematopoietic stem cell repopulation in NOD/Shi-scid/IL-2Ra (null) mice." Transplant Proc **43**(5): 2004-2008.

Mesenchymal stem cells (MSCs) are progenitors that are capable of differentiating into mesenchymal tissues. They are known to support allogeneic hematopoietic stem cell transplantation by facilitating engraftment without increasing the risk of graft-versus-host disease. We optimized culture conditions for human fetal liver-derived MSCs (hFL-MSCs) to investigate the role of hFL-MSCs on repopulation of hematopoietic stem cells in NOD/Shi-scid/IL-2Rgamma (null) (NOG) mice using CD34(+) hematopoietic stem cells (HSCs) derived from umbilical cord blood (UCB). FL-MSCs and CD34(+) HSCs were prepared from fetal liver and UCB, respectively. Twenty-four hours after irradiation, CD34(+) HSCs and hFL-MSCs were injected intravenously and intratibially into NOG mice. During 24 weeks posttransplantation, engraftment levels of human cells were analyzed in bone marrow, peripheral blood, and spleen of transplanted mice by flow cytometry. hFL-MSCs showed a fibroblast-like morphology and immunophenotypic characteristics appropriate for MSCs. hFL-MSCs prolonged the survival of NOG mice that had been cotransplanted with UCB CD34(+) cells. Fluorescence-activated cell-sorting analysis showed that engraftment of human cells was increased by cotransplantation of hFL-MSCs. However, significant enhancement of human cell engraftment was not detected in NOG mice regardless of the number of cotransplanted MSCs. Although survival of repopulating NOG mice and engraftment of human cells were prolonged by cotransplantation of hFL-MSCs, 8.0 x 10(6) MSCs were not sufficient to increase HSC engraftment in irradiated NOG mice in vivo.

Yang, L., et al. (2014). "An IL6-STAT3 loop mediates resistance to PI3K inhibitors by inducing epithelial-mesenchymal transition and cancer stem cell expansion in human breast cancer cells." Biochem Biophys Res Commun **453**(3): 582-587.

Recently, a new generation of PI3K-specific inhibitors, such as GDC0941 and BKM120, are being investigated in clinical trials for treatment against tumors harboring PIK3CA mutations. Nevertheless, not all patients benefit from such treatment, suggesting that their tumors may be resistant to PI3K inhibitors. The investigation of the underlying mechanisms and efficacious personalized treatment remain a large unmet need. In this study, we revealed an IL6-STAT3 positive feedback loop that mediated the resistance to PI3K inhibitors. We found that breast cancer cells with acquired resistance to PI3K inhibitors displayed epithelial-mesenchymal transition (EMT) features and an highly enriched cancer stem cells (CSCs), secreting approximately 1000-fold more IL6 than parental cells. Further studies elucidated that activation of the IL6-STAT3 signaling effectively triggered EMT action, expanded the CSCs population, and reduced sensitivity to PI3K inhibitors. Pharmacological inhibition of STAT3 disrupted the IL6-STAT3 signaling and overcome resistance to PI3K inhibitors partially due to increased apoptosis induction. Taken together, our results demonstrated that feedback activation of the IL6-STAT3 loop lead to acquired resistance to PI3K inhibitors by promoting EMT and CSC-like features, and suggested that targeting this loop may be an efficient strategy to overcome resistance to PI3K inhibitors.

Yang, R. F., et al. (2014). "Enhancement of mouse germ cell-associated genes expression by injection of human umbilical cord mesenchymal stem cells into the testis of chemical-induced azoospermic mice." Asian J Androl **16**(5): 698-704.

Various methods are currently under investigation to preserve fertility in males treated with high-dose chemotherapy and radiation for malignant and nonmalignant disorders. Human umbilical cord mesenchymal stem cells (HUC-MSCs), which possess potent immunosuppressive function and secrete various cytokines and growth factors, have the potential clinical applications. As a potential alternative, we investigate whether injection of HUC-MSCs into the interstitial compartment of the testes to promote spermatogenic regeneration efficiently. HUC-MSCs were isolated from different sources of umbilical cords and injected into the interstitial space of one testis from 10 busulfan-treated mice (saline and HEK293 cells injections were performed in a separate set of mice) and the other testis remained uninjected. Three weeks after MSCs injection, Relative quantitative reverse transcription polymerase chain reaction was used to identify the expression of 10 of germ cell associated, which are all related to meiosis, demonstrated higher levels of spermatogenic gene expression (2-8 fold) in HUC-MSCs injected testes compared to the contralateral uninjected testes (five mice). Protein levels for germ cell-specific genes, miwi, vasa and synaptonemal complex protein (Scp3) were also higher in MSC-treated testes compared to injected controls 3 weeks after treatment. However, no different expression was detected in saline water and HEK293 cells injection control group. We have demonstrated HUC-MSCs could affect mouse germ cell-specific genes expression. The results also provide a possibility that the transplanted HUC-MSCs may promote the recovery of spermatogenesis. This study provides further evidence for preclinical therapeutic effects of HUC-MSCs, and explores a new approach to the treatment of azoospermia.

Yang, S., et al. (2013). "Pleiotrophin is involved in the amniotic epithelial cell-induced differentiation of human umbilical cord blood-derived mesenchymal stem cells into dopaminergic neuron-like cells." Neurosci Lett **539**: 86-91.

We have reported that human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) are capable of differentiating into dopaminergic (DA) neuron-like cells upon being induced by amniotic epithelial cells (AECs). However, what factor (s) is involved in the differentiation process has not been explored out thoroughly. Because pleiotrophin (PTN) is known to exert important trophic effects on DA neurons, in the present study, we investigated whether PTN is released by AECs and whether it is involved in the differentiation of hUCB-MSCs into DA neuron-like cells. The expression and secretion of PTN by AECs were detected by immunofluorescence, RT-PCR and ELISA. The hUCB-MSCs were isolated and treated with AEC-conditioned medium (ACM) or recombinant human PTN. Compared to the controls, a higher proportion of treated cells differentiated into DA neuron-like cells, indicated by the increased expression of TH and DAT and the increased dopamine content. These results indicate that PTN released by AECs acts as a synergetic factor with other neurotrophic factors and is involved in the differentiation of hUCB-MSCs into DA neuron-like cells. We suggest that ACM, which contains PTN and other neurotrophic factors, could potentially be used as an agent to promote the differentiation of DA neuron-like cells from hUCB-MSCs for cell therapy of Parkinson's disease without creating legal or ethical issues.

Yang, X., et al. (2012). "WITHDRAWN: The mesenchymal stem cell potential of human dental pulp derived cells transfected with embryonic transcription factor Oct-4." Biomaterials.

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Yang, Y., et al. (2016). "Conditioned umbilical cord tissue provides a natural three-dimensional storage compartment as in vitro stem cell niche for human mesenchymal stroma/stem cells." Stem Cell Res Ther **7**: 28.

BACKGROUND: The use of large amounts of human multipotent mesenchymal stroma/stem cells (MSC) for cell therapies represents a desirable property in tissue engineering and banking in the field of regenerative medicine. METHODS AND RESULTS: Whereas cryo-storage of umbilical cord (UC) tissue pieces in liquid nitrogen without ingredients was associated with predominant appearance of apoptotic cells after thawing and re-culture, progressive growth of MSC was observed following use of cryo-medium. Moreover, conditioning of UC tissue pieces by initial explant culture and subsequent cryo-storage with cryo-medium accelerated a further MSC culture after thawing. These findings suggested that conditioning of UC tissue pieces provides an in vitro stem cell niche by maintenance of a 3-dimensional natural microenvironment for continuous MSC outgrowth and expansion. Indeed, culture of GFP-labeled UC tissue pieces was accompanied by increased outgrowth of

GFP-labeled cells which was accelerated in conditioned UC tissue after cryo-storage. Moreover, cryopreserved conditioned UC tissue pieces in cryo-medium after thawing and explant culture could be cryopreserved again demonstrating renewed MSC outgrowth after repeated thawing with similar population doublings compared to the initial explant culture. Flow cytometry analysis of outgrowing cells revealed expression of the typical MSC markers CD73, CD90, and CD105. Furthermore, these cells demonstrated little if any senescence and cultures revealed stem cell-like characteristics by differentiation along the adipogenic, chondrogenic and osteogenic lineages. CONCLUSIONS: Expression of MSC markers was maintained for at least 10 freeze/thaw/explant culture cycles demonstrating that repeated cryopreservation of conditioned UC tissue pieces provided a reproducible and enriched stem cell source.

Yang, Y., et al. (2015). "Human mesenchymal stroma/stem cells exchange membrane proteins and alter functionality during interaction with different tumor cell lines." Stem Cells Dev **24**(10): 1205-1222.

To analyze effects of cellular interaction between human mesenchymal stroma/stem cells (MSC) and different cancer cells, direct co-cultures were performed and revealed significant growth stimulation of the tumor populations and a variety of protein exchanges. More than 90% of MCF-7 and primary human HBCEC699 breast cancer cells as well as NIH:OVCAR-3 ovarian adenocarcinoma cells acquired CD90 proteins during MSC co-culture, respectively. Furthermore, SK-OV-3 ovarian cancer cells progressively elevated CD105 and CD90 proteins in co-culture with MSC. Primary small cell hypercalcemic ovarian carcinoma cells (SCCOHT-1) demonstrated undetectable levels of CD73 and CD105; however, both proteins were significantly increased in the presence of MSC. This co-culture-mediated protein induction was also observed at transcriptional levels and changed functionality of SCCOHT-1 cells by an acquired capability to metabolize 5'cAMP. Moreover, exchange between tumor cells and MSC worked bidirectional, as undetectable expression of epithelial cell adhesion molecule (EpCAM) in MSC significantly increased after co-culture with SK-OV-3 or NIH:OVCAR-3 cells. In addition, a small population of chimeric/hybrid cells appeared in each MSC/tumor cell co-culture by spontaneous cell fusion. Immune fluorescence demonstrated nanotube structures and exosomes between MSC and tumor cells, whereas cytochalasin-D partially abolished the intercellular protein transfer. More detailed functional analysis of FACS-separated MSC and NIH:OVCAR-3 cells after co-culture revealed the acquisition of epithelial cell-specific properties by MSC, including increased gene expression for cytokeratins and epithelial-like differentiation factors. Vice versa, a variety of transcriptional regulatory genes were down-modulated in NIH:OVCAR-3 cells after co-culture with MSC. Together, these mutual cellular interactions contributed to functional alterations in MSC and tumor cells.

Yao, J., et al. (2012). "Tissue inhibitor of matrix metalloproteinase-3 or vascular endothelial growth factor transfection of aged human mesenchymal stem cells enhances cell therapy after myocardial infarction." Rejuvenation Res **15**(5): 495-506.

Mesenchymal stem cell (MSC) transplantation has been proposed as a potential therapeutic approach for ischemic heart disease, but the regenerative capacity of these cells decreases with age. In this study, we genetically engineered old human MSCs (O-hMSCs) with tissue inhibitor of matrix metalloproteinase-3 (TIMP3) and vascular endothelial growth factor (VEGF) and evaluated the effects on the efficacy of cell-based gene therapy in a rat myocardial infarction (MI) model. Cultured O-hMSCs were transfected with TIMP3 (O-TIMP3) or VEGF (O-VEGF) and compared with young hMSCs (Y-hMSCs) and non-transfected O-hMSCs for growth, clonogenic capacity, and differentiation potential. In vivo, rats were subjected to left coronary artery ligation with subsequent injection of Y-hMSCs, O-hMSCs, O-TIMP3, O-VEGF, or medium. Echocardiography was performed prior to and at 1, 2, and 4 weeks after MI. Myocardial levels of matrix metalloproteinase-2 (MMP2), MMP9, TIMP3, and VEGF were assessed at 1 week. Hemodynamics, morphology, and histology were measured at 4 weeks. In vitro, genetically modified O-hMSCs showed no changes in growth, colony formation, or multi-differentiation capacity.

Yen, B. L., et al. (2009). "Brief report--human embryonic stem cell-derived mesenchymal progenitors possess strong immunosuppressive effects toward natural killer cells as well as T lymphocytes." Stem Cells **27**(2): 451-456.

The derivation of mesenchymal progenitors from human embryonic stem cells (hESCs) has recently been reported. We studied the immune characteristics of these hESC-derived mesenchymal progenitors (EMPs) and their interactions with T lymphocytes and natural killer cells (NKs), two populations of lymphocytes with important roles in transplantation immunology. EMPs express a number of bone marrow mesenchymal stromal cell (BMMSC) markers, as well as the hESC marker SSEA-4. Immunologically, EMPs do not express HLA-DR or costimulatory molecules. On the other hand, HLA-G, a nonclassic MHC I

protein involved in mediating maternal-fetal tolerance, can be found on the surface of EMPs, and its expression is increased after interferon-gamma stimulation. EMPs can suppress CD4(+) or CD8(+) lymphocyte proliferation, similar to BMMSCs. However, EMPs are more resistant to NK-mediated lysis than BMMSCs and can suppress the cytotoxic effects of activated NKs, as well as downregulating the NK-activating receptors NKp30 and NKp46. With their broad immunosuppressive properties, EMPs may represent a new potential cell source for therapeutic use.

Yiang, G. T., et al. (2015). "Acetaminophen induces JNK/p38 signaling and activates the caspase-9-3-dependent cell death pathway in human mesenchymal stem cells." Int J Mol Med **36**(2): 485-492.

Acetaminophen (APAP) is a widely used analgesic and antipyretic drug. Generally, the therapeutic dose of APAP is clinically safe, however, high doses of APAP can cause acute liver and kidney injury. Therefore, the majority of previous studies have focussed on elucidating the mechanisms of APAP-induced hepatotoxicity and nephrotoxicity, in addition to examining ways to treat these conditions in clinical cases. However, few studies have reported APAP-induced intoxication in human stem cells. Stem cells are important in cell proliferation, differentiation and repair during human development, particularly during fetal and child development. At present, whether APAP causes cytotoxic effects in human stem cells remains to be elucidated, therefore, the present study aimed to investigate the cellular effects of APAP treatment in human stem cells. The results of the present study revealed that high-dose APAP induced more marked cytotoxic effects in human mesenchymal stem cells (hMSCs) than in renal tubular cells. In addition, increased levels of hydrogen peroxide (H2O2), phosphorylation of c-Jun N-terminal kinase and p38, and activation of caspase-9/-3 cascade were observed in the APAP-treated hMSCs. By contrast, antioxidants, including vitamin C reduced APAP-induced augmentations in H2O2 levels, but did not inhibit the APAP-induced cytotoxic effects in the hMSCs. These results suggested that high doses of APAP may cause serious damage towards hMSCs.

Yokokura, T., et al. (2017). "The Short-Stature Homeobox-Containing Gene (shox/SHOX) Is Required for the Regulation of Cell Proliferation and Bone Differentiation in Zebrafish Embryo and Human Mesenchymal Stem Cells." Front Endocrinol (Lausanne) **8**: 125.

The short-stature homeobox-containing gene (SHOX) was originally discovered as one of genes responsible for idiopathic short-stature syndromes in humans. Previous studies in animal models have shown the evolutionarily conserved link between this gene and skeletal formation in early embryogenesis. Here, we characterized developmental roles of shox/SHOX in zebrafish embryos and human mesenchymal stem cells (hMSCs) using loss-of-function approaches. Morpholino oligo-mediated knockdown of zebrafish shox markedly hindered cell proliferation in the anterior region of the pharyngula embryos, which was accompanied by reduction in the dlx2 expression at mesenchymal core sites for future pharyngeal bones. In addition, the impaired shox expression transiently increased expression levels of skeletal differentiation genes in early larval stage. In cell culture studies, we found that hMSCs expressed SHOX; the siRNA-mediated blockade of SHOX expression significantly blunted cell proliferation in undifferentiated hMSCs but the loss of SHOX expression did augment the expressions of subsets of early osteogenic genes during early osteoblast differentiation.

Yoshida, S., et al. (2018). "Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes by Soluble Factors from Human Mesenchymal Stem Cells." Mol Ther.

In this study, we proposed that the functionality or phenotype of differentiated cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) might be modified by co-culture with mesenchymal stem cells (MSCs), resulting in an improved therapeutic potential for failing myocardial tissues. Structural, motility, electrophysiological, and metabolic analyses revealed that iPSC-CMs co-cultured with MSCs displayed aligned myofibrils with A-, H-, and I-bands that could contract and relax quickly, indicating the promotion of differentiation and the establishment of the iPSC-CM structural framework, and showed clear gap junctions and an electric pacing of >2 Hz, indicating enhanced cell-cell interactions. In addition, soluble factors excreted by MSCs, including several cytokines and exosomes, enhanced cardiomyocyte-specific marker production, produced more energy under normal and stressed conditions, and reduced reactive oxygen species production by iPSC-CMs under stressed condition. Notably, gene ontology and pathway analysis revealed that microRNAs and proteins in the exosomes impacted the functionality and maturation of iPSC-CMs.

Yu, B., et al. (2011). "Simulated microgravity using a rotary cell culture system promotes chondrogenesis of human adipose-derived

mesenchymal stem cells via the p38 MAPK pathway." Biochem Biophys Res Commun **414**(2): 412-418.

Mesenchymal stem cells (MSCs) are multi-potent, and the chondrogenesis of MSCs is affected by mechanical stimulation. The aim of this study was to investigate, using a rotary cell culture system (RCCS) bioreactor, the effects of microgravity on the chondrogenic differentiation of human adipose-derived MSCs (ADSCs), which were cultured in pellets with or without the chondrogenic growth factor TGF-beta1. In addition, we evaluated the role of the p38 MAPK pathway in this process. The real-time PCR and histological results show that microgravity has a synergistic effect on chondrogenesis with TGF-beta1. The p38 MAPK pathway was activated by TGF-beta1 alone and was further stimulated by microgravity. Inhibition of p38 activity with SB203580 suppressed chondrocyte-specific gene expression and matrix production. These findings suggest that the p38 MAPK signal acts as an essential mediator in the microgravity-induced chondrogenesis of ADSCs.

Yu, J. M., et al. (2008). "Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo." Stem Cells Dev **17**(3): 463-473.

Mesenchymal stem cells (MSCs) have generated a great deal of interest in clinical situations, due principally to their potential use in regenerative medicine and tissue engineering applications. However, the therapeutic application of MSCs remains limited, unless the favorable effects of MSCs for tumor growth in vivo and the long-term safety of the clinical applications of MSCs can be understood more thoroughly. In this study, MSCs derived from human adipose tissues (hASCs) together with tumor cells were transplanted subcutaneously or intracranially into BALB/c nude mice to observe tumor outgrowth. The results indicated that hASCs with H460 or U87MG cells promoted tumor growth in nude mice. Our histopathological analyses indicated that the co-injection of tumor cells with hASCs exerted no influence on the formation of intratumoral vessels. Co-culture of tumor cells with hASCs or the addition of conditioned medium (CM) from hASCs effected an increase in the proliferation of H460 or U87MG cells. Co-injection of hASCs with tumor cells effected an increase in tumor cell viability in vivo, and also induced a reduction in apoptotic cell death. CM from hASCs inhibited hydrogen peroxide-induced cell death in H460 or U87MG cells.

Yuan, L., et al. (2016). "Human embryonic mesenchymal stem cells alleviate pathologic changes of MRL/Lpr mice by regulating Th7 cell differentiation." Ren Fail **38**(9): 1432-1440.

Recent evidence indicates that mesenchymal stem cells (MSC) derived from early embryonic tissues have better therapeutic ability as compared with adult tissue-derived stem cells. In the present study, we transplanted human early embryonic MSC (hMSC) into MRL/Lpr mice via tail vein injection to observe the therapeutic efficacy of hMSC and their impact on T helper 17 (Th17) cell differentiation in MRL/Lpr mice. Animals in hMSC treatment group received hMSC (1 x 10(6)/200 muL) via the tail vein at the age of 16 and 19 weeks. We found that hMSC treatment prolonged the survival of MRL/Lpr mice without inducing tumorigenesis, reduced urine protein, and alleviated the renal pathologic changes. In addition, it reduced the proportion of Th17 cells in the spleen of MRL/Lpr mice and the serum interleukin 17 (IL-17) concentration. Our in vitro experiment also demonstrated that hMSC could secrete Th17 differentiation-related cytokines of PGE2, IL-10 and TGF-beta, and IFN-gamma stimulation up-regulated the secretion of these immune regulating factors. The results of the present study suggest that hMSC therapy could alleviate systemic and local renal lesions in MRL/Lpr mice, probably by secreting immune regulating factors and regulating Th17 cell differentiation in MRL/Lpr mice.

Yuan, X., et al. (2017). "Extracellular vesicles from human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs) protect against renal ischemia/reperfusion injury via delivering specificity protein (SP1) and transcriptional activating of sphingosine kinase 1 and inhibiting necroptosis." Cell Death Dis **8**(12): 3200.

Renal ischemia-reperfusion is a main cause of acute kidney injury (AKI), which is associated with high mortality. Here we show that extracellular vesicles (EVs) secreted from hiPSC-MSCs play a critical role in protection against renal I/R injury. hiPSC-MSCs-EVs can fuse with renal cells and deliver SP1 into target cells, subsequently active SK1 expression and increase S1P formation. Chromatin immunoprecipitation (ChIP) analyses and luciferase assay were used to confirm SP1 binds directly to the SK1 promoter region and promote promoter activity. Moreover, SP1 inhibition (MIT) or SK1 inhibition (SKI-II) completely abolished the renal protective effect of hiPSC-MSCs-EVs in rat I/R injury mode. However, pre-treatment of necroptosis inhibitor Nec-1 showed no difference with the administration of hiPSC-MSCs-EVs only.

Yuan, Y., et al. (2013). "Forced expression of indoleamine-2,3-dioxygenase in human umbilical cord-derived mesenchymal stem cells abolishes their

anti-apoptotic effect on leukemia cell lines in vitro." In Vitro Cell Dev Biol Anim **49**(10): 752-758.

The ability of mesenchymal stem cells (MSCs) to preserve cancer cells potentially constitutes the adverse effect of MSC-based cell therapy in the context of hematologic malignancy. In an effort to reverse this undesirable feature of MSCs, we manipulated human umbilical cord-derived MSCs (UC-MSCs) to express indoleamine-2,3-dioxygenase (IDO), an enzyme that induces immune suppression by inhibiting T cell proliferation and triggering apoptosis in immune cells. Cultures of human UC-MSCs were generated by plastic adherence method. Full-length cDNA of human IDO was cloned into adenovirus shuttle vector. Then, the recombinant virus harboring IDO gene was produced in 293 cells and used to infect UC-MSCs. Expression of IDO protein was detected within infected UC-MSCs, and accumulation of kynurenine was observed in the supernatant. Two human leukemia cell lines, Jurkat and HL-60, were cultured on the monolayer of native or infected UC-MSCs, respectively. It was observed that forced IDO expression abolished the anti-apoptotic effect of UC-MSCs on these leukemia cells and enhanced their proliferation inhibitory effect on activated human lymphocytes as well as leukemia cells. These results suggested that equipping MSCs with IDO could be one of the reasonable strategies to reverse their cancer-supportive effect unfavorable for clinical applications.

Yuan, Y., et al. (2018). "Suppression of tumor cell proliferation and migration by human umbilical cord mesenchymal stem cells: A possible role for apoptosis and Wnt signaling." Oncol Lett **15**(6): 8536-8544.

Human umbilical cord-derived mesenchymal stem cells (hUCMSCs) represent potential therapeutic tools for solid tumors. However, there are numerous inconsistent results regarding the effects of hUCMSCs on tumors, and the mechanisms underlying this remain poorly understood. The present study further examined this controversial issue by analyzing the molecular mechanisms of the inhibitory effects of hUCMSCs on the proliferation and migration of the human lung cancer A549 cell line and the human hepatocellular carcinoma (HCC) BEL7402 cell line in vitro. Flow cytometric analysis demonstrated that hUCMSCs arrested tumor cells in specific phases of the cell cycle and induced the apoptosis of tumor cells by using the hUCMSC-conditioned medium (hUCMSC-CM). The hUCMSC-CM also attenuated the migratory abilities of the two tumor cell types.

Zanichelli, F., et al. (2012). "Dose-dependent effects of R-sulforaphane isothiocyanate on the biology of human mesenchymal stem cells, at dietary amounts, it promotes cell proliferation and reduces senescence and apoptosis, while at anti-cancer drug doses, it has a cytotoxic effect." Age (Dordr) **34**(2): 281-293.

Brassica vegetables are attracting a great deal of attention as healthy foods because of the fact that they contain substantial amounts of secondary metabolite glucosinolates that are converted into isothiocyanates, such as sulforaphane [(-)1-isothiocyanato-4R-(methylsulfinyl)-butane] (R-SFN), through the actions of chopping or chewing the vegetables. Several studies have analyzed the biological and molecular mechanisms of the anti-cancer activity of synthetic R,S-sulforaphane, which is thought to be a result of its antioxidant properties and its ability to inhibit histone deacetylase enzymes (HDAC). Few studies have addressed the possible antioxidant effects of R-SFN, which could protect cells from the free radical damage that strongly contribute to aging. Moreover, little is known about the effect of R-SFN on stem cells whose longevity is implicated in human aging. We evaluated the effects of R-SFN on the biology on human mesenchymal stem cells (MSCs), which, in addition to their ability to differentiate into mesenchymal tissues, support hematopoiesis, and contribute to the homeostatic maintenance of many organs and tissues. Our investigation found evidence that low doses of R-SFN promote MSCs proliferation and protect them from apoptosis and senescence, while higher doses have a cytotoxic effect, leading to the induction of cell cycle arrest, programmed cell death and senescence. The beneficial effects of R-SFN may be ascribed to its antioxidant properties, which were observed when MSC cultures were incubated with low doses of R-SFN.

Zemel'ko, V. I., et al. (2011). "[Multipotent mesenchymal stem cells of desquamated endometrium: isolation, characterization and use as feeder layer for maintenance of human embryonic stem cell lines]." Tsitologiia **53**(12): 919-929.

In this study, we characterize new multipotent human mesenchymal stem cell (MSC) lines derived from desquamated (shedding) endometrium in menstrual blood. The isolated endometrial MSC (eMSC) is an adhesive to plastic heterogeneous population composed mainly of endometrial glandular and stromal cells. The established cell lines meet the criteria of the International Society for Cellular Therapy for defining multipotent human MSC of any origin. The eMSCs have positive expression of CD73, CD90, CD105, CD13, CD29, CD44 markers and the absence of expression of the hematopoietic cell surface antigens CD19, CD34, CD45, CD117, CD130 and HLA-DR (class II). Multipotency of the established eMSC is confirmed by their ability to differentiate into

other mesodermal cell types such as osteocytes and adipocytes. Besides, the isolated eMSC lines partially (over 50%) express the pluripotency marker SSEA-4, but do not express Oct-4. Immunofluorescent analysis of the derived cells revealed the expression of the neural precursor markers nestin and beta-III-tubulin. This suggests a neural predisposition of the established eMSC. These cells are characterized by high rate of cell proliferation (doubling time 22-23 h) and high cloning efficiency (about 60%). In vitro the eMSCs undergo more than 45 population doublings revealing normal karyotype without karyotipic abnormalilies. We demonstrate, that the mititotically inactivated eMSCs are perfect feeder cells for human embryonic stem cell lines (hESC) C612 and C910. The eMSC being a feeder culture maintain the pluripotent status of the hESC, which is revealed by the expression of Oct-4, alkaline phosphatase and SSEA-4.

Zeng, Y., et al. (2013). "Electrophysiological characterisation of human umbilical cord blood-derived mesenchymal stem cells induced by olfactory ensheathing cell-conditioned medium." Neurochem Res **38**(12): 2483-2489.

Umbilical cord blood-derived marrow stromal cells (UCB-MSCs) with high proliferation capacity and immunomodulatory properties are considered to be a good candidate for cell-based therapies. But until now, little work has been focused on the differentiation of UCB-MSCs. In this work, UCB-MSCs were demonstrated to be negative for CD34 and CD45 expression but positive for CD90 and CD105 expression. The gate values of UCB-MSCs for CD90 and CD105 were 99.3 and 98.6 %, respectively. Two weeks after treatment, the percentage of neuron-like cells differentiated from UCB-MSCs was increased to 84 +/- 12 % in the experimental group [treated with olfactory ensheathing cells (OECs)-conditioned medium] and they were neuron-specific enolase positive; few neuron-like cells were found in the control group (without OECs-conditioned medium). Using whole-cell recording, sodium and potassium currents were recorded in UCB-MSCs after differentiation by OECs. Thus, human UCB-MSCs could be differentiated to neural cells by secreted secretion from OECs and exhibited electrophysiological properties similar to mature neurons after 2 weeks post-induction. These results imply that OECs can be used as a new strategy for stem cell differentiation and provide an alternative neurogenesis pathway for generating sufficient numbers of neural cells for cell therapy.

Zhang, A. Z., et al. (2017). "Cell Toxicity in Fibroblasts, Tenocytes, and Human Mesenchymal Stem Cells-A Comparison of Necrosis and Apoptosis-Inducing Ability in Ropivacaine, Bupivacaine, and Triamcinolone." Arthroscopy **33**(4): 840-848.

PURPOSE: To analyze the ability of ropivacaine, bupivacaine, and triamcinolone to induce apoptosis and necrosis in fibroblasts, tenocytes, and human mesenchymal stem cells. METHODS: Human dermal fibroblasts, adipose-derived human mesenchymal stem cells (hMSCs), and tenocytes gained from the rotator cuff tendon were seeded with a cell density of 0.5 x 10(4)/cm (2). One specimen of ropivacaine, bupivacaine, and triamcinolone was tested separately on the cells with separate concentrations of 0.5%, 0.25%, and 0.125% for each specimen. The negative control received no agent, only a change of medium. The incubation period for each agent was 30 minutes. After a change of medium and 1 hour, 24 hours, and 7 days of incubation, 10(4) cells were harvested and analyzed via fluorescence-activated cell sorting with double-staining with annexin V and propidium iodide. Statistical analysis to determine significant difference ($P < .05$) between the groups with SPSS statistics 23 through one-way analysis of variance with a univariate general linear model was performed. RESULTS: Bupivacaine showed necrosis-inducing effects on fibroblasts and tenocytes, with the necrotic effect peaking at 0.5% and 0.25%. Ropivacaine and triamcinolone caused no significant necrosis. Compared with fibroblasts and tenocytes, hMSCs did not show significant necrotic or apoptotic effects after exposure to bupivacaine.

Zhang, D. Y., et al. (2018). "Sirtuin3 protects aged human mesenchymal stem cells against oxidative stress and enhances efficacy of cell therapy for ischaemic heart diseases." J Cell Mol Med.

Sirtuin3 (SIRT3) is associated with oxidative stress and lifespan. However, the possible mechanisms underlying its influence are unknown. We hypothesized that SIRT3 increases the antioxidant capacity of aged cells and improves the efficacy of human mesenchymal stem cell (hMSC) therapy for ischaemic heart diseases in aged patients. In vitro, the antioxidant capacity of old hMSCs (O-hMSCs) was increased after SIRT3 overexpression using a gene transfection technique, while the antioxidant capacity of young hMSCs (Y-hMSCs) was decreased by SIRT3 silencing. The levels of forkhead box O3a (FoxO3a) in the nucleus, and antioxidant enzymes Mn-superoxide dismutase (MnSOD) and catalase (CAT) increased in SIRT3-overexpressed O-hMSCs while they decreased in SIRT3-silenced Y-hMSCs after oxidative stress. Following myocardial infarction in adult rats in vivo, infarct size decreased and cardiac function was significantly enhanced after cell transplantation with SIRT3 overexpressed O-hMSCs.

Zhang, F., et al. (2017). "Preservation media, durations and cell concentrations of short-term storage affect key features of human adipose-derived mesenchymal stem cells for therapeutic application." PeerJ **5**: e3301.

BACKGROUND: Adipose-derived mesenchymal stem cells (ADSCs) have shown great potential in the treatment of various diseases. However, the optimum short-term storage condition of ADSCs in 2 approximately 8 degrees C is rarely reported. This study aimed at optimizing a short-term storage condition to ensure the viability and function of ADSCs before transplantation. METHODS: Preservation media and durations of storage were evaluated by cell viability, apoptosis, adhesion ability and colony-forming unit (CFU) capacity of ADSCs. The abilities of cell proliferation and differentiation were used to optimize cell concentrations. Optimized preservation condition was evaluated by cell surface markers, cell cycle and immunosuppressive capacity. RESULTS: A total of 5% human serum albumin in multiple electrolytes (ME + HSA) was the optimized medium with high cell viability, low cluster rate, good adhesion ability and high CFU capacity of ADSCs. Duration of storage should be limited to 24 h to ensure the quality of ADSCs before transplantation. A concentration of 5 x 10(6) cells/ml was the most suitable cell concentration with low late stage apoptosis, rapid proliferation and good osteogenic and adipogenic differentiation ability. This selected condition did not change surface markers, cell cycle, indoleamine 2, 3-dioxygenase 1 (IDO1) gene expression and kynurenine (Kyn) concentration significantly. DISCUSSION: In this study, ME + HSA was found to be the best medium, most likely due to the supplement of HSA which could protect cells, the physiological pH (7.4) of ME and sodium gluconate ingredient in ME which could provide energy for cells. Duration should be limited to 24 h because of reduced nutrient supply and increased waste and lactic acid accumulation during prolonged storage.

Zhang, J., et al. (2012). "Regulation of cell proliferation of human induced pluripotent stem cell-derived mesenchymal stem cells via ether-a-go-go 1 (hEAG1) potassium channel." Am J Physiol Cell Physiol **303**(2): C115-125.

The successful generation of a high yield of mesenchymal stem cells (MSCs) from human induced pluripotent stem cells (iPSCs) may represent an unlimited cell source with superior therapeutic benefits for tissue regeneration to bone marrow (BM)-derived MSCs. We investigated whether the differential expression of ion channels in iPSC-MSCs was responsible for their higher proliferation capacity than BM-MSCs. The expression of ion channels for K (+), Na (+), Ca (2+), and Cl (-) was examined by RT-PCR. The electrophysiological properties of iPSC-MSCs and BM-MSCs were then compared by patch-clamp experiments to verify their functional roles. Significant mRNA expression of ion channel genes including KCa1.1, KCa3.1, KCNH1, Kir2.1, SCN9A, CACNA1C, and Clcn3 was observed in both human iPSC-MSCs and BM-MSCs, whereas Kir2.2 and Kir2.3 were only detected in human iPSC-MSCs. Five types of currents [big-conductance Ca (2+)-activated K (+) current (BK (Ca)), delayed rectifier K (+) current (IK (DR)), inwardly rectifying K (+) current (I (Kir)), Ca (2+)-activated K (+) current (IK (Ca)), and chloride current (I (Cl))] were found in iPSC-MSCs (83%, 47%, 11%, 5%, and 4%, respectively) but only four of them (BK (Ca), IK (DR), I (Kir), and IK (Ca)) were identified in BM-MSCs (76%, 25%, 22%, and 11%, respectively). Cell proliferation was examined with MTT or bromodeoxyuridine assay, and doubling times were 2.66 and 3.72 days for iPSC-MSCs and BM-MSCs, respectively, showing a 1.4-fold discrepancy. Blockade of IK (DR) with short hairpin RNA or human ether-a-go-go 1 (hEAG1) channel blockers, 4-AP and astemizole, significantly reduced the rate of proliferation of human iPSC-MSCs.

Zhang, J., et al. (2014). "Overexpression of myocardin induces partial transdifferentiation of human-induced pluripotent stem cell-derived mesenchymal stem cells into cardiomyocytes." Physiol Rep **2**(2): e00237.

Mesenchymal stem cells (MSCs) derived from human-induced pluripotent stem cells (iPSCs) show superior proliferative capacity and therapeutic potential than those derived from bone marrow (BM). Ectopic expression of myocardin further improved the therapeutic potential of BM-MSCs in a mouse model of myocardial infarction. The aim was of this study was to assess whether forced myocardin expression in iPSC-MSCs could further enhance their transdifferentiation to cardiomyocytes and improve their electrophysiological properties for cardiac regeneration. Myocardin was overexpressed in iPSC-MSCs using viral vectors (adenovirus or lentivirus). The expression of smooth muscle cell and cardiomyocyte markers, and ion channel genes was examined by reverse transcription-polymerase chain reaction (RT-PCR), immunofluorescence staining and patch clamp. The conduction velocity of the neonatal rat ventricular cardiomyocytes cocultured with iPSC-MSC monolayer was measured by multielectrode arrays recording plate. Myocardin induced the expression of alpha-MHC, GATA4, alpha-actinin, cardiac MHC, MYH11, calponin, and SM alpha-actin, but not cTnT, beta-MHC, and MLC2v in iPSC-MSCs. Overexpression of myocardin in iPSC-MSC enhanced

the expression of SCN9A and CACNA1C, but reduced that of KCa3.1 and Kir2.2 in iPSC-MSCs. Moreover, BKCa, IKir, ICl, Ito and INa.TTX were detected in iPSC-MSC with myocardin overexpression; while only BKCa, IKir, ICl, IKDR, and IKCa were noted in iPSC-MSC transfected with green florescence protein. Furthermore, the conduction velocity of iPSC-MSC was significantly increased after myocardin overexpression. Overexpression of myocardin in iPSC-MSCs resulted in partial transdifferentiation into cardiomyocytes phenotype and improved the electrical conduction during integration with mature cardiomyocytes.

Zhang, S., et al. (2013). "Neonatal desensitization supports long-term survival and functional integration of human embryonic stem cell-derived mesenchymal stem cells in rat joint cartilage without immunosuppression." Stem Cells Dev **22**(1): 90-101.

Immunological response hampers the investigation of human embryonic stem cells (hESCs) or their derivates for tissue regeneration in vivo. Immunosuppression is often used after surgery, but exhibits side effects of significant weight loss and allows only short-term observation. The purpose of this study was to investigate whether neonatal desensitization supports relative long-term survival of hESC-derived mesenchymal stem cells (hESC-MSCs) and promotes cartilage regeneration. hESC-MSCs were injected on the day of birth in rats. Six weeks after neonatal injection, a full-thickness cylindrical cartilage defect was created and transplanted with a hESC-MSC-seeded collagen bilayer scaffold (group d+s+c) or a collagen bilayer scaffold (group d+s). Rats without neonatal injection were transplanted with the hESC-MSC-seeded collagen bilayer scaffold to serve as controls (group s+c). Cartilage regeneration was evaluated by histological analysis, immunohistochemical staining, and biomechanical test.

Zhang, Y., et al. (2017). "Systemic administration of cell-free exosomes generated by human bone marrow derived mesenchymal stem cells cultured under 2D and 3D conditions improves functional recovery in rats after traumatic brain injury." Neurochem Int **111**: 69-81.

Multipotent human bone marrow derived mesenchymal stem cells (hMSCs) improve functional outcome after experimental traumatic brain injury (TBI). The present study was designed to investigate whether systemic administration of cell-free exosomes generated from hMSCs cultured in 2-dimensional (2D) conventional conditions or in 3-dimensional (3D) collagen scaffolds promote functional recovery and neurovascular remodeling in rats after TBI. Wistar rats were subjected to TBI induced by controlled cortical impact; 24 h later tail vein injection of exosomes derived from hMSCs cultured under 2D or 3D conditions or an equal number of liposomes as a treatment control were performed. The modified Morris water maze, neurological severity score and footfault tests were employed to evaluate cognitive and sensorimotor functional recovery. Animals were sacrificed at 35 days after TBI. Histological and immunohistochemical analyses were performed for measurements of lesion volume, neurovascular remodeling (angiogenesis and neurogenesis), and neuroinflammation. Compared with liposome-treated control, exosome-treatments did not reduce lesion size but significantly improved spatial learning at 33-35 days measured by the Morris water maze test, and sensorimotor functional recovery, i.e., reduced neurological deficits and footfault frequency, observed at 14-35 days post injury ($p < 0.05$). Exosome treatments significantly increased the number of newborn endothelial cells in the lesion boundary zone and dentate gyrus, and significantly increased the number of newborn mature neurons in the dentate gyrus as well as reduced neuroinflammation. Exosomes derived from hMSCs cultured in 3D scaffolds provided better outcome in spatial learning than exosomes from hMSCs cultured in the 2D condition. In conclusion, hMSC-generated exosomes significantly improve functional recovery in rats after TBI, at least in part, by promoting endogenous angiogenesis and neurogenesis and reducing neuroinflammation. Thus, exosomes derived from hMSCs may be a novel cell-free therapy for TBI, and hMSC-scaffold generated exosomes may selectively enhance spatial learning.

Zhang, Y., et al. (2015). "Potent Paracrine Effects of human induced Pluripotent Stem Cell-derived Mesenchymal Stem Cells Attenuate Doxorubicin-induced Cardiomyopathy." Sci Rep **5**: 11235.

Transplantation of bone marrow mesenchymal stem cells (BM-MSCs) can protect cardiomyocytes against anthracycline-induced cardiomyopathy (AIC) through paracrine effects. Nonetheless the paracrine effects of human induced pluripotent stem cell-derived MSCs (iPSC-MSCs) on AIC are poorly understood. In vitro studies reveal that doxorubicin (Dox)-induced reactive oxidative stress (ROS) generation and cell apoptosis in neonatal rat cardiomyocytes (NRCMs) are significantly reduced when treated with conditioned medium harvested from BM-MSCs (BM-MSCs-CdM) or iPSC-MSCs (iPSC-MSCs-CdM). Compared with BM-MSCs-CdM, NRCMs treated with iPSC-MSCs-CdM exhibit significantly less ROS and cell apoptosis in a dose-dependent manner. Transplantation of BM-MSCs-CdM or iPSC-MSCs-CdM into mice with AIC remarkably attenuated left ventricular (LV)

dysfunction and dilatation. Compared with BM-MSCs-CdM, iPSC-MSCs-CdM treatment showed better alleviation of heart failure, less cardiomyocyte apoptosis and fibrosis.

Zhang, Y., et al. (2012). "Improved cell survival and paracrine capacity of human embryonic stem cell-derived mesenchymal stem cells promote therapeutic potential for pulmonary arterial hypertension." Cell Transplant **21**(10): 2225-2239.

Although transplantation of adult bone marrow mesenchymal stem cells (BM-MSCs) holds promise in the treatment for pulmonary arterial hypertension (PAH), the poor survival and differentiation potential of adult BM-MSCs have limited their therapeutic efficiency. Here, we compared the therapeutic efficacy of human embryonic stem cell-derived MSCs (hESC-MSCs) with adult BM-MSCs for the treatment of PAH in an animal model. One week following monocrotaline (MCT)-induced PAH, mice were randomly assigned to receive phosphate-buffered saline (MCT group); 3.0x10(6) human BM-derived MSCs (BM-MSCs group) or 3.0x10(6) hESC-derived MSCs (hESC-MSCs group) via tail vein injection. At 3 weeks post-transplantation, the right ventricular systolic pressure (RVSP), degree of RV hypertrophy, and medial wall thickening of pulmonary arteries were lower=, and pulmonary capillary density was higher in the hESC-MSC group as compared with BM-MSC and MCT groups (all $p < 0.05$). At 1 week post-transplantation, the number of engrafted MSCs in the lungs was found significantly higher in the hESC-MSC group than in the BM-MSC group (all $p < 0.01$). At 3 weeks post-transplantation, implanted BM-MSCs were undetectable whereas hESC-MSCs were not only engrafted in injured pulmonary arteries but had also undergone endothelial differentiation. In addition, protein profiling of hESC-MSC- and BM-MSC-conditioned medium revealed a differential paracrine capacity. Classification of these factors into bioprocesses revealed that secreted factors from hESC-MSCs were preferentially involved in early embryonic development and tissue differentiation, especially blood vessel morphogenesis. We concluded that improved cell survival and paracrine capacity of hESC-MSCs provide better therapeutic efficacy than BM-MSCs in the treatment for PAH.

Zhang, Y., et al. (2011). "Plasticity of marrow mesenchymal stem cells from human first-trimester fetus: from single-cell clone to neuronal differentiation." Cell Reprogram **13**(1): 57-64.

Recent results have shown that bone marrow mesenchymal stem cells (BMSCs) from human first-trimester abortus (hfBMSCs) are closer to embryonic stem cells and perform greater telomerase activity and faster propagation than mid- and late-prophase fetal and adult BMSCs. However, no research has been done on the plasticity of hfBMSCs into neuronal cells using single-cell cloned strains without cell contamination. In this study, we isolated five single cells from hfBMSCs and obtained five single-cell cloned strains, and investigated their biological property and neuronal differentiation potential. We found that four of the five strains showed similar expression profile of surface antigen markers to hfBMSCs, and most of them differentiated into neuron-like cells expressing Nestin, Pax6, Sox1, beta-III Tubulin, NF-L, and NSE under induction. One strain showed different expression profile of surface antigen markers from the four strains and hfBMSCs, and did not differentiate toward neuronal cells. We demonstrated for the first time that some of single-cell cloned strains from hfBMSCs can differentiate into nerve tissue-like cell clusters under induction in vitro, and that the plasticity of each single-cell cloned strain into neuronal cells is different.

Zhang, Y. Y., et al. (2014). "BKCa and hEag1 channels regulate cell proliferation and differentiation in human bone marrow-derived mesenchymal stem cells." J Cell Physiol **229**(2): 202-212.

Human bone marrow-derived mesenchymal stem cells (MSCs) serve as a reservoir for the continuous renewal of various mesenchymal tissues; however, cellular physiology of ion channels is not fully understood. The present study investigated potential roles of large-conductance Ca (2+) -activated potassium (BKCa) channels and ether-a-go-go potassium (hEag1 or Kv10.1) channels in regulating cell proliferation and differentiation in human MSCs. We found that inhibition of BKCa with paxilline or hEag1 with astemizole, or knockdown of BKCa with shRNAs targeting KCa1.1 or hEag1 channels with shRNAs targeting KCNH1 arrested the cells at G0/G1 phase. In addition, silencing BKCa or hEag1 channels significantly reduced adipogenic differentiation with decrease of lipid accumulation and expression of the adipocyte marker PPARgamma, and decreased osteogenic differentiation with reduction of mineral precipitation and osteocalcin. These effects were accompanied with a reduced cyclin D1, cyclin E, p-ERK1/2, and p-Akt. Our results demonstrate that BKCa and hEag1 channels not only regulate cell proliferation, but also participate in the adipogenic and osteogenic differentiations in human MSCs, which indicates that BKCa and hEag1 channels may be essential in maintaining bone marrow physiological function and bone regeneration.

Zhang, Z., et al. (2017). "Human Umbilical Cord Mesenchymal Stem Cells Inhibit T Follicular Helper

Cell Expansion Through the Activation of iNOS in Lupus-Prone B6.MRL-Fas (lpr) Mice." Cell Transplant **26**(6): 1031-1042.

The aberrant generation or activation of T follicular helper (Tfh) cells contributes to the pathogenesis of systemic lupus erythematosus (SLE), yet little is known about how these cells are regulated. In this study, we demonstrated that the frequency of Tfh cells was increased in lupus-prone B6.MRL-Faslpr (B6.lpr) mice and positively correlated to plasma cell proportions and serum total IgG as well as anti-dsDNA antibody levels. Transplantation of mesenchymal stem cells derived from Wharton's jelly of human umbilical cords (hUC-MSCs) ameliorated lupus symptoms in B6.lpr mice, along with decreased percentages of Tfh cells. In vitro studies showed that the differentiation and proliferation of Tfh cells were markedly suppressed by hUC-MSCs. The production of inducible nitric oxide synthase (iNOS) was dramatically upregulated in hUC-MSCs when cocultured with CD4+ T cells directly, while adding the specific inhibitor of iNOS into the coculture system significantly reversed the inhibitory effect of hUC-MSCs on Tfh cell generation. Interestingly, the efficacy of hUC-MSCs in inhibiting Tfh cells was impaired in the Transwell system, with the reduction of iNOS in both mRNA and protein levels. Taken together, our findings suggest that hUC-MSCs could effectively inhibit Tfh cell expansion through the activation of iNOS in lupus-prone B6.lpr mice, which is highly dependent on cell-to-cell contacts.

Zhao, Y., et al. (2013). "Hydrogen sulfide augments the proliferation and survival of human induced pluripotent stem cell-derived mesenchymal stromal cells through inhibition of BKCa." Cytotherapy **15**(11): 1395-1405.

BACKGROUND: Hydrogen sulfide (H2S) is an endogenously generated gaseous transmitter known for its cytoprotective effect mediated by the PI3K-Akt signaling pathway. Human induced pluripotent stem cell (hiPSC)-derived mesenchymal stromal cells (MSCs), or hiPSC-MSCs, represent an alternative source of MSCs for autologous cell therapy. The big-conductance Ca (2+)-activated outward K (+) currents (BKCa), known to mediate cell proliferation, have been detected in >80% of hiPSC-MSCs. The present study aimed to explore the effect of H2S on survival and proliferation of hiPSC-MSCs and investigate the mediatory role of BKCa. METHODS: Effects of H2S on proliferation and survival of hiPSC-MSCs were measured by 5-bromo-2-deoxyuridine incorporation, population doubling and cell cycle assays, and by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide assay and 4'-6-diamidino-2-phenylindole staining, respectively. BKCa was recorded by means of the whole-cell patch-clamp technique. The expressions of KCa 1.1 (encoding BKCa) and apoptosis-related genes were measured by reverse transcriptase-polymerase chain reaction. The phosphorylation of Akt was assessed by Western blot analysis. RESULTS: Exogenously administered NaHS (an H2S donor, 50-300 mumol/L) significantly promoted proliferation of hiPSC-MSCs. NaHS prevented the hypoxia-induced apoptosis and suppressed BKCa currents without altering the expression levels of alpha- and beta-KCa 1.1. In addition, NaHS increased the phosphorylation of Akt and decreased the expression of Caspase 8 and Bax in hiPSC-MSCs. Paxilline (1 mumol/L), a BKCa blocker, showed similar effects on promoting cell proliferation and phosphorylation of Akt and suppression of apoptotic genes in hiPSC-MSCs. CONCLUSIONS: Our data confirmed that H2S arguments the proliferation and survival of hiPSC-MSCs through activation of the PI3K-Akt pathway and that such effects could be mediated through inhibition of BKCa.

Zhao, Y. M., et al. (2008). "Cell cycle dependent telomere regulation by telomerase in human bone marrow mesenchymal stem cells." Biochem Biophys Res Commun **369**(4): 1114-1119.

Human bone marrow mesenchymal stem cells (hMSCs) are a promising source for clinical stem cell transplantation. However, telomere regulation mechanisms, as one of the possible major mechanisms by which hMSCs sustain their stem cell characteristics, remain unknown. We isolated hMSCs by plastic adhesion and characterized these cells by morphology, immune phenotype and differentiation capacity. Telomerase was found negative in hMSCs, but slightly up-regulated in hMSC-derived adipocytes by the Telomeric Repeat Amplification Protocol (TRAP) assay. Moreover, hMSCs lack the alternative lengthening of telomeres (ALT) mechanism, because the hallmarks of ALT, such as very long and heterogeneous telomeres, extra-chromosome telomere repeat DNA (ECTR), and ALT-associated promyelocytic leukemia bodies (APBs), were not evident. However, when hMSCs were arrested in S phase with a combination of serum deprivation and aphidicolin, previously undetectable telomerase activity became predominantly positive.

Zhau, H. E., et al. (2011). "Human prostate cancer harbors the stem cell properties of bone marrow mesenchymal stem cells." Clin Cancer Res **17**(8): 2159-2169.

PURPOSE: Prostate tumor cells frequently show the features of osteoblasts, which are differentiated from bone marrow mesenchymal stem cells. We examined human prostate cancer cell lines and clinical

prostate cancer specimens for additional bone marrow mesenchymal stem cell properties. EXPERIMENTAL DESIGN: Prostate cancer cell lines were induced for osteoblastogenic and adipogenic differentiation, detected by standard staining methods and confirmed by lineage-specific marker expression. Abnormal expression of the markers was then assessed in clinical prostate cancer specimens. RESULTS: After osteoblastogenic induction, cells of the LNCaP lineage, PC-3 lineage, and DU145 displayed osteoblastic features. Upon adipogenic induction, PC-3 lineage and DU145 cells differentiated into adipocyte-like cells. The adipocyte-like cancer cells expressed brown adipocyte-specific markers, suggesting differentiation along the brown adipocyte lineage. The adipogenic differentiation was accompanied by growth inhibition, and most of the adipocyte-like cancer cells were committed to apoptotic death. During cyclic treatments with adipogenic differentiation medium and then with control medium, the cancer cells could commit to repeated adipogenic differentiation and retrodifferentiation. In clinical prostate cancer specimens, the expression of uncoupling protein 1 (UCP1), a brown fat-specific marker, was enhanced with the level of expression correlated to disease progression from primary to bone metastatic cancers. CONCLUSIONS: This study thus revealed that prostate cancer cells harbor the stem cell properties of bone marrow mesenchymal stem cells.

Zhou, J., et al. (2012). "Neural cell injury microenvironment induces neural differentiation of human umbilical cord mesenchymal stem cells." Neural Regen Res **7**(34): 2689-2697.

This study aimed to investigate the neural differentiation of human umbilical cord mesenchymal stem cells (hUCMSCs) under the induction of injured neural cells. After in vitro isolation and culture, passage 5 hUCMSCs were used for experimentation. hUCMSCs were co-cultured with normal or Abeta1-40-injured PC12 cells, PC12 cell supernatant or PC12 cell lysate in a Transwell co-culture system. Western blot analysis and flow cytometry results showed that choline acetyltransferase and microtubule-associated protein 2, a specific marker for neural cells, were expressed in hUCMSCs under various culture conditions, and highest expression was observed in the hUCMSCs co-cultured with injured PC12 cells. Choline acetyltransferase and microtubule-associated protein 2 were not expressed in hUCMSCs cultured alone (no treatment). Cell Counting Kit-8 assay results showed that hUCMSCs under co-culture conditions promoted the proliferation of injured PC12 cells. These findings suggest that the microenvironment during neural tissue injury can effectively induce neural cell differentiation of hUCMSCs. These differentiated hUCMSCs likely accelerate the repair of injured neural cells.

Zhou, L., et al. (2018). "SIRT1-dependent anti-senescence effects of cell-deposited matrix on human umbilical cord mesenchymal stem cells." J Tissue Eng Regen Med **12**(2): e1008-e1021.

Human umbilical cord-derived mesenchymal stem cells (UC-MSCs) are considered an attractive cell source for tissue regeneration. However, environmental oxidative stress can trigger premature senescence in MSCs and thus compromises their regenerative potential. Extracellular matrix (ECM) derived from MSCs has been shown to facilitate cell proliferation and multi-lineage differentiation. This investigation evaluated the effect of cell-deposited decellularized ECM (DECM) on oxidative stress-induced premature senescence in UC-MSCs. Sublethal dosages of H2 O2, ranging from 50 mum to 200 mum, were used to induce senescence in MSCs. We found that DECM protected UC-MSCs from oxidative stress-induced premature senescence. When treated with H2 O2 at the same concentration, cell proliferation of DECM-cultured UC-MSCs was twofold higher than those on standard tissue culture polystyrene (TCPS). After exposure to 100 mum H2 O2, fewer senescence-associated beta-galactosidase-positive cells were observed on DECM than those on TCPS (17.6 +/- 4.0% vs. 60.4 +/- 6.2%). UC-MSCs cultured on DECM also showed significantly lower levels of senescence-related regulators, such as p16(INK4alpha) and p21. Most importantly, DECM preserved the osteogenic differentiation potential of UC-MSCs with premature senescence. The underlying molecular mechanisms involved the silent information regulator type 1 (SIRT1)-dependent signalling pathway, confirmed by the fact that the SIRT1 inhibitor nicotinamide counteracted the DECM-mediated anti-senescent effect. Collagen type I, rather than fibronectin, partially contributed to the protective effect of decellularized matrix. These findings provide a new strategy of using stem cell-deposited matrix to overcome the challenge of cellular senescence and to facilitate the clinical application of MSCs in regenerative medicine. Copyright (c) 2017 John Wiley & Sons, Ltd.

Zhou, S., et al. (2017). "CD109 released from human bone marrow mesenchymal stem cells attenuates TGF-beta-induced epithelial to mesenchymal transition and stemness of squamous cell carcinoma." Oncotarget **8**(56): 95632-95647.

Although there is increasing evidence that human bone marrow mesenchymal stem cells (hBM-MSCs) play an important role in cancer progression, the underlying mechanisms are poorly understood.

Transforming growth factor beta (TGF-beta) is an important pro-metastatic cytokine. We have previously shown that CD109, a glycosylphosphatidylinositol-anchored protein, is a TGF-beta co-receptor and a strong inhibitor of TGF-beta signalling. Moreover, CD109 can be released from the cell surface. In the current study, we examined whether hBM-MSCs regulate the malignant properties of squamous cell carcinoma cells, and whether CD109 plays a role in mediating the effect of hBM-MSCs on cancer cells. Here we show that hBM-MSC-conditioned medium decreases proliferation and induces apoptosis in human squamous carcinoma cell lines, A431 and FaDu. Importantly, hBM-MSC-conditioned medium markedly suppresses markers of epithelial-to-mesenchymal transition and stemness, and concomitantly decreases cell migration, invasion, and spheroid formation in A431 and FaDu cells. In addition, knockdown of CD109 in hBM-MSCs abrogates the anti-malignant activity of hBM-MSC-conditioned medium on A431 and FaDu cells. Furthermore, overexpression of CD109 in A431 cells decreases their malignant traits. Together, our findings suggest that hBM-MSCs inhibit the malignant traits of squamous cell carcinoma cells by a paracrine effect via released factors and that CD109 released from hBM-MSCs, at least partially, mediates these effects.

Zhu, S. F., et al. (2013). "Comparison of cell proliferation, apoptosis, cellular morphology and ultrastructure between human umbilical cord and placenta-derived mesenchymal stem cells." Neurosci Lett **541**: 77-82.

Research in mesenchymal stem cells (MSCs) is mainly focused on applications for treatments of brain and spinal cord injury as well as mechanisms underlying effects of MSCs. However, due to numerous limitations, there is little information on selection of appropriate sources of MSCs for transplantation in clinical applications. Therefore, in this study we compared various properties of human umbilical cord-derived MSCs (HUCMSCs) with human placenta-derived MSCs (HPDMSCs), including cell proliferation, apoptosis, cellular morphology, ultrastructure, and their ability to secrete various growth factors (i.e. vascular endothelial growth factor, insulin-like growth factors-1, and hepatocyte growth factor), which will allow us to select appropriate MSC sources for cellular therapy. Cell culture, flow cytometry, transmission electron microscope (TEM) and atomic force microscope (AFM) were used for assessment of HUCMSCs and HPDMSCs. Results showed that the two types of cells appeared slightly different when they were observed under AFM. HUCMSCs appeared more fibroblast-like, whereas HPDMSCs appeared as large flat cells. HUCMSCs had higher proliferative rate and lower rate of apoptosis than HPDMSCs ($p<0.05$). However, HPDMSCs secreted more of the three growth factors than HUCMSCs ($p<0.05$). Results of TEM revealed that the two types of MSCs underwent active metabolism and had low degree of differentiation, especially HUCMSCs. Results of AFM showed that HUCMSCs had stronger ability of mass transport and cell migration than HPDMSCs. However, HPDMSCs displayed stronger adhesive properties than HUCMSCs. Our findings indicate that different sources of MSCs have different properties, and that care should be taken when choosing the appropriate sources of MSCs for stem cell transplantation.

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

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

Zhuo, H. L., et al. (2016). "[Effects of retinol on expressions of epidermal growth factor, stem cell factor, colony-stimulating factor 1 and leukemia inhibitory factor in human umbilical cord-derived mesenchymal stem cells]." Nan Fang Yi Ke Da Xue Xue Bao **37**(2): 221-225.

OBJECTIVE: To investigate effects of retinol on the expressions of epidermal growth factor (EGF), stem cell factor (SCF), colony-stimulating factor 1 (CSF1) and leukemia inhibitory factor (LIF) in cultured human umbilical-derived mesenchymal stem cells (UCMSCs). METHODS: Human UCMSCs were isolated from human umbilical cord and identified for immunophenotypes. The cells were then cultured in

DMEM/F12 media supplemented with 12% fetal bovine serum (FBS), 12% FBS+1 micromol/L retinol, 15% knockout serum replacement (KSR) and 15% KSR+ 1 micromol/L retinol. The expressions of the cytokines EGF, SCF, CSF1 and LIF in the cells were detected using RT-PCR and ELISA. RESULTS: The isolated cells exhibited characteristic immunophenotypes of human UCMSCs and expressed EGF, CSF1 and SCF at both mRNA and protein levels but not LIF protein. Retinol (1 micromol/L) significantly promoted the expressions of SCF and CSF1 at both mRNA and protein levels but did not result in changes of EGF and LIF expressions in human UCMSCs. CONCLUSION: Retinol at the concentration of 1 micromol/L can promote expression of SCF and CSF1 in human UCMSCs in vitro.

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11/18/2018