Stem Cell and Clone Research Literatures

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

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Key words: stem cell; life; research; literature

Introduction

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

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

Akazawa, Y., et al. (2015). "Recruitment of mesenchymal stem cells by stromal cell-derived factor 1alpha in pulp cells from deciduous teeth." Int J Mol Med **36**(2): 442-448.

Dental pulp cells (DPCs), including dental pulp (DP) stem cells, play a role in dentine repair under certain conditions caused by bacterial infections associated with caries, tooth fracture and injury. Mesenchymal stem cells (MSCs) have also been shown to be involved in this process of repair. However, the mechanisms through which MSCs are recruited to the DP have not yet been elucidated. Therefore, the aim of the present in vitro study was to investigate whether stromal cell-derived factor 1alpha (SDF1)-C-X-C chemokine receptor type 4 (CXCR4) signaling is involved in tissue repair in the DP of deciduous teeth. A single-cell clone from DPCs (SDP11) and UE7T-13 cells were used as pulp cells and MSCs, respectively. The MG-63 and HuO9 cells, two osteosarcoma cell lines, were used as positive control cells. Reverse transcription polymerase chain reaction (RT-PCR) revealed that all cell lines (SDP11, UE7T-13 MG-63 and HuO9) were positive for both SDF1 and CXCR4 mRNA expression. Moreover, immunocytochemical analysis indicated that SDF1 and CXCR4 proteins were expressed in the SDP11 and UE7T-13 cells. SDF1 was also detected in the cell lysates (CLs) and conditioned medium (CM) collected from the SDP11 and UE7T-13 cells, and AMD3100, a specific antagonist of CXCR4, inhibited the migration of the UE7T-13 cells; this migration was induced by treatment with CM, which was collected from the SDP11 cells. In addition, real-time PCR showed that the expression of SDF1 in the SDP11 cells was inhibited by treatment with 20 ng/ml fibroblast growth factor (FGF)-2, and exposure to AZD4547, an inhibitor of the FGF receptor, blocked this inhibition. Collectively, these data suggest that SDF1 produced by DP plays an important role in homeostasis, repair and regeneration via the recruitment of MSCs.

Bocker, W., et al. (2008). "Introducing a single-cell-derived human mesenchymal stem cell line expressing hTERT after lentiviral gene transfer." J Cell Mol Med **12**(4): 1347-1359.

Human mesenchymal stem cells (hMSCs) can be readily isolated from bone marrow and differentiate into multiple tissues, making them a promising target for future cell and gene therapy applications. The low frequency of hMSCs in bone marrow necessitates their isolation and expansion in vitro prior to clinical use, but due to senescence-associated growth arrest during culture, limited cell numbers can be generated. The lifespan of hMSCs has been extended by ectopic expression of human telomerase reverse transcriptase (hTERT) using retroviral vectors. Since malignant transformation was observed in hMSCs and retroviral

vectors cause insertional mutagenesis, we ectopically expressed hTERT using lentiviral gene transfer. Single-cell-derived hMSC clones expressing hTERT did not show malignant transformation in vitro and in vivo after extended culture periods. There were no changes observed in the expression of tumour suppressor genes and karyotype. Cultured hMSCs lack telomerase activity, but it was significantly increased by ectopic expression of hTERT. HTERT expression prevented hMSC senescence and the cells showed significantly higher and unlimited proliferation capacity. Even after an extended culture period, hMSCs expressing hTERT preserved their stem cells character as shown by osteogenic, adipogenic and chondrogenic differentiation. In summary, extending the lifespan of human mesenchymal stem cells by ectopic expression of hTERT using lentiviral gene transfer may be an attractive and safe way to generate appropriate cell numbers for cell and gene therapy applications.

Burns, J. S., et al. (2008). "The histopathology of a human mesenchymal stem cell experimental tumor model: support for an hMSC origin for Ewing's sarcoma?" Histol Histopathol **23**(10): 1229-1240.

Sarcomas display varied degrees of karyotypic abnormality, vascularity and mesenchymal differentiation. We have reported that a strain of telomerized adult human bone marrow mesenchymal stem cells (hMSC-TERT20) spontaneously evolved a tumorigenic phenotype after long-term continuous culture. We asked to what extent our hMSC-TERT20 derived tumors reflected events found in human sarcomas using routine histopathological procedures. Early versus late passage hMSC-TERT20 cultures persistently expressed mesenchymal lineage proteins e.g. CD105, CD44, CD99 and vimentin. However, late passage cultures, showed increased immunohistochemical staining for CyclinD1 and p21WAF1/Cip1, whereas p27Kip1 staining was reduced. Notably, spectral karyotyping showed that tumorigenic hMSC-TERT20 cells retained a normal diploid karyotype, with no detectable chromosome abnormalities. Consistent with the bone-forming potential of early passage hMSC-TERT20 cells, tumors derived from late passage cells expressed early biomarkers of osteogenesis. However, hMSC-TERT20 cells were heterogeneous for alpha smooth muscle actin (ASMA) expression and one out of six hMSC-TERT20 derived single cell clones was strongly ASMA positive. Tumors from this ASMA+ clone had distinctive vascular qualities with hot spots of high CD34+ murine endothelial cell density, together with CD34- regions with a branching periodic acid Schiff reaction pattern. Such clone-specific differences in host vascular response provide novel models to explore interactions between mesenchymal stem and endothelial cells. Despite the lack of a characteristic chromosomal translocation, the histomorphology, biomarkers and oncogenic changes were similar to those prevalent for Ewing's sarcomas. The phenotype and ontogenesis of hMSC-TERT20 tumors was consistent with the hypothesis that sarcomas may arise from hMSC, providing a unique diploid model for exploring human sarcoma biology.

Da, C., et al. (2017). "Effects of irradiation on radioresistance, HOTAIR and epithelial-mesenchymal transition/cancer stem cell marker expression in esophageal squamous cell carcinoma." Oncol Lett **13**(4): 2751-2757.

Radiotherapy is a common therapeutic strategy used to treat esophageal squamous cell carcinoma (ESCC). However, tumor cells often develop radioresistance, thereby reducing treatment efficacy. Here, we aimed to identify the mechanisms through which ESCC cells develop radioresistance and identify associated biomarkers. Eca109 cells were exposed to repeated radiation at 2 Gy/fraction for a total dose of 60 Gy (Eca109R60/2Gy cells). MTT and colony formation assays were performed to measure cell proliferation and compare the radiation biology parameters of Eca109 and Eca109R60/2Gy cells. Cell cycle distributions and apoptosis were assessed by flow cytometry. Reverse transcription-quantitative polymerase chain reaction and western blotting were employed to analyze the expression of HOX transcript antisense RNA (HOTAIR), in addition to biomarkers of the epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs). Eca109R60/2Gy cells exhibited increased cell proliferation and clone formation, with significantly higher radiobiological parameters compared with the parental Eca109 cells. The Eca109R60/2Gy cells also exhibited significantly decreased accumulation in G2 phase and increased accumulation in S phase. Additionally, the apoptosis rate was significantly lower in Eca109R60/2Gy cells than in parental Eca109 cells. Finally, HOTAIR expression levels and SNAI1 and beta-catenin mRNA and protein expression levels were significantly higher, whereas E-cadherin levels were significantly lower in Eca109R60/2Gy cells than in Eca109 cells. Therefore, our findings demonstrated that radioresistance was affected by the expression of HOTAIR and biomarkers of the EMT and CSCs.

Fang, N., et al. (2008). "[Separation of immortalized mesenchymal stem cell like stromal cells of mouse embryonic aorta-gonad-mesonephros region and their biological characteristics]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **16**(3): 584-588.

To investigate the effects of microenvironment of aorta-gonad-mesonephros (AGM) on embryonic hematopoiesis, mesenchymal stem cell like stromal cells (MSC like stromal cells) derived from dorsal aorta (DA) in AGM region were separated and identified by their growth characteristics, related molecules expression and mesenchymal lineage potentials. Stromal cells from DA region in mouse embryos (E11.5) were isolated and cultured in vitro. After transfected by pSV3neo-SV40, the clones with G418 resistance were selected, and their growth characteristics were studied. The related molecules were analyzed by flow cytometry, and each clone was induced to differentiate into adipocytes, osteocytes, and chondrocytes. The results showed that most clones (20 clones) selected in the mouse DA region held the morphology of fibroblastoid cells. mDAF3 and mDAF18 could be grown in culture for more than 50 passages with G418 resistance, both have the potential to differentiate into adipocytes, osteocytes, and chondrocytes. At the logarithmic growth period, the cell population doubling time is about 24 hours. Surface markers, such as CD29, CD44, CD105 and Sca-1 were positively detected, while low levels of CD34, CD45, and CD31 were detected. It is concluded that immortalized mDAF3 and mDAF18 have the specific phenotype and differential potency of MSC, which suggests that MSC maybe exist in mouse embryonic DA region, where the MSC like stromal cells can be used as a cell model for further research on the modulation activity of DA microenvironment for embryonic hematopoiesis.

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.

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

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

Hamidouche, Z., et al. (2017). "Bistable Epigenetic States Explain Age-Dependent Decline in Mesenchymal Stem Cell Heterogeneity." Stem Cells **35**(3): 694-704.

The molecular mechanisms by which heterogeneity, a major characteristic of stem cells, is achieved are yet unclear. We here study the expression of the membrane stem cell antigen-1 (Sca-1) in mouse bone marrow mesenchymal stem cell (MSC) clones. We show that subpopulations with varying Sca-1 expression profiles regenerate the Sca-1 profile of the mother population within a few days. However, after extensive replication in vitro, the expression profiles

shift to lower values and the regeneration time increases. Study of the promoter of Ly6a unravels that the expression level of Sca-1 is related to the promoter occupancy by the activating histone mark H3K4me3. We demonstrate that these findings can be consistently explained by a computational model that considers positive feedback between promoter H3K4me3 modification and gene transcription. This feedback implicates bistable epigenetic states which the cells occupy with an age-dependent frequency due to persistent histone (de-)modification. Our results provide evidence that MSC heterogeneity, and presumably that of other stem cells, is associated with bistable epigenetic states and suggest that MSCs are subject to permanent state fluctuations. Stem Cells 2017;35:694-704.

He, Q., et al. (2017). "Enhanced Hematopoietic Stem Cell Self-Renewal-Promoting Ability of Clonal Primary Mesenchymal Stromal/Stem cells Versus Their Osteogenic Progeny." Stem Cells **35**(2): 473-484.

Long-term self-renewing hematopoietic stem cell (LT-HSC) homeostasis within the bone marrow (BM) of adult mammals is regulated by complex interactions between LT-HSC and a number of niche-associated cell types including mesenchymal stromal/stem cells (MSC), osteoblasts (OB), macrophage, and neuronal cells in close proximity with the vasculature. Here, we cloned and functionally characterized a murine BM MSC subpopulation that was uniformly Nestin (+) Lepr (+) Sca-1(+) CD146(+) and could be stably propagated with high colony-forming unit fibroblast re-cloning efficiency. MSC synergized with SCF and IL-11 to support a 20-fold expansion in true LT-HSC after 10-days of in vitro coculture. Optimal stimulation of LT-HSC expansion was minimally dependent on Notch signaling but was significantly enhanced by global inhibition of Wnt signaling. The self-renewal-promoting activity of MSC was progressively lost when MSC clones were differentiated into mature OB. This suggests that the stage of osteoblast development may significantly impact the ability of osteolineage cells to support LT-HSC homeostasis in vivo. Stem Cells 2017;35:473-484.

Huss, R., et al. (2000). "Evidence of peripheral blood-derived, plastic-adherent CD34(-/low) hematopoietic stem cell clones with mesenchymal stem cell characteristics." Stem Cells **18**(4): 252-260.

The hematopoietic system of vertebrates can be completely reconstituted with hematopoietic stem cells derived from the bone marrow, fetal liver, or cord blood, or even from peripheral-blood-derived cells. A cellular marker to identify those cells is the proteoglycan CD34, although we have shown that the earliest identifiable hematopoietic stem cell is a CD34(-) fibroblast-like cell which can differentiate into CD34(+) hematopoietic precursors. Peripheral blood mononuclear cells were isolated from the heparinized blood of a dog and incubated in tissue culture in the presence of interleukin 6. After 10-14 days, an adherent layer of fibroblast-like cells had developed and cells were immortalized using the SV-40 large T antigen. Cells were cloned and subcloned by measures of limiting dilution, and various fibroblast-like clones were established. These fibroblast-like cells either do not express the CD34 antigen or express CD34 on a low level, although transcribing CD34. The CD34(-/low) cells express osteocalcin as a mesenchymal cell marker. The fibroblast-like cells eventually differentiate spontaneously in vitro into CD34(+) precursors and show colony formation. Prior to autologous stem cell transplantation, one clone of choice (IIIG7) was transfected with a retroviral construct containing the green-fluorescence protein (GFP). The recipient dog was totally irradiated with 300 cGy and received a stem cell transplant with GFP-containing, immortalized, fibroblast-like monoclonal autologous stem cells (0.5 x 10(8)/kg dog). No additional growth factors were applied. The peripheral blood counts recovered after 23 days (WBC >500; platelets >10,000). A peripheral blood smear showed some dim but definite, although timely, limited expression of the GFP protein in nucleated peripheral blood cells just five weeks after transplantation. A bone marrow biopsy showed GFP-positive cells in the marrow cavity predominantly as "bone-lining cells."

Kaback, L. A., et al. (2008). "Osterix/Sp7 regulates mesenchymal stem cell mediated endochondral ossification." J Cell Physiol **214**(1): 173-182.

We investigated the expression and regulation of the zinc finger protein Osterix (Osx) during endochondral ossification in mice. In studies to determine the temporal and spatial regulation of Osx mRNA and protein during embryogenesis we found it to be present throughout development, but its expression is restricted to the immature chondro/osteoprogenitor cells and mature osteoblasts, excluding hypertrophic chondrocytes. Using a fracture model, we show a consistent pattern of Osx protein expression in mesenchymal progenitor cells in the periosteum and immature chondrocytes and osteoblasts embedded in the fracture callus. In contrast, hypertrophic chondrocytes, vessels and fibrous tissue were devoid of Osx expression. Additionally, using RNA isolated from fracture callus throughout the healing process, we observe that Osx transcripts parallel that of Runx2 and differentially overlap both cartilage and bone phenotypic markers. Furthermore,

using limb bud-derived MLB13MYC Clone 17 cells, we show that PTHrP inhibited chondrocyte maturation while it enhanced mRNA levels of Osx in these chondro/osteoprogenitor cells. Gain and loss of function of Osx function experiments with these cells demonstrated that Osx serves as an inhibitor of chondrogenesis and chondrocyte maturation, while it promotes osteoblast maturation. Together, our findings provide the first demonstration of the molecular mechanisms underlying Osx inhibition of chondrocyte differentiation, and further suggest a role for this transcription factor in mediating endochondral ossification during bone repair.

Komine, A., et al. (2012). "Establishment of adipose-derived mesenchymal stem cell lines from a p53-knockout mouse." Biochem Biophys Res Commun **426**(4): 468-474.

Mesenchymal stem cells (MSCs) can differentiate into a variety of cell types. MSCs exist in several tissues such as the bone marrow, adipose, muscle, cartilage, and tendon. This differentiation potential makes MSCs candidates for cell-based therapeutic strategies for mesenchymal tissue injuries. MSCs can be prepared from bone marrow (BM-MSCs) and adipose (AD-MSCs); however, these MSCs exhibit senescence-associated growth arrest and display inevitable heterogeneity. We established several AD-MSC cell lines from a p53-knockout (KO) mouse. These cell lines were immortalized, but no cell lines grew anchorage-independently, suggesting that they are not cancerous. They differentiated into adipocytes, osteoblasts, and chondrocytes by treatment with certain stimuli. Moreover, following injection into the tail vein, the cells migrated into the wounded region of the liver and differentiated into hepatocytes. We succeeded in establishing several AD-MSC clonal cell lines that maintain the tissue-specific markers and characteristics of the developmental phase. These clonal cell lines will serve as important tools to study the mechanism of differentiation of MSCs.

Lai, R. C., et al. (2013). "Exosomes for drug delivery - a novel application for the mesenchymal stem cell." Biotechnol Adv **31**(5): 543-551.

Exosomes are the most extensively characterized class of secreted membrane vesicles that carry proteins and RNAs for intercellular communication. They are increasingly seen as possible alternatives to liposomes as drug delivery vehicles. Like liposomes, they could deliver their cargo across the plasma membrane and provide a barrier against premature transformation and elimination. In addition, these naturally-occurring secreted membrane vesicles are less toxic and better tolerated in the body as evidenced by their ubiquitous presence in biological fluids, and have an intrinsic homing ability. They are also amenable to in vivo and in vitro loading of therapeutic agents, and membrane modifications to enhance tissue-specific homing. Here we propose human mesenchymal stem cells as the ideal cell source of exosomes for drug delivery. Mesenchymal stem cell transplantation for various disease indications has been extensively tested and shown to be safe in numerous clinical trials. These cells are also prolific producers of immunologically inert exosomes. Immortalization of these cells does not compromise the quantity or quality of exosome production, thus enabling infinite and reproducible exosome production from a single cell clone.

Lan, L., et al. (2013). "[Epithelial-mesenchymal transition induces cancer stem cell generation in human thyroid cancer cells in vitro]." Zhonghua Yi Xue Za Zhi **93**(16): 1261-1265.

OBJECTIVE: Epithelial-mesenchymal transition (EMT) has been implicated in the initiation and conversion of early stage tumors into invasive malignancies and is associated with the "stemness" of cancer cells. The present study was designed to identify whether EMT induces cancer stem cell generation and tumor progression in human thyroid cancer cells in vitro. METHODS: FTC133 cells, as EMT-negative cells, were used for EMT induction by hypoxia-inducible factor-1alpha (HIF-1alpha) transfection. And EMT features were then examined by Western blot, immunofluorescent staining, invasion and proliferation assays. Moreover, stem-like side population (SP) cells were sorted with flow cytometry from FTC133 cells before and after EMT. The proportion of SP was compared and stemness, self-renewal and tumorigenicity in vitro were identified in SP cells. RESULTS: Overexpression of HIF-1alpha induced FTC133 cells to undergo EMT. And it down-regulated epithelial marker E-cadherin, up-regulated mesenchymal marker vimentin and caused nucleus translocation of beta-catenin and highly invasive and metastatic properties. Most importantly, the induction of EMT promoted proportion of stem-like side population cells (0.70% vs 0.03%, $P < 0.05$) with higher sphere formation and clone forming capability in contrast to non-side population cells. CONCLUSIONS: EMT can induce cancer stem cell generation and tumor progression in thyroid cancers. Further understanding the role of EMT and cancer stem cells in cancer progression may reveal new preventive and therapeutic targets for thyroid cancers.

Letchford, J., et al. (2006). "Isolation of C15: a novel antibody generated by phage display against mesenchymal stem cell-enriched fractions of adult human marrow." J Immunol Methods **308**(1-2): 124-137.

Adult bone marrow stroma contains a source of mesenchymal stem cells (MSC) that have the capacity to self-renew and differentiate into multiple stromal lineages. These rare cells can be visualised indirectly by the formation of heterogeneous colonies, containing stem cells and their differentiated progeny in long-term culture. If MSC and their associated progenitor and precursor populations are to reach their full therapeutic potential, markers will be required to identify and characterize specific bone marrow stromal subsets. We sought to use phage display to generate antibodies against bone marrow mononuclear cells (BMMNC) enriched for colony forming cells. Initially, we identified our target cell population by comparing the colony forming efficiency (CFE) of CD49a-positive, STRO-1-positive and CD45-negative BMMNC subpopulations with unseparated BMMNC. Selection with anti-CD49a gave the greatest enrichment (19-fold) of colony forming cells and in light of these findings, we generated phage antibodies against CD49a-positive BMMNC by simultaneous positive/negative selection. A dominant clone (C15), generated after 3 rounds of selection, has been isolated and sequenced, then characterized for cell and tissue specificity. Sequence analysis showed that the V (H) and V (L) gene segments of C15 aligned most closely to the VH26/DP-47 and IGLV3S1/DPL16 germline V segments found in the synthetic repertoire. C15 bound to 4% of freshly isolated BMMNC and localized to osteoblastic cells and proximal marrow cells in areas of active bone formation in sections of osteophyte. C15 binding was upregulated in cultured bone marrow stromal cells (BMSC) and was also detected on bone-derived cell lines. This report demonstrates that phage display is a powerful tool for the isolation of antibodies against rare cell populations, and provides a platform for the future application of this technology in the search for antigens on MSC and other rare cell populations.

Li, Y., et al. (2006). "[Screening of differentially expressed genes in rats with cardiomyopathy after bone marrow mesenchymal stem cell transplantation]." Zhonghua Er Ke Za Zhi **44**(10): 787-791.

OBJECTIVE: Earlier studies have confirmed that mesenchymal stem cells (MSCs) can transdifferentiate into myocytes and improve heart function in 2 weeks. But the mechanism is not clear. In this study, the mechanism of improvement of heart function after transplantation of MSCs was examined with suppression subtractive hybridization (SSH). METHODS: MSCs were isolated from thighone and tibia of Wistar rats, purified by adhesion-screening method, and expanded in vitro. Intraperitoneal injection of doxorubicin (at 2.5 mg/kg/time and total doses of 15 mg/kg) established cardiomyopathy models. MSCs were transplanted into cardiomyocytes. The differential genes between tester (rats with cardiomyopathy that were injected with MSCs) and driver (rats with cardiomyopathy that were injected with equivalent volume of culture medium) were screened with suppression subtractive hybridization. RESULTS: After 4 weeks of intraperitoneal injection of doxorubicin, left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) decreased by 26.48% and 40.61%, respectively ($P < 0.01$), as compared with those of normal group. Cardiomyopathy model was established successfully. And the heart function of the rats with cardiomyopathy was significantly improved after transplantation. Sixteen gene fragments were detected, and 12 of them were up-regulated in testers. They were rattus norvegicus mitochondrial BN/SsNHsdMCW, rattus norvegicus strain mitochondrial F344 X BN F1, rattus norvegicus mitochondrion H (+)-ATP synthase alphase subunit (Atp5al) mRNA, rattus norvegicus BHE/Cdb tRNA-Lys gene, rat mitochondrial H (+)-ATP synthase alpha subunit mRNA, rattus norvegic (wild-caught animal) complete mitochondrial genome, rattus norvegic clone BB.1.4.1 unknown Glu-Pro dipeptide repeat protein mRNA, Arabidopsis thaliana transgenic line C DNA, rat mitochondrial ATP synthase beta subunit mRNA, rattus norvegic mitochondrial genome, rat cardiac troponin T mRNA and rat mRNA for beta-globin. Four gene fragments were down-regulated in testers. They were rat mRNA for sarcomeric mitochondrial creatine kinase, rat mRNA for ribosomal phosphoprotein P2, rat alpha-crystallin B chain mRNA and rattus norvegicus NADH-ubiquinone oxidoreductase Fe-S protein 7 mRNA. CONCLUSION: The expression of the genes relating to mitochondrial synthesizing and contracting proteins synthesizing increased after MSC transplantation. The genes might enhance energy synthesis and promote MSC transdifferentiate into myocytes, and then improve heart function of rats with cardiomyopathy.

Li, Z., et al. (2013). "Bone marrow mesenchymal stem cells are an attractive donor cell type for production of cloned pigs as well as genetically modified cloned pigs by somatic cell nuclear transfer." Cell Reprogram **15**(5): 459-470.

The somatic cell nuclear transfer (SCNT) technique has been widely applied to clone pigs or to produce genetically modified pigs. Currently, this technique relies mainly on using terminally differentiated fibroblasts as donor cells. To improve cloning efficiency, only partially differentiated multipotent mesenchymal stem cells (MSCs), thought to be more easily reprogrammed to a pluripotent state, have been used as nuclear donors in pig SCNT.

Although in vitro-cultured embryos cloned from porcine MSCs (MSCs-embryos) were shown to have higher preimplantation developmental ability than cloned embryos reconstructed from fibroblasts (Fs-embryos), the difference in in vivo full-term developmental rate between porcine MSCs-embryos and Fs-embryos has not been investigated so far. In this study, we demonstrated that blastocyst total cell number and full-term survival abilities of MSCs-embryos were significantly higher than those of Fs-embryos cloned from the same donor pig. The enhanced developmental potential of MSCs-embryos may be associated with their nuclear donors' DNA methylation profile, because we found that the methylation level of imprinting genes and repeat sequences differed between MSCs and fibroblasts. In addition, we showed that use of transgenic porcine MSCs generated from transgene plasmid transfection as donor cells for SCNT can produce live transgenic cloned pigs. These results strongly suggest that porcine bone marrow MSCs are a desirable donor cell type for production of cloned pigs and genetically modified cloned pigs via SCNT.

Lin, J. R., et al. (2003). "In vitro culture of human bone marrow mesenchymal stem cell clonies and induced differentiation into neuron-like cells." Di Yi Jun Yi Da Xue Xue Bao **23**(3): 251-253, 264.

OBJECTIVE: To study the long-term in vitro culture of human bone marrow mesenchymal stem cells (hMSC) and their phenotypical and functional properties. METHODS: Adherent hMSC colonies were digested by 0.25% trypsin-EDTA with a clone cycle for in vitro subculture. Flow cytometry was employed to examine the phenotypes of the cells. Their committed differentiation potential to neurons, clone-forming ability and growth curves were all investigated. RESULTS: hMSCs could be subcultured under this culture condition for 20 passages, expressing CD13, CD29 and CD59 but not CD11, CD14, CD31, CD34, CD45, CD80, CD86, CD117 and HLA-DR. The cells could be induced to differentiate into neurons when subcultured for 17 passages. CONCLUSION: hMSCs can be efficiently expanded under this culture condition, and the colony-derived hMSCs can maintain the differentiation potentials and retain their biological characteristics.

Mareddy, S., et al. (2010). "Stem cell-related gene expression in clonal populations of mesenchymal stromal cells from bone marrow." Tissue Eng Part A **16**(2): 749-758.

Decline in the frequency of potent mesenchymal stem cells (MSCs) has been implicated in ageing and degenerative diseases. Increasing the circulating stem cell population can lead to renewed recruitment of these potent cells at sites of damage. Therefore, identifying the ideal cells for ex vivo expansion will form a major pursuit of clinical applications. This study is a follow-up of previous work that demonstrated the occurrence of fast-growing multipotential cells from the bone marrow samples. To investigate the molecular processes involved in the existence of such varying populations, gene expression studies were performed between fast- and slow-growing clonal populations to identify potential genetic markers associated with stemness using the quantitative real-time polymerase chain reaction comprising a series of 84 genes related to stem cell pathways. A group of 10 genes were commonly overrepresented in the fast-growing stem cell clones. These included genes that encode proteins involved in the maintenance of embryonic and neural stem cell renewal (sex-determining region Y-box 2, notch homolog 1, and delta-like 3), proteins associated with chondrogenesis (aggrecan and collagen 2 A1), growth factors (bone morphogenetic protein 2 and insulin-like growth factor 1), an endodermal organogenesis protein (forkhead box a2), and proteins associated with cell-fate specification (fibroblast growth factor 2 and cell division cycle 2). Expression of diverse differentiation genes in MSC clones suggests that these commonly expressed genes may confer the maintenance of multipotentiality and self-renewal of MSCs.

Marini, I., et al. (2017). "Antitumor Activity of a Mesenchymal Stem Cell Line Stably Secreting a Tumor-Targeted TNF-Related Apoptosis-Inducing Ligand Fusion Protein." Front Immunol **8**: 536.

Mesenchymal stem cells (MSCs) are currently exploited as gene delivery systems for transient in situ expression of cancer therapeutics. As an alternative to the prevailing viral expression, we here describe a murine MSC line stably expressing a therapeutic protein for up to 42 passages, yet fully maintaining MSC features. Because of superior antitumoral activity of hexavalent TNF-related apoptosis-inducing ligand (TRAIL) formats and the advantage of a tumor-targeted action, we choose expression of a dimeric EGFR-specific diabody single-chain TRAIL (Db-scTRAIL) as a model. The bioactivity of Db-scTRAIL produced from an isolated clone (MSC.TRAIL) was revealed from cell death induction in Colo205 cells treated with either culture supernatants from or cocultured with MSC.TRAIL. In vivo, therapeutic activity of MSC.TRAIL was shown upon peritumoral injection in a Colo205 xenograft tumor model. Best antitumor activity in vitro and in vivo was observed upon combined treatment of MSC.TRAIL with bortezomib. Importantly, in vivo combination treatment did not cause apparent hepatotoxicity, weight loss, or behavioral changes. The development

of well characterized stocks of stable drug-producing human MSC lines has the potential to establish standardized protocols of cell-based therapy broadly applicable in cancer treatment.

Martin, J., et al. (2008). "Adult lung side population cells have mesenchymal stem cell potential." Cytotherapy **10**(2): 140-151.

BACKGROUND: The development of stem cell therapy for pulmonary diseases remains a challenge. Many diverse cell types reside within the lung and a common stem cell has not yet been identified. A basic understanding of lung stem cell fate during disease may prove important for drug intervention as well as autologous therapies. Niches for resident mesenchymal stem cells (MSC) have been identified in many adult tissues and more recently in the lung. We present data to confirm the observation that non-hematopoietic CD45(neg) lung side population (SP) cells contain MSC, single cells capable of multilineage differentiation. METHODS We carried these observations forward by analyzing the MSC potential of single-cell clones, as well as their chromosomal stability and telomerase activity. RESULTS: The expression of MSC markers was characterized in mouse CD45(neg) lung SP by flow cytometry on freshly isolated or cultured clonal populations. The karyotype of these cells was subsequently assayed by banding analysis, and telomerase activity was assessed using quantitative polymerase chain reaction. MSC differentiation potential was confirmed by the characteristic ability of single-cell clones to differentiate into cells of three mesenchymal lineages, chondrocytes, adipocytes and osteocytes. Differentiation was confirmed by histochemical analysis. All analyzed populations of CD45(neg) lung SP expressed mesenchymal markers (CD44, CD90, CD105, CD106, CD73 and Sca-I) and lacked hematopoietic markers (CD45, c-kit, CD11b, CD34 and CD14). The cultured and clonal CD45(neg) lung SP had normal chromosomal structures and expressed high levels of telomerase. After being expanded and cultured in differentiation medium, all populations of CD45(neg) lung SP demonstrated adipogenic, osteogenic and chrondrogenic potential. Adult CD45(neg) lung SP cells are a source of MSC. DISCUSSION: In defining this tissue-specific stem cell population in the lung, we are now better able to clarify a potential role for them in lung diseases.

Masaka, T., et al. (2008). "Derivation of hepato-pancreatic intermediate progenitor cells from a clonal mesenchymal stem cell line of rat bone marrow origin." Int J Mol Med **22**(4): 447-452.

We have recently established a clonal mesenchymal stem cell line (rBM25/S3) from adult rat bone marrow. The cells have practically unlimited proliferation capacity (over 300 PDL), maintaining multipotency for differentiation. In the present study, we examined the potential for rBM25/S3 cells to differentiate into insulin-secreting cells. When cultured in the presence of HGF and FGF-4 on Matrigel, rBM25/S3 cells expressed genes specific to pancreatic beta-cells as well as those specific to hepatocytes. They still maintained proliferation capacity with a doubling time of approximately 30 h. These hepato-pancreatic intermediate progenitor cells, but not the original undifferentiated rBM25/S3 cells, were induced by the overexpression of PDX-1 to produce significant amounts of insulin in a manner responding to glucose concentration in medium. The present culture system indicates a direction for further studies aimed at the realization of cell transplantation therapy for type I diabetes mellitus.

Mihaly, D., et al. (2017). "First cloned human immortalized adipose derived mesenchymal stem-cell line with chimeric SS18-SSX1 gene (SS-iASC)." Cancer Genet **216-217**: 52-60.

The SS18-SSX chimeric gene is unique to synovial sarcoma. Multiple model systems including mouse cell lines expressing SS18-SSX, and genetically engineered mouse models of synovial sarcoma have been developed to elucidate the role of the chimeric gene in synovial sarcomagenesis. Although several cell lines stably expressing human SS18-SSX exist, there is an ongoing need for cell culture models enabling researchers to investigate the molecular mechanism of SS18-SSX action in a relevant cellular context. Here we report the establishment of a novel SS18-SSX1-expressing cell line created from immortalized human adipose tissue-derived mesenchymal stem cells via lentiviral transduction of the chimeric gene. Our cell line, termed SS-iASC, has been characterized by karyotyping and cell line identification, and stable expression of SS18-SSX1 has been verified using real-time PCR (RT-PCR), nested PCR, immunofluorescence, and immunoblotting. Focal cytokeratin positivity characteristic of synovial sarcoma but no beta-Catenin, Bcl-2 or cyclin D1 expression was observed in SS-iASC. The novel cell line expressing SS18-SSX1 on a human adipose-derived stromal cell background is expected to be helpful in addressing the question whether the chimeric gene alone is sufficient to trigger the formation of synovial sarcoma.

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

BACKGROUND: Induced pluripotent stem (iPS) cells are generated from mouse and human somatic

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

Osathanon, T., et al. (2012). "Notch signalling inhibits the adipogenic differentiation of single-cell-derived mesenchymal stem cell clones isolated from human adipose tissue." Cell Biol Int **36**(12): 1161-1170.

ADSCs (adipose-derived mesenchymal stem cells) are candidate adult stem cells for regenerative medicine. Notch signalling participates in the differentiation of a heterogeneous ADSC population. We have isolated, human adipose tissue-derived single-cell clones using a cloning ring technique and characterized for their stem cell characteristics. The role of Notch signalling in the differentiation capacity of these adipose-derived single-cell-clones has also been investigated. All 14 clones expressed embryonic and mesenchymal stem cell marker genes. These clones could differentiate into both osteogenic and adipogenic lineages. However, the differentiation potential of each clone was different. Low adipogenic clones had significantly higher mRNA expression levels of Notch 2, 3 and 4, Jagged1, as well as Delta1, compared with those of high adipogenic clones. In contrast, no changes in expression of Notch signalling component mRNA between low and high osteogenic clones was found. Notch receptor mRNA expression decreased with the adipogenic differentiation of both low and high adipogenic clones. The gamma-secretase inhibitor, DAPT (N-[N-(3,5-difluorophenacetyl)-l-alanyl]-(S)-phenylglycine t-butyl ester), enhanced adipogenic differentiation. Correspondingly, cells seeded on a Notch ligand (Jagged1) bound surface showed lower intracellular lipid accumulation. These results were noted in both low and high adipogenic clones, indicating that Notch signalling inhibited the adipogenic differentiation of adipose ADSC clones, and could be used to identify an adipogenic susceptible subpopulation for soft-tissue augmentation application.

Peng, H. H., et al. (2012). "DNA methylation patterns of imprinting centers for H19, SNRPN, and KCNQ1OT1 in single-cell clones of human amniotic fluid mesenchymal stem cell." Taiwan J Obstet Gynecol **51**(3): 342-349.

OBJECTIVE: To test the hypothesis that human amniotic fluid mesenchymal stem cells contain a unique epigenetic signature in imprinting centers of H19, SNRPN, and KCNQ1OT1 during in vitro cell culture. MATERIALS AND METHODS: By bisulfite genomic sequencing, we analyzed the imprinting centers of three imprinted genes (including H19, SNRPN, and KCNQ1OT/) in a total of six single-cell clones of human amniotic fluid mesenchymal stem cells at cell passages 7, 8, 9, and 10 during in vitro cell culture. RESULTS: The imprinting centers of H19 and KCNQ1OT1 showed hypermethylation at passage 7 in all single-cell clones of human amniotic fluid mesenchymal stem cells, and there was no significant change in DNA methylation patterns during in vitro cell culture. The imprinting centers of SNRPN showed variable methylation patterns at passage 7 in six single-cell clones, and DNA methylation patterns varied during in vitro cell culture from passages 8 to 10. CONCLUSION: In conclusion, human amniotic fluid mesenchymal stem cells contain a unique epigenetic signature during in vitro cell culture. H19 and KCNQ1OT1 possessed a substantial degree of hypermethylation status, and variable DNA methylation patterns of SNRPN was observed during in vitro cell culture of human amniotic fluid mesenchymal stem cells. Our results urge further understanding of epigenetic status of human amniotic fluid mesenchymal stem cells before it is applied in cell replacement therapy.

Pontikoglou, C., et al. (2013). "Study of the quantitative, functional, cytogenetic, and immunoregulatory properties of bone marrow mesenchymal stem cells in patients with B-cell chronic lymphocytic leukemia." Stem Cells Dev **22**(9): 1329-1341.

The bone marrow (BM) microenvironment has clearly been implicated in the pathogenesis of B-cell chronic lymphocytic leukemia (B-CLL). However, the potential involvement of BM stromal progenitors, the mesenchymal stem cells (MSCs), in the

pathophysiology of the disease has not been extensively investigated. We expanded in vitro BM-MSCs from B-CLL patients (n=11) and healthy individuals (n=16) and comparatively assessed their reserves, proliferative potential, differentiation capacity, and immunoregulatory effects on T- and B-cells. We also evaluated the anti-apoptotic effect of patient-derived MSCs on leukemic cells and studied their cytogenetic characteristics in comparison to BM hematopoietic cells. B-CLL-derived BM MSCs exhibit a similar phenotype, differentiation potential, and ability to suppress T-cell proliferative responses as compared with MSCs from normal controls. Furthermore, they do not carry the cytogenetic abnormalities of the leukemic clone, and they exert a similar anti-apoptotic effect on leukemic cells and healthy donor-derived B-cells, as their normal counterparts. On the other hand, MSCs from B-CLL patients significantly promote normal B-cell proliferation and IgG production, in contrast to healthy-donor-derived MSCs. Furthermore, they have impaired reserves, defective cellular growth due to increased apoptotic cell death and exhibit aberrant production of stromal cell-derived factor 1, B-cell activating factor, a proliferation inducing ligand, and transforming growth factor beta1, cytokines that are crucial for the survival/nourishing of the leukemic cells. We conclude that ex vivo expanded B-CLL-derived MSCs harbor intrinsic qualitative and quantitative abnormalities that may be implicated in disease development and/or progression.

Qi, Z. M., et al. (2008). "[Differentiation of human telomerase reverse transcriptase immortalized human marrow mesenchymal stem cell into chondrocyte]." Zhonghua Wai Ke Za Zhi **46**(9): 697-699.

OBJECTIVE: To establish an immortalized marrow mesenchymal stem cell line to facilitate advances in cartilage engineering research. METHODS: Human telomerase reverse transcriptase (hTERT) cDNA was transferred into primary human marrow mesenchymal stem cells (hMSC) by retroviral vector pLEGFP-C1-hTERT. Subsequently G418 resistant cell clone was screened and expanded for further studies. hMSC biomarkers and hTERT expression were confirmed by examination. Transfected hMSC was induced to differentiate into chondrocyte using TGF-P1 and dexamethasone. RESULTS: Up-regulated hTERT expression was detected in transfected hMSC. hMSC-hTERT cells could be induced to differentiate into chondrocyte. Higher telomerase activity in transfected cells was maintained for 50 population doublings so far. Collagen II could be detected in induced transfected hMSC by immunocytochemical and hybridization in situ. CONCLUSIONS: Ectopic expression of hTERT can effectively immortalize hMSC in vitro. Immortalized hMSC can be induced to differentiate into chondrocyte under certain condition. It may be an ideal target of further studies in cartilage engineering.

Quang, T., et al. (2014). "Dosage and cell line dependent inhibitory effect of bFGF supplement in human pluripotent stem cell culture on inactivated human mesenchymal stem cells." PLoS One **9**(1): e86031.

Many different culture systems have been developed for expanding human pluripotent stem cells (hESCs and hiPSCs). In general, 4-10 ng/ml of bFGF is supplemented in culture media in feeder-dependent systems regardless of feeder cell types, whereas in feeder-free systems, up to 100 ng/ml of bFGF is required for maintaining long-term culture on various substrates. The amount of bFGF required in native hESCs growth niche is unclear. Here we report using inactivated adipose-derived human mesenchymal stem cells as feeder cells to examine long-term parallel cultures of two hESCs lines (H1 and H9) and one hiPSCs line (DF19-9-7T) in media supplemented with 0, 0.4 or 4 ng/ml of bFGF for up to 23 passages, as well as parallel cultures of H9 and DF19 in media supplemented with 4, 20 or 100 ng/ml bFGF for up to 13 passages for comparison. Across all cell lines tested, bFGF supplement demonstrated inhibitory effect over growth expansion, single cell colonization and recovery from freezing in a dosage dependent manner. In addition, bFGF exerted differential effects on different cell lines, inducing H1 and DF19 differentiation at 4 ng/ml or higher, while permitting long-term culture of H9 at the same concentrations with no apparent dosage effect. Pluripotency was confirmed for all cell lines cultured in 0, 0.4 or 4 ng/ml bFGF excluding H1-4 ng, as well as H9 cultured in 4, 20 and 100 ng/ml bFGF. However, DF19 demonstrated similar karyotypic abnormality in both 0 and 4 ng/ml bFGF media while H1 and H9 were karyotypically normal in 0 ng/ml bFGF after long-term culture. Our results indicate that exogenous bFGF exerts dosage and cell line dependent effect on human pluripotent stem cells cultured on mesenchymal stem cells, and implies optimal use of bFGF in hESCs/hiPSCs culture should be based on specific cell line and its culture system.

Saadi, G., et al. (2013). "Mesenchymal stem cell transfusion for desensitization of positive lymphocyte cross-match before kidney transplantation: outcome of 3 cases." Cell Prolif **46**(2): 121-126.

OBJECTIVES: Donor specific antibodies (DSA) and a positive cross-match are contraindications for kidney transplantation. Trials of allograft

transplantation across the HLA barrier have employed desensitization strategies, including the use of plasmapheresis, intravenous immunoglobulins, anti-B-cell monoclonal antibodies and splenectomy, associated with high-intensity immunosuppressive regimens. Our case 1 report suffered from repeatedly positive lymphocyte cross match after 1st renal transplantation. Graft nephrectomy could not correct the state of sensitization. Splenectomy was done in a trial to get rid of the antibody producing clone. Furthermore plasmapheresis with low dose IVIG could not as well revert the state of sensitization for the patient. MATERIAL AND METHODS: About 50 millions donor specific MSCs were injected to the patient. RESULTS: MSCs transfusion proved to be the only procedure which could achieve successful desensitization before performing the second transplantation owing to their immunosuppressive properties. CONCLUSION: This case indicates that DS-MSCs is a potential option for anti-HLA desensitization. In cases 2 and 3 IV DS-MSCs transfusion was selected from the start as a successful line of treatment for pre renal transplantation desensitization to save other unnecessary lines of treatment that were tried in case 1.

Salingcarnboriboon, R., et al. (2003). "Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property." Exp Cell Res **287**(2): 289-300.

Development of the musculoskeletal system requires coordinated formation of distinct types of tissues, including bone, cartilage, muscle, and tendon. Compared to muscle, cartilage, and bone, cellular and molecular bases of tendon development have not been well understood due to the lack of tendon cell lines. The purpose of this study was to establish and characterize tendon cell lines. Three clonal tendon cell lines (TT-E4, TT-G11, and TT-D6) were established using transgenic mice harboring a temperature-sensitive mutant of SV40 large T antigen. Proliferation of these cells was significantly enhanced by treatment with bFGF and TGF-beta but not BMP2. Tendon phenotype-related genes such as those encoding scleraxis, Six1, EphA4, COMP, and type I collagen were expressed in these tendon cell clones. In addition to tendon phenotype-related genes, expression of osteopontin and Cbfal was observed. These clonal cell lines formed hard fibrous connective tissue when implanted onto chorioallantoic membrane in ovo. Furthermore, these cells also formed tendon-like tissues when they were implanted into defects made in patella tendon in mice. As these tendon cell lines also produced fibrocartilaginous tissues in tendon defect implantation experiments, mesenchymal stem cell properties were examined. Interestingly, these cells expressed genes related to osteogenic, chondrogenic, and adipogenic lineages at low levels when examined by RT-PCR. TT-G11 and TT-E4 cells differentiated into either osteoblasts or adipocytes, respectively, when they were cultured in cognate differentiation medium. These observations indicated that the established tendon cell line possesses mesenchymal stem cell-like properties, suggesting the existence of mesenchymal stem cell in tendon tissue.

Schwarz, S., et al. (2014). "Bone marrow-derived mesenchymal stem cells migrate to healthy and damaged salivary glands following stem cell infusion." Int J Oral Sci **6**(3): 154-161.

Xerostomia is a severe side effect of radiation therapy in head and neck cancer patients. To date, no satisfactory treatment option has been established. Because mesenchymal stem cells (MSCs) have been identified as a potential treatment modality, we aimed to evaluate stem cell distribution following intravenous and intraglandular injections using a surgical model of salivary gland damage and to analyse the effects of MSC injections on the recruitment of immune cells. The submandibular gland ducts of rats were surgically ligated. Syngeneic adult MSCs were isolated, immortalised by simian virus 40 (SV40) large T antigen and characterized by flow cytometry. MSCs were injected intravenously and intraglandularly. After 1, 3 and 7 days, the organs of interest were analysed for stem cell recruitment. Inflammation was analysed by immunohistochemical staining. We were able to demonstrate that, after intravenous injection, MSCs were recruited to normal and damaged submandibular glands on days 1, 3 and 7. Unexpectedly, stem cells were recruited to ligated and non-ligated glands in a comparable manner. After intraglandular injection of MSCs into ligated glands, the presence of MSCs, leucocytes and macrophages was enhanced, compared to intravenous injection of stem cells. Our data suggest that injected MSCs were retained within the inflamed glands, could become activated and subsequently recruited leucocytes to the sites of tissue damage.

Selich, A., et al. (2016). "Massive Clonal Selection and Transiently Contributing Clones During Expansion of Mesenchymal Stem Cell Cultures Revealed by Lentiviral RGB-Barcode Technology." Stem Cells Transl Med **5**(5): 591-601.

UNLABELLED: Mesenchymal stem (or stromal) cells (MSCs) have been used in more than 400 clinical trials for the treatment of various diseases. The clinical benefit and reproducibility of results, however, remain extremely variable. During the in vitro expansion phase, which is necessary to achieve clinically relevant cell numbers, MSCs show signs of aging accompanied

by different contributions of single clones to the mass culture. Here we used multicolor lentiviral barcode labeling to follow the clonal dynamics during in vitro MSC expansion from whole umbilical cord pieces (UCPs). The clonal composition was analyzed by a combination of flow cytometry, fluorescence microscopy, and deep sequencing. Starting with highly complex cell populations, we observed a massive reduction in diversity, transiently dominating populations, and a selection of single clones over time. Importantly, the first wave of clonal constriction already occurred in the early passages during MSC expansion. Consecutive MSC cultures from the same UCP implied the existence of more primitive, MSC culture-initiating cells. Our results show that microscopically homogenous MSC mass cultures consist of many subpopulations, which undergo clonal selection and have different capabilities. Among other factors, the clonal composition of the graft might have an impact on the functional properties of MSCs in experimental and clinical settings. SIGNIFICANCE: Mesenchymal stem cells (MSCs) can easily be obtained from various adult or embryonal tissues and are frequently used in clinical trials. For their clinical application, MSCs have to be expanded in vitro. This unavoidable step influences the features of MSCs, so that clinical benefit and experimental results are often highly variable. Despite a homogenous appearance under the microscope, MSC cultures undergo massive clonal selection over time. Multicolor fluorescence labeling and deep sequencing were used to demonstrate the dynamic clonal composition of MSC cultures, which might ultimately explain the variable clinical performance of the cells.

Somoza, R., et al. (2008). "Neuropotency of human mesenchymal stem cell cultures: clonal studies reveal the contribution of cell plasticity and cell contamination." Biol Blood Marrow Transplant **14**(5): 546-555.

Various studies have shown neuropotency of bone marrow-derived human mesenchymal stem cells (hMSC) based on the appearance of cells with neural phenotype before or after neural induction protocols. However, to date, it is unclear which mechanisms account for this observation. We hypothesized that neural phenotypes observed in hMSC cultures can be because of both intrinsic cell plasticity and contamination by cells of neural origin. Therefore, we characterized 38 clones from hMSC cultures by assessing their adipogenic/osteogenic potential with specific mesenchymal differentiation protocols, and their molecular neural phenotype by RT-PCR analysis before and after exposure to a defined neural stem cell (NSC) medium for 8 days (neural protocol). We found 33 clones with mesenchymal potential and 15 of them also showed a neural phenotype. As neural phenotypes were maintained during the neural protocol, this suggested neural cell plasticity in 39% of all clones through pluripotency. Importantly, we were able to induce neural phenotypes in 11 of mesenchymal clones applying the neural protocol, demonstrating neural cell plasticity in 29% of all clones through the mechanism of transdifferentiation. Finally, 2 of 5 nonmesenchymal clones (5% of all clones) displayed a neural phenotype indicating neural cell contamination of hMSC cultures. In conclusion, we found 2 different ways of neuropotency of hMSC cultures: cell plasticity and cell contamination.

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.

Szabo, E., et al. (2015). "Licensing by Inflammatory Cytokines Abolishes Heterogeneity of Immunosuppressive Function of Mesenchymal Stem Cell Population." Stem Cells Dev **24**(18): 2171-2180.

When mesenchymal stem cells (MSCs) are used for therapy of immunological pathologies, they get into an inflammatory environment, altering the effectiveness of the treatment. To establish the impact of environmental inflammatory factors on MSCs'

immunofunction in the mirror of intrinsic heterogeneity of mouse MSC population, individual MSC clones were generated and characterized. Adipogenic but not osteogenic differentiation and pro-angiogenic activity of five independent MSC cell lines were similar. Regarding osteogenic differentiation, clones MSC3 and MSC6 exhibited poorer capacity than MSC2, MSC4, and MSC5. To study the immunosuppressive heterogeneity, in vitro and in vivo experiments have been carried out using T-cell proliferation assay and delayed-type hypersensitivity (DTH) response, respectively. A remarkable difference was found between the clones in their ability to inhibit T-cell proliferation in the following order: MSC2>/=MSC5>MSC4>MSC3 >> MSC6. Nevertheless, the differences between the immunosuppressive activities of the individual clones disappeared on pretreatment of the cells with pro-inflammatory cytokines, a procedure called licensing. Stimulation of all clones with IFN-gamma and TNF-alpha resulted in elevation of their inhibitory capability to a similar level. Nitric oxide (NO) and prostaglandin E2 (PGE2) were identified as major mediators of immunofunction of the MSC clones. The earlier findings were also supported by in vivo results. Without licensing, MSC2 inhibited DTH response, while MSC6 did not affect DTH response. In contrast, prestimulation of MSC6 with inflammatory cytokines resulted in strong suppression by this clone as well. Here, we have showed that MSC population is functionally heterogeneous in terms of immunosuppressive function; however, this variability is largely reduced under pro-inflammatory conditions.

Tan, Y. F., et al. (2010). "[Stem cell factor secretion by bone mesenchymal stem cells stimulated with astragaloside IV]." Zhongguo Dang Dai Er Ke Za Zhi **12**(4): 290-292.

OBJECTIVE: To study the effect of astragaloside IV on the expression of cytokines in bone mesenchymal stem cells (MSCs) in rats. METHODS: MSCs were isolated from Wistar rats by the method of adhesive cultiration and clone, and then their biological activities were assessed using indirect immunofluorescence. Proliferation of MSCs stimulated with astragaloside IV was ascertained by the MTT method. Expression of cytokines was ascertained using RT-PCR in MSCs with astragaloside IV stimulation or not. RESULTS: MSCs were effectively isolated and purified in vitro, and had expression of many cytokines except IL-3, such as stem cell factor (SCF), thrombopoietin (TPO), granulocyte macrophage colony stimulating factor (GM-CSF) and transforming growth factor (TGF-beta1). Astragaloside IV stimulation promoted MSCs proliferation, and 200 mg/mL astragaloside IV treatment produced a peak effect 72 hrs after culture. The SCF expression in MSCs stimulated with astragaloside IV increased significantly compared with that in MSCs without astragaloside IV stimulation. CONCLUSIONS: Astragaloside IV may promote MSCs proliferation and increase SCF secretion in vitro.

Varga, G., et al. (2007). "Inappropriate Notch activity and limited mesenchymal stem cell plasticity in the bone marrow of patients with myelodysplastic syndromes." Pathol Oncol Res **13**(4): 311-319.

Myelodysplastic syndromes (MDSs) are a heterogeneous group of hematological disorders characterized by ineffective hematopoiesis, enhanced bone marrow apoptosis and frequent progression to acute myeloid leukemia. Several recent studies suggested that, besides the abnormal development of stem cells, microenvironmental alterations are also present in the MDS bone marrow. In this study, we have examined the relative frequencies of stem and progenitor cell subsets of MDS and normal hematopoietic cells growing on stromal cell layers established from MDS patients and from normal donors. When hematopoietic cells from MDS patients were co-cultured with normal stromal cells, the frequency of either early or late cobblestone area-forming cells (CAFC) was significantly lower compared to the corresponding normal control values in 4 out of 8 patients. In the opposite situation, when normal hematopoietic cells were incubated on MDS stromal cells, the CAFC frequencies were decreased in 5 out of 6 patients, compared to normal stromal layer-containing control cultures. Moreover, a soluble Notch ligand (Jagged-1 protein) was an inhibitor of day-35-42 CAFC when normal hematopoietic cells were cultured with normal or MDS stromal cells, but was unable to inhibit MDS stem and early progenitor cell growth (day-35-42 CAFC) on pre-established stromal layers. These findings suggest that in early hematopoietic cells isolated from MDS patients the Notch signal transduction pathway is disrupted. Furthermore, there was a marked reduction in the plasticity of mesenchymal stem cells of MDS patients compared with those of normal marrow donors, in neurogenic and adipogenic differentiation ability and hematopoiesis supporting capacity in vitro. These results are consistent with the hypothesis that when alterations are present in the myelodysplastic stroma environment along with intrinsic changes in a hematopoietic stem/progenitor cell clone, both factors might equally contribute to the abnormal hematopoiesis in MDS.

Venkatachalam, S., et al. (2018). "Potential risk of clonally expanded amnion mesenchymal stem cell

transplants in contused spinal cords." Restor Neurol Neurosci **36**(3): 387-396.

OBJECTIVE: Mesenchymal stem/stromal cells (MSC) promote recovery after spinal cord injury (SCI) using adult bone marrow MSC (BM-MSC). Newborn tissues are a convenient source of MSC that does not involve an invasive procedure for cell collection. In this study the authors tested the effects of rat amnion MSC clone (rAM-MSC) in SCI. METHODS: We tested intra-parenchymal injection of a GFP+ rat rAM-MSC clone derived from E18.5 rats in rat SCI and measured behavioral recovery (BBB scores), histology and X-ray opacity. Expression of aggrecan was measured in culture after treatment with TGFss. RESULTS: Injection of rAM-MSC after SCI did not improve BBB scores compared to control vehicle injections; rather they reduced scores significantly over 6 weeks. Spinal cords injected with rAM-MSC were hard in regions surrounding the SCI site, which was confirmed by X-ray opacity. Whole mount imaging of these cords showed minimal tissue loss in the SCI site that occurred in SCI controls, and persistence of GFP+ rAM-MSC. Mason's Trichrome staining of tissue sections showed more intense staining for extracellular matrix (ECM) surrounding and extending beyond the SCI site with injections of rAM-MSC but not in controls. In response to TGF-ss treatment in culture, chondrogenic aggrecan was expressed at higher levels in rAM-MSC than in rBM-MSC, suggesting that the upregulation of TGF-ss in SCI sites may promote chondrogenic differentiation. CONCLUSION: Acute injection after SCI of a clonally expanded rAM-MSC resulted in aberrant differentiation towards a chondrocytic phenotype that disrupts the spinal cord and inhibits behavioral recovery after SCI. It will be critical to ensure that injection of extensively expanded neonatal cells do not differentiate aberrantly in traumatic CNS tissue and disrupt recovery.

Wang, M., et al. (2010). "Cloning and characterization of a novel gene with alternative splicing in murine mesenchymal stem cell line C3H/10T1/2 by gene trap screening." BMB Rep **43**(12): 789-794.

A novel gene, designated mgt-6, containing four splicing variants, was isolated from a gene trap clone library of C3H/10T1/2 cells transfected with retroviral promoterless gene-trap vector, ROSAFARY. The transcript variants were differentially expressed in murine tissues and cell lines and differentially responded to diverse stimuli including TGF-beta1 and mitogen-activated protein kinase (MAPK) inhibitors. The mgt-6 gene encoded a protein of 37 or 11 amino acid residuals with cytoplasmic distribution. However, when C3H/10T1/2 cells were treated with 5-azacytidine, the protein translocated into cell nucleus as indicated by fused LacZ or C-terminally tagged EGFP. Our preliminary results suggest that further study on the role of mgt-6 gene in cell transformation and differentiation may be of significance.

Yoshida, K., et al. (2007). "TPA-induced multinucleation of a mesenchymal stem cell-like clone is mediated primarily by karyokinesis without cytokinesis, although cell-cell fusion also occurs." Eur J Cell Biol **86**(8): 461-471.

The 5F9A cell, which is a mesenchymal stem cell-like clone established from rat bone marrow substrate adherent cells, can differentiate into adipocytes and osteoblasts in vitro under the appropriate conditions. Multinucleated cells could be also induced by 12-O-tetradecanoylphorbol 13-acetate (TPA) in 5F9A cells. This effect was mediated by protein kinase C. Possible mechanisms of multinucleation by TPA were hypothesized to be either karyokinesis without cytokinesis or cell-cell fusion. By observation using time-lapse phase-contrast microscopy, we determined that the multinucleated cells were generated mainly by karyokinesis without cytokinesis. Cell fusion was studied using time-lapse photography, and confocal laser scanning microscopy using two differentially labeled cells. These techniques demonstrated that multinucleated 5F9A cells could be produced by cell fusion, albeit at a low frequency. We conclude that multinucleated 5F9A cells are formed primarily by karyokinesis without cytokinesis, although some cells are also formed by cell-cell fusion.

Yoshida, K., et al. (2007). "Oligopotent mesenchymal stem cell-like clone becomes multinucleated following phorbol ester, TPA stimulation." Anat Rec (Hoboken) **290**(10): 1256-1267.

We established a mesenchymal stem cell clone, 5F9A, from rat bone marrow substrate adherent cells by repeated limiting dilutions. The cells have a fibroblastic shape and form intimate contacts with adjacent cells with interdigitations and junctions similar to adherence and tight junctions in a semi-confluent culture. Analysis of the phenotypes of these cells by RT-PCR and FACS demonstrated that they resembled mesenchymal stem cells, and the cells could differentiate into adiopocytes and osteoblasts under appropriate conditions in vitro showing their oligopotency. Furthermore, the cells were induced to become multinuclear cells by TPA (12-o-tetradecanoylphorbol 13-acetate) stimulation.

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.

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