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Adipose-derived mesenchymal stem cell exosomes: a novel pathway for tissues repair.

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Author information

Abstract

The well-characterized curative effect of transplanted mesenchymal stem cells has been mainly attributed to their homing and subsequent differentiation for the repair and regeneration of damaged tissue. Adipose-derived mesenchymal stem cells (ADMSCs) are not only multipotent and plastic, but also abundant as they can be easily harvested with minimally invasive surgical techniques. This makes ADMSCs conducive for clinical applications. Recently, the secretory function of ADMSCs has been regarded as the primary mediator of MSC-based therapy. Exosomes are one kind of small cell extracellular membrane vesicles, which are primarily used to deliver cell-specific proteins, as well as nucleic acids secreted by various cell types. This review will introduce and characterize exosomes-derived ADMSCs (ADMSCs-Exo) and look at new therapies and prospective, including the limitations and outlook for therapeutic strategy. We will describe the latest research progress on myocardial repair, neuroprotection and neurotrophic effects, hepatic repair, renal repair, cutaneous repair, regeneration and other aspects using these cells

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Conditioned media from adipocytes promote proliferation, migration, and invasion in melanoma and colorectal cancer cells.

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Abstract

Epidemiological evidence suggests that obesity can significantly increase the risk of various cancers, although the mechanisms underlying this link are completely unknown. Here, we analyzed the effect of adipocytes on melanoma and colon cancer cells proliferation, migration, and invasion. The potential effects of conditioned media (CM) obtained from differentiated mouse 3T3-L1 cells and human adipose tissue-derived mesenchymal stem cells (hAMSC) on the proliferation, migration, and invasion of B16BL6 melanoma and colon 26-L5 cancer cells were investigated. The 3T3-L1 and hAMSC CM increased cell proliferation, migration, and invasion in both the cell lines. In addition, adipocytes CM increased matrix metalloproteinase 9 (MMP-9) and MMP-2 activity in both B16BL6 and colon 26-L5 cells. These effects were found to be associated with an increased expression of various oncogenic proteins in B16BL6 and colon 26-L5 cells. Also, adipocyte CM induced Akt and mTOR activation in both tumor cell lines, and the pharmacological inhibition of Akt and mTOR blocked the CM induced Akt as

well as mTOR activation and CM-stimulated melanoma and colon cancer cell proliferation, migration, and invasion. These data suggest that adipocyte promotes melanoma and colon cancer progression through modulating the expression of diverse proteins associated with cancer growth and metastasis as well as modulation of the Akt/mTOR signaling.

Cancers (Basel). 2019 Mar 6;11(3). pii: E313. doi: 10.3390/cancers11030313.

Anatomic Origin of Osteochondrogenic Progenitors Impacts Sensitivity to EWS-FLI1-Induced Transformation.

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Abstract

Ewing sarcomas predominantly arise in pelvic and stylopod bones (i.e., femur and humerus), likely as a consequence of *EWS-FLI1*oncogene-induced transformation of mesenchymal stem/progenitor cells (MSCs). MSCs located in the embryonic superficial zone cells (eSZ) of limbs express anatomically distinct posterior *Hox* genes. Significantly, high expression of posterior *HOXD* genes, especially *HOXD13*, is a hallmark of Ewing sarcoma. These data drove our hypothesis that *Hox* genes in posterior skeleton MSCs contribute to Ewing sarcoma tumorigenesis. We isolated eSZ cells from stylopod and zeugopod (i.e., tibia/fibula, radius/ulna) bones, from wild-type and *Hoxd13* mutant embryos, and tested the impact of *EWS-FLI1* transduction on cell proliferation, gene expression, and tumorigenicity. Our data demonstrate that both stylopod and zeugopod eSZ cells tolerate EWS-FLI1 but that stylopod eSZ cells are relatively more susceptible, demonstrating changes in proliferation and gene expression consistent with initiation of malignant transformation. Significantly, loss of *Hoxd13* had no impact, showing that it is dispensable for the initiation of *EWS-FLI1*-induced transformation in mouse MSCs. These findings show that MSCs from anatomically distinct sites are differentially susceptible to EWS-FLI1-induced transformation, supporting the premise that the dominant presentation of Ewing sarcoma in pelvic and stylopod bones is attributable to anatomically-defined differences in MSCs.

Acta Biomater. 2019 Mar 4. pii: S1742-7061(19)30167-9. doi: 10.1016/j.actbio.2019.03.001. [Epub ahead of print]

Culture of hybrid spheroids composed of calcium phosphate materials and mesenchymal stem cells on an oxygen-permeable culture device to predict in vivo bone forming capability.

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Abstract

Three-dimensional (3-D) cell culture can better mimic physiological conditions in which cells interact with adjacent cells and the extracellular matrix than monolayer culture. We have developed a 3-D cell culture device, the Oxy chip, which can be used to generate and supply oxygen to cell spheroids to

prevent hypoxia. Here, we used the Oxy chip to generate hybrid spheroids comprising calcium phosphate (CaP) particles (hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) or octacalcium phosphate (OCP)) and mesenchymal stem cells (MSCs, C3H10T1/2 cells or D1 cells) that can be used to analyze cell differentiation mechanisms. We showed that the 3-D cell-cell and cell-material interactions and oxygenation offered by the Oxy chip promoted osteoblastic differentiation of MSCs. We also used histomorphometric analysis of hematoxylin and eosin staining, quality analyses by μCT and collagen orientation observation with picrosirius red staining in bone regeneration following implantation of three CaPs in a critical-sized defect in mouse calvaria. The in vivo bone formation capacity of the three tested CaP materials was OCP ≥ β-TCP > HA: the newly formed bone by OCP had a structure relatively close to that of the calvaria intact bone. When MSCs were 3-D cultured with the CaP materials using the Oxy chip, the in vitro osteogenic capacity of these materials was highly similar to trends observed in vivo. The in vitro alkaline phosphatase activity of D1 cells had the highest correlation with in vivo bone volume (R = 0.900). Chemical and FTIR spectroscopic analyses confirmed that differentiation of D1 cells could be associated with amorphous calcium phosphate (ACP) precipitation concomitant with OCP hydrolysis. Taken together, hybrid spheroid cultures using the Oxy chip can be used to screen and predict bone forming potential of bone substitute materials. STATEMENT OF SIGNIFICANCE: An oxygen permeable-culture chip (Oxy chip) can be used to induce formation of cell spheroids by mesenchymal stem cells (MSCs). Use of the Oxy chip avoids hypoxia in the spheroid core and enhances MSC osteoblastic differentiation relative to conventional spheroid culture methods. The present study showed that the Oxy chip mimics the in vivo environment associated with bone formation and can be used to generate hybrid spheroids consisting of calcium phosphates and MSCs that are useful for analyzing cell differentiation mechanisms. Bone formation analysis following implantation of calcium phosphate materials in mouse calvaria defects showed positive correlation with the in vitro results. We propose that hybrid spheroids cultured on the Oxy chip can be used to screen and predict the bone forming potential of bone substitute materials.

Int J Mol Sci. 2019 Mar 5;20(5). pii: E1108. doi: 10.3390/ijms20051108.

Identification of miRNA Reference Genes in Extracellular Vesicles from Adipose Derived Mesenchymal Stem Cells for Studying Osteoarthritis.

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Abstract

Osteoarthritis (OA) leads to chronic pain and disability, and traditional conservative treatments are not effective in the long term. The intra-articular injection of mesenchymal stem cells (MSCs) is considered a novel therapy for OA whose efficacy mainly relies on the adaptive release of paracrine molecules which are either soluble or extracellular vesicles (EVs) embedded. The correct quantification of EV-miRNAs using reliable reference genes (RGs) is a crucial step in optimizing this future therapeutic cell-

free approach. The purpose of this study is to rate the stabilities of literature-selected proposed RGs for EV-miRNAs in adipose derived-MSCs (ASCs). EVs were isolated by ultracentrifugation from ASCs cultured with or without inflammatory priming mimicking OA synovial fluid condition. Expression of putative RGs (let-7a-5p, miR-16-5p, miR-23a-3p, miR-26a-5p, miR-101-3p, miR-103a-3p, miR-221-3p, miR-423-5p, miR-425-5p, U6 snRNA) was scored by using the algorithms geNorm, NormFinder, BestKeeper and ΔCt method. miR-16a-5p/miR-23a-3p yielded the most stable RGs, whereas let-7a-5p/miR-425-5p performed poorly. Outcomes were validated by qRT-PCR on miR-146a-5p, reported to be ASC-EVs enriched and involved in OA. Incorrect RG selection affected the evaluation of miR-146a-5p abundance and modulation by inflammation, with both values resulting strongly donor-dependent. Our findings demonstrated that an integrated approach of multiple algorithms is necessary to identify reliable, stable RGs for ASC-EVs miRNAs evaluation. A correct approach would increase the accuracy of embedded molecule assessments aimed to develop therapeutic strategies for the treatment of OA based on EVs.

Methods Mol Biol. 2019 Mar 6. doi: 10.1007/7651_2018_174. [Epub ahead of print]

Methods and Strategies for Procurement, Isolation, Characterization, and Assessment of Senescence of Human Mesenchymal Stem Cells from Adipose Tissue.

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Abstract

Human adipose-derived mesenchymal stem (stromal) cells (hADSC) represent an attractive source of the cells for numerous therapeutic applications in regenerative medicine. These cells are also an efficient model to study biological pathways of stem cell action, tissue injury and disease. Like any other primary somatic cells in culture, industrial-scale expansion of mesenchymal stromal cells (MSC) leads to the replicative exhaustion/senescence as defined by the "Hayflick limit." The senescence is not only greatly effecting in vivo potency of the stem cell cultures but also might be the cause and the source of clinical inconsistency arising from infused cell preparations. In this light, the characterization of hADSC replicative and stressor-induced senescence phenotypes is of great interest.This chapter summarizes some of the essential protocols and assays used at our laboratories and clinic for the human fat procurement, isolation, culture, differentiation, and characterization of mesenchymal stem cells from adipose tissue and the stromal vascular fraction. Additionally, we provide manuals for characterization of hADSC senescence in a culture based on stem cells immunophenotype, proliferation rate, migration potential, and numerous other well-accepted markers of cellular senescence. Such methodological framework will be immensely helpful to design standards and surrogate measures for hADSC-based therapeutic applications.

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Platelet lysate outperforms FCS and human serum for co-culture of primary human macrophages and hMSCs.

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Abstract

In vitro co-cultures of different primary human cell types are pivotal for the testing and evaluation of biomaterials under conditions that are closer to the human in vivo situation. Especially co-cultures of macrophages and mesenchymal stem cells (MSCs) are of interest, as they are both present and involved in tissue regeneration and inflammatory reactions and play crucial roles in the immediate inflammatory reactions and the onset of regenerative processes, thus reflecting the decisive early phase of biomaterial contact with the host. A co-culture system of these cell types might thus allow for the assessment of the biocompatibility of biomaterials. The establishment of such a co-culture is challenging due to the different in vitro cell culture conditions. For human macrophages, medium is usually supplemented with human serum (hS), whereas hMSC culture is mostly performed using fetal calf serum (FCS), and these conditions are disadvantageous for the respective other cell type. We demonstrate that human platelet lysate (hPL) can replace hS in macrophage cultivation and appears to be the best option for co-cultivation of human macrophages with hMSCs. In contrast to FCS and hS, hPL maintained the phenotype of both cell types, comparable to that of their respective standard culture serum, as well as the percentage of each cell population. Moreover, the expression profile and phagocytosis activity of macrophages was similar to hS.

Stem Cells Transl Med. 2019 Mar 5. doi: 10.1002/sctm.18-0122. [Epub ahead of print]

Intra-Articular Injection of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis: A Phase IIb, Randomized, Placebo-Controlled Clinical Trial.

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Abstract

Mesenchymal stem cells (MSCs) have been the focus of an emerging treatment for osteoarthritis. However, few studies reported about outcomes of an intra-articular injection of autologous adipose-derived mesenchymal stem cells (AD-MSCs). This study aimed to assess the efficacy and safety of a single intra-articular injection of AD-MSCs for patients with knee osteoarthritis. It was a prospective double-blinded, randomized controlled, phase IIb clinical trial. AD-MSCs were administered for 12 patients (MSC group), and the group was compared with 12 knees with injection of normal saline (control group) up to 6 months. All procedures were performed in the outpatient clinic. Primary outcome measure was the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) score. Secondary outcome measure included various clinical and radiologic examination, and safety after injection. Change of cartilage defect after injection was evaluated using magnetic resonance imaging (MRI). Single injection of AD-MSCs led to a significant improvement of the WOMAC score at 6 months.

In the control group, there was no significant change in the WOMAC score at 6 months. No serious adverse events were observed in both groups during the follow-up period. In MRI, there was no significant change of cartilage defect at 6 months in MSC group whereas the defect in the control group was increased. An intra-articular injection of autologous AD-MSCs provided satisfactory functional improvement and pain relief for patients with knee osteoarthritis in the outpatient setting, without causing adverse events at 6 months' follow-up. Larger sample size and long-term follow-up are required

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Survival/adaptation of Bone Marrow derived Mesenchymal Stem Cells after long term starvation through selective processes.

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Abstract

After in vivo transplantation Mesenchymal Stem Cells (MSC) face an ischemic microenvironment, characterized by nutrient deprivation and reduced oxygen tension, which reduces their viability and thus their therapeutic potential. Therefore, MSC response to models of in vitro ischemia is of relevance for improving their survival and therapeutic efficacy. The aim of this study is to understand the survival/adaptive response mechanism that MSC use to respond to extreme culture conditions. Specifically, the effect of a long term starvation on human bone marrow derived mesenchymal stem cells cultured in a chemically defined medium (fetal bovine serum-free and human serum-free), either in hypoxic or normoxic conditions. We observed that human Bone Marrow Mesenchymal Stem Cells (hBM-MSC) that were isolated and cultured in SF medium and subjected to a complete starvation for up to 75 days transiently changed their behavior and phenotype. However, at the end of that period, hBM-MSC retained their characteristics as determined by their morphology, DNA damage resistance, proliferation kinetic and differentiation potential. This survival mode involved a quiescent state, confirmed by increased expression of cell cycle regulators p16, p27, p57 and decreased expression of PCNA, Ki-67, mTOR and Nanog. In addition, Jak/STAT (STAT6) anti-apoptotic activity selected which cells conserved stemness and that supported metabolic, bioenergetic and scavenging requirements. We also demonstrated that hBM-MSC exploited an autophagic process which induced lipid β-oxidation as an alternative energy source. Priming MSCs by concomitant starvation and culture in hypoxic conditions to induce their quiescence, would be of benefit to increase MSC survival when transplanted in vivo. SIGNIFICANCE STATEMENT: MSC response to models of in vitro ischemia is of great interest for improving their survival and therapeutic efficacy. Understanding the effect of cell priming in serum-free medium, by concomitant starvation and culture in hypoxic conditions to induce their quiescence, would be of benefit to increase their survival rate when transplanted in vivo, as well as to enable the development of new improved culture protocols before MSC transplantation. MSC primed in this way

may not only have the capacity to secrete trophic factors, increasing angiogenesis and reducing inflammation but also survive, fully integrate and differentiate into the regenerating site.

BMC Med. 2019 Mar 5;17(1):53. doi: 10.1186/s12916-019-1289-6.

Advanced cell therapeutics are changing the clinical landscape: will mesenchymal stromal cells be a part of it?

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Abstract

During the past 15 years there have been dramatic changes in the medical landscape, particularly in oncology and regenerative medicine. Cell therapies have played a substantial part in this progress. Cellular immunotherapies can use immune cells, such as T cells or natural killer cells that, after functional modification ex vivo, exert powerful anti-cancer effects when given to the patient. Innovative technologies, such as re-programming terminally differentiated cells into pluripotent stem cells or into other cell types and applying specific enzymes to more precisely edit the human genome, are paving the way towards more potent cell and gene therapies.Mesenchymal stromal cells are promising cellular immunotherapeutics, which also have potential for use in tissue engineering strategies and other regenerative medicine applications. However, substantial gaps in our knowledge of their biology and therapeutic efficacy present major challenges to their sustainable implementation in the clinical routine.In this article, progress in the field of cell therapeutics during the past 15 years will be briefly discussed, with a focus on mesenchymal stromal cells, highlighting the impact of this field on patient care.

J Clin Invest. 2019 Feb 25;130. pii: 123191. doi: 10.1172/JCI123191. eCollection 2019 Feb 25.

Bone marrow stromal cells from β-thalassemia patients have impaired hematopoietic supportive capacity.

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Author information

Abstract

BACKGROUND:

The human bone marrow (BM) niche contains a population of mesenchymal stromal cells (MSCs) that provide physical support and regulate hematopoietic stem cell (HSC) homeostasis. β-Thalassemia (BT) is a hereditary disorder characterized by altered hemoglobin beta-chain synthesis amenable to allogeneic HSC transplantation and HSC gene therapy. Iron overload (IO) is a common complication in BT patients affecting several organs. However, data on the BM stromal compartment are scarce.

METHODS:

MSCs were isolated and characterized from BM aspirates of healthy donors (HDs) and BT patients. The state of IO was assessed and correlated with the presence of primitive MSCs in vitro and in vivo.

Hematopoietic supportive capacity of MSCs was evaluated by transwell migration assay and 2D coculture of MSCs with human CD34+ HSCs. In vivo, the ability of MSCs to facilitate HSC engraftment was tested in a xenogenic transplant model, whereas the capacity to sustain human hematopoiesis was evaluated in humanized ossicle models.

RESULTS:

We report that, despite iron chelation, BT BM contains high levels of iron and ferritin, indicative of iron accumulation in the BM niche. We found a pauperization of the most primitive MSC pool caused by increased ROS production in vitro which impaired MSC stemness properties. We confirmed a reduced frequency of primitive MSCs in vivo in BT patients. We also discovered a weakened antioxidative response and diminished expression of BM niche-associated genes in BT-MSCs. This caused a functional impairment in MSC hematopoietic supportive capacity in vitro and in cotransplantation models. In addition, BT-MSCs failed to form a proper BM niche in humanized ossicle models.

CONCLUSION:

Our results suggest an impairment in the mesenchymal compartment of BT BM niche and highlight the need for novel strategies to target the niche to reduce IO and oxidative stress before transplantation.

Nanoscale. 2019 Mar 14;11(11):4904-4910. doi: 10.1039/c8nr10490e.

Stem cell-mediated delivery of nanogels loaded with ultrasmall iron oxide nanoparticles for enhanced tumor MR imaging.

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Abstract

The development of new nanoplatforms with enhanced tumor accumulation for accurate diagnosis still remains a great challenge in current precision nanomedicine. Herein, we report the design of stem cell-mediated delivery of nanogels (NGs) loaded with ultrasmall iron oxide (Fe_3O_4) nanoparticles (NPs) for enhanced magnetic resonance (MR) imaging of tumors. In this study, sodium citrate-stabilized ultrasmall Fe_3O_4 NPs with a size of 3.16 ± 1.30 nm were first synthesized using a solvothermal route, coated with polyethyleneimine (PEI), and used as crosslinkers to crosslink alginate (AG) NGs formed via a double emulsion approach, where the AG carboxyl groups were pre-activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. The thus prepared Fe_3O_4 NP-loaded NGs (AG/PEI-Fe_3O_4 NGs) with a size of 47.68 ± 3.41 nm are water-dispersible, colloidally stable, cytocompatible in a given concentration range, display a relatively high r1 relaxivity (r_1 = 1.5 mM^{-1} s^{-1}), and are able to be taken up by bone mesenchymal stem cells without compromising cell viability and stem cell characteristics. Due to the tumor-chemotaxis or tumor tropism, the BMSCs are able to mediate the enhanced delivery of AG/PEI-Fe_3O_4 NGs to the tumor site after intravenous injection, thus enabling significantly strengthened MR imaging of tumors when compared to free NGs. These findings suggest

that the developed AG/PEI-Fe3O4NGs, once mediated by stem cells may serve as a novel, safe, effective and targeted platform for enhanced MR imaging of tumors.