The cannabinoid receptor type 2 as mediator of mesenchymal stromal cell immunosuppressive properties.


Source
Department of Women, Child and General and Specialistic Surgery, Second University of Naples, Naples, Italy; Department of Onco-Haematology, IRCCS “Bambino Gesù” Children Hospital, Rome, Italy.

Abstract
Mesenchymal stromal cells are non-hematopoietic, multipotent progenitor cells producing cytokines, chemokines, and extracellular matrix proteins that support hematopoietic stem cell survival and engraftment, influence immune effector cell development, maturation, and function, and inhibit alloreactive T-cell responses. The immunosuppressive properties of human mesenchymal stromal cells have attracted much attention from immunologists, stem cell biologists and clinicians. Recently, the presence of the endocannabinoid system in hematopoietic and neural stem cells has been demonstrated. Endocannabinoids, mainly acting through the cannabinoid receptor subtype 2, are able to modulate cytokine release and to act as immunosuppressant when added to activated T lymphocytes. In the present study, we have investigated, through a multidisciplinary approach, the involvement of the endocannabinoids in migration, viability and cytokine release of human mesenchymal stromal cells. We show, for the first time, that cultures of human mesenchymal stromal cells express all of the components of the endocannabinoid system, suggesting a potential role for the cannabinoid CB2 receptor as a mediator of anti-inflammatory properties of human mesenchymal stromal cells, as well as of their survival pathways and their capability to home and migrate towards endocannabinoid sources.

Challenges facing in vivo tracking of mesenchymal stem cells used for tissue regeneration.

Hossain MA, Frampton AE, Bagul A.

Source
Renal Transplant and Vascular Access Department, St. George's Healthcare NHS Trust, Blackshaw Road, London SW17 0QT, UK.

Abstract
Evaluation of: Li X-X, Li K-A, Qin J-B et al. In vivo MRI tracking of iron oxide nanoparticle-labeled human mesenchymal stem cells in limb ischemia. Int. J. Nanomedicine 8, 1063-1073 (2013). Bone marrow-derived mesenchymal stem cells (MSCs) are increasingly being investigated in the field of regenerative medicine. In vivo monitoring of MSCs can be performed with MRI, which is a non-invasive, non-toxic and clinically acceptable modality. In order to track these MSCs, cells must be labeled with detectable magnetic nanoparticles. However, they 'leak' from labeled cells, limiting their surveillance to a 3-week period. Li et al. developed a rodent model in order to evaluate MRI monitoring of intramuscularly injected aminopropyltriethoxysilane iron oxide-labeled MSCs. Both in vivo tracking and histological analysis were undertaken. Seeded MSCs demonstrated increased MRI signal in the labeled test group over 3 weeks compared with the unlabeled controls. Histological Prussian blue staining of posttermination tissues confirmed these findings. The authors conclude that successful labeling of MSCs is possible with aminopropyltriethoxysilane - magnetic nanoparticles and that these cells can be monitored in vivo. They offer this form of labeling as an alternative to more common dextran-coated magnetic nanoparticles.


Adipose-Derived Mesenchymal Cells for Bone Regeneration: State of the Art.

Barba M, Cicione C, Bernardini C, Michetti F, Lattanzi W.

Source
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Abstract
Adipose tissue represents a hot topic in regenerative medicine because of the tissue source abundance, the relatively easy retrieval, and the inherent biological properties of mesenchymal stem cells residing in its stroma. Adipose-derived mesenchymal stem cells (ASCs) are indeed multipotent somatic stem cells exhibiting growth kinetics and plasticity, proved to induce efficient tissue regeneration in several biomedical applications. A defined consensus for their isolation, classification, and characterization has been very recently achieved. In particular, bone tissue reconstruction and regeneration based on ASCs has emerged as a promising approach to restore structure and function of bone compromised by injury or disease. ASCs have been used in combination with osteoinductive biomaterial and/or osteogenic molecules, in either static or dynamic culture systems, to improve bone regeneration in several animal models. To date, few clinical trials on ASC-based bone reconstruction have been concluded and proved effective. The aim of this review...
is to dissect the state of the art on ASC use in bone regenerative applications in the attempt to provide a comprehensive coverage of the topics, from the basic laboratory to recent clinical applications.

**Sox2 Suppression by miR-21 Governs Human Mesenchymal Stem Cell Properties.**

*Trohatou O, Zagoura D, Bitsika V, Pappa KI, Antsaklis A, Anagnostou NP, Roubelakis MG.*

**Source**

Laboratory of Biology, School of Medicine, University of Athens, Athens, Greece; Cell and Gene Therapy Laboratory, Center of Basic Research II, and Biotechnology Laboratory, Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece; First Department of Obstetrics and Gynecology, School of Medicine, University of Athens, Athens, Greece.

**Abstract**

MicroRNAs (miRNAs) have recently been shown to act as regulatory signals for maintaining stemness and for determining the fate of adult and fetal stem cells, such as human mesenchymal stem cells (hMSCs). hMSCs constitute a population of multipotent stem cells that can be expanded easily in culture and are able to differentiate into many lineages. We have isolated two subpopulations of fetal mesenchymal stem cells (MSCs) from amniotic fluid (AF) known as spindle-shaped (SS) and round-shaped (RS) cells and characterized them on the basis of their phenotypes, pluripotency, proliferation rates, and differentiation potentials. In this study, we analyzed the miRNA profile of MSCs derived from AF, bone marrow (BM), and umbilical cord blood (UCB). We initially identified 67 different miRNAs that were expressed in all three types of MSCs but at different levels, depending on the source. A more detailed analysis revealed that miR-21 was expressed at higher levels in RS-AF-MSCs and BM-MSCs compared with SS-AF-MSCs. We further demonstrated for the first time a direct interaction between miR-21 and the pluripotency marker Sox2. The induction of miR-21 strongly inhibited Sox2 expression in SS-AF-MSCs, resulting in reduced clonogenic and proliferative potential and cell cycle arrest. Strikingly, the opposite effect was observed upon miR-21 inhibition in RS-AF-MSCs and BM-MSCs, which led to an enhanced proliferation rate. Finally, miR-21 induction accelerated osteogenesis and impaired adipogenesis and chondrogenesis in SS-AF-MSCs. Therefore, these findings suggest that miR-21 might specifically function by regulating Sox2 expression in human MSCs and might also act as a key molecule determining MSC proliferation and differentiation.

**Stem Cells.** 2013 Dec 5. doi: 10.1002/stem.1615. [Epub ahead of print]

**MicroRNA-34a Inhibits osteoblast differentiation and in vivo bone formation of human stromal stem cells.**

*Chen L, Holmstrøm K, Qiu W, Ditzel N, Shi K, Hokland LB, Kassem M.*

**Source**

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**Abstract**

Osteoblast differentiation and bone formation (osteogenesis) are regulated by transcriptional and post-transcriptional mechanisms. Recently, microRNAs (miRNAs) were identified as novel key regulators of human stromal (skeletal, mesenchymal) stem cells (hMSCs) differentiation. Here, we identified miRNA-34a (miR-34a) and its target protein networks as modulator of osteoblastic (OB) differentiation of hMSC. miRNA array profiling and further validation by quantitative RT-PCR revealed that miR-34a was up-regulated during OB differentiation of hMSC and in-situ hybridization confirmed its OB expression in vivo. Overexpression of miR-34a inhibited early commitment and late OB differentiation of hMSC in vitro, whereas inhibition of miR-34a by anti-miR-34a enhanced these processes. Target prediction analysis and experimental validation confirmed Jagged1 (JAG1), a ligand for Notch 1, as a bona fide target of miR-34a. siRNA-mediated reduction of JAG1 expression inhibited OB differentiation. Moreover, a number of known cell cycle regulator and cell proliferation proteins, such as Cyclin D1, Cyclin-dependent kinase 4 & 6 (CDK4 & CDK6), E2F transcription factor 3 (E2F3) and cell division cycle 25 homolog A (CDC25A) were among miR-34a targets. Furthermore, in a preclinical model of in vivo bone formation, overexpression of miR-34a in hMSC reduced heterotopic bone formation by 60%, and conversely, in vivo bone formation was increased by 200% in miR-34a-deficient hMSC. miRNA-34a exhibited unique dual regulatory effects controlling both hMSC proliferation and OB differentiation. Tissue-specific inhibition of miR-34a might be a potential novel therapeutic strategy for enhancing in vivo bone formation.

**Cytotechnology.** 2013 Dec 4. [Epub ahead of print]

**Optimization of the isolation and expansion method of human mediastinal-adipose tissue derived mesenchymal stem cells with virally inactivated GMP-grade platelet lysate.**


**Source**

Department of Medical-Surgical Science and Biotechnologies, Faculty of Pharmacy and Medicine, University of Rome “Sapienza”, C.so della Repubblica 79, 04100, Latina, Italy.

**Abstract**


Mesenchymal stem cells (MSCs) are adult multipotent cells currently employed in several clinical trials due to their immunomodulating, angiogenic and repairing features. The adipose tissue is certainly considered an eligible source of MSCs. Recently, putative adipose tissue derived MSCs (ADMSCs) have been isolated from the mediastinal depots. However, very little is known about the properties, the function and the potential of human mediastinal ADMSCs (hmADMSCs). However, the lack of standardized methodologies to culture ADMSCs prevents comparison across. Herein for the first time, we report a detailed step by step description to optimize the isolation and the expansion methodology of hmADMSCs using a virally inactivated good manufacturing practice (GMP)-grade platelet lysate, highlighting the critical aspects of the procedure and providing useful troubleshooting suggestions. Our approach offers a reproducible system which could provide standardization across laboratories. Moreover, our system is time and cost effective, and it can provide a reproducible source of adipose stem cells to enable future studies to unravel new insights regarding this promising stem cell population.

Cytotechnology, 2013 Dec 5. [Epub ahead of print]
The utility of human dedifferentiated fat cells in bone tissue engineering in vitro.
Sakamoto F, Hashimoto Y, Kishimoto N, Honda Y, Matsumoto N.
Source
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Abstract
We compared the osteoblastic differentiation abilities of dedifferentiated fat cells (DFATs) and human bone marrow mesenchymal stem cells (hMSCs) as a cell source for bone regeneration therapies. In addition, the utility of DFATs in bone tissue engineering in vitro was assessed by an alpha-tricalcium phosphate (α-TCP)/collagen sponge (CS). Human DFATs were isolated from the submandibular of a patient by ceiling culture. DFATs and hMSCs at passage 3 were cultured in control medium or osteogenic medium (OM) for 14 days. Runx2 gene expression, alkaline phosphatase (ALP) activity, as well as osteocalcin (OCN) and calcium contents were analyzed to evaluate the osteoblastic differentiation ability of both cell types. DFATs seeded in an α-TCP/CS and cultured in OM for 14 days were analyzed by scanning electron microscopy (SEM) and histologically. Compared with hMSCs, DFATs cultured in OM generally underwent superior osteoblastogenesis by higher Runx2 gene expression at all days tested, as well as higher ALP activity at day 3 and 7, OCN expression at day 14, and calcium content at day 7. In SEM analyses, DFATs seeded in a α-TCP/CS were well spread and covered the α-TCP/CS by day 7. In addition, numerous spherical deposits were found to almost completely cover the α-TCP/CS on day 14. Von Kossa staining showed that DFATs differentiated into osteoblasts in the α-TCP/CS and formed cultured bone by deposition of a mineralized extracellular matrix. The combined use of DFATs and an α-TCP/CS may be an attractive option for bone tissue engineering.
Quantitative metabolic imaging using endogenous fluorescence to detect stem cell differentiation.
Quinn KP, Sridharan GV, Hayden RS, Kaplan DL, Lee K, Georgakoudi I.
Source
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Abstract
The non-invasive high-resolution spatial mapping of cell metabolism within tissues could provide substantial advancements in assessing the efficacy of stem cell therapy and understanding tissue development. Here, using two-photon excited fluorescence microscopy, we elucidate the relationships among endogenous cell fluorescence, cell redox state, and the differentiation of human mesenchymal stem cells into adipogenic and osteoblastic lineages. Using liquid chromatography/mass spectrometry and quantitative PCR, we evaluate the sensitivity of an optical redox ratio of FAD/(NADH + FAD) to metabolic changes associated with stem cell differentiation. Furthermore, we probe the underlying physiological mechanisms, which relate a decrease in the redox ratio to the onset of differentiation. Because traditional assessments of stem cells and engineered tissues are destructive, time consuming, and logistically intensive, the development and validation of a non-invasive, label-free approach to defining the spatiotemporal patterns of cell differentiation can offer a powerful tool for rapid, high-content characterization of cell and tissue cultures.
Increase of mesenchymal stem cell migration by Cannabidiol via activation of p42/44 MAPK.
Schmuhl E, Ramer R, Salamon A, Peters K, Hinz B.
Source
Institute of Toxicology and Pharmacology, University of Rostock, Schillingallee 70, D-18057 Rostock, Germany; Department of Cell Biology, University of Rostock, Schillingallee 69, D-18057 Rostock, Germany.
Abstract
Migration and differentiation of mesenchymal stem cells (MSCs) are known to be involved in various regenerative processes such as bone healing. However, little is known about the pharmacotherapeutical
options aiming at the mobilization and differentiation of MSCs. The present study therefore focussed on cannabinoids which have been demonstrated to exhibit tissue healing properties. Using Boyden chamber assays, the non-psychoactive phytocannabinoid cannabidiol (CBD) was found to increase the migration of adipose-derived MSCs in a time- and concentration-dependent manner. CBD-induced migration was inhibited by AM-630 (CB2 receptor antagonist) and O-1602 (G protein-coupled receptor 55 [GRP55] agonist). Moreover, the promigratory effect of CBD was antagonized by inhibition of the p42/44 mitogen-activated protein kinase (MAPK) pathway which became activated upon CBD treatment. In line with this data, AM-630 and O-1602 attenuated CBD-induced p42/44 MAPK phosphorylation. A p42/44 MAPK-dependent promigratory effect was likewise demonstrated for the GPR55 antagonist O-1918 and the selective CB2 receptor agonist JWH-133. Additional evidence for a functional effect of CBD on MSCs was provided by experiments demonstrating long-term stimulation with CBD to induce differentiation of MSCs into the osteoblastic lineage as evidenced by increased mineralization assessed by cresolphthalein complexone assay and enhanced activity of alkaline phosphatase. Collectively, this study demonstrates CBD to promote the migration of MSCs via activation of the CB2 receptor and inhibition of GPR55 and to induce osteoblastic differentiation. CBD may therefore recruit MSCs to sites of calcifying tissue regeneration and subsequently support bone regeneration via an osteoanabolic action on MSCs.


Human omental-derived adipose stem cells increase ovarian cancer proliferation, migration, and chemoresistance.


Source
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Abstract
OBJECTIVES:
Adipose tissue contains a population of multipotent adipose stem cells (ASCs) that form tumor stroma and can promote tumor progression. Given the high rate of ovarian cancer metastasis to the omental adipose, we hypothesized that omental-derived ASC may contribute to ovarian cancer growth and dissemination.

MATERIALS AND METHODS:
We isolated ASCs from the omentum of three patients with ovarian cancer, with (O-ASC4, O-ASC5) and without (O-ASC1) omental metastasis. BM-MSCs, SQ-ASCs, O-ASCs were characterized with gene expression arrays and metabolic analysis. Stromal cells effects on ovarian cancer cells proliferation, chemoresistance and radiation resistance was evaluated using co-culture assays with luciferase-labeled human ovarian cancer cell lines. Transwell migration assays were performed with conditioned media from O-ASCs and control cell lines. SKOV3 cells were intraperitoneally injected with or without O-ASC1 to track in-vivo engraftment.

RESULTS:
O-ASCs significantly promoted in vitro proliferation, migration chemotherapy and radiation response of ovarian cancer cell lines. O-ASC4 had more marked effects on migration and chemotherapy response on OVCA 429 and OVCA 433 cells than O-ASC1. Analysis of microarray data revealed that O-ASC4 and O-ASC5 have similar gene expression profiles, in contrast to O-ASC1, which was more similar to BM-MSCs and subcutaneous ASCs in hierarchical clustering. Human O-ASCs were detected in the stroma of human ovarian cancer murine xenografts but not uninvolved ovaries.

CONCLUSIONS:
ASCs derived from the human omentum can promote ovarian cancer proliferation, migration, chemoresistance and radiation resistance in-vitro. Furthermore, clinical O-ASCs isolates demonstrate heterogenous effects on ovarian cancer in-vitro.