ML 10-17 (30/03/2017)

Int Immunol. 2017 Feb 18. doi: 10.1093/intimm/dxx008. [Epub ahead of print]

Mesenchymal stromal cells and autoimmunity.

Pistoia V[1], Raffaghello L[2].
Author information

Abstract

Mesenchymal stromal cells (MSCs) are committed progenitors of mesodermal origin that are found virtually in every organ and exhibit multilineage differentiation into osteocytes, adipocytes and chondrocytes. MSCs also mediate a wide spectrum of immunoregulatory activities that usually dampen innate and adaptive immune responses. These features have attracted interest in the perspective of developing novel cell therapies for autoimmune disease. However, depending on the microenvironmental conditions, MSCs may show a plastic behavior and switch to an immunostimulatory phenotype. After thorough characterization of the effects of MSCs on the immune system, MSC cell therapy has been tested in animal models of autoimmunity using different cell sources, protocols of in vitro expansion and routes and schedules of administration. The pre-clinical results have been encouraging in some models [e.g. Crohn's disease (CD), multiple sclerosis] and heterogeneous in others (e.g. graft-versus-host disease, systemic lupus erythematosus, rheumatoid arthritis). Clinical trials have been carried out and many are ongoing. As discussed, the results obtained are too preliminary to draw any conclusion, with the only exception of topical administration of MSCs in CD that has proven efficacious. The mechanism of action of infused MSCs is still under investigation, but the apparent paradox of a therapeutic effect achieved in spite of the very low number of cells reaching the target organ has been solved by the finding that MSC-derived extracellular vesicles (EVs) closely mimic the therapeutic activity of MSCs in pre-clinical models. These issues are critically discussed in view of the potential clinical use of MSC-derived EVs.

Stem Cells Int. 2017;2017:8482326. doi: 10.1155/2017/8482326. Epub 2017 Feb 28.

Gut Mesenchymal Stromal Cells in Immunity.

Messina V[1], Buccione C[2], Marotta G[2], Zicchedda G[2], Signore M[2], Mattia G[2], Puglisi R[2], Sacchetti B[2], Biancone L[3], Valtieri M[4].
Author information

Abstract

Mesenchymal stromal cells (MSCs), first found in bone marrow (BM), are the structural architects of all organs, participating in most biological functions. MSCs possess tissue-specific signatures that allow their discrimination according to their origin and location. Among their multiple functions, MSCs closely interact with immune cells, orchestrating their activity to maintain overall homeostasis. The phenotype of tissue MSCs residing in the bowel overlaps with myofibroblasts, lining the bottom walls of intestinal crypts (pericryptal) or interspersed within intestinal submucosa (intercryptal). In Crohn's disease, intestinal MSCs are tightly stacked in a chronic inflammatory milieu, which causes their enforced expression of Class II major histocompatibility complex (MHC). The absence of Class II MHC is a

hallmark for immune-modulator and tolerogenic properties of normal MSCs and, vice versa, the expression of HLA-DR is peculiar to antigen presenting cells, that is, immune-activator cells. Interferon *gamma* (IFN*γ*) is responsible for induction of Class II MHC expression on intestinal MSCs. The reversal of myofibroblasts/MSCs from an immune-modulator to an activator phenotype in Crohn's disease results in the formation of a fibrotic tube subverting the intestinal structure. Epithelial metaplastic areas in this context can progress to dysplasia and cancer.

Stem Cells Int. 2017;2017:3738071. doi: 10.1155/2017/3738071. Epub 2017 Feb 27.

A Conditioned Medium of Umbilical Cord Mesenchymal Stem Cells Overexpressing Wnt7a Promotes Wound Repair and Regeneration of Hair Follicles in Mice.

Dong L[1], Hao H[1], Liu J[1], Ti D[1], Tong C[1], Hou Q[1], Li M[1], Zheng J[1], Liu G[1], Fu X[1], Han W[1].
Author information

Abstract

Mesenchymal stem cells (MSCs) can affect the microenvironment of a wound and thereby accelerate wound healing. Wnt proteins act as key mediators of skin development and participate in the formation of skin appendages such as hair. The mechanisms of action of MSCs and Wnt proteins on skin wounds are largely unknown. Here, we prepared a Wnt7a-containing conditioned medium (Wnt-CM) from the supernatant of cultured human umbilical cord-MSCs (UC-MSCs) overexpressing Wnt7a in order to examine the effects of this CM on cutaneous healing. Our results revealed that Wnt-CM can accelerate wound closure and induce regeneration of hair follicles. Meanwhile, Wnt-CM enhanced expression of extracellular matrix (ECM) components and cell migration of fibroblasts but inhibited the migratory ability and expression of K6 and K16 in keratinocytes by enhancing expression of c-Myc. However, we found that the CM of fibroblasts treated with Wnt-CM ($HF^{Wnt\text{-}CM}$-CM) can also promote wound repair and keratinocyte migration; but there was no increase in the number of hair follicles of regeneration. These data indicate that Wnt7a and UC-MSCs have synergistic effects: they can accelerate wound repair and induce hair regeneration via cellular communication in the wound microenvironment. Thus, this study opens up new avenues of research on the mechanisms underlying wound repair.

Sci Rep. 2017 Mar 23;7:45018. doi: 10.1038/srep45018.

Bioreactor mechanically guided 3D mesenchymal stem cell chondrogenesis using a biocompatible novel thermo-reversible methylcellulose-based hydrogel.

Cochis A[1,2], Grad S[3], Stoddart MJ[3,4], Farè S[2,5], Altomare L[2,5], Azzimonti B[2,6], Alini M[3], Rimondini L[1,2].
Author information

Abstract

Autologous chondrocyte implantation for cartilage repair represents a challenge because strongly limited by chondrocytes' poor expansion capacity in vitro. Mesenchymal stem cells (MSCs) can differentiate into chondrocytes, while mechanical loading has been proposed as alternative strategy to induce chondrogenesis excluding the use of exogenous factors. Moreover, MSC supporting material

selection is fundamental to allow for an active interaction with cells. Here, we tested a novel thermo-reversible hydrogel composed of 8% w/v methylcellulose (MC) in a 0.05 M Na_2SO_4 solution. MC hydrogel was obtained by dispersion technique and its thermo-reversibility, mechanical properties, degradation and swelling were investigated, demonstrating a solution-gelation transition between 34 and 37 °C and a low bulk degradation (<20%) after 1 month. The lack of any hydrogel-derived immunoreaction was demonstrated in vivo by mice subcutaneous implantation. To induce in vitro chondrogenesis, MSCs were seeded into MC solution retained within a porous polyurethane (PU) matrix. PU-MC composites were subjected to a combination of compression and shear forces for 21 days in a custom made bioreactor. Mechanical stimulation led to a significant increase in chondrogenic gene expression, while histological analysis detected sulphated glycosaminoglycans and collagen II only in loaded specimens, confirming MC hydrogel suitability to support load induced MSCs chondrogenesis.

Stem Cell Reports. 2017 Mar 7. pii: S2213-6711(17)30075-9. doi: 10.1016/j.stemcr.2017.02.008. [Epub ahead of print]

CD54-Mediated Interaction with Pro-inflammatory Macrophages Increases the Immunosuppressive Function of Human Mesenchymal Stromal Cells.

Espagnolle N[1], Balguerie A[1], Arnaud E[2], Sensebé L[3], Varin A[4].
Author information

Abstract

Mesenchymal stromal cells (MSCs) sense and modulate inflammation and represent potential clinical treatment for immune disorders. However, many details of the bidirectional interaction of MSCs and the innate immune compartment are still unsolved. Here we describe an unconventional but functional interaction between pro-inflammatory classically activated macrophages (M1MΦ) and MSCs, with CD54 playing a central role. CD54 was upregulated and enriched specifically at the contact area between M1MΦ and MSCs. Moreover, the specific interaction induced calcium signaling and increased the immunosuppressive capacities of MSCs dependent on CD54 mediation. Our data demonstrate that MSCs can detect an inflammatory microenvironment via a direct and physical interaction with innate immune cells. This finding opens different perspectives for MSC-based cell therapy

J Extracell Vesicles. 2017 Jan 18;6(1):1265291. doi: 10.1080/20013078.2017.1265291. eCollection 2017.

TRAIL delivery by MSC-derived extracellular vesicles is an effective anticancer therapy.

Yuan Z[1], Kolluri KK[1], Gowers KH[1], Janes SM[1].
Author information

Abstract

Extracellular vesicles (EVs) are lipid membrane-enclosed nanoparticles released by cells. They mediate intercellular communication by transferring biological molecules and therefore have potential as innovative drug delivery vehicles. TNF-related apoptosis-inducing ligand (TRAIL) selectively induces

apoptosis of cancer cells. Unfortunately, the clinical application of recombinant rTRAIL has been hampered by its low bioavailability and resistance of cancer cells. EV-mediated TRAIL delivery may circumvent these problems. Mesenchymal stromal cells (MSCs) produce EVs and could be a good source for therapeutic EV production. We investigated if TRAIL could be expressed in MSC-derived EVs and examined their cancer cell-killing efficacy. EVs were isolated by ultracentrifugation and were membranous particles of 50-70 nm in diameter. Both MSC- and TRAIL-expressing MSC (MSCT)-derived EVs express CD63, CD9 and CD81, but only MSCT-EVs express surface TRAIL. MSCT-EVs induced apoptosis in 11 cancer cell lines in a dose-dependent manner but showed no cytotoxicity in primary human bronchial epithelial cells. Caspase activity inhibition or TRAIL neutralisation blocked the cytotoxicity of TRAIL-positive EVs. MSCT-EVs induced pronounced apoptosis in TRAIL-resistant cancer cells and this effect could be further enhanced using a CDK9 inhibitor. These data indicate that TRAIL delivery by MSC-derived EVs is an effective anticancer therapy.

Proc Natl Acad Sci U S A. 2017 Mar 21. pii: 201700622. doi: 10.1073/pnas.1700622114. [Epub ahead of print]

CRISPR-Cas9-guided oncogenic chromosomal translocations with conditional fusion protein expression in human mesenchymal cells.

Vanoli F[1], Tomishima M[1], Feng W[1,2], Lamribet K[3], Babin L[3], Brunet E[3], Jasin M[4,2].
Author information

Abstract

Gene editing techniques have been extensively used to attempt to model recurrent genomic rearrangements found in tumor cells. These methods involve the induction of double-strand breaks at endogenous loci followed by the identification of breakpoint junctions within a population, which typically arise by nonhomologous end joining. The low frequency of these events, however, has hindered the cloning of cells with the desired rearrangement before oncogenic transformation. Here we present a strategy combining CRISPR-Cas9 technology and homology-directed repair to allow for the selection of human mesenchymal stem cells harboring the oncogenic translocation *EWSR1-WT1* found in the aggressive desmoplastic small round cell tumor. The expression of the fusion transcript is under the control of the endogenous *EWSR1* promoter and, importantly, can be conditionally expressed using Cre recombinase. This method is easily adapted to generate any cancer-relevant rearrangement

Stem Cell Res Ther. 2017 Mar 20;8(1):69. doi: 10.1186/s13287-017-0528-z.

Human and feline adipose-derived mesenchymal stem cells have comparable phenotype, immunomodulatory functions, and transcriptome.

Clark KC[1], Fierro FA[2], Ko EM[1], Walker NJ[1], Arzi B[3], Tepper CG[4], Dahlenburg H[2], Cicchetto A[2], Kol A[1], Marsh L[1], Murphy WJ[5], Fazel N[5], Borjesson DL[6].

Author information

Abstract

BACKGROUND:

Adipose-derived mesenchymal stem cells (ASCs) are a promising cell therapy to treat inflammatory and immune-mediated diseases. Development of appropriate pre-clinical animal models is critical to determine safety and attain early efficacy data for the most promising therapeutic candidates. Naturally occurring diseases in cats already serve as valuable models to inform human clinical trials in oncologic, cardiovascular, and genetic diseases. The objective of this study was to complete a comprehensive side-by-side comparison of human and feline ASCs, with an emphasis on their immunomodulatory capacity and transcriptome.

METHODS:

Human and feline ASCs were evaluated for phenotype, immunomodulatory profile, and transcriptome. Additionally, transwells were used to determine the role of cell-cell contact in ASC-mediated inhibition of lymphocyte proliferation in both humans and cats.

RESULTS:

Similar to human ASCs, feline ASCs were highly proliferative at low passages and fit the minimal criteria of multipotent stem cells including a compatible surface protein phenotype, osteogenic capacity, and normal karyotype. Like ASCs from all species, feline ASCs inhibited mitogen-activated lymphocyte proliferation in vitro, with or without direct ASC-lymphocyte contact. Feline ASCs mimic human ASCs in their mediator secretion pattern, including prostaglandin E2, indoleamine 2,3 dioxygenase, transforming growth factor beta, and interleukin-6, all augmented by interferon gamma secretion by lymphocytes. The transcriptome of three unactivated feline ASC lines were highly similar. Functional analysis of the most highly expressed genes highlighted processes including: 1) the regulation of apoptosis; 2) cell adhesion; 3) response to oxidative stress; and 4) regulation of cell differentiation. Finally, feline ASCs had a similar gene expression profile to noninduced human ASCs.

CONCLUSIONS:

Findings suggest that feline ASCs modulate lymphocyte proliferation using soluble mediators that mirror the human ASC secretion pattern. Uninduced feline ASCs have similar gene expression profiles to uninduced human ASCs, as revealed by transcriptome analysis. These data will help inform clinical trials using cats with naturally occurring diseases as surrogate models for human clinical trials in the regenerative medicine arena.

Res Vet Sci. 2017 Feb 27;114:51-58. doi: 10.1016/j.rvsc.2017.02.019. [Epub ahead of print]

Immunophenotypical characterization of canine mesenchymal stem cells from perivisceral and subcutaneous adipose tissue by a species-specific panel of antibodies.

Ivanovska A[1], Grolli S[1], Borghetti P[1], Ravanetti F[2], Conti V[1], De Angelis E[1], Macchi F[1], Ramoni R[1], Martelli P[1], Gazza F[1], Cacchioli A[1].

Author information

Abstract

Immunophenotypical characterization of mesenchymal stem cells is fundamental for the design and execution of sound experimental and clinical studies. The scarce availability of species-specific antibodies for canine antigens has hampered the immunophenotypical characterization of canine mesenchymal stem cells (MSC). The aim of this study was to select a panel of species-specific direct antibodies readily useful for canine mesenchymal stem cells characterization. They were isolated from perivisceral and subcutaneous adipose tissue samples collected during regular surgeries from 8 dogs. Single color flow cytometric analysis of mesenchymal stem cells (P3) deriving from subcutaneous and perivisceral adipose tissue with a panel of 7 direct anti-canine antibodies revealed two largely homogenous cell populations with a similar pattern: $CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$, $CD34^-$, $CD45^-$ and $MHC\text{-}II^-$ with no statistically significant differences among them. Antibody reactivity was demonstrated on canine peripheral blood mononuclear cells. The similarities are reinforced by their in vitro cell morphology, trilineage differentiation ability and RT-PCR analysis ($CD90^+$, $CD73^+$, $CD105^+$, $CD44^+$, $CD13^+$, $CD29^+$, $Oct\text{-}4^+$ gene and $CD31^-$ and $CD45^-$ expression). Our results report for the first time a comparison between the immunophenotypic profile of canine MSC deriving from perivisceral and subcutaneous adipose tissue. The substantial equivalence between the two populations has practical implication on clinical applications, giving the opportunity to choose the source depending on the patient needs. The results contribute to routine characterization of MSC populations grown in vitro, a mandatory process for the definition of solid and reproducible laboratory and therapeutic procedures.

Differentiation. 2017 Mar 16;95:44-53. doi: 10.1016/j.diff.2017.03.001. [Epub ahead of print]

Different combinations of growth factors for the tenogenic differentiation of bone marrow mesenchymal stem cells in monolayer culture and in fibrin-based three-dimensional constructs.

Bottagisio M[1], Lopa S[1], Granata V[2], Talò G[1], Bazzocchi C[2], Moretti M[3], Barbara Lovati A[4].
Author information

Abstract

Tendon injuries are severe burdens in clinics. The poor tendon healing is related to an ineffective response of resident cells and inadequate vascularization. Thanks to the high proliferation and multi-lineage differentiation capability, bone marrow-derived mesenchymal stem cells (BMSCs) are a promising cell source to support the tendon repair. To date, the association of various growth factors to induce the in vitro tenogenic differentiation of multipotent progenitor cells is poorly investigated. This study aimed to investigate the tenogenic differentiation of rabbit BMSCs by testing the combination of bone morphogenetic proteins (BMP-12 and 14) with transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF) both in 2D and 3D cultures within fibrin-based constructs. After 7 and 14 days, the tenogenic differentiation was assessed by analyzing cell metabolism and collagen content, the gene expression of tenogenic markers and the histological cell distribution and collagen deposition within 3D constructs. Our results demonstrated that the association of BMP-14 with

TGF-β3 and VEGF enhanced the BMSC tenogenic differentiation both in 2D and 3D cultures. This study supports the use of fibrin as hydrogel-based matrix to generate spheroids loaded with tenogenic differentiated BMSCs that could be used to treat tendon lesions in the future.

J Craniomaxillofac Surg. 2017 Feb 17. pii: S1010-5182(17)30068-9. doi: 10.1016/j.jcms.2017.02.009. [Epub ahead of print]

Influence of TGF-β1 on tumor transition in oral cancer cell and BMSC co-cultures.

Böhrnsen F[1], Godek F[2], Kiesel J[3], Kramer FJ[3], Brockmeyer P[3], Schliephake H[3].

Author information

Abstract

OBJECTIVES:

TGF-β1 signaling modulates epithelial mesenchymal transitions (EMT) of head and neck squamous cell carcinoma (HNSCC). Bone marrow mesenchymal stromal cells (BMSC) are able to exert a regulating influence on the expression of markers of EMT in HNSCC cells. It was thus the aim of this study to test the hypothesis that TGF-β1 modulates the interactions of tumor transition between BMSCs and HNSCC, affecting the expression of E-cadherin, Vimentin, Snail, Twist, MMP14 and beta-catenin. Furthermore, we analyzed alterations in the AKT-signaling of tumor and stroma cells.

MATERIALS AND METHODS:

BMSCs were isolated from iliac bone marrow aspirates and co-cultured in trans-well permeable membrane wells with tumor cells of the established HNSCC cell line PCI-13. Following the induction with TGF-β1 under serum free conditions the expression of Vimentin and E-Cadherin was assessed via immunofluorescence. A quantitative RT-PCR analysis of tumor transition markers E-cadherin, Vimentin, Snail, Twist, MMP14 and beta-catenin was performed. Changes in AKT-Signaling were identified via protein analysis.

RESULTS:

In non-induced co-cultures, BMSC were able to suppress Vimentin in PCI-13 as a marker of tumor transition. In TGF-β1 induced co-cultures PCI-13 significantly increased the expression of Vimentin, Twist, Snail, MMP14, GSK3a, PRAS40, 4E-BP1, and AMPKa compared to monolayer controls. TGF-β1 co-cultured BMSC demonstrated a significant increase of Snail, PRAS40, mTOR, GSK3a/b, Bad, PDK1 and 4E-BP1.

CONCLUSIONS:

TGF-β1 was able to attenuate the modulating influence of BMSC in co-culture and drive the co-culture towards a progressive tumor transition, affecting the expression of markers of EMT, AKT-Signaling and proliferative checkpoints.

Biotech Histochem. 2017 Mar 20:1-11. doi: 10.1080/10520295.2017.1292366. [Epub ahead of print]

Subchondral bone response to injected adipose-derived stromal cells for treating osteoarthritis using an experimental rabbit model.

Parrilli A[1], Giavaresi G[2], Ferrari A[2], Salamanna F[1], Desando G[3], Grigolo B[4], Martini L[2], Fini M[2].
Author information

Abstract

Although articular cartilage is the target of osteoarthritis (OA), its deterioration is not always clearly associated with patient symptoms. Because a functional interaction between cartilage and bone is crucial, the pathophysiology of OA and its treatment strategy must focus also on subchondral bone. We investigated whether adipose-derived stromal cells (ASCs) injected into a joint at two different concentrations could prevent subchondral bone damage after the onset of mild OA in a rabbit model. We measured both volumetric and densitometric aspects of bone remodeling. Although OA can stimulate bone remodeling either catabolically or anabolically over time, the accelerated turnover does not allow complete mineralization of new bone and therefore gradually reduces its density. We measured changes in morphometric and densitometric bone parameters using micro-CT analysis and correlated them with the corresponding parameters in cartilage and meniscus. We found that ASCs promoted cartilage repair and helped counteract the accelerated bone turnover that occurs with OA.

Phytomedicine. 2017 Apr 15;27:39-51. doi: 10.1016/j.phymed.2017.02.003. Epub 2017 Feb 14.

Osteoinductive effects of glyceollins on adult mesenchymal stromal/stem cells from adipose tissue and bone marrow.

Bateman ME[1], Strong AL[1], Hunter RS[1], Bratton MR[2], Komati R[3], Sridhar J[3], Riley KE[3], Wang G[3], Hayes DJ[4], Boue SM[5], Burow ME[6], Bunnell BA[7].

Author information

Abstract

BACKGROUND:

While current therapies for osteoporosis focus on reducing bone resorption, the development of therapies to regenerate bone may also be beneficial. Promising anabolic therapy candidates include phytoestrogens, such as daidzein, which effectively induce osteogenesis of adipose-derived stromal cells (ASCs) and bone marrow stromal cells (BMSCs).

PURPOSE:

To investigate the effects of glyceollins, structural derivatives of daidzein, on osteogenesis of ASCs and BMSCs.

STUDY DESIGN:

Herein, the osteoinductive effects of glyceollin I and glyceollin II were assessed and compared to estradiol in ASCs and BMSCs. The mechanism by which glyceollin II induces osteogenesis was further examined.

METHODS:

The ability of glyceollins to promote osteogenesis of ASCs and BMSCs was evaluated in adherent and scaffold cultures. Relative deposition of calcium was analyzed using Alizarin Red staining, Bichinchoninic acid Protein Assay, and Alamar Blue Assay. To further explore the mechanism by which glyceollin II exerts its osteoinductive effects, docking studies of glyceollin II, RNA isolation, cDNA synthesis, and quantitative RT-PCR (qPCR) were performed.

RESULTS:

In adherent cultures, ASCs and BMSCs treated with estradiol, glyceollin I, or glyceollin II demonstrated increased calcium deposition relative to vehicle-treated cells. During evaluation on PLGA scaffolds seeded with ASCs and BMSCs, glyceollin II was the most efficacious in inducing ASC and BMSC osteogenesis compared to estradiol and glyceollin I. Dose-response analysis in ASCs and BMSCs revealed that glyceollin II has the highest potency at 10nM in adherent cultures and 1μM in tissue scaffold cultures. At all doses, osteoinductive effects were attenuated by fulvestrant, suggesting that glyceollin II acts at least in part through estrogen receptor-mediated pathways to induce osteogenesis. Analysis of gene expression demonstrated that, similar to estradiol, glyceollin II induces upregulation of genes involved in osteogenic differentiation.

CONCLUSION:

The ability of glyceollin II to induce osteogenic differentiation in ASCs and BMSCs indicates that glyceollins hold the potential for the development of pharmacological interventions to improve clinical outcomes of patients with osteoporosis.