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Conditioned Medium of the Osteosarcoma Cell Line U2OS Induces hBMSCs to Exhibit Characteristics of Carcinoma-Associated Fibroblasts via Activation of IL-6/STAT3 Signaling.

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Author information

Abstract

As a research hotspot in recent years, bone mesenchymal stem cells (BMSCs) play an important role in the process of a variety of human diseases, including cancers. However, in osteosarcoma, the role of bone mesenchymal stem cells and their communication with tumor cells are not clear. In this study, we validated the communication of osteosarcoma cells with BMSCs. The results showed that the conditioned medium of osteosarcoma cell line U2OS (U2OS-CM) induces the Carcinoma-Associated Fibroblasts(CAFs)-like transformation of BMSCs and promotes the proliferation, migration and invasion of BMSCs. Mechanistically, treatment of human bone mesenchymal stem cells(hBMSCs) with U2OS-CM results in a significant increase in the IL-6 expression and phosphorylation of STAT3.Furthermore, blockade of the IL-6/STAT3 signaling in hBMSCs rescues the transformation of CAF phenotype induced by U2OS-CM. And, human IL-6 can directly increase the expression of the CAF marker genes in hMSCs. Meanwhile, IL-6/STAT3 signaling involves in promoting effects of U2OS-CM on the proliferation, migration and invasion of BMSCs. In summary, our results suggest that BMSCs communicate with osteosarcoma cells through IL-6/STAT3 signaling and play an important role in the progress of osteosarcoma.

Aging (Albany NY). 2020 Apr 14;12. doi: 10.18632/aging.103027. [Epub ahead of print]

Leptin acts on mesenchymal stem cells to promote chemoresistance in osteosarcoma cells.

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Abstract

Leptin signaling influences osteoblastogenesis and modulates the fate of mesenchymal stem cells (MSCs) during bone and cartilage regeneration. Although MSCs abound in the osteosarcoma (OS) microenvironment, and leptin exhibits pro-tumorigenic properties, leptin's influence on OS progression and chemoresistant signaling in MSCs remains unclear. Using cell viability and apoptosis assays, we showed that medium conditioned by leptin-treated human MSCs promotes cisplatin resistance in cultured human OS cells. Moreover, GFP-LC3 expression and chloroquine treatment experiments showed that this effect is mediated by stimulation of autophagy in OS cells. TGF-β expression in MSCs was upregulated by leptin and suppressed by leptin receptor knockdown. Silencing TGF-β in MSCs

also abolished OS cell chemoresistance induced by leptin-conditioned medium. Cisplatin resistance was also induced when leptin-conditioned MSCs were co-injected with MG-63 OS cells to generate subcutaneous xenografts in nude mice. Finally, we observed a significant correlation between autophagy-associated gene expression in OS clinical samples and patient prognosis. We conclude that leptin upregulates TGF-β in MSCs, which promotes autophagy-mediated chemoresistance in OS cells.

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Mesenchymal Stem Cells Enhance Pulmonary Antimicrobial Immunity and Prevent Following Bacterial Infection.

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Abstract

BACKGROUND:

Immunosuppressants such as cyclophosphamide (CTX) have been employed to treat a wide array of autoimmune diseases. The most unfavourable side effects of these drugs are their suppression on the antimicrobial immunity and increasing the risk of infection. As a promising substitution/adjunct, mesenchymal stem cells (MSCs) are currently being tested in several clinical trials. However, their influence on the recipients' antimicrobial immunity remains unclear.

METHODS:

In this study, C57BL/6 mice were treated with either CTX or MSCs, and then both the innate and adaptive immunity of the lung were determined. To investigate the influence of CTX and MSCs on the immune defence against infection, the treated mice were intranasally infected with opportunistic pathogen *Haemophilus influenzae* (Hi). Bacterial clearance and antibacterial immune responses were analysed.

RESULTS:

Our data showed that CTX strongly inhibited the proliferation of lung immune cells, including alveolar macrophages (AMs) and T cells, whereas MSCs increased the numbers of these cells. CTX suppressed the phagocytic activity of AMs; on the contrary, MSCs enhanced it. Notably, infusion of MSCs led to a remarkable increase of regulatory T cells and Th1 cells in the lung. When infected by Hi, CTX did not significantly impair the elimination of invaded bacteria. However, MSC-treated mice exhibited accelerated bacterial clearance and moderate inflammation and tissue damage.

CONCLUSION:

Our study reported that unlike traditional immunosuppressants, modulation of MSCs on the recipient's immune response is more elegant. It could preserve and even enhance the antimicrobial defence, suggesting that MSCs are better choice for patients with high risk of infection or those who need long-term immunosuppressive regimen.

Artif Cells Nanomed Biotechnol. 2020 Dec;48(1):770-776. doi: 10.1080/21691401.2020.1748641.

Natural polymeric nanoparticles as a non-invasive probe for mesenchymal stem cell labelling.

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Abstract

Non-invasive tracking of stem cells after transplant is necessary for cell therapy and tissue engineering field. Herein, we introduce natural and biodegradable nanoparticle to develop a highly efficient nanoprobe with the ability to penetrate the stem cell for tracking. Based on the use of (Gd^{3+}) to label stem cells for magnetic resonance imaging (MRI) we synthesized nanoparticle-containing Gd^{3+}. Gd^{3+} could be used as t_1-weighted MRI contrast agents. In this study, chitosan-alginate nanoparticles were synthesized as a clinical Dotarem® carrier for decreased t_1-weighted. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), and Fourier-transform infrared spectroscopy (FTIR) were utilized for nanoprobe characterization and ICP analysis was performed for Gd^{3+} concentration measurement. The results illustrate that nanoprobes with spherical shape and with a size of 80 nm without any aggregation were obtained. Relaxivity results suggest that r_1 in the phantom was 12.8 $mM^{-1}s^{-1}$ per Gd^{3+} ion, which is 3.5 times larger than that for Dotarem® (r_1 ~3.6 $mM^{-1}s^{-1}$ per Gd^{3+} ion) and this result for synthesized nanoprobe in stem cells 3.56 $mM^{-1}s^{-1}$ per Gd^{3+} ion with 2.16 times larger than that for Dotarem® was reported and also enhanced signal in *in-vivo* imaging was observed. Chitosan-alginate nanoparticles as a novel biocompatible probe for stem cell tracking can be utilized in tissue engineering approach.

Med Drug Discov. 2020 Mar;5:100019. doi: 10.1016/j.medidd.2020.100019. Epub 2020 Mar 19.

Mesenchymal stem cells and management of COVID-19 pneumonia.

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Abstract

Human coronavirus, hCoV-19, is highly pathogenic with severe pneumonia associated with rapid virus replication. Arising in Wuhan China December 2019, the current COVID-19 epidemic has rapidly grown with person-to-person infection expanding to become a global health emergency now on pandemic scale. Governments will not be able to minimise both deaths from COVID-19 and the economic impact of viral spread in mitigation of this current COVID-19 pandemic, according to Anderson et al. 2020 [1], Keeping mortality as low as possible will be the highest priority for individuals; hence governments must put in place measures to ameliorate the inevitable economic downturn. The current global picture shows small chains of transmission in many countries and large chains resulting in extensive spread in a few countries, such as Italy, Iran, South Korea, and Japan. Most countries are likely to have spread of COVID-19, at least in the early stages, before any mitigation measures have an impact. The scale of the problem is massive. Here I consider new approaches to improve patient's biological resistance to

COVID-19 using stem cells, and how benefit might be scaled and simplified using synthetic stem cells to meet logistical needs within a short time frame.

Front Bioeng Biotechnol. 2020 Mar 31;8:243. doi: 10.3389/fbioe.2020.00243. eCollection 2020.

Sodium Hyaluronate Supplemented Culture Media as a New hMSC Chondrogenic Differentiation Media-Model for *in vitro/ex vivo* Screening of Potential Cartilage Repair Therapies.

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Abstract

Surgical strategies to treat articular cartilage injury such as microfracture, expose human bone marrow stem cells (hMSCs) to synovial fluid and its components. High molecular weight hyaluronan (hMwt HA) is one of the most abundant bioactive macromolecules of healthy synovial fluid (hSF) and it plays an important role in the protection of opposing articular cartilage surfaces within the synovial joint. Although hMwt HA has been extensively used to attempt the engineering of the cartilage tissue, its effect as media supplement has not been established. Indeed, current media are often simple in their composition and doesn't recapitulate the rheological and biological features of hSF. In addition, critical *in vivo* molecules that can potentially change the chondrogenic behavior of hBMSCs to make the *in vitro* results more predictive of the real *in vivo* outcome, are lacking. In order to be one step closer to the *in vivo* physiology of hSF, a new culture media supplemented with physiological level of hMwt HA was developed and the effect of the hMwt HA on the chondrogenesis of hMSCs that would be present in a traumatic defect after marrow stimulation techniques, was investigated. hBMSC-seeded fibrin-polyurethane constructs were cultured in a serum free chondropermissive control medium (HA- TGFβ-). This medium was further supplemented with 10 ng/mL TGFβ1 (HA- TGFβ+) or 2 mg/ml hMwt HA 1.8 MDa (HA+ TGFβ-) or both (HA+ TGFβ+). Alternatively, 1 MDa HA was mixed with the fibrin at 0.2 mg/ml (HASc TGFβ+). The effect of hMwt HA on hMSC differentiation was investigated at the gene expression level by RT-qPCR and total DNA, sulfated glycosaminoglycans and Safranin O staining were evaluated. Addition of hMwt HA to the culture media, significantly increased the synthesis of sulfated glycosaminoglycans, especially in the early days of chondrogenesis, and reduced the upregulation of the hypertrophic cartilage marker collagen X. hMwt HA added inside the fibrin gel(HASc TGF+) led to the best matrix deposition. hMwt HA can be one key medium component in a more reliable *in vitro/ex vivo* system to reduce *in vitro* artifacts, enable more accurate pre-screening of potential cartilage repair therapies and reduce the need for animal studies.

Front Bioeng Biotechnol. 2020 Mar 31;8:230. doi: 10.3389/fbioe.2020.00230. eCollection 2020.

A Developmental Engineering-Based Approach to Bone Repair: Endochondral Priming Enhances Vascularization and New Bone Formation in a Critical Size Defect.

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Abstract

There is a distinct clinical need for new therapies that provide an effective treatment for large bone defect repair. Herein we describe a developmental approach, whereby constructs are primed to mimic certain aspects of bone formation that occur during embryogenesis. Specifically, we directly compared the bone healing potential of unprimed, intramembranous, and endochondral primed MSC-laden polycaprolactone (PCL) scaffolds. To generate intramembranous constructs, MSC-seeded PCL scaffolds were exposed to osteogenic growth factors, while endochondral constructs were exposed to chondrogenic growth factors to generate a cartilage template. Eight weeks after implantation into a cranial critical sized defect in mice, there were significantly more vessels present throughout defects treated with endochondral constructs compared to intramembranous constructs. Furthermore, 33 and 50% of the animals treated with the intramembranous and endochondral constructs respectively, had full bone union along the sagittal suture line, with significantly higher levels of bone healing than the unprimed group. Having demonstrated the potential of endochondral priming but recognizing that only 50% of animals completely healed after 8 weeks, we next sought to examine if we could further accelerate the bone healing capacity of the constructs by pre-vascularizing them *in vitro* prior to implantation. The addition of endothelial cells alone significantly reduced the healing capacity of the constructs. The addition of a co-culture of endothelial cells and MSCs had no benefit to either the vascularization or mineralization potential of the scaffolds. Together, these results demonstrate that endochondral priming alone is enough to induce vascularization and subsequent mineralization in a critical-size defect.

Microfluid Nanofluidics. 2019 Aug;23(8). pii: 99. doi: 10.1007/s10404-019-2261-7. Epub 2019 Jul 6.

Bone-chip system to monitor osteogenic differentiation using optical imaging.

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Abstract

Human organoids and organ-on-chip systems to predict human responses to new therapies and for the understanding of disease mechanisms are being more commonly used in translational research. We have developed a bone-chip system to study osteogenic differentiation in vitro, coupled with optical imaging approach which provides the opportunity of monitoring cell survival, proliferation and differentiation in vitro without the need to terminate the culture. We used the mesenchymal stem cell (MSC) line over-expressing bone morphogenetic protein-2 (BMP-2), under Tet-Off system, and *luciferase* reporter gene under constitutive promoter. Cells were seeded on chips and supplemented with osteogenic medium. Flow of media was started 24 h later, while static cultures were performed using media reservoirs. Cells grown on the bone-chips under constant flow of media showed enhanced survival/proliferation, comparing to the cells grown in static conditions; *luciferase* reporter

gene expression and activity, reflecting the cell survival and proliferation, was quantified using bioluminescence imaging and a significant advantage to the flow system was observed. In addition, the flow had positive effect on osteogenic differentiation, when compared with static cultures. Quantitative fluorescent imaging, performed using the osteogenic extra-cellular matrix-targeted probes, showed higher osteogenic differentiation of the cells under the flow conditions. Gene expression analysis of osteogenic markers confirmed the osteogenic differentiation of the MSC-BMP2 cells. Immunofluorescent staining performed against the Osteocalcin, Col1, and BSP markers illustrated robust osteogenic differentiation in the flow culture and lessened differentiation in the static culture. To sum, the bone-chip allows monitoring cell survival, proliferation, and osteogenic differentiation using optical imaging.

Stem Cells Dev. 2020 Apr 16. doi: 10.1089/scd.2020.0019. [Epub ahead of print]

Longtime outcome after intraosseous application of autologous mesenchymal stromal cells in pediatric patients and young adults with avascular necrosis after steroid or chemotherapy.

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Abstract

Avascular necrosis (AVN) is a severe complication of immunosuppressant therapy or chemotherapy. A beneficial AVN therapy with core decompression (CD) and intraosseous infusion of mesenchymal stromal cells (MSC) has been described in adult patients, but there are only few data on MSC applications in pediatric and young adult patients (PYAP). Between 2006 and 2015, 14 AVN lesions of 10 PYAP (6 females) with a median age of 16.9 years (range 8.5 - 25.8 years) received CD and intraosseous application of autologous MSC. Data of these patients were analyzed regarding efficacy, safety and feasibility of this procedure as AVN therapy and compared to a control group of 13 AVN lesions of 11 PYAP (5 females) with a median age of 17.9 years (range 13.5 - 27.5 years) who received CD only. During the follow-up analysis (MSC group: median 3.1 (1.6 - 5.8) years after CD; CD group: median 2.0 (1.5 - 8.5) years after CD), relative lesion sizes (as assessed by MRI) compared to the initial lesion volume, were significantly lower ($p<0.05$) in the MSC group (volume reduction to a median of 18.5%) when compared to the CD group (58.0%). One lesion in the MSC group comprised a complete remission. Size progression was not observed in either group. Clinical improvement (pain, mobility) was not significantly different between the two groups. None of the patients experienced treatment-related adverse effects. CD and additional MSC application was regarded safe, effective, feasible and superior in reducing the lesion size when compared to CD only. Prospective, randomized clinical trials are needed to further evaluate these findings.

Polymers (Basel). 2020 Apr 14;12(4). pii: E905. doi: 10.3390/polym12040905.

Natural and Synthetic Polymers for Bone Scaffolds Optimization.

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Author information

Abstract

Bone tissue is the structural component of the body, which allows locomotion, protects vital internal organs, and provides the maintenance of mineral homeostasis. Several bone-related pathologies generate critical-size bone defects that our organism is not able to heal spontaneously and require a therapeutic action. Conventional therapies span from pharmacological to interventional methodologies, all of them characterized by several drawbacks. To circumvent these effects, tissue engineering and regenerative medicine are innovative and promising approaches that exploit the capability of bone progenitors, especially mesenchymal stem cells, to differentiate into functional bone cells. So far, several materials have been tested in order to guarantee the specific requirements for bone tissue regeneration, ranging from the material biocompatibility to the ideal 3D bone-like architectural structure. In this review, we analyse the state-of-the-art of the most widespread polymeric scaffold materials and their application in in vitro and in vivo models, in order to evaluate their usability in the field of bone tissue engineering. Here, we will present several adopted strategies in scaffold production, from the different combination of materials, to chemical factor inclusion, embedding of cells, and manufacturing technology improvement.

Bioelectricity. 2019 Mar 1;1(1):56-66. doi: 10.1089/bioe.2018.0005. Epub 2019 Mar 21.

Membrane Potential Depolarization Alters Calcium Flux and Phosphate Signaling During Osteogenic Differentiation of Human Mesenchymal Stem Cells.

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Abstract

Background: Membrane potential (V_{mem}) changes accompany important events in embryonic development and organ regeneration. Recent studies have pointed to its function as a potent regulator of cell proliferation, differentiation, migration, and tissue regeneration. We have previously reported that V_{mem} depolarization and hyperpolarization control the osteogenic (OS) differentiation potential of human mesenchymal stem cells (hMSCs). ***Materials and Methods:*** In this study, we sought to understand the mechanism(s) underlying voltage regulation of hMSC differentiation. We investigated the role of calcium and phosphate ion flux in the depolarization response of OS-differentiating hMSCs, as these ions are the two major inorganic components of the bone mineral matrix and are indicative of mature osteoblast function. ***Results:*** Our results suggest that inorganic phosphate levels play a larger role than calcium flux in mediating hMSC response to depolarization and that the expression of stanniocalcin 1 (STC1), a protein that regulates calcium and phosphate homeostasis in osteoblasts, is functionally required for the depolarization response during the early stages of differentiation. ***Conclusion:*** Depolarization alters

hMSC differentiation through a phosphate signaling pathway involving STC1. This study enriches our mechanistic understanding of hMSC response to endogenous voltage cues.

Cells. 2020 Apr 9;9(4). pii: E924. doi: 10.3390/cells9040924.

Mesenchymal Stromal Cell Secretome for Severe COVID-19 Infections: Premises for the Therapeutic Use.

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Author information

Abstract

From the end of 2019, the world population has been faced the spread of the novel coronavirus SARS-CoV-2 responsible for COVID-19 infection. In approximately 14% of the patients affected by the novel coronavirus, the infection progresses with the development of pneumonia that requires mechanical ventilation. At the moment, there is no specific antiviral treatment recommended for the COVID-19 pandemic and the therapeutic strategies to deal with the infection are only supportive. In our opinion, mesenchymal stem cell secretome could offer a new therapeutic approach in treating COVID-19 pneumonia, due to the broad pharmacological effects it shows, including anti-inflammatory, immunomodulatory, regenerative, pro-angiogenic and anti-fibrotic properties.

KEYWORDS:

COVID-19; SARS-CoV-2; acute respiratory distress syndrome; exosomes; extracellular ve