**Cancer-Associated Fibroblasts Share Characteristics and Pro-tumorigenic Activity with Mesenchymal Stromal Cells.**

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**Author information**

**Abstract**

Cancer-associated fibroblasts (CAF) have been suggested to originate from mesenchymal stromal cells (MSC), but their relationship to MSC is not clear. Here we have isolated from primary human neuroblastoma (NB) tumors a population of αFAP- and FSP-1-expressing CAF that share phenotypic and functional characteristics with bone marrow-derived MSC (BM-MSC). Analysis of human NB tumors also confirmed the presence of αFAP- and FSP-1-positive cells in the tumor stroma, and their presence correlated with that of M2 tumor-associated macrophages. These cells (designated CAF-MSC) enhanced in vitro NB cell proliferation, survival, and resistance to chemotherapy and stimulated NB tumor engraftment and growth in immunodeficient mice, indicating an effect independent of the immune system. The pro-tumorigenic activity of MSC in vitro and in xenografted mice was dependent on the co-activation of JAK2/STAT3 and MEK/ERK1/2 in NB cells. In a mouse model of orthotopically implanted NB cells, inhibition of JAK2/STAT3 and MEK/ERK1/2 by ruxolitinib and trametinib potentiated tumor response to etoposide and increased overall survival. These data point to a new type pro-tumorigenic CAF in the tumor microenvironment (TME) of NB and to STAT3 and ERK1/2 as mediators of their activity.

**Effect of monocytes/macrophages on the osteogenic differentiation of adipose-derived mesenchymal stromal cells in 3D co-culture spheroids.**

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**Author information**

**Abstract**

This study aimed to investigate the distinctive roles of the monocytes and macrophages on osteogenic differentiation of adipose-derived mesenchymal stromal cells (ADMSCs) in 3D spheroid co-cultures. We hypothesized that monocytes or macrophages (subtypes pro-inflammatory M1 and pro-wound healing M2) would affect the osteogenic differentiation of ADMSCs in 3D spheroids and that cell-cell interactions between monocytes/macrophages and ADMSCs play an important role in the osteogenic differentiation process of ADMSCs. The obtained results indicated that the osteogenic differentiation of ADMSCs was inhibited by monocytes and both macrophage subtypes in 3D spheroids. Monocytes and M2 macrophages had a stronger inhibiting effect than M1 macrophages. Cell-cell interactions mediated...
by N-cadherin likely played a role in the inhibiting effect of monocytes/macrophages on the osteogenic differentiation of ADMSCs.


Arthroscopic airbrush-assisted cell spraying for cartilage repair - Design, development and characterization of custom-made arthroscopic spray nozzles.

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Author information

Abstract

INTRODUCTION:

Airbrush-assisted cell spraying would facilitate fully arthroscopic filling of cartilage defects, thereby providing a minimally invasive procedure for cartilage repair. This study provides the development and characterisation of custom-made spray nozzles that could serve as a foundation for the development of a BioAirbrush, a platform technology for the arthroscopic application of (cell-laden) hydrogels.

METHODS:

Custom-made spray nozzles were designed and produced with 3D printing technology. A commercially available spraying system was used for comparison. Sprays were characterised based on spray angle, cone width, droplet size, velocity and density. This was performed with conventional and high-speed imaging. Furthermore, cell survival of chondrocytes and mesenchymal stromal cells (MSCs) as well as the chondrogenic capacity of chondrocytes after spraying were evaluated.

RESULTS:

Changing nozzle design from internal to external mixing significantly increased cell survival after spraying. Custom-made spray nozzles provide larger droplets compared to the current commercially available technology, potentially improving cell survival. Sufficient mixing of two gel components was confirmed for the custom-made nozzles. Overall, custom-made nozzles improved cell survival after spraying, without significantly affecting the chondrogenic capacity of the cells.

CONCLUSIONS:

This study provides a platform for the development of a BioAirbrush for spray-assisted cell implantations in arthroscopic cartilage repair procedures. Evaluation of the fundamental characteristics of a spray as well as a study of cell survival after spraying has further expanded the knowledge regarding cell spraying for cartilage repair. Nozzle design and air pressure characteristics are essential parameters to consider for the clinical implementation of spray-assisted cell implantations.


Cellular Changes of Stem Cells in 3-Dimensional Culture.

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PURPOSE:
During various operations and procedures, such as distraction osteogenesis and orthodontics, skeletal tissues use mechanotransduction. Mechanotransduction is important for maintaining bone health and converting mechanical forces into biochemical signals. We hypothesized that cells put under mechanical stress would adapt and change morphologically and respond with a decrease in cellular proliferation to accommodate the stress differences. These differences will be measured at the molecular and genetic level. We also wanted to test the practicality of an in vitro 3-dimensional gel model system.

MATERIALS AND METHODS:
We implemented a 3-dimensional cell culture model. The sample was composed of isolated mouse mesenchymal prefibroblast bone marrow cells from the femurs and tibias of 6- to 8-week-old wild-type C57BL6 mice. The cells were seeded on fibronectin-coated hydrogels along with fibrin and nodulin growth factors. The variables tested were a no-force model (control) and a force model. The force model required two 0.1-mm suture pins put through one 0.25-cm length of cell-gel matrix. After the experiments were run to completion, the samples were fixed with 4% paraformaldehyde and embedded in paraffin. Serial sections were cut at a thickness of 5 μm along the long axis for the force construct and encompassing the entire circular area of the control construct. Descriptive and bivariate statistics were computed, and the P value was set at 5%.

RESULTS:
There was a statistically significant difference between the 2 models. The force model had longer and straighter primary cilia, less apoptosis, and an increase in cell proliferation. In addition, the shape of the cells was markedly different after the experiment.

CONCLUSIONS:
The results of the study suggest cells put under tensile stress have the ability to mechanically sense the environment to provide improved adaptation. Our work also confirms the usefulness of the in vitro 3-dimensional gel model system to mimic in vivo applications.


AML-induced osteogenic differentiation in mesenchymal stromal cells supports leukemia growth.


Author information

Abstract

Genotypic and phenotypic alterations in the bone marrow (BM) microenvironment, in particular in osteoprogenitor cells, have been shown to support leukemogenesis. However, it is unclear how
leukemia cells alter the BM microenvironment to create a hospitable niche. Here, we report that acute myeloid leukemia (AML) cells, but not normal CD34+ or CD33+ cells, induce osteogenic differentiation in mesenchymal stromal cells (MSCs). In addition, AML cells inhibited adipogenic differentiation of MSCs. Mechanistic studies identified that AML-derived BMPs activate Smad1/5 signaling to induce osteogenic differentiation in MSCs. Gene expression array analysis revealed that AML cells induce connective tissue growth factor (CTGF) expression in BM-MSCs irrespective of AML type. Overexpression of CTGF in a transgenic mouse model greatly enhanced leukemia engraftment in vivo. Together, our data suggest that AML cells induce a preosteoblast-rich niche in the BM that in turn enhances AML expansion.


An In Vitro Potency Assay for Monitoring the Immunomodulatory Potential of Stromal Cell-Derived Extracellular Vesicles.

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Author information

Abstract

The regenerative and immunomodulatory activity of mesenchymal stromal cells (MSCs) is partially mediated by secreted vesicular factors. Extracellular vesicles (EVs) exocytosed by MSCs are gaining increased attention as prospective non-cellular therapeutics for a variety of diseases. However, the lack of suitable in vitro assays to monitor the therapeutic potential of EVs currently restricts their application in clinical studies. We have evaluated a dual in vitro immunomodulation potency assay that reproducibly reports the inhibitory effect of MSCs on induced T-cell proliferation and the alloantigen-driven mixed leukocyte reaction of pooled peripheral blood mononuclear cells in a dose-dependent manner. Phytohemagglutinin-stimulated T-cell proliferation was inhibited by MSC-derived EVs in a dose-dependent manner comparable to MSCs. In contrast, inhibition of alloantigen-driven mixed leukocyte reaction was only observed for MSCs, but not for EVs. Our results support the application of a cell-based in vitro potency assay for reproducibly determining the immunomodulatory potential of EVs. Validation of this assay can help establish reliable release criteria for EVs for future clinical studies.


Challenging abdominal incisional hernia repaired with platelet-rich plasma and bone marrow-derived mesenchymal stromal cells. A case report.

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Author information

Abstract
INTRODUCTION:
The necessity to develop new treatment options for challenging procedures in hernia surgery is becoming even more evident and tissue engineering and biological technologies offer even newer strategies to improve fascial healing. The present case reports a patient-tailored surgical technique performed to repair a grade IV abdominal incisional hernia, with a combined use of platelet-rich plasma and bone marrow-derived mesenchymal stromal cells, implanted on a biological mesh.

PRESENTATION OF THE CASE:
A 71 year-old female patient complained of an abdominal incisional hernia, complicated by enterocutaneous fistula, four-months following laparostomy. Contrast enhanced computed tomography showed an incisional hernia defect of 15.5×20cm, with a subcutaneous abscess and an intestinal loop adherent to the anterior abdominal wall, with a concomitant enterocutaneous fistula. Surgery involved abdominal wall standardized technique closure, with in addition platelet-rich plasma and bone marrow-derived mesenchymal stromal cells implanted on a biological mesh. Two years follow up showed no recurrences of incisional hernia.

DISCUSSION:
Coating surgical meshes with patient’s own cells may improve biocompatibility, by reducing inflammation and adhesion formation. Moreover, platelet-rich plasma is a good source of growth factors for wound healing, as well as a good medium for bone marrow multinucleate cells introduction into fascial repair.

CONCLUSION:
This approach is likely to improve abdominal wall repair in high grade (IV) incisional hernia, with the real possibility of improving prosthetic compatibility and reducing future recurrences. The authors agree with the necessity of further studies and trials to assure the safety profile and superiority of this procedure.


One-stage Positron Emission Tomography and Magnetic Resonance Imaging to Assess Mesenchymal Stem Cell Survival in a Canine Model of Intervertebral Disc Degeneration.


Abstract
Intervertebral disc (IVD) degeneration is a major health problem. Although mesenchymal stem cells (MSCs) have been used to promote IVD regeneration, the actual survival time of implanted MSCs in IVDs has never been studied non-invasively and continuously in vivo. To investigate survival of implanted MSCs in vivo, this study used a canine model of degenerated IVD and MSCs transfected with a mutant herpes simplex type-1 virus thymidine kinase (HSV1-sr39tk) and labelled with magnetic iron oxide nanoparticles (MION). One-stage positron emission tomography (PET) and magnetic
resonance (MR) imaging was carried out 3 days and 2, 3, and 4 weeks after implantation of MSCs into IVDs with surgically-induced degeneration. Pfirrmann disc degeneration grade determined from the MR images indicated that the repair progress of degenerated IVD stopped 3 weeks after MSC implantation. Meanwhile, MION signal strength, signal contrast ratio (%), and low signal area (mm2) did not change significantly from that seen 3 days after cell implantation until 4 weeks (751.43 (4 wks) ± 52.67 (3 days) vs. 225.34 ± 35.62; 47.37 ± 5.01 vs. 85.37 ± 10.54; 1.78 ± 0.31 vs. 5.29 ± 1.35; p<0.01, respectively).

Accumulation of the PET reporter probe, 9-(4-[18F]-fluoro-3-hydroxymethylbutyl)-guanine, ([18F]-FHBG), was dramatically decreased at 3 weeks after MSC implantation. These results demonstrated that MSCs could survive no more than 3 weeks after implantation into IVDs with surgically-induced degeneration, suggesting that MSCs could contribute to IVD repair for the first three weeks after implantation. The results also indicate that PET imaging could be used reliably to quantify the survival of implanted MSCs, whereas MION with MR imaging would likely be unsuitable for long-term tracking of MSCs in IVDs.


**Stem cell therapy for reconstruction of alveolar cleft and trauma defects in adults: A randomized controlled, clinical trial.**


**Author information**

**Abstract**

**BACKGROUND:**
Stem cell therapy with bone marrow-derived mesenchymal stem cells is a promising tissue engineering strategy to promote regeneration of craniofacial bone.

**PURPOSE:**
To determine whether cell therapy with ex vivo expanded stem cell populations would be safe and efficacious in the regeneration of large alveolar defects in patients with a history of cleft palate or craniofacial trauma.

**MATERIALS AND METHODS:**
Eighteen patients (10 patients with traumatic injury and 8 patients with cleft palate) presenting with missing teeth associated with horizontal alveolar bone deficiencies were included in this randomized controlled clinical trial. Patients were randomized to receive either conventional autogenous block grafts or stem cell therapy. After a healing period of 4 months the treated sites were re-entered and the bone width re-assessed prior to implant placement. Implant stability was evaluated through torque testing of the implant upon insertion and at 6 months postloading.
RESULTS:
The mean gain in bone width was 1.5 ± 1.5 mm in the stem cell therapy group and 3.3 ± 1.4 mm in the control group. Overall, bone gain was higher in trauma patients as compared to patients with cleft palate, for both the control and the stem cell therapy groups. Most postoperative complications were wound dehiscences and incision line openings. Implants were placed successfully in 5 out of 10 patients in the stem cell therapy group and in all 8 patients in the control group. One implant from the control/cleft palate group failed before loading, while the rest of the implants were loaded successfully and remained stable at 6 months. The patients who did not receive implants were re-treated with autogenous block bone graft.

CONCLUSION:
The ability of stem cells to treat large alveolar defects is safe, yet, their ability to completely reconstitute large alveolar defects is limited. This approach requires further optimization to meet the outcomes seen using current methods to treat large defects, particularly those resultant of cleft palate

Impact of starting material (fresh versus cryopreserved marrow) on mesenchymal stem cell culture.
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BACKGROUND:
Mesenchymal stem cells (MSCs) continue to be investigated in multiple clinical trials as potential therapy for different disorders. There is ongoing controversy surrounding the clinical use of cryopreserved versus fresh MSCs. However, little is known about how cryopreservation affects marrow as starting material. The growth kinetics of MSC cultures derived from fresh versus cryopreserved marrow were compared.

STUDY DESIGN AND METHODS:
Data were reviewed on the growth kinetics of MSCs derived from fresh versus cryopreserved marrow of nine donors. Marrow harvested from each donor was separated into four aliquots (one fresh and three cryopreserved for culture). Data on the date of mononuclear cell cryopreservation/thaw, MSC counts at Passages 1 and 2, MSC doubling, MSC fold expansion, viability (of mononuclear cells and final MSCs), and on flow cytometry markers of mononuclear cells and final MSCs were analyzed for the fresh and cryopreserved marrow groups.

RESULTS:
In total, 21 MSC lots (seven fresh and 14 cryopreserved) were obtained. The average age of cryopreserved mononuclear cell product was 295 days (range, 18-1241 days). There were no significant differences between MSC numbers at Passage 1 (p = 0.1), final MSC numbers (p = 0.5), MSC doubling (p = 0.7), or MSC fold expansion (p = 0.7). A significant difference was observed in
viability by flow cytometry for both mononuclear cells ($p = 0.002$) and final MSCs ($p = 0.009$), with higher viability in the fresh marrow group.

**CONCLUSION:**
This study demonstrates that MSCs derived from cryopreserved marrow have the same growth characteristics as fresh marrow-derived MSCs. Further studies are needed to explore potential differences in clinical efficacy.

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