Int J Mol Sci. 2018 Aug 29;19(9). pii: E2564. doi: 10.3390/ijms19092564.

Tracking of Adipose-Derived Mesenchymal Stromal/Stem Cells in a Model of Cisplatin-Induced Acute Kidney Injury: Comparison of Bioluminescence Imaging versus qRT-PCR.

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Author information

Abstract

Determining the cell fate and the distribution of mesenchymal stromal/stem cells (MSCs) after transplantation are essential parts of characterizing the mechanisms of action and biosafety profile of stem cell therapy. Many recent studies have shown that MSCs migrate into injured tissues, but are only detectable at extremely low frequencies. We investigated the cell fate of MSCs after transplantation in an acute kidney injury (AKI) mouse model using in vivo bioluminescence imaging (BLI) and subsequent verification of cell migration using quantitative real-time polymerase chain reaction (qRT-PCR). The AKI was induced by a single injection of cisplatin (8 or 12 mg/kg). One day later, adipose-derived mesenchymal stromal/stem cells isolated from luciferase transgenic mice (Luc^+-mASCs, 5×10^5) were intravenously transplanted. Migration kinetics of the cells was monitored using BLI on day 1, 3, and 6, and finally via quantitative real-time PCR at the endpoint on day 6. Using BLI, infused Luc^+-mASCs could only be detected in the lungs, but not in the kidneys. In contrast, PCR endpoint analysis revealed that Luc-specific mRNA could be detected in injured renal tissue; compared to the control group, the induction was 2.2-fold higher for the 8 mg/kg cisplatin group ($p < 0.05$), respectively 6.1-fold for the 12 mg/kg cisplatin group ($p < 0.001$). In conclusion, our study demonstrated that Luc-based real-time PCR rather than BLI is likely to be a better tool for cell tracking after transplantation in models such as cisplatin-induced AKI.

Vet Rec. 2018 Aug 29. pii: vetrec-2018-104867. doi: 10.1136/vr.104867. [Epub ahead of print]

Allogeneic adipose-derived mesenchymal stem cell therapy in dogs with refractory atopic dermatitis: clinical efficacy and safety.

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Abstract

Canine atopic dermatitis (AD) is a common skin disease with a 10-15 per cent prevalence. Current treatments vary in their efficacy and safety. The immunomodulatory properties of mesenchymal stem cells (MSCs) make them a promising alternative treatment. The aim of this study was to evaluate the therapeutic efficacy and safety of allogeneic canine adipose MSCs (cAd-MSCs) in dogs with refractory AD. Twenty-six dogs, suffering from AD for at least 12 months, not responding to conventional therapy, received an intravenous dose of 1.5×10^6 cAd-MSCs/kg bodyweight. Clinical signs, haematological and

biochemistry profiles, and AD severity were assessed in a six-month follow-up using a validated scoring system (Canine Atopic Dermatitis Extent and Severity Index, version 4 (CADESI-04)). The degree of pruritus was quantified using a validated visual analogue scale, and also owner's global assessment of treatment efficacy. Twenty-two animals completed the study. Pruritus and CADESI-04 scores decreased significantly after one week or month of treatment, respectively, and remained stable for six months. Owner's global assessment score was 2.15±1.15 for all the animals in the study. In conclusion, systemic administration of allogeneic cAd-MSCs appeared to be a simple therapy with positive outcome in the remission of clinical signs for AD refractory to conventional medications, for at least six months and with no adverse events.

Cytotherapy. 2018 Aug 29. pii: S1465-3249(18)30556-5. doi: 10.1016/j.jcyt.2018.07.001. [Epub ahead of print]

Production via good manufacturing practice of exofucosylated human mesenchymal stromal cells for clinical applications.

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Abstract

BACKGROUND:

The regenerative and immunomodulatory properties of human mesenchymal stromal cells (hMSCs) have raised great hope for their use in cell therapy. However, when intravenously infused, hMSCs fail to reach sites of tissue injury. Fucose addition in α(1,3)-linkage to terminal sialyllactosamines on CD44 creates the molecule known as hematopoietic cell E-/L-selectin ligand (HCELL), programming hMSC binding to E-selectin that is expressed on microvascular endothelial cells of bone marrow (BM), skin and at all sites of inflammation. Here we describe how this modification on BM-derived hMSCs (BM-hMSCs) can be adapted to good manufacturing practice (GMP) standards.

METHODS:

BM-hMSCs were expanded using xenogenic-free media and exofucosylated using α(1,3)-fucosyltransferases VI (FTVI) or VII (FTVII). Enforced fucosylation converted CD44 into HCELL, and HCELL formation was assessed using Western blot, flow cytometry and cell-binding assays. Untreated (unfucosylated), buffer-treated and exofucosylated BM-hMSCs were each analyzed for cell viability, immunophenotype and differentiation potential, and E-selectin binding stability was assessed at room temperature, at 4°C, and after cryopreservation. Cell product safety was evaluated using microbiological testing, karyotype analysis, and c-Myc messenger RNA (mRNA) expression, and potential effects on genetic reprogramming and in cell signaling were analyzed using gene expression microarrays and receptor tyrosine kinase (RTK) phosphorylation arrays.

RESULTS:

Our protocol efficiently generates HCELL on clinical-scale batches of BM-hMSCs. Exofucosylation yields stable HCELL expression for 48 h at 4°C, with retained expression after cell cryopreservation. Cell viability and identity are unaffected by exofucosylation, without changes in gene expression or RTK phosphorylation.

DISCUSSION:

The described exofucosylation protocol using xenogenic-free reagents enforces HCELL expression on hMSCs endowing potent E-selectin binding without affecting cell viability or native phenotype. This described protocol is readily scalable for GMP-compliant clinical production.

Toxicol Sci. 2018 Sep 1;165(1):40-49. doi: 10.1093/toxsci/kfy176.

Arsenic Alters Exosome Quantity and Cargo to Mediate Stem Cell Recruitment Into a Cancer Stem Cell-Like Phenotype.

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Abstract

Inorganic arsenic is a human carcinogen that can target the prostate. Accumulating evidence suggests arsenic can disrupt stem cell (SC) dynamics during the carcinogenic process. Previous work demonstrated arsenic-transformed prostate epithelial (CAsE-PE) cells can recruit prostate SCs into rapidly acquiring a cancer SC (CSC) phenotype via the secretion of soluble factors. Exosomes are small, membrane-derived vesicles that contain lipids, RNA, and proteins, and actively contribute to cancer initiation and progression when taken up by target cells. Here we hypothesized that CAsE-PE cells are recruiting SCs to a CSC-like phenotype via exosomal signaling. CAsE-PE cells secreted 700% more exosomes than parental RWPE-1 cells. CAsE-PE exosomes were enriched with oncogenic factors, including oncogenes (KRAS, NRAS, VEFGA, MYB, and EGFR), inflammation-related (cyclooxygenase-2, interleukin 1B (IL1B), IL6, transforming growth factor-β, and tumor necrosis factor-A), and apoptosis-related (CASP7, CASP9, and BCL2) transcripts, and oncogenesis-associated microRNAs. When compared with SCs cultured in exosome-depleted conditioned medium (CM), SCs cultured in CM containing CAsE-PE-derived exosomes showed increased (198%) matrix metalloproteinase activity and underwent an epithelial-to-mesenchymal transition in morphology, suggesting an exosome-mediated transformation. KRAS plays an important role in arsenic carcinogenesis. Although KRAS transcript (>24 000%) and protein (866%) levels were elevated in CAsE-PE exosomes, knock-down of KRAS in these cells only partially mitigated the CSC-like phenotype in cocultured SCs. Collectively, these results suggest arsenic impacts both exosomal quantity and cargo. Exosomal KRAS is only minimally involved in this recruitment, and additional factors (eg, cancer-associated miRNAs) likely also play a role. This work furthers our mechanistic understanding of how arsenic disrupts SC dynamics and influences the tumor microenvironment during carcinogenesis.

MethodsX. 2018 Aug 10;5:924-932. doi: 10.1016/j.mex.2018.08.001. eCollection 2018.

A 3D printed mechanical bioreactor for investigating mechanobiology and soft tissue mechanics.

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Abstract

Mechanical loading is an important cue for directing stem cell fate and engineered tissue formation *in vitro*. Stem cells cultured on 2-dimensional (D) substrates and in 3D scaffolds have been shown to differentiate toward bone, tendon, cartilage, ligament, and skeletal muscle lineages depending on their exposure to mechanical stimuli. To apply this mechanical stimulus *in vitro*, mechanical bioreactors are needed. However, current bioreactor systems are challenged by their high cost, limited ability for customization, and lack of force measurement capabilities. We demonstrate the use of 3-dimensional printing (3DP) technology to design and fabricate a low-cost custom bioreactor system that can be used to apply controlled mechanical stimuli to cells in culture and measure the mechanical properties of small soft tissues. The results of our *in vitro* studies and mechanical evaluations show that 3DP technology is feasible as a platform for developing a low-cost, customizable, and multifunctional mechanical bioreactor system. • 3DP technology was used to print a multifunctional bioreactor system/tensile load frame for a fraction of the cost of commercial systems. • The system mechanically stimulated cells in culture and evaluated the mechanical properties of soft tissues. • This system is easily customizable and can be used to evaluate multiple types of soft tissues.

J Biomed Nanotechnol. 2018 Nov 1;14(11):1906-1920. doi: 10.1166/jbn.2018.2639.

The Regulation of Mesenchymal Stem Cell Therapy Through Magnetic Resonance Imaging Agents-Based Cellular Condition and Oxygen Environment.

Lu Y, Wei L, Zhang X, Cai J, Zhu Y, Xiao J, Duan Y, Liu H, Wang Z, Li S.

Abstract

Repairing articular cartilage defects is difficult due to the hypovascular biostructure and poor self-repairing capacity of articular cartilage. Currently, mesenchymal stem cells (MSCs) with excellent differentiation potential are considered as a promising biological approach for cartilage regeneration. The effect, however, remains far from satisfactory for clinical applications owing to the main drawbacks of tracking the retention of cells and a low differentiation efficiency. As known, the nanoparticles with superparamagnetic properties has been used to monitor the MSCs in vivo through magnetic resonance imaging (MRI) in clinical application. In this study, different external and internal bio-conditions were applied to regulate the biological behavior of cells. Here, intracellular MRI contrast agents, superparamagnetic iron oxide nanocrystals (SPIONs), and a hypoxic culture environment were found to exert synergistic effects on gene and protein expression, and the cell viability, cell cycle, apoptosis, reactive oxygen species and the stem cell differentiations were measured. The levels of chondrogenic and migrant markers (including collagen II, collagen X, aggrecan, SOX9, MMPs and CXCR4)

increased, triggering directional differentiation and enhancing cell migration to the inflammatory site. Moreover, SPION-labeled hypoxia-preconditioned MSCs were found without reactive oxygen species generation and transplanted into rat models with articular cartilage disorders. Interestingly, MRI and histological identification confirmed that new cartilage-like tissue was regenerated and that defects were repaired, and this method is more efficient for cartilage regeneration than SPION-labeled normoxia MSCs. The synergistic effect of hypoxia-precondition and SPIONs based cellular iron source could improve the cell migration and facilitate chondrogenic differentiation.

Tissue Eng Part B Rev. 2018 Aug 31. doi: 10.1089/ten.TEB.2018.0118. [Epub ahead of print]

MSC functionalization for enhanced therapeutic applications.

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Abstract

To date, the therapeutic efficacy of human mesenchymal stem cells (hMSCs) has been investigated in various clinical trials with moderate or in some cases inconsistent results. The still elusive reproducibility relates in part with constitutive differences in the cell preparation, translated into variable "cell potencies". Other factors include poor cell homing and survival, and age/disease-associated host tissue impairment. It is well accepted that within in vivo niches MSCs exist as heterogeneous cell populations with different stemness propensities and supportive functions. Phenotype-based MSC purification of homogeneous subsets can result in cell populations with distinct biological functions. In addition, preclinical studies have shown that MSC functionalization in vitro, via cell priming, can boost their immunomodulatory, trophic and reparative capacities in vivo. Therefore, in the present review we discuss how phenotype-based MSC purification and MSC priming technologies can contribute to an improved MSC-based product for safer and more effective therapeutic applications.

Knee Surg Sports Traumatol Arthrosc. 2018 Aug 29. doi: 10.1007/s00167-018-5118-9. [Epub ahead of print]

Injective mesenchymal stem cell-based treatments for knee osteoarthritis: from mechanisms of action to current clinical evidences.

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Abstract

PURPOSE:

Osteoarthritis (OA) represents a relevant social and economic burden worldwide. "Mesenchymal stem cells" or, as recently proposed, "medicinal signaling cells" (MSCs) have been recently introduced as injective treatments for OA with the aim of restoring joint homeostasis. The aim of this review is to provide the reader with the tools necessary to interpret the currently available clinical data, focusing on the MSC mechanisms of action which might help to clarify what we should expect from this treatment.

METHODS:

Clinical studies reporting MSC injections for the treatment of knee OA, either freshly isolated or culture-expanded cells, have been included and commented in relation to the supposed therapeutic effect that MSCs might exert giving their supposed mode of actions.

RESULTS:

The majority of the studies reports significant improvements in terms of pain and knee function compared to baseline values, up to 24 months of follow-up. Although these data support the expected therapeutic effect of this therapy giving the features of these cells, only 14% of the studies present a control group and more than one-third of them report the results on less than ten patients.

CONCLUSIONS:

Despite the constant presence of positive and satisfactory results in the studies analyzed, the complexity of MSC metabolism and related therapeutic effects as well as the weakness of most of the studies do not allow withdrawing definitive conclusions about the superiority of one tissue source over another, as well as about the best cell dose and the long-term durability of the effects of these procedures. Given the high potential value of these therapies in the treatment of OA, further studies accurately designed, carefully defining the type of patients to be included and pursuing minimal standard requirements in terms of follow-up, number of patients, and types of measurements should be conducted to finally assess the efficacy of MSC-based injective treatments.

Stem Cells Int. 2018 Aug 6;2018:7309031. doi: 10.1155/2018/7309031. eCollection 2018.

Differential Proteomic Analysis Predicts Appropriate Applications for the Secretome of Adipose-Derived Mesenchymal Stem/Stromal Cells and Dermal Fibroblasts.

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Abstract

The adult stem cell secretome is currently under investigation as an alternative to cell-based therapy in regenerative medicine, thanks to the remarkable translational opportunity and the advantages in terms of handling and safety. In this perspective, we recently demonstrated the efficient performance of the adipose-derived mesenchymal stem/stromal cell (ASC) secretome in contrasting neuroinflammation in a murine model of diabetic neuropathy, where the administration of factors released by dermal fibroblasts (DFs) did not exert any effect. Up to now, the complex mixture of the constituents of the conditioned medium from ASCs has not been fully deepened, although its appropriate characterization is required in the perspective of a clinical use. Herein, we propose the differential proteomic approach for the identification of the players accounting for the functional effects of the cell secretome with the aim to unravel its appropriate applications. Out of 967 quantified proteins, 34 and 62 factors were found preponderantly or exclusively secreted by ASCs and DFs, respectively. This approach led to the recognition of distinct functions related to the conditioned medium of ASCs and DFs, with the former being involved in the regulation of neuronal death and apoptosis and the latter in bone metabolism and

ossification. The proosteogenic effect of DF secretome was validated *in vitro* on human primary osteoblasts, providing a proof of concept of its osteoinductive potential. Besides discovering new applications of the cell type-specific secretome, the proposed strategy could allow the recognition of the cocktail of bioactive factors which might be responsible for the effects of conditioned media, thus providing a solid rationale to the implementation of a cell-free approach in several clinical scenarios involving tissue regeneration.

Adv Exp Med Biol. 2018 Aug 29. doi: 10.1007/5584_2018_256. [Epub ahead of print]

Promotion of Cell-Based Therapy: Special Focus on the Cooperation of Mesenchymal Stem Cell Therapy and Gene Therapy for Clinical Trial Studies.

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Abstract

Regenerative medicine (RM) is a promising new field of medicine that has mobilized several new tools to repair or replace lost or damaged cells or tissues by stimulating natural regenerative mechanisms nearby cell and tissue-based therapy approaches. However, mesenchymal stem cell (MSC) based therapy has been shown to be safe and effective to a certain degree in multiple clinical trial studies (CTSs) of several diseases, in most MSC CTSs the efficacy of treatment has been reported low. Therefore, researchers have focused on efficacy enhancing of MSC to improve migratory and homing, survival, stemness, differentiation and other therapeutic applicable properties by using different approaches. Gene therapy is one of the experimental technique tools that uses genes to change cells for therapeutic and investigation purposes. In this study has been focused on genetically modified MSCs for use in RM with an emphasis on CTSs. We highlight the basic concept of genetic modifications and also discuss recent clinical studies aspects. Recently reviewed studies show that MSC therapy with assistant gene therapy can be used in cancer therapy, heart diseases, Fanconi anemia and several other diseases.

Stem Cells Int. 2018 Jul 31;2018:7357213. doi: 10.1155/2018/7357213. eCollection 2018.

Induction of Expression of CD271 and CD34 in Mesenchymal Stromal Cells Cultured as Spheroids.

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Abstract

Cultured mesenchymal stromal cells (MSCs) are cells that can be used for tissue engineering or cell therapies owing to their multipotency and ability to secrete immunomodulatory and trophic molecules. Several studies suggest that MSCs can become pericytes when cocultured with endothelial cells (ECs) but failed to use pericyte markers not already expressed by MSCs. We hypothesized ECs could instruct MSCs to express the molecules CD271 or CD34, which are expressed by pericytes in situ but not by MSCs. CD271 is a marker of especial interest because it is associated with multipotency, a

characteristic that wanes in MSCs as they are culture expanded. Consequently, surface expression of CD271 and CD34 was detected in roughly half of the MSCs cocultured with ECs as spheroids in the presence of insulin-like growth factor 1 (IGF-1). Conversely, expression of CD271 and CD34 was detected in a similar proportion of MSCs cultured under these conditions without ECs, and expression of these markers was low or absent when no IGF-1 was added. These findings indicate that specific culture conditions including IGF-1 can endow cultured MSCs with expression of CD271 and CD34, which may enhance the multipotency of these cells when they are used for therapeutic purposes.

Int J Mol Sci. 2018 Aug 25;19(9). pii: E2526. doi: 10.3390/ijms19092526.

Autologous Mesenchymal Stroma Cells Are Superior to Allogeneic Ones in Bone Defect Regeneration.

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Abstract

The application of autologous mesenchymal stem cells (MSC) for the treatment of bone defects requires two invasive procedures and several weeks of ex vivo cell expansion. To overcome these limitations, the administration of allogeneic MSC may be attractive, because they are anticipated to be immunoprivileged. Because preclinical studies using various animal models are conflicting with respect to the efficacy of allogeneic MSC, we investigated whether autologous and allogeneic human MSC (hMSC) are equally effective in regenerating bone in a humanized mouse model resembling the human immune system. Applying autologous and allogeneic hMSC in critically sized femoral defects, we found that allogeneic hMSC elicited a mild immune response early after implantation, whereas early angiogenic processes were similar in both treatments. At later healing time points, the transplantation of allogeneic hMSC resulted in less bone formation than autologous hMSC, associated with a reduced expression of the osteogenic factor Runx2 and impaired angiogenesis. We found by species-specific staining for collagen-type-1α2 that MSCs of either source did not synthesize new bone matrix, indicating an indirect contribution of transplanted hMSC to bone regeneration. In conclusion, our data suggest that the application of autologous hMSC is superior to that of allogeneic cells for bone defect treatment.