ML 24-20 (23/06/2020)

Stem Cells Transl Med. 2020 Jun 15.
doi: 10.1002/sctm.20-0161. Online ahead of print.

Cross Talk Between Mesenchymal and Glioblastoma Stem Cells: Communication Beyond Controversies

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- PMID: 32543030

- DOI: 10.1002/sctm.20-0161

Free article

Abstract

Mesenchymal stem cells (MSCs) can be isolated from bone marrow or other adult tissues (adipose tissue, dental pulp, amniotic fluid, and umbilical cord). In vitro, MSCs grow as adherent cells, display fibroblast-like morphology, and self-renew, undergoing specific mesodermal differentiation. High heterogeneity of MSCs from different origin, and differences in preparation techniques, make difficult to uniform their functional properties for therapeutic purposes. Immunomodulatory, migratory, and differentiation ability, fueled clinical MSC application in regenerative medicine, whereas beneficial effects are currently mainly ascribed to their secretome and extracellular vesicles. MSC translational potential in cancer therapy exploits putative anti-tumor activity and inherent tropism toward tumor sites to deliver cytotoxic drugs. However, controversial results emerged evaluating either the therapeutic potential or homing efficiency of MSCs, as both antitumor and protumor effects were reported. Glioblastoma (GBM) is the most malignant brain tumor and its development and aggressive nature is sustained by cancer stem cells (CSCs) and the identification of effective therapeutic is required. MSC dualistic action, tumor-promoting or tumor-targeting, is dependent on secreted factors and extracellular vesicles driving a complex cross talk between MSCs and GBM CSCs. Tumor-tropic ability of MSCs, besides providing an alternative therapeutic approach, could represent a tool to understand the biology of GBM CSCs and related paracrine mechanisms, underpinning MSC-GBM interactions. In this review, recent findings on the complex nature of MSCs will be highlighted, focusing on their elusive impact on GBM progression and aggressiveness by

direct cell-cell interaction and via secretome, also facing the perspectives and challenges in treatment strategies.

Bioengineering (Basel). 2020 Jun 17;7(2):E59.
doi: 10.3390/bioengineering7020059.

Human Mesenchymal Stem Cells Overexpressing Interleukin 2 Can Suppress Proliferation of Neuroblastoma Cells in Co-Culture and Activate Mononuclear Cells In Vitro

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- PMID: 32560387

- DOI: 10.3390/bioengineering7020059

Free article

Abstract

High-dose recombinant interleukin 2 (IL2) therapy has been shown to be successful in renal cell carcinoma and metastatic melanoma. However, systemic administration of high doses of IL2 can be toxic, causing capillary leakage syndrome and stimulating pro-tumor immune response. One of the strategies to reduce the systemic toxicity of IL2 is the use of mesenchymal stem cells (MSCs) as a vehicle for the targeted delivery of IL2. Human adipose tissue-derived MSCs were transduced with lentivirus encoding *IL2* (hADSCs-IL2) or blue fluorescent protein (BFP) (hADSCs-BFP). The proliferation, immunophenotype, cytokine profile and ultrastructure of hADSCs-IL2 and hADSCs-BFP were determined. The effect of hADSCs on activation of peripheral blood mononuclear cells (PBMCs) and proliferation and viability of SH-SY5Y neuroblastoma cells after co-culture with native hADSCs, hADSCs-BFP or hADSCs-IL2 on plastic and Matrigel was evaluated. Ultrastructure and cytokine production by hADSCs-IL2 showed modest changes in comparison with hADSCs and hADSCs-BFP. Conditioned medium from hADSC-IL2 affected tumor cell

proliferation, increasing the proliferation of SH-SY5Y cells and also increasing the number of late-activated T-cells, natural killer (NK) cells, NKT-cells and activated T-killers. Conversely, hADSC-IL2 co-culture led to a decrease in SH-SY5Y proliferation on plastic and Matrigel. These data show that hADSCs-IL2 can reduce SH-SY5Y proliferation and activate PBMCs in vitro. However, IL2-mediated therapeutic effects of hADSCs could be offset by the increased expression of pro-oncogenes, as well as the natural ability of hADSCs to promote the progression of some tumors.

World J Stem Cells

. 2020 May 26;12(5):368-380.
doi: 10.4252/wjsc.v12.i5.368.

Safety of Menstrual Blood-Derived Stromal Cell Transplantation in Treatment of Intrauterine Adhesion

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- PMID: 32547685
- PMCID: PMC7280865
- DOI: 10.4252/wjsc.v12.i5.368

Free PMC article

Abstract

Background: Intrauterine adhesion (IUA) can cause serious damage to women's reproductive health, yet current treatment methods are difficult to achieve satisfactory results. In our previous studies, we demonstrated that menstrual-derived stromal stem cells (MenSCs), with high proliferative capacity and self-renewal ability, have a powerful therapeutic effect in patients with severe IUA. However, safety assessment of MenSCs transplantation is essential for its further application.

Aim: To evaluate the short-, medium-, and long-term biosafety of MenSCs *via* intrauterine transplantation in a rat model of IUA, with a focus on toxicity and tumorigenicity.

Methods: MenSCs were injected into the sub-serosal layer of the uterus in an IUA rat model, for 3 d, 3 mo, and 6 mo separately, to monitor the corresponding acute, sub-chronic, and chronic effects. Healthy rats of the same age served as negative controls. Toxicity effects were evaluated by body weight, organ weight, histopathology, hematology, and biochemistry tests. Tumorigenicity of MenSCs was investigated in Balb/c-nu mice *in vivo* and by colony formation assays *in vitro*.

Results: Compared with the same week-old control group, all of the IUA rats receiving MenSC transplantation demonstrated no obvious changes in body weight, main organ weight, or blood cell composition during the acute, sub-chronic, and chronic observation periods. At the same time, serum biochemical tests showed no adverse effects on metabolism or liver and kidney function. After 4 wk of subcutaneous injection of MenSCs in Balb/c-nu nude mice, no tumor formation or cell metastasis was observed. Moreover, there was no tumor colony formation of MenSCs during soft agar culture *in vitro*.

Conclusion: There is no acute, sub-chronic, or chronic poisoning, infection, tumorigenesis, or endometriosis in rats with IUA after MenSC transplantation. The above results suggest that intrauterine transplantation of MenSCs is safe for endometrial treatment.

Int J Mol Sci. 2020 Jun 16;21(12):E4298.
doi: 10.3390/ijms21124298.

A Dynamic Hanging-Drop System for Mesenchymal Stem Cell Culture

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- PMID: 32560269

- DOI: 10.3390/ijms21124298

Free article

Abstract

There have been many microfluid technologies combined with hanging-drop for cell culture gotten developed in the past decade. A common problem within these devices is that the cell suspension introduced at the central inlet could cause a number of cells in each microwell to not regularize. Also, the instability of droplets during the spheroid formation remains an unsolved ordeal. In this study, we designed a microfluidic-based

hanging-drop culture system with the design of taper-tube that can increase the stability of droplets while enhancing the rate of liquid exchange. A ring is surrounding the taper-tube. The ring can hold the cells to enable us to seed an adequate amount of cells before perfusion. Moreover, during the period of cell culture, the mechanical force around the cell is relatively low to prevent stem cells from differentiate and maintain the phenotype. As a result of our hanging system design, cells are designed to accumulate at the bottom of the droplet. This method enhances convenience for observation activities and analysis of experiments. Thus, this microfluid chip can be used as an in vitro platform representing in vivo physiological conditions, and can be useful in regenerative therapy.

Arthroscopy

. 2020 Jun 15;S0749-8063(20)30525-9.
doi: 10.1016/j.arthro.2020.06.009. Online ahead of print.

Proximal Humerus and Ilium Are Reliable Sources of Bone Marrow Aspirates for Biologic Augmentation During Arthroscopic Surgery

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- PMID: 32554079

- DOI: 10.1016/j.arthro.2020.06.009

Abstract

Purpose: The purpose of this study was to evaluate the number of colony-forming units (CFUs) derived from concentrated bone marrow aspirates (BMAs) that were processed following arthroscopic harvest from either the proximal humerus or the body of the ilium during biologic augmentation of the rotator cuff and acetabular labral repairs.

Methods: Between November 2014 and January 2019, BMA was harvested from the proximal humerus (n = 89) and the body of the ilium (n = 30) during arthroscopic surgery. Following concentration of the aspirate, a 0.5 ml aliquot was further processed and the number of nucleated cells (NC) was counted. Each aliquot was cultured until CFUs were

quantifiable. Fluorescence-activated cell sorting analysis and quantitative polymerase chain reaction was performed to confirm presence of mesenchymal stem cells. BMA harvest sites were prospectively assessed and evaluated for differences in age, sex, volume of aspirated BM, and CFUs per ml of BMA.

Results: The prevalence ($38.57\pm27.92_{ilium}$ vs. $56.00\pm25.60_{humerus}$ CFUs per 10^6 nucleated cells) and concentration ($979.17\pm740.31_{ilium}$ vs. $1,516.62\pm763.63_{humerus}$ CFUs per 1.0 ml BMA) of CFUs was significantly higher ($p<0.001$, respectively) for BMA harvested from the proximal humerus. Additionally, the estimated total number of cells was significantly higher ($p=0.013$) in BMA from the proximal humerus ($97,529.00\pm91,064.01_{ilium}$ vs. $130,552.4\pm85,294.2_{humerus}$). There was no significant difference between groups regarding BMA volume ($91.67\pm18.77_{ilium}$ vs. $85.63\pm35.61_{humerus}$ ml; $p=0.286$) and NC count ($24.01\pm5.13_{ilium}$ vs. $27.07 \pm 6.28_{humerus}$ x 10^6 per ml BMA; $p=0.061$). The mean age was significantly lower ($p<0.001$) in patients with BMA being harvested from the ilium ($30.18\pm7.63_{ilium}$ vs. $56.82\pm7.08_{humerus}$ years). Patient sex and age had no significant influence on cellular measures within groups ($p>0.05$, respectively).

Conclusion: Both proximal humerus and the body of the ilium can be considered reliable sources of bone marrow aspirate for the use in biologic augmentation during their respective arthroscopic surgery. Samples of bone marrow aspirate from the proximal humerus yielded a significantly higher amount of colony-forming units when compared to samples of bone marrow aspirate obtained from the ilium.

Clinical relevance: This study may assist orthopaedic surgeons, who are performing biologic augmentation at the shoulder or hip joint, to better understand the properties of BMA by harvest site and facilitate decision-making between utilizing a local donor site or the iliac crest.

Gene X. 2020 Mar 14;5:100031.
doi: 10.1016/j.gene.2020.100031. eCollection 2020 Dec.

ALCAM (CD166) as a Gene Expression Marker for Human Mesenchymal Stromal Cell Characterisation

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- PMID: 32550557

- PMCID: PMC7285916

- DOI: 10.1016/j.gene.2020.100031

Free PMC article

Abstract

Background: Human mesenchymal stromal cells (MSCs) phenotypically share their positive expression of the International Society for Cell and Gene Therapy (ISCT) markers CD73, CD90 and CD105 with fibroblasts. Fibroblasts are often co-isolated as an unwanted by-product from biopsy and they can rapidly overgrow the MSCs in culture. Indeed, many other surface markers have been proposed, though no unique MSC specific marker has been identified yet. Quantitative PCR (qPCR) is a precise, efficient and rapid method for gene expression analysis. To identify a marker suitable for accurate MSC characterisation, qPCR was exploited.

Methods and results: Two commercially obtained bone marrow (BM) derived MSCs and an hTERT immortalised BM-MSC line (MSC-TERT) have been cultured for different days and at different oxygen levels before RNA extraction. Together with RNA samples previous extracted from umbilical cord derived MSCs and MSC-TERT cells cultured in 2D or 3D, this heterogeneous sample set was quantitatively analysed for the expression levels of 18 candidate MSC marker genes. The expression levels in MSCs were compared with the expression levels in fibroblasts to verify the differentiation capability of these genes between MSCs and fibroblasts. None of the ISCT markers could differentiate between fibroblasts and MSCs. A total of six other genes (*ALCAM*, *CLIC1*, *EDIL3*, *EPHA2*, *NECTIN2*, and *TMEM47*) were identified as possible biomarkers for accurate identification of MSCs.

Conclusion: Justified by considerations on expression level, reliability and specificity, Activated-Leukocyte Cell Adhesion Molecule (*ALCAM*) was the best candidate for improving the biomarker set of MSC identification.

Keywords: (q)PCR, (quantitative) polymerase chain reaction; AD, adipose; AF, Amniotic Fluid; ALCAM, Activated-Leukocyte Cell Adhesion Molecule; Activated-leukocyte cell adhesion molecule; BM, bone marrow; BSG, Basigin; Biomarker; CD, cluster of differentiation; CLIC1, chloride intracellular channel 1; CLIC4, chloride intracellular channel 4; Cq, Quantification cycle; DF, Dermal Fibroblasts; DP, Dental Pulp; EDIL3, EGF like repeats and discoidin domains 3; ENG, Endoglin; EPHA2, EPH receptor A2; ER, Endoplasmatic Reticulum; FACS, Fluorescence Assisted Cell Sorting; FN1, Fibronectin 1; IGFBP7, insulin like growth factor binding protein 7; ISCT, International Society for Cell and Gene Therapy; ITGA1, integrin subunit alpha 1; LAMP1, lysosomal associated membrane protein 1; LRRC59, leucine rich repeat containing 59; MCAM, melanoma cell adhesion molecule; MM, Multiple Myeloma; MPC, Mesenchymal Progenitor Cell; MSC; MSC, Mesenchymal Stromal Cells; NECTIN2, nectin cell adhesion molecule 2; NK, Natural Killer; NT5E, 5′-nucleotidase ecto; OS, Osteosarcoma; PL, Placenta; PPIA, peptidylprolyl isomerase A; PUM1, pumilio

RNA binding family member 1; RM, Regenerative Medicine; RNA; RNA-seq, RNA sequencing; RT, Reverse Transcriptase; Regenerative medicine; SEM, Standard Error of the Mean; TBP, TATA-box binding protein; TCF, Tissue Culture Plate; TE, Tissue Engineering; TFRC, transferrin receptor; THY1, Thy-1 cell surface antigen; TLN1, Talin 1; TMEM47, transmembrane protein 47; UC, umbilical cord; YWHAZ, tyrosine 3-monooxygenase/t

J Transl Med

. 2020 Jun 16;18(1):241.
doi: 10.1186/s12967-020-02410-z.

Mesenchymal Stem Cells Activate Notch Signaling to Induce Regulatory Dendritic Cells in LPS-induced Acute Lung Injury

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- PMID: 32546185

- PMCID: PMC7298963

- DOI: 10.1186/s12967-020-02410-z

Free PMC article

Abstract

Background: Mesenchymal stem cells (MSCs) have been shown to alleviate acute lung injury (ALI) and induce the production of regulatory dendritic cells (DCregs), but the potential link between these two cell types remains unclear. The goal of this study was to investigate the effect and mechanism of MSC-induced regulatory dendritic cells in ALI mice.

Material/methods: In vivo experiments, C57BL/6 wild-type male mice were sacrificed at different times after intratracheal injection of LPS to observe changes in lung DC maturation and pathological damage. MSCs, DCregs or/and carboxyfluorescein diacetate

succinimidyl ester (CFSE)-labeled DCs were administered to the mice by tail vein, and flow cytometry was performed to measure the phenotype of lung DCs and T cells. Lung injury was estimated by the lung wet weight/body weight ratio and histopathological analysis. In vitro, Western blotting or flow cytometry was used to detect the expression of Notch ligand or receptor in MSCs or DCs after coculture or LPS stimulation. Finally, in vivo and in vitro, we used the Notch signaling inhibitor DAPT to verify the effect of the Notch pathway on MSC-induced DCregs and their pulmonary protection.

Results: We showed significant accumulation and maturation of lung DCs 2 h after intratracheal injection of LPS, which were positively correlated with the lung pathological injury score. MSC treatment alleviated ALI lung injury, accompanied by a decrease in the number and maturity of classical DCs in the lungs. CFSE-labeled DCs migrated to the lungs of ALI mice more than those of the normal group, and the elimination of CFSE-labeled DCs in the blood was slower. MSCs inhibited the migration of CFSE-labeled DCs to the lung and promoted their elimination in the blood. DCregs, which are obtained by contact coculture of mDCs with MSCs, expressed reduced levels of MHCII, CD86, CD40 and increased levels of PD-L1, and had a reduced ability to stimulate lymphocyte proliferation and activation (expression of CD44 and CD69). mDCs expressing Notch2 significantly increased after coculture with MSCs or rhJagged1, and MSCs expressed more Jagged1 after LPS stimulation. After stimulation of mDCs with recombinant Jagged1, DCs with low expression of MHCII, CD86 and CD40 were also induced, and the effects of both rhJagged1 and MSCs on DCs were blocked by the Notch inhibitor DAPT. Intra-airway DAPT reversed the inhibitory effect of mesenchymal stem cells on DC recruitment to the lungs and its maturation.

Conclusions: Our results suggested that the recruitment and maturation of lung DCs is an important process in early ALI, MSCs attenuate LPS-induced ALI by inducing the production of DCregs by activating Notch signaling.

Cells

. 2020 Jun 11;9(6):E1453.
doi: 10.3390/cells9061453.

Veterinary Regenerative Medicine for Musculoskeletal Disorders: Can Mesenchymal Stem/Stromal Cells and Their Secretome Be the New Frontier?

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- PMID: 32545382

- DOI: 10.3390/cells9061453

Free article

Abstract

Regenerative medicine aims to restore the normal function of diseased or damaged cells, tissues, and organs using a set of different approaches, including cell-based therapies. In the veterinary field, regenerative medicine is strongly related to the use of mesenchymal stromal cells (MSCs), which belong to the body repair system and are defined as multipotent progenitor cells, able to self-replicate and to differentiate into different cell types. This review aims to take stock of what is known about the MSCs and their use in the veterinary medicine focusing on clinical reports on dogs and horses in musculoskeletal diseases, a research field extensively reported in the literature data. Finally, a perspective regarding the use of the secretome and/or extracellular vesicles (EVs) in the veterinary field to replace parental MSCs is provided. The pharmaceuticalization of EVs is wished due to the realization of a Good Manufacturing Practice (GMP product suitable for clinical trials.