Spheroidal formation preserves human stem cells for prolonged time under ambient conditions for facile storage and transportation.

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Abstract
Human stem cells are vulnerable to unfavorable conditions, and their transportation relies on costly and inconvenient cryopreservation. We report here that human mesenchymal stem cells (MSC) in spheroids survived ambient conditions (AC) many days longer than in monolayer. Under AC, the viability of MSC in spheroids remained >90% even after seven days, whereas MSC in monolayer mostly died fast. AC-exposed MSC spheroids, after recovery under normal monolayer culture conditions with controlled carbon dioxide and humidity contents, resumed typical morphology and proliferation, and retained differentiating and immnosuppressive capabilities. RNA-sequencing and other assays demonstrate that reduced cell metabolism and proliferation correlates to the enhanced survival of AC-exposed MSC in spheroids versus monolayer. Moreover, AC-exposed MSC, when injected as either single cells or spheroids, retained therapeutic effects in vivo in mouse colitis models. Spheroidal formation also prolonged survival and sustained pluripotency of human embryonic stem cells kept under AC. Therefore, this work offers an alternative and relatively simple method termed spheropreservation versus the conventional method cryopreservation. It shall remarkably simplify long-distance transportation of stem cells of these and probably also other types within temperature-mild areas, and facilitate therapeutic application of MSC as spheroids without further processing.

Hyaluronan keeps mesenchymal stem cells quiescent and maintains the differentiation potential over time.

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Abstract
Hyaluronan (HA), an abundant polysaccharide found in human bodies, plays a role in the mesenchymal stem cells (MSCs) maintenance. We had previously found that HA prolonged the lifespan, and prevented the cellular aging of murine adipose-derived stromal cells. Recently, we had also summarized the potential pathways associated with HA regulation in human MSCs. In this study, we used the human placenta-derived MSCs (PDMSC) to investigate the effectiveness of HA in maintaining the PDMSC. We found that coating the culture surface coated with 30 μg cm⁻² of HA (C) led to cluster growth of PDMSC, and maintained a higher number of PDMSC in quiescence compared to those grown on the normal tissue culture surface (T). PDMSC were treated for either 4 (short-term) or 19
(long-term) consecutive passages. PDMSC which were treated with HA for 19 consecutive passages had reduced cell enlargement, preserved MSCs biomarker expressions and osteogenic potential when compared to those grown only on T. The PDMSC transferred to T condition after long-term HA treatment showed preserved replicative capability compared to those on only T. The telomerase activity of the HA-treated PDMSC was also higher than that of untreated PDMSC. These data suggested a connection between HA and MSC maintenance. We suggest that HA might be regulating the distribution of cytoskeletal proteins on cell spreading in the event of quiescence to preserve MSC stemness. Maintenance of MSCs stemness delayed cellular aging, leading to the anti-aging phenotype of PDMSC.


Effects of altered CXCL12/CXCR4 axis on BMP2/Smad/Runx2/Osterix axis and osteogenic gene expressions during osteogenic differentiation of MSCs.

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Author information

Abstract

This study investigated the effects of altered CXCL12/CXCR4 axis on the bone morphogenetic protein 2 (BMP-2)/Smad/runt-related transcription factor 2 (Runx2)/Osterix (Osx) signal axis and osteogenic gene expression during osteogenic differentiation of mesenchymal stem cells (MSCs), to gain understanding of the link between migration and osteogenic differentiation signal axis and MSCs osteogenic differentiation mechanisms. The pHBA-MCMV-CXCL12-GFP vector (Ad-CXCL12) was constructed and quantitative polymerase chain reaction (qPCR)/western blotting used to determine CXCL12 expression in Ad-CXCL12-transfected MSCs. MSCs were treated with Ad-CXCL12 and AMD3100 (CXCL12 inhibitor) to detect BMP-2/Smad/Runx2/Osterix expression, bone sialoprotein (BSP), osteocalcin (OCN) and osteopontin (OPN) mRNA expression, and alkaline phosphatase (ALP) activity. PCR and sequencing confirmed successful construction of Ad-CXCL12. qPCR and enzyme-linked immunosorbent assay indicated that Ad-CXCL12 transfection promoted CXCL12 expression in MSCs. At 72 hours, Runx2 and Osterix, and Smad1/5/8 mRNA and protein expressions were significantly higher in the Ad-CXCL12 group than in the control group (P < 0.01). At 1 and 2 weeks, ALP activity and BSP mRNA expression were significantly higher in the Ad-CXCL12 group than in the control group (P < 0.01), respectively. No significant difference in OCN and OPN mRNA expression was determined between Ad-CXCL12 and control groups (P > 0.05). At 3 weeks, no significant difference in mineralized nodule staining was observed between groups (P > 0.05). Changes in the CXCL12/CXCR4 migration axis affected the BMP-2/Smad/Runx2/Osterix axis and BSP, OCN and OPN mRNA expression in early-stage, but not mid-/latestage, MSCs osteogenic differentiation, therefore affecting the ability of MSCs to undergo osteogenic differentiation.

Stemness Maintenance Properties in Human Oral Stem Cells after Long-Term Passage.

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Abstract

Background. Neural crest-derived mesenchymal stem cells (MSCs) from human oral tissues possess immunomodulatory and regenerative properties and are emerging as a potential therapeutic tool to treat diverse diseases, such as multiple sclerosis, myocardial infarction, and connective tissue damages. In addition to cell-surface antigens, dental MSCs express embryonic stem cell markers as neural crest cells originate from the ectoderm layer. In vitro passages may eventually modify these embryonic marker expressions and other stemness properties, including proliferation. In the present study, we have investigated the expression of proteins involved in cell proliferation/senescence and embryonic stem cell markers during early (passage 2) and late passages (passage 15) in MSCs obtained from human gingiva, periodontal, and dental pulp tissues. Methods. Cell proliferation assay, beta galactosidase staining, immunocytochemistry, and real-time PCR techniques were applied. Results. Cell proliferation assay showed no difference between early and late passages while senescence markers p16 and p21 were considerably increased in late passage. Embryonic stem cell markers including SKIL, MEIS1, and JARID2 were differentially modulated between P2 and P15 cells. Discussion. Our results suggest that the presence of embryonic and proliferation markers even in late passage may potentially endorse the application of dental-derived MSCs in stem cell therapy-based clinical trials.


Aging of bone marrow- and umbilical cord-derived mesenchymal stromal cells during expansion.

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Abstract

BACKGROUND AIMS:

Mesenchymal stromal cells (MSCs) are used as experimental immunotherapy. Extensive culture expansion is necessary to obtain clinically relevant cell numbers, although the impact on MSCs stability and function is unclear. This study investigated the effects of long-term in vitro expansion on the stability and function of MSCs.

METHODS:

Human bone marrow-derived (bmMSCs) and umbilical cord-derived (ucMSCs) MSCs were in vitro expanded. During expansion, their proliferative capacity was examined. At passages 4, 8 and 12,
analyses were performed to investigate the ploidy, metabolic stability, telomere length and immunophenotype. In addition, their potential to suppress lymphocyte proliferation and susceptibility to natural killer cell lysis was examined.

RESULTS:
BmMSCs and ucMSCs showed decreasing proliferative capacity over time, while their telomere lengths and mitochondrial activity remained stable. Percentage of aneuploidy in cultures was unchanged after expansion. Furthermore, expression of MSC markers and markers associated with stress or aging remained unchanged. Reduced capacity to suppress CD4 and CD8 T-cell proliferation was observed for passage 8 and 12 bmMSCs and ucMSCs. Finally, susceptibility of bmMSCs and ucMSCs to NK-cell lysis remained stable.

CONCLUSIONS:
We showed that after long-term expansion, phenotype of bmMSCs and ucMSCs remains stable and cells exhibit similar immunogenic properties compared with lower passage cells. However, immunosuppressive properties of MSCs are reduced. These findings reveal the consequences of application of higher passage MSCs in the clinic, which will help increase the yield of therapeutic MSCs but may interfere with their efficacy.


The production method affects the efficacy of platelet derivatives to expand mesenchymal stromal cells in vitro.

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Abstract

BACKGROUND:
The use of fetal bovine serum (FBS) as a media supplement for the ex vivo expansion of bone-marrow derived mesenchymal stromal cells (BM-MSC) has been discouraged by regulatory agencies, due to the risk of transmitting zoonoses and to elicit immune reactions in the host once transplanted. Platelet derivatives are valid FBS substitutes due to their content of growth factors that can be released disrupting the platelets by physical methods or physiological stimuli. We compared platelet derivatives produced by freezing/thawing (platelet lysates, PL) or after CaCl2 activation (platelet releasate surfatant rich in growth factors, PR-SRGF) for their content in growth factors and their ability to support the ex vivo expansion of BM-MSC.

METHODS:
The cytokine content in the two platelet derivatives was evaluated. BM-MSC were expanded in complete medium containing 10, 7.5 and 5% PL or PR-SRGF and the cell phenotype, clonogenic capacity, immunomodulation properties and tri-lineage differentiation potential of the expanded cells in both media were investigated.
RESULTS:
The concentration of PDGF-AB, PDGF-AA, PDGF-BB in PR-SRGF resulted to be respectively 5.7×, 1.7× and 2.3× higher compared to PL. PR-SRGF promoted a higher BM-MSC proliferation rate compared to PL not altering BM-MSC phenotype. Colony forming efficiency of BM-MSC expanded in PR-SRGF showed a frequency of colonies significantly higher than cells expanded in PL. BM-MSC expanded in PL or PR-SRGF maintained their immunomodulatory properties against activated lymphocytes even if BM-MSC expanded in FBS performed significantly better.

CONCLUSIONS:
The method used to release platelet factors significantly affects the enrichment in growth factors and overall product performance. The standardization of the production process of platelet derivatives and the definition of their release criteria requires further investigation.

Intratumoral acidosis fosters cancer-induced bone pain through the activation of the mesenchymal tumor-associated stroma in bone metastasis from breast carcinoma.

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Abstract
Cancer-induced bone pain (CIBP) is common in patients with bone metastases (BM), significantly impairing quality of life. The current treatments for CIBP are limited since they are often ineffective. Local acidosis derived from glycolytic carcinoma and tumor-induced osteolysis is only barely explored cause of pain. We found that breast carcinoma cells that prefer bone as a metastatic site have very high extracellular proton efflux and expression of pumps/ion transporters associated with acid-base balance (MCT4, CA9, and V-ATPase). Further, the impairment of intratumoral acidification via V-ATPase targeting in xenografts with BM significantly reduced CIBP, as measured by incapacitance test. We hypothesize that in addition to the direct acid-induced stimulation of nociceptors in the bone, a novel mechanism mediated by the acid-induced and tumor-associated mesenchymal stroma might ultimately lead to nociceptor sensitization and hyperalgesia. Consistent with this, short-term exposure of cancer-associated fibroblasts, mesenchymal stem cells, and osteoblasts to pH 6.8 promotes the expression of inflammatory and nociceptive mediators (NGF, BDNF, IL6, IL8, IL1b and CCL5). This is also consistent with a significant correlation between breakthrough pain, measured by pain questionnaire, and combined high serum levels of BDNF and IL6 in patients with BM, and also by immunofluorescence staining showing IL8 expression that was more in mesenchymal stromal cells rather than in tumors cells, and close to LAMP-2 positive acidifying carcinoma cells in BM tissue sections. In summary, intratumoral acidification in BM might promote CIBP also by activating the tumor-associated stroma, offering a new target for palliative treatments in advanced cancer.
Bone Marrow-Derived Mesenchymal Stem Cells as Autologous Therapy in Dogs with Naturally Occurring Intervertebral Disc Disease: Feasibility, Safety and Preliminary Results.

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Author information

Abstract

The intradiscal application of mesenchymal stem cells (MSC) is considered a promising strategy for intervertebral disc (IVD) regeneration. Although many studies have been published, the feasibility and regenerative effects of intradiscal MSC application has not been evaluated in an animal model suffering from naturally occurring degenerative disc disease. Six German Shepherd Dogs suffering from naturally occurring degenerative IVD disease were included. Autologous MSC were isolated from bone marrow (iliac crest) and cultured for 3 weeks. After decompressive spinal surgery, 3 dogs received an intradiscal injection of MSC, while the other 3 dogs received an intradiscal injection of saline (control). Clinical status, disc height index, Pfirrmann grading, and disc volumetry were evaluated at 1, 5, 6, and 12 months after treatment. Autologous application of canine MSC was feasible and successful in all dogs. No evident complications were found. Surgery resulted in an equal improvement in clinical status in the treatment and control dogs. In the MSC group, the Pfirrmann grade increased in all patients over time, whereas in the control group the Pfirrmann grade remained stable. The volume of the L7-S1 IVD gradually increased during the 12-month study period in all dogs, with no evident difference between the MSC and control group. On the basis of this preliminary study, it can be concluded that intradiscal injection of autologous MSC in dogs with spontaneous degenerative IVD disease is well tolerated without any adverse effects, does not affect clinical outcome, and does not have any evident regenerative effects.