Stem Cells Int. 2018 Mar 11;2018:5034679. doi: 10.1155/2018/5034679. eCollection 2018.

Sphingosine 1-Phosphate Receptor 1 Is Required for MMP-2 Function in Bone Marrow Mesenchymal Stromal Cells: Implications for Cytoskeleton Assembly and Proliferation.

Sassoli C[1], Pierucci F[2], Tani A[1], Frati A[2], Chellini F[1], Matteini F[2], Vestri A[2], Anderloni G[2], Nosi D[1], Zecchi-Orlandini S[1], Meacci E[2].
Author information

Abstract

Bone marrow-derived mesenchymal stromal cell- (BM-MSC-) based therapy is a promising option for regenerative medicine. An important role in the control of the processes influencing the BM-MSC therapeutic efficacy, namely, extracellular matrix remodelling and proliferation and secretion ability, is played by matrix metalloproteinase- (MMP-) 2. Therefore, the identification of paracrine/autocrine regulators of MMP-2 function may be of great relevance for improving BM-MSC therapeutic potential. We recently reported that BM-MSCs release the bioactive lipid sphingosine 1-phosphate (S1P) and, here, we demonstrated an impairment of MMP-2 expression/release when the S1P receptor subtype S1PR1 is blocked. Notably, active S1PR1/MMP-2 signalling is required for F-actin structure assembly (lamellipodia, microspikes, and stress fibers) and, in turn, cell proliferation. Moreover, in experimental conditions resembling the damaged/regenerating tissue microenvironment (hypoxia), S1P/S1PR1 system is also required for HIF-1*α* expression and vinculin reduction. Our findings demonstrate for the first time the trophic role of S1P/S1PR1 signalling in maintaining BM-MSCs' ability to modulate MMP-2 function, necessary for cytoskeleton reorganization and cell proliferation in both normoxia and hypoxia. Altogether, these data provide new perspectives for considering S1P/S1PR1 signalling a pharmacological target to preserve BM-MSC properties and to potentiate their beneficial potential in tissue repair.

Eur Radiol Exp. 2017;1(1):6. doi: 10.1186/s41747-017-0010-9. Epub 2017 Jun 29.

In vitro labelling and detection of mesenchymal stromal cells: a comparison between magnetic resonance imaging of iron-labelled cells and magnetic resonance spectroscopy of fluorine-labelled cells.

Rizzo S[1], Petrella F[2,3], Zucca I[4], Rinaldi E[5], Barbaglia A[4], Padelli F[4], Baggi F[5], Spaggiari L[2,3], Bellomi M[1,3], Bruzzone MG[6].
Author information

Abstract

BACKGROUND:

Among the various stem cell populations used for cell therapy, adult mesenchymal stromal cells (MSCs) have emerged as a major new cell technology. These cells must be tracked after transplantation to monitor their migration within the body and quantify their accumulation at the target site. This study assessed whether rat bone marrow MSCs can be labelled with superparamagnetic iron

oxide (SPIO) nanoparticles and perfluorocarbon (PFC) nanoemulsion formulations without altering cell viability and compared magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) results from iron-labelled and fluorine-labelled MSCs, respectively.

METHODS:

Of MSCs, 2×10^6 were labelled with Molday ION Rhodamine-B (MIRB) and 2×10^6 were labelled with Cell Sense. Cell viability was evaluated by trypan blue exclusion method. Labelled MSCs were divided into four samples containing increasing cell numbers (0.125×10^6, 0.25×10^6, 0.5×10^6, 1×10^6) and scanned on a 7T MRI: for MIRB-labelled cells, phantoms and cells negative control, T1, T2 and T2* maps were acquired; for Cell Sense labelled cells, phantoms and unlabelled cells, a ^{19}F non-localised single-pulse MRS sequence was acquired.

RESULTS:

In total, 86.8% and 83.6% of MIRB-labelled cells and Cell Sense-labelled cells were viable, respectively. MIRB-labelled cells were visible in all samples with different cell numbers; pellets containing 0.5×10^6 and 1×10^6 of Cell Sense-labelled cells showed a detectable ^{19}F signal.

CONCLUSIONS:

Our data support the use of both types of contrast material (SPIO and PFC) for MSCs labelling, although further efforts should be dedicated to improve the efficiency of PFC labelling.

Stem Cell Res Ther. 2018 May 2;9(1):124. doi: 10.1186/s13287-018-0863-8.

Human platelet lysate in mesenchymal stromal cell expansion according to a GMP grade protocol: a cell factory experience.

Becherucci V[1], Piccini L[2], Casamassima S[2], Bisin S[2], Gori V[2], Gentile F[2], Ceccantini R[2], De Rienzo E[2], Bindi B[3], Pavan P[3], Cunial V[3], Allegro E[3], Ermini S[3], Brugnolo F[2], Astori G[4], Bambi F[2].

Author information

Abstract

BACKGROUND:

The use of platelet lysate (PL) for the ex-vivo expansion of mesenchymal stromal/stem cells (MSCs) was initially proposed by Doucet et al. in 2005, as an alternative to animal serum. Moreover, regulatory authorities discourage the use of fetal bovine serum (FBS) or other animal derivatives, to avoid risk of zoonoses and xenogeneic immune reactions. Even if many studies investigated PL composition, there still are some open issues related to its use in ex-vivo MSC expansion, especially according to good manufacturing practice (GMP) grade protocols.

METHODS:

As an authorized cell factory, we report our experience using standardized PL produced by Azienda Ospedaliero Universitaria Meyer Transfusion Service for MSC expansion according to a GMP grade clinical protocol. As suggested by other authors, we performed an in-vitro test on MSCs versus MSCs cultured with FBS that still represents the best way to test PL batches. We compared 12 MSC batches cultured with DMEM 5% PL with similar batches cultured with DMEM 10% FBS, focusing on the MSC

proliferation rate, MSC surface marker expression, MSC immunomodulatory and differentiation potential, and finally MSC relative telomere length.

RESULTS:

Results confirmed the literature data as PL increases cell proliferation without affecting the MSC immunophenotype, immunomodulatory potential, differentiation potential and relative telomere length.

CONCLUSIONS:

PL can be considered a safe alternative to FBS for ex-vivo expansion of MSC according to a GMP grade protocol. Our experience confirms the literature data: a large number of MSCs for clinical applications can be obtained by expansion with PL, without affecting the MSC main features. Our experience underlines the benefits of a close collaboration between the PL producers (transfusion service) and the end users (cell factory) in a synergy of skills and experiences that can lead to standardized PL production.

KEYWORDS:

Advanced therapy medicinal products; Cell factory; Fetal bovine serum; Good manufacturing practice; Mesenchymal stromal/stem cells; Platelet lysate

Oncotarget. 2018 Apr 10;9(27):19328-19341. doi: 10.18632/oncotarget.25039. eCollection 2018 Apr 10.

Stress and stem cells: adult Muse cells tolerate extensive genotoxic stimuli better than mesenchymal stromal cells.

Alessio N[1], Squillaro T[1], Özcan S[2], Di Bernardo G[1], Venditti M[1], Melone M[3,4], Peluso G[5], Galderisi U[2,1,3].

Author information

Abstract

Mesenchymal stromal cells (MSCs) are not a homogenous population but comprehend several cell types, such as stem cells, progenitor cells, fibroblasts, and other types of cells. Among these is a population of pluripotent stem cells, which represent around 1-3% of MSCs. These cells, named multilineage-differentiating stress enduring (Muse) cells, are stress-tolerant cells. Stem cells may undergo several rounds of intrinsic and extrinsic stresses due to their long life and must have a robust and effective DNA damage checkpoint and DNA repair mechanism, which, following a genotoxic episode, promote the complete recovery of cells rather than triggering senescence and/or apoptosis. We evaluated how Muse cells can cope with DNA damaging stress in comparison with MSCs. We found that Muse cells were resistant to chemical and physical genotoxic stresses better than non-Muse cells. Indeed, the level of senescence and apoptosis was lower in Muse cells. Our results proved that the DNA damage repair system (DDR) was properly activated following injury in Muse cells. While in non-Muse cells some anomalies may have occurred because, in some cases, the activation of the DDR persisted by 48 hr post damage, in others no activation took place. In Muse cells, the non-homologous end joining (NHEJ) enzymatic activity increases compared to other cells, while single-strand repair activity (NER, BER) does not. In conclusion, the high ability of Muse cells to cope with genotoxic stress is related to their quick and efficient sensing of DNA damage and activation of DNA repair systems.

J Mol Neurosci. 2018 Apr 28. doi: 10.1007/s12031-018-1070-x. [Epub ahead of print]

Mesenchymal Stem Cells Form 3D Clusters Following Intraventricular Transplantation.

Jungwirth N[1,2], Salinas Tejedor L[2,3], Jin W[1,2], Gudi V[2,3], Skripuletz T[3], Stein VM[4], Tipold A[2,4], Hoffmann A[5], Stangel M[2,3], Baumgärtner W[6,7], Hansmann F[1,2].
Author information

Abstract

Mesenchymal stem cells (MSCs) are regarded as an immune privileged cell type with numerous regeneration-promoting effects. The in vivo behavior of MSC and underlying mechanisms leading to their regenerative effects are largely unknown. The aims of this study were to comparatively investigate the in vivo behavior of canine (cMSC), human (hMSC), and murine MSC (mMSC) following intra-cerebroventricular transplantation. At 7 days post transplantation (dpt), clusters of cMSC, hMSC, and mMSC were detected within the ventricular system. At 49 dpt, cMSC-transplanted mice showed clusters mostly consisting of extracellular matrix lacking transplanted MSC. Similarly, hMSC-transplanted mice lacked MSC clusters at 49 dpt. Xenogeneic MSC transplantation was associated with a local T lymphocyte-dominated immune reaction at both time points. Interestingly, no associated inflammation was observed following syngeneic mMSC transplantation. In conclusion, transplanted MSC formed intraventricular cell clusters and exhibited a short life span in vivo. Xenogeneically in contrast to syngeneically transplanted MSC triggered a T cell-mediated graft rejection indicating that MSCs are not as immune privileged as previously assumed. However, MSC may mediate their effects by a "hit and run" mechanism and future studies will show whether syngeneically or xenogeneically transplanted MSCs exert better therapeutic effects in animals with CNS disease.

Res Vet Sci. 2018 Mar 21;118:423-430. doi: 10.1016/j.rvsc.2018.03.012. [Epub ahead of print]

Allogeneic mesenchymal stem cell transplantation in healthy equine superficial digital flexor tendon: A study of the local inflammatory response.

Brandão JS[1], Alvarenga ML[1], Pfeifer JPH[1], Dos Santos VH[1], Fonseca-Alves CE[2], Rodrigues M[1], Laufer-Amorim R[2], Castillo JAL[1], Alves ALG[3].
Author information

Abstract

The superficial digital flexor tendon (SDFT) is a structure frequently affected by injuries in high-performance athletic horses, and there are limited therapeutic options. Regenerative medicine has evolved significantly in treating different illnesses. However, understanding the cellular behaviour during mesenchymal stem cell (MSC) transplantation in healthy tissues is not fully known yet. To address the inflammatory response induced by allogeneic MSC transplantation, this study evaluated the local inflammatory response after the application of allogeneic adipose tissue-derived mesenchymal stem cells (AT-MSCs) in the equine tendon compared to an autologous transplant and the control group. Eighteen thoracic limbs (TL) in nine animals were divided into three groups and subjected to the

application of AT-MSCs in the healthy tendon. In the allogeneic group (Gallog), the animals received an allogeneic AT-MSC application in the TL. The autologous group (Gauto) received an application of autologous cells in the TL, and in the control group (Gcont), phosphate-buffered saline (PBS) was applied. There were no significant differences among the evaluated groups in the physical, morphological, thermography, and ultrasonography analyses. A higher number of CD3-positive lymphocytes was observed in the Gauto group compared to the control ($P < 0.05$). Additionally, we did not observe different expressions of CD172 and microvascular density among the groups. The allogeneic transplantation of AT-MSCs did not result in an adverse or inflammatory reaction that compromised the use of these cells in this experiment. Their behaviour was similar to that of autologous transplantation.

BioDrugs. 2018 Apr 27. doi: 10.1007/s40259-018-0276-3. [Epub ahead of print]

Tissue Engineering in Osteoarthritis: Current Status and Prospect of Mesenchymal Stem Cell Therapy.

Im GI[1].
Author information

Abstract

Osteoarthritis (OA) is the most common form of arthritis. Over the last 20 years, attempts have been made to regenerate articular cartilage to overcome the limitations of conventional treatments. As OA is generally associated with larger and diffuse involvement of articular surfaces and alteration of joint homeostasis, a tissue engineering approach for cartilage regeneration is more difficult than in simple chondral defects. Autologous and allogeneic mesenchymal stem cells (MSCs) have rapidly emerged as investigational products for cartilage regeneration. This review outlines points to consider in MSC-based approaches for OA treatment, including allogeneic MSCs, sources of MSCs, dosages, feasibility of multiple injections, indication according to severity of OA lesion and patient age, and issues regarding implantation versus injection. We introduce possible mechanisms of action of implanted or injected MSCs as well as the immunological aspects of MSC therapy and provide a summary of clinical trials of MSCs in the treatment of OA. Given current knowledge, it is too early to draw conclusions on the ultimate effectiveness of intra-articular application of MSCs in terms of regenerative effects. Further radiological and histological data will be needed, with a larger pool of patients, before this question can be answered.

J Transl Med. 2018 Apr 27;16(1):113. doi: 10.1186/s12967-018-1484-9.

Effects of mesenchymal stem cells on solid tumor metastasis in experimental cancer models: a systematic review and meta-analysis.

Li JH[1,2,3], Fan WS[4], Wang MM[1,2], Wang YH[1,2], Ren ZG[5,6].
Author information

Abstract

BACKGROUND:

It has been reported mesenchymal stem cells (MSCs) are recruited to and become integral parts of the tumor microenvironment. MSCs might have an active role in solid tumor progression, especially cancer metastasis. However, the contribution of MSCs in the process of cancer metastasis is still controversial. In this review, we performed a meta-analysis on the effects of MSCs administration on cancer metastasis based on published preclinical studies.

METHODS:

The PRISMA guidelines were used. A total of 42 publications met the inclusion criteria. Outcome data on the incidence and the number of cancer metastasis as well as study characteristics were extracted. Quality of the studies was assessed according to SYRCLE Risk of Bias tool. Random-effects meta-analysis was used to pool estimates.

RESULTS:

Of the 42 studies included, 32 reported that MSCs administration promoted outcome events (numbers or incidences of cancer metastasis), and 39 reported data suitable for meta-analysis. The median effect size (RR) was 2.04 for the incidence of cancer metastasis (95% CI 1.57-2.65, $I^2 = 21\%$), and the median effect size (SMD) was 1.23 for the number of cancer metastasis (95% CI 0.43-2.03, $I^2 = 89\%$). Heterogeneity was observed, with the greater impact based on study length and different ways of metastasis measurement and MSCs administration.

CONCLUSION:

Our results suggested MSCs administration increased the number and the incidence of cancer metastasis in experimental cancer models. High heterogeneity and poor reported risk of bias limit the quality of these findings. Further preclinical studies with better design and adequate reporting are still needed.

Biochem Biophys Res Commun. 2018 May 4. pii: S0006-291X(18)31015-5. doi: 10.1016/j.bbrc.2018.04.218. [Epub ahead of print]

Mesenchymal stem cells drive paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells via paracrine of neuregulin 1.

Chen J[1], Ren Q[2], Cai Y[3], Lin T[2], Zuo W[4], Wang J[2], Lin R[2], Zhu L[2], Wang P[2], Dong H[2], Zhao H[2], Huang L[2], Fu Y[2], Yang S[2], Tan J[2], Lan X[5], Wang S[6].

Author information

Abstract

We had previously demonstrated that increased expression of ErbB3 is required for ErbB2-mediated paclitaxel resistance in breast cancer cells. In the present study, we have explored the possible role of mesenchymal stem cells (MSCs) in regulating the paclitaxel-sensitivity of ErbB2/ErbB3-coexpressing breast cancer cells. We show that human umbilical cord-derived MSCs express significantly higher level of neuregulin-1 as compared with ErbB2/ErbB3-coexpressing breast cancer cells themselves. Coculture or treatment with conditioned medium of MSCs not only decreases the anti-proliferation effect of paclitaxel on ErbB2/ErbB3-coexpressing breast cancer cells, but also significantly inhibits paclitaxel-

induced apoptosis. We further demonstrate that this MSCs-drived paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells could be attributed to upregulation of Survivin via paracrine effect of NRG-1/ErbB3/PI-3K/Akt signaling, as either specific knockdown expression of ErbB3, or blocking of downstream PI-3K/Akt signaling, or specific inhibition of Survivin can completely reverse this effect. Moreover, targeted knockdown of NRG-1 expression in MSCs abrogates theirs effect on paclitaxel sensitivity of ErbB2/ErbB3-coexpressing breast cancer cells. Taken together, our study indicate that paracrine of NRG-1 by MSCs induces paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells through PI-3K/Akt signaling-dependent upregulation of Survivin. Our findings suggest that simultaneously targeting mesenchymal stem cells in tumor microenvironment may be a novel strategy to overcome paclitaxel resistance in patients with ErbB2/ErbB3-coexpressing breast cancer.

J Extracell Vesicles. 2018 Apr 26;7(1):1463778. doi: 10.1080/20013078.2018.1463778. eCollection 2018.

Extracellular vesicles from bone marrow-derived mesenchymal stromal cells support *ex vivo*survival of human antibody secreting cells.

Nguyen DC[1], Lewis HC[2], Joyner C[3], Warren V[1], Xiao H[4], Kissick HT[5], Wu R[4], Galipeau J[6], Lee FE[1].
Author information

Abstract

Extracellular vesicles (EVs) from bone marrow (BM)-derived mesenchymal stromal cells (BM-MSC) are novel mechanisms of cell-cell communication over short and long distances. BM-MSC have been shown to support human antibody secreting cells (ASC) survival *ex vivo*, but whether the crosstalk between the MSC-ASC interaction can occur via EVs is not known. Thus, we evaluated the role of EVs in ASC survival and IgG secretion. EVs were isolated from irradiated and non-irradiated primary BM-MSC and were quantified. They were further characterized by electron microscopy (EM) and CD63 and CD81 immuno-gold EM staining. Human ASC were isolated via fluorescence-activated cell sorting (FACS) and cultured *ex vivo* with the EV fractions, the EV-reduced fractions, or conventional media. IgG Elispots were used to measure the survival and functionality of the ASC. Contents of the EV fractions were evaluated by proteomics. We saw that both irradiated and non-irradiated MSC secretome preparations afforded vesicles of a size consistent with EVs. Both preparations appeared comparable in EM morphology and CD63 and CD81 immuno-gold EM. Both irradiated and non-irradiated EV fractions supported ASC function, at 88% and 90%, respectively, by day 3. In contrast, conventional media maintained only 4% ASC survival by day 3. To identify the specific factors that provided *in vitro* ASC support, we compared proteomes of the irradiated and non-irradiated EV fractions with conventional media. Pathway analysis of these proteins identified factors involved in the vesicle-mediated delivery of integrin signalling proteins. These findings indicate that BM-MSC EVs provide an effective support system for ASC survival and IgG secretion.

Stem Cell Res Ther. 2018 May 2;9(1):121. doi: 10.1186/s13287-018-0867-4.

Early passaging of mesenchymal stem cells does not instigate significant modifications in their immunological behavior.

Sareen N[1], Sequiera GL[1], Chaudhary R[1], Abu-El-Rub E[1], Chowdhury SR[2], Sharma V[3], Surendran A[1], Moudgil M[1], Fernyhough P[2], Ravandi A[1], Dhingra S[4].
Author information

Abstract

BACKGROUND:

Bone marrow-derived allogeneic mesenchymal stem cells (MSCs) from young healthy donors are immunoprivileged and their clinical application for regenerative medicine is under evaluation. However, data from preclinical and initial clinical trials indicate that allogeneic MSCs after transplantation provoke a host immune response and are rejected. In the current study, we evaluated the effect of an increase in passage number in cell culture on immunoprivilege of the MSCs. Since only limited numbers of MSCs can be sourced at a time from a donor, it is imperative to expand them in culture to meet the necessary numbers required for cell therapy. Presently, the most commonly used passages for transplantation include passages (P)3-7. Therefore, in this study we included clinically relevant passages, i.e., P3, P5, and P7, for evaluation.

METHODS:

The immunoprivilege of MSCs was assessed with the mixed leukocyte reaction assay, where rat MSCs were cocultured with peripheral blood leukocytes for 72 h. Leukocyte-mediated cytotoxicity, apoptosis (Bax/Bcl-xl ratio), leukocyte proliferation, and alterations in cellular bioenergetics in MSCs were assessed after the coculture. Furthermore, the expression of various oxidized phospholipids (oxidized phosphatidylcholine (ox-PC)) was analyzed in MSCs using a lipidomic platform. To determine if the ox-PCs were acting in tandem with downstream intracellular protein alterations, we performed proteome analysis using a liquid chromatography/mass spectrometry (LC/MS) proteomic platform.

RESULTS:

Our data demonstrate that MSCs were immunoprivileged at all three passages since coculture with leukocytes did not affect the survival of MSCs at P3, P5, and P7. We also found that, with an increase in the passage number of MSCs, leukocytes did not cause any significant effect on cellular bioenergetics (basal respiration rate, spare respiratory capacity, maximal respiration, and coupling efficiency). Interestingly, in our omics data, we detected alterations in some of the ox-PCs and proteins in MSCs at different passages; however, these changes were not significant enough to affect their immunoprivilege.

CONCLUSIONS:

The outcome of this study demonstrates that an increase in passage number (from P3 to P7) in the cell culture does not have any significant effect on the immunoprivilege of MSCs.

Sports Med Arthrosc Rev. 2018 Jun;26(2):59-63. doi: 10.1097/JSA.0000000000000191.

Platelet-rich Plasma and Mesenchymal Stem Cells: Exciting, But … are we there Yet?

Andia I[1], Martin JI[1,2], Maffulli N[3,4].
Author information

Abstract

Joint conditions incapacitate free movement driving to a sedentary lifestyle, a major risk factor for chronic diseases. Regenerative procedures, involving the use of mesenchymal stem/stromal cells along with platelet-rich plasma (PRP), can help patients with these conditions. We describe the main characteristics of cellular products (bone marrow concentrate, stromal vascular fraction of adipose tissue, and mesenchymal stem/stromal cells derived from these tissues), and the potential benefits of combination with PRP in 3 scenarios: PRP lysates used during laboratory cell expansion; PRP to prime cellular products or the host tissue before cell implantation; PRP used as a vehicle for cell transplantation and to provide trophic signals. Clinical studies exploring the benefits of combination products are limited to case series and few controlled studies, involving either arthroscopy or percutaneous injections. Combination products are making their way to clinics but further experimental and clinical research is needed to establish protocols and indications.

Vet Res Commun. 2018 May 2. doi: 10.1007/s11259-018-9720-6. [Epub ahead of print]

Effects of three-dimensional spheroid culture on equine mesenchymal stem cell plasticity.

Park MJ[1], Lee J[1], Byeon JS[1], Jeong DU[1], Gu NY[1], Cho IS[1], Cha SH[2].
Author information

Abstract

Mesenchymal stem cells (MSCs) are useful candidates for tissue engineering and cell therapy fields. We optimize culture conditions of equine adipose tissue-derived MSCs (eAD-MSCs) for treatment of horse fractures. To investigate enhancing properties of three-dimensional (3D) culture system in eAD-MSCs, we performed various sized spheroid formation and determined changes in gene expression levels to obtain different sized spheroid for cell therapy. eAD-MSCs were successfully isolated from horse tailhead. Using hanging drop method, spheroid formation was generated for three days. Quantitative real-time PCR was performed to analyze gene expression. As results, expression levels of pluripotent markers were increased depending on spheroid size and the production of PGE_2 was increased in spheroid formation compared to that in monolayer. Ki-67 showed a remarkable increase in the spheroid formed with 2.0×10^5 cells/drop as compared to that in the monolayer. Expression levels of angiogenesis-inducing factors such as VEGF, IL-6, IL-8, and IL-18 were significantly increased in spheroid formation compared to those in the monolayer. Expression levels of bone morphogenesis-inducing factors such as Cox-2 and TGF-β1 were also significantly increased in spheroid formation compared to those in the monolayer. Expression levels of osteocyte-specific markers such as RUNX2, osteocalcin, and differentiation potential were also significantly increased in spheroid formation compared to those in the monolayer. Therefore, spheroid formation of eAD-MSCs through the hanging

drop method can increases the expression of angiogenesis-inducing and bone morphogenesis-inducing factors under optimal culture conditions.

Biomaterials. 2018 Apr 16;171:230-246. doi: 10.1016/j.biomaterials.2018.04.030. [Epub ahead of print]

Humanization of bone and bone marrow in an orthotopic site reveals new potential therapeutic targets in osteosarcoma.

Wagner F[1], Holzapfel BM[2], McGovern JA[3], Shafiee A[3], Baldwin JG[3], Martine LC[3], Lahr CA[3], Wunner FM[3], Friis T[3], Bas O[3], Boxberg M[4], Prodinger PM[5], Shokoohmand A[3], Moi D[6], Mazzieri R[6], Loessner D[7], Hutmacher DW[8].
Author information

Abstract

BACKGROUND:

Existing preclinical murine models often fail to predict effects of anti-cancer drugs. In order to minimize interspecies-differences between murine hosts and human bone tumors of in vivo xenograft platforms, we tissue-engineered a novel orthotopic humanized bone model.

METHODS:

Orthotopic humanized tissue engineered bone constructs (ohTEBC) were fabricated by 3D printing of medical-grade polycaprolactone scaffolds, which were seeded with human osteoblasts and embedded within polyethylene glycol-based hydrogels containing human umbilical vein endothelial cells (HUVECs). Constructs were then implanted at the femur of NOD-scid and NSG mice. NSG mice were then bone marrow transplanted with human $CD34^+$ cells. Human osteosarcoma (OS) growth was induced within the ohTEBCs by direct injection of Luc-SAOS-2 cells. Tissues were harvested for bone matrix and marrow morphology analysis as well as tumor biology investigations. Tumor marker expression was analyzed in the humanized OS and correlated with the expression in 68 OS patients utilizing tissue micro arrays (TMA).

RESULTS:

After harvesting the femurs micro computed tomography and immunohistochemical staining showed an organ, which had all features of human bone. Around the original mouse femur new bone trabeculae have formed surrounded by a bone cortex. Staining for human specific (hs) collagen type-I (hs Col-I) showed human extracellular bone matrix production. The presence of nuclei staining positive for human nuclear mitotic apparatus protein 1 (hs NuMa) proved the osteocytes residing within the bone matrix were of human origin. Flow cytometry verified the presence of human hematopoietic cells. After injection of Luc-SAOS-2 cells a primary tumor and lung metastasis developed. After euthanization histological analysis showed pathognomic features of osteoblastic OS. Furthermore, the tumor utilized the previously implanted HUVECS for angiogenesis. Tumor marker expression was similar to human patients. Moreover, the recently discovered musculoskeletal gene C12orf29 was expressed in the most common subtypes of OS patient samples.

CONCLUSION:

OhTEBCs represent a suitable orthotopic microenvironment for humanized OS growth and offers a new translational direction, as the femur is the most common location of OS. The newly developed and validated preclinical model allows controlled and predictive marker studies of primary bone tumors and other bone malignancies.