AMNIOTIC FLUID CHORIONIC VILLI AND PLACENTA DERIVED

STEM CELLS

SCIENTIFIC PAPER REVIEW

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Biocell Center S.p.A. – Viale Stelvio 125, 21052
Busto Arsizio (Va), Italy – Tel. +39 0331 386028
Fax +39 0331 367321 – N. Verde 800 042433
www.biocellcenter.it – info@biocellcenter.it

Biocell Center Corporation – 4th Strathmore Rd,
Natick 01760,MA, USA – Tel. +1 3912040
Fax +1 7813950302 – Toll Free 866 BIO 2720
www.biocellcenter.com – info@biocellcenter.com

Introduction

This review is a collection of the most important articles related to amniotic fluid, chorionic villi and placenta derived stem cells. It includes articles published from April 2014 to July 2015. The summary section includes the titles both in English and Italian, then for each article the scientific journal, the publication date, the authors and the abstract are reported.

Introduzione

In questo fascicolo sono stati raccolti gli articoli più significativi relativi alle cellule staminali da liquido amniotico, da villi coriali e placenta. La rassegna contiene articoli pubblicati da Aprile 2014 a Luglio 2015; l'indice generale riporta i titoli delle ricerche in inglese e in italiano. Nel dettaglio vengono riportati la rivista scientifica sulla quale è stato pubblicato l'articolo, la data di pubblicazione, gli autori ed il riassunto.

SUMMAR

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1. Human amniotic fluid stem cells possess the potential to differentiate into primordial follicle oocytes in vitro.

Yu X[1], Wang N, Qiang R, Wan Q, Qin M, Chen S, Wang H.

Biol Reprod. 2014 Apr 3

Previous reports have demonstrated that embryonic stem cells were capable of differentiating into primordial germ cells through the formation of embryoid bodies that subsequently generated oocyte-like cells (OLCs). Such a process could facilitate studies of primordial follicle oocyte development in vitro and regenerative medicine. To investigate the pluripotency of human amniotic fluid stem cells (hAFSCs) and their ability to differentiate into germ cells, we isolated a CD117(+)/CD44(+)

hAFSC line that showed fibroblastoid morphology and intrinsically expressed both stem cell markers (OCT4, NANOG, SOX2) and germ cell markers (DAZL, STELLA). To encourage differentiation into OLCs, the hAFSCs were first cultured in a medium supplemented with 5% porcine follicular fluid for 10 days. During the induction period, cell aggregates formed and syntheses of steroid hormones were detected; some OLCs and granulosa cell-like cells could be loosened from the surface of the culture dish. Cell aggregates were collected and replated in oocyte culture medium for an additional 7-10 days. OLCs ranging from 50 to 120 μm presenting zona pellucida were observed in cumulus-oocyte complexes; some OLCs developed spontaneously into multicell structures similar to preimplantation embryos. Approximately

2% of the hAFSCs differentiated to meiotic germ cells that expressed folliculogenesis- and oogenesis-associated markers. Although the in vitro maturation and fertilization potentials are as yet unproven, short-term (<25 days) and high-efficiency (>2%) derivation of OLCs from hAFSCs might provide a new approach to the study of human germ cell development in vitro.

2. Isolation of mesenchymal stromal cells from extraembryonic tissues and their characteristics.

Veryasov VN[1], Savilova AM, Buyanovskaya OA, Chulkina MM, Pavlovich SV, Sukhikh GT.

Bull Exp Biol Med. 2014 May

We describe a method of isolation of human mesenchymal stromal cells from the umbilical cord (Wharton's jelly) and human placenta: amnion, placental villi, and trophoblast. Morphology, immunophenotypic characteristics, and differentiation potencies of isolated cells were studied. The capacity of mesenchymal stromal cells from extraembryonic tissues to osteogenic, adipogenic, and chondrogenic differentiation was demonstrated and the dynamics of this process was described. The isolated cells met

the criteria for multipotent mesenchymal stem cells.

3. Fms-related tyrosine kinase 3 ligand promotes proliferation of placenta amnion and chorion mesenchymal stem cells in vitro.

Li F, Xu Y, Xu X, Xu B, Zhao J, Zhang X. Mol Med Rep. 2014 Jul

Placental mesenchymal stem cells (PMSCs) have important biological properties and the potential for application in numerous clinical fields, including hematopoietic stem cell transplantation and myocardial repair. There are two types of MSCs in the placenta, amniotic mesenchymal stem cells (AMSCs) and chorion mesenchymal stem cells (CMSCs). By comparing the biological characteristics of human placental AMSCs with CMSCs, the present study identified that CD90- and CD166-positive cells were located

in the amniotic stroma and chorion stroma surrounding the vessels. In addition, the cultured AMSCs and CMSCs expressed high levels of CD73, CD90, CD105, CD29 and CD44; however they did not express CD14, CD34, CD45 and HLA-DR. Furthermore, the amplification of the fms-related tyrosine kinase 3 ligand (FL) in AMSCs and CMSCs was investigated in vitro. The results demonstrated that FL is able to promote the proliferation of AMSCs and CMSCs effectively in vitro, particularly that of CMSCs. In the FL group, the phenotype and the ability of AMSCs and CMSCs to differentiate into mesenchymal lineages did not change. Flt3, the receptor of FL, is expressed in AMSCs and CMSCs. In conclusion, mesenchymal stem cells with low immunogenicity were identified in the placental amniotic membrane and around the

chorion axis. Furthermore, FL has a positive effect on the proliferation of AMSCs and CMSCs in vitro; however, does not affect their differentiation potential. It is particularly promising that FL is able to stimulate CMSCs to proliferate in vitro.

4. Human placenta-derived adherent cells induce tolerogenic immune responses.

Liu W, Morschauser A, Zhang X, Lu X, Gleason J, He S, Chen HJ, Jankovic V, Ye Q, Labazzo K, Herzberg U, Albert VR, Abbot SE[1], Liang B[1], Hariri R[1].

Clin Transl Immunology. 2014 May 2

Human placenta-derived adherent cells (PDAC cells) are a culture expanded, undifferentiated mesenchymal-like population derived from full-term placental tissue, with immunomodulatory and anti-inflammatory properties. PDA-001 (cenplacel-L), an intravenous formulation of PDAC cells, is in clinical development for the treatment of autoimmune and inflammatory diseases. To elucidate the mechanisms underlying the immunoregulatory properties of PDAC cells,

we investigated their effects on immune cell populations, including T cells and dendritic cells (DC) in vitro and in vivo. PDAC cells suppressed T-cell proliferation in an OT-II T-cell adoptive transfer model, reduced the severity of myelin oligodendrocyte glycoprotein peptide-induced experimental autoimmune encephalomyelitis and ameliorated inflammation in a delayed type hypersensitivity response model. In vitro, PDAC cells suppressed T-cell proliferation and inhibited Th1 and Th17 differentiation. Analysis of tissues derived from PDAC cell-treated animals revealed diminished CD86 expression on splenic DC, suggesting that they can also modulate DC populations. Furthermore, PDAC cells modulate the differentiation and maturation of mouse bone marrow-derived DC. Similarly, human DC differentiated from CD14(+) monocytes in the

presence of PDAC cells acquired a tolerogenic phenotype. These tolerogenic DC failed to induce allogeneic T-cell proliferation and differentiation toward Th1, but skewed T-cell differentiation toward Th2. Inhibition of cyclo-oxygenase-2 activity resulted in a significant, but not complete, abrogation of PDAC cells' effects on DC phenotype and function, implying a role for prostaglandin E2 in PDAC-mediated immunomodulation. This study identifies modulation of DC differentiation toward immune tolerance as a key mechanism underlying the immunomodulatory activities of PDAC cells.

5. Osteogenic differentiation of placenta-derived multipotent cells in vitro.

Cheng CC, Chung CA, Su LC, Chien CC, Cheng YC.
Taiwan J Obstet Gynecol. 2014 Jun

OBJECTIVE:

Stem cells offer great potential for clinical therapeutic use because of their ability to rejuvenate and to differentiate into numerous types of cells. We isolated multipotent cells from the human term placenta that were capable of differentiation into cells of all three germ layers.

MATERIALS AND METHODS:

We examined the ability of these placenta-derived multipotent cells (PDMCs) to differentiate into osteoblasts (OBs) or OB-

like cells. The PDMCs were treated with osteogenic medium (OM) consisting of dexamethasone, β-glycerol phosphate, and ascorbic acid. At sequential time intervals (0 day, 3 days, 6 days, 9 days, and 12 days) we measured several parameters. These included alkaline phosphatase (ALP) activity, alizarin red staining (ARS) to measure calcium deposition, messenger RNA (mRNA) expressions of osteogenesis-related transcription factor (Cbfa1), and calcium coordination protein (osteocalcin). These variables were used as indicators of PDMC osteodifferentiation.

RESULTS:

We showed that ALP activity in the early stage of differentiation and calcium deposition were both significantly increased in PDMCs after OM induction. Moreover, the

Cbfa1 and osteocalcin gene expressions were upregulated. The results suggested that OM induced an osteodifferentiation potential in PDMCs.

CONCLUSION:

PDMC-derived osteocytes provide a useful model to evaluate the mechanisms of key biomolecules and bioengineering processes.

6. Two-way regulation between cells and aligned collagen fibrils: local 3D matrix formation and accelerated neural differentiation of human decidua parietalis placental stem cells.

Li W, Zhu B, Strakova Z, Wang R.
Biochem Biophys Res Commun.
Biochem Biophys Res Commun. 2014 Aug 8

It has been well established that an aligned matrix provides structural and signaling cues to guide cell polarization and cell fate decision. However, the modulation role of cells in matrix remodeling and the feedforward effect on stem cell differentiation have not been studied extensively. In this study, we report on the concerted changes of human decidua parietalis placental stem cells (hdpPSCs) and the highly ordered collagen

fibril matrix in response to cell-matrix interaction. With high-resolution imaging, we found the hdpPSCs interacted with the matrix by deforming the cell shape, harvesting the nearby collagen fibrils, and reorganizing the fibrils around the cell body to transform a 2D matrix to a localized 3D matrix. Such a unique 3D matrix prompted high expression of β-1 integrin around the cell body that mediates and facilitates the stem cell differentiation toward neural cells. The study offers insights into the coordinated, dynamic changes at the cell-matrix interface and elucidates cell modulation of its matrix to establish structural and biochemical cues for effective cell growth and differentiation.

7. Paracrine regulation of fetal lung morphogenesis using human placenta-derived mesenchymal stromal cells.

Di Bernardo J, Maiden MM, Jiang G, Hershenson MB, Kunisaki SM.
J Surg Res. 2014 Jul

BACKGROUND:

Recent experimental work suggests the therapeutic role of mesenchymal stromal cells (MSC) during perinatal lung morphogenesis. The purpose of this study was to investigate the potential paracrine effects of human placenta-derived mesenchymal stromal cells (PL-MSCs) on pulmonary development.

METHODS:

Human MSCs were isolated from preterm placental chorion. Normal E14.5-15.5 fetal rat

lungs were subsequently harvested and cultured ex vivo in the presence of conditioned media from PL-MSCs for 72 h. The lungs were analyzed morphometrically and by quantitative DNA, protein, and gene expression. Postnatal human bone marrow-derived mesenchymal stromal cells and neonatal foreskin fibroblasts (FF) were used as controls.

RESULTS:

The MSC phenotype of the isolated placental cells was confirmed. Compared with lungs cultured in the absence of PL-MSCs, fetal lung growth was markedly accelerated on exposure to PL-MSC conditioned media as demonstrated by increases in Δlung surface area, terminal bud formation, and Δterminal bud formation. Pulmonary growth was predominantly impacted by enhanced

branching morphogenesis, as shown by 73.5±6.1 terminal buds after stimulation with PL-MSCs compared with 46.7±5.7 terminal buds in control unconditioned media ($P<0.05$). Significant differences were noted favoring PL-MSCs over FFs based on terminal bud formation and Δterminal bud formation ($P<0.05$). There was significant upregulation of club cell secretory protein in lungs exposed to PL-MSCs compared with all other groups.

CONCLUSIONS:

These data suggest that human PL-MSCs are potent paracrine stimulators of pulmonary morphogenesis in a fetal organ culture model. Cell therapies based on autologous or donor-derived PL-MSCs may represent a novel strategy for enhancing perinatal lung growth.

8. Mesenchymal stromal cells from the human placenta promote neovascularization in a mouse model in vivo.

Kinzer M, Hingerl K, König J, Reinisch A, Strunk D, Huppertz B, Lang I.
Placenta. 2014 Jul

Cell transplantation is a promising strategy in regenerative medicine for revascularization of ischemic tissues. Based on our observation that placental mesenchymal stromal cells (PMSC) enhance endothelial cell viability in vitro via secretion of angiogenic factors, we asked whether PMSC support vascular growth in vivo. PMSC were isolated from amnion and placental endothelial cells (PLEC) from chorion and either separately or co-transplanted subcutaneously into immune-

deficient mice. Co-transplantation resulted in a higher number of perfused human vessels (CD31+/vimentin+) containing mouse glycophorin A+ erythrocytes. Results indicate positive effects of PMSC on neovascularization in vivo, making them attractive candidates to create autologous PMSC/PLEC pairs for research and transplantation.

9. Improvement of cardiac function by placenta-derived mesenchymal stem cells does not require permanent engraftment and is independent of the insulin signaling pathway.

Passipieri JA, Kasai-Brunswick TH, Suhett G, Martins AB, Brasil GV, Campos DB, Rocha NN, Ramos IP, Mello DB[15], Rodrigues DC, Christie BB, Silva-Mendes BJ, Balduíno A, Sá RM, Lopes LM, Goldenberg RC, Campos de Carvalho AC, Carvalho AB.

Stem Cell Res Ther. 2014 Aug 21

INTRODUCTION:

The objective of this work was to evaluate the efficacy of placenta-derived mesenchymal stem cell (MSC) therapy in a mouse model of myocardial infarction (MI). Since MSCs can be obtained from two different regions of the

human term placenta (chorionic plate or villi), cells obtained from both these regions were compared so that the best candidate for cell therapy could be selected.

METHODS:

For the in vitro studies, chorionic plate MSCs (cp-MSCs) and chorionic villi MSCs (cv-MSCs) were extensively characterized for their genetic stability, clonogenic and differentiation potential, gene expression, and immunophenotype. For the in vivo studies, C57Bl/6 mice were submitted to MI and, after 21 days, received weekly intramyocardial injections of cp-MSCs for 3 weeks. Cells were also stably transduced with a viral construct expressing luciferase, under the control of the murine stem cell virus (MSCV) promoter, and were used in a bioluminescence assay. The expression of

genes associated with the insulin signaling pathway was analyzed in the cardiac tissue from cp-MSCs and placebo groups.

RESULTS:

Morphology, differentiation, immunophenotype, and proliferation were quite similar between these cells. However, cp-MSCs had a greater clonogenic potential and higher expression of genes related to cell cycle progression and genome stability. Therefore, we considered that the chorionic plate was preferable to the chorionic villi for the isolation of MSCs. Sixty days after MI, cell-treated mice had a significant increase in ejection fraction and a reduction in end-systolic volume. This improvement was not caused by a reduction in infarct size. In addition, tracking of cp-MSCs transduced with luciferase revealed that cells remained in

the heart for 4 days after the first injection but that the survival period was reduced after the second and third injections. Quantitative reverse transcription-polymerase chain reaction revealed similar expression of genes involved in the insulin signaling pathway when comparing cell-treated and placebo groups.

CONCLUSIONS:

Improvement of cardiac function by cp-MSCs did not require permanent engraftment and was not mediated by the insulin signaling pathway.

10. Osteogenic differentiation of amniotic epithelial cells: synergism of pulsed electromagnetic field and biochemical stimuli.

Wang Q, Wu W, Han X, Zheng A, Lei S, Wu J, Chen H, He C, Luo F, Liu X .
BMC Musculoskelet Disord. 2014 Aug 11

BACKGROUND:
Pulsed electromagnetic field (PEMF) is a non-invasive physical therapy used in the treatment of fracture nonunion or delayed healing. PEMF can facilitate the osteogenic differentiation of bone marrow mesenchymal stem cells in vitro. Amniotic epithelial cells (AECs) have been proposed as a potential source of stem cells for cell therapy. However, whether PEMF could modulate the osteogenic differentiation of AECs is

unknown. In the present study, the effects of PEMF on the osteogenic differentiation of AECs were investigated.

METHODS:

AECs were isolated from amniotic membrane of human placenta by trypsin digestion and were induced by PEMF and/or osteo-induction medium. After 21 days we used real time RT-PCR and immunocytochemistry to study the expression of osteoblast markers. The signal transduction of osteogenesis was further investigated.

RESULTS:

The PEMF stimulation, or osteo-induction medium alone could induce osteogenic differentiation of AECs, as shown by expression of osteoblast specific genes and proteins including alkaline phosphatase and osteocalcin. Furthermore, a combination of

PEMF and osteo-induction medium had synergy effects on osteogenic differentiation. In our study, the gene expression of BMP-2, Runx2, β-catenin, Nrf2, Keap1 and integrinβ1 were up-regulated in the osteogenic differentiation of AECs induced by PEMF and/or osteo-induction medium.

CONCLUSIONS:

Combined application of PEMF and osteo-induction medium is synergistic for the osteogenic differentiation of AECs. It might be a novel approach in the bone regenerative medicine.

11. Fetal stem cells and skeletal muscle regeneration: a therapeutic approach.

Pozzobon M, Franzin C, Piccoli M, De Coppi P.

Front Aging Neurosci. 2014 Aug 27

More than 40% of the body mass is represented by muscle tissue, which possesses the innate ability to regenerate after damage through the activation of muscle-specific stem cells, namely satellite cells. Muscle diseases, in particular chronic degenerative states of skeletal muscle such as dystrophies, lead to a perturbation of the regenerative process, which causes the premature exhaustion of satellite cell reservoir due to continuous cycles of degeneration/regeneration. Nowadays, the research is focused on different therapeutic approaches, ranging

from gene and cell to pharmacological therapy, but still there is no definitive cure in particular for genetic muscle disease. Keeping this in mind, in this article, we will give special consideration to muscle diseases and the use of fetal derived stem cells as a new approach for therapy. Cells of fetal origin, from cord blood to placenta and amniotic fluid, can be easily obtained without ethical concern, expanded and differentiated in culture, and possess immune-modulatory properties. The in vivo approach in animal models can be helpful to study the mechanism underneath the operating principle of the stem cell reservoir, namely the niche, which holds great potential to understand the onset of muscle pathologies.

12. miR-375 induces human decidua basalis-derived stromal cells to become insulin-producing cells.

Shaer A, Azarpira N, Vahdati A, Karimi MH, Shariati M.

Cell Mol Biol Lett. 2014 Sep

This paper focuses on the development of renewable sources of isletreplacement tissue for the treatment of type I diabetes mellitus. Placental tissue-derived mesenchymal stem cells (MSCs) are a promising source for regenerative medicine due to their plasticity and easy availability. They have the potential to differentiate into insulin-producing cells. miR-375 is a micro RNA that is expressed in

the pancreas and involved in islet development. Human placental decidua basalis MSCs (PDB-MSCs) were cultured from full-term human placenta. The immunophenotype of the isolated cells was checked for CD90, CD105, CD44, CD133 and CD34 markers. The MSCs (P3) were chemically transfected with hsa-miR-375. Total RNA was extracted 4 and 6 days after transfection. The expressions of insulin, NGN3, GLUT2, PAX4, PAX6, KIR6.2, NKX6.1, PDX1, and glucagon genes were evaluated using real-time qPCR. On day 6, we tested the potency of the clusters in response to the high glucose challenge and assessed the presence of insulin and NGN3 proteins via immunocytochemistry. Flow cytometry analysis confirmed that more than 90% of the cells were positive for CD90, CD105 and CD44 and negative for CD133

and CD34. Morphological changes were followed from day 2. Cell clusters formed during day 6. Insulin-producing clusters showed a deep red color with DTZ. The expression of pancreatic-specific transcription factors increased remarkably during the four days after transfection and significantly increased on day 7. The clusters were positive for insulin and NGN3 proteins, and C-peptide and insulin secretion increased in response to changes in the glucose concentration (2.8 mM and 16.7 mM). In conclusion, the MSCs could be programmed into functional insulin-producing cells by transfection of miR-375.

13. A phase 1b study of placenta-derived mesenchymal stromal cells in patients with idiopathic pulmonary fibrosis.

Chambers DC, Enever D, Ilic N, Sparks L, Whitelaw K, Ayres J, Yerkovich ST, Khalil D, Atkinson KM, Hopkins PM.

Respirology. 2014 Oct

BACKGROUND AND OBJECTIVE:

Idiopathic pulmonary fibrosis (IPF) is a degenerative disease characterized by fibrosis following failed epithelial repair. Mesenchymal stromal cells (MSC), a key component of the stem cell niche in bone marrow and possibly other organs including lung, have been shown to enhance epithelial repair and are effective in preclinical models of inflammation-induced pulmonary fibrosis, but may be profibrotic in some circumstances.

METHODS:

In this single centre, non-randomized, dose escalation phase 1b trial, patients with moderately severe IPF (diffusing capacity for carbon monoxide (DLCO) ≥ 25% and forced vital capacity (FVC) ≥ 50%) received either $1 \times 10(6)$ ($n = 4$) or $2 \times 10(6)$ ($n = 4$) unrelated-donor, placenta-derived MSC/kg via a peripheral vein and were followed for 6 months with lung function (FVC and DLCO), 6-min walk distance (6MWD) and computed tomography (CT) chest.

RESULTS:

Eight patients (4 female, aged 63.5 (57-75) years) with median (interquartile range) FVC 60 (52.5-74.5)% and DLCO 34.5 (29.5-40)% predicted were treated. Both dose schedules were well tolerated with only minor and transient acute adverse effects. MSC infusion

was associated with a transient (1% (0-2%)) fall in SaO2 after 15 min, but no changes in haemodynamics. At 6 months FVC, DLCO , 6MWD and CT fibrosis score were unchanged compared with baseline. There was no evidence of worsening fibrosis.

CONCLUSIONS:

Intravenous MSC administration is feasible and has a good short-term safety profile in patients with moderately severe IPF.

14. Transcriptomic portrait of human Mesenchymal Stromal/Stem Cells isolated from bone marrow and placenta.

Roson-Burgo B, Sanchez-Guijo F, Del Cañizo C, De Las Rivas J. BMC Genomics. 2014 Oct

BACKGROUND:

Human Mesenchymal Stromal/Stem Cells (MSCs) are adult multipotent cells that behave in a highly plastic manner, inhabiting the stroma of several tissues. The potential utility of MSCs is nowadays strongly investigated in the field of regenerative medicine and cell therapy, although many questions about their molecular identity remain uncertain.

RESULTS:

MSC primary cultures from human bone marrow (BM) and placenta (PL) were derived and verified by their immunophenotype standard pattern and trilineage differentiation potential. Then, a broad characterization of the transcriptome of these MSCs was performed using RNA deep sequencing (RNA-Seq). Quantitative analysis of these data rendered an extensive expression footprint that includes 5,271 protein-coding genes. Flow cytometry assays of canonical MSC CD-markers were congruent with their expression levels detected by the RNA-Seq. Expression of other recently proposed MSC markers (CD146, Nestin and CD271) was tested in the placenta samples, finding only CD146 and Nestin. Functional analysis revealed enrichment in stem cell related genes and mesenchymal regulatory transcription

factors (TFs). Analysis of TF binding sites (TFBSs) identified 11 meta-regulators, including factors KLF4 and MYC among them. Epigenetically, hypomethylated promoter patterns supported the active expression of the MSC TFs found. An interaction network of these TFs was built to show up their links and relations. Assessment of dissimilarities between cell origins (BM versus PL) disclosed two hundred differentially expressed genes enrolled in microenvironment processes related to the cellular niche, as regulation of bone formation and blood vessel morphogenesis for the case of BM-MSCs. By contrast genes overexpressed in PL-MSCs showed functional enrichment on mitosis, negative regulation of cell-death and embryonic morphogenesis that supported the higher growth rates observed in the cultures of these

fetal cells and their closer links with development processes.

CONCLUSIONS:

The results present a transcriptomic portrait of the human MSCs isolated from bone marrow and placenta. The data are released as a cell-specific resource, providing a comprehensive expression footprint of the MSCs useful to better understand their cellular and molecular biology and for further investigations on the isolation and biomedical use of these multipotent cells.

15. Biotechnological and biomedical applications of mesenchymal stem cells as a therapeutic system.

Rahimzadeh A, Tabatabaei Mirakabad FS, Movassaghpour A, Shamsasenjan K, Kariminekoo S, Talebi M, Shekari A, Zeighamian V, Gandomkar Ghalhar M, Akbarzadeh A.

Artif Cells Nanomed Biotechnol. 2014 Oct 23

Mesenchymal stem cells (MSCs) are non-hematopoietic, multipotent progenitor cells which reside in bone marrow (BM), support homing of hematopoietic stem cells (HSCs) and self-renewal in the BM. These cells have the potential to differentiate into tissues of mesenchymal origin, such as fibroblasts, adipocytes, cardiomyocytes, and stromal

cells. MSCs can express surface molecules like CD13, CD29, CD44, CD73, CD90, CD166, CXCL12 and toll-like receptors (TLRs). Different factors, such as TGF-β, IL-10, IDO, PGE-2, sHLA-G5, HO, and Galectin-3, secreted by MSCs, induce interaction in cell to cell immunomodulatory effects on innate and adaptive cells of the immune system. Furthermore, these cells can stimulate and increase the TH2 and regulatory T-cells through inhibitory effects on the immune system. MSCs originate from the BM and other tissues including the brain, adipose tissue, peripheral blood, cornea, thymus, spleen, fallopian tube, placenta, Wharton's jelly and umbilical cord blood. Many studies have focused on two significant features of MSC therapy: (I) MSCs can modulate T-cell-mediated immunological responses, and (II) systemically administered

MSCs home in to sites of ischemia or injury. In this review, we describe the known mechanisms of immunomodulation and homing of MSCs. As a result, this review emphasizes the functional role of MSCs in modulating immune responses, their capability in homing to injured tissue, and their clinical therapeutic potential.

16. Identification and isolation of putative stem cells from the murine placenta.

Proudfit CL, Chan MK, Basch RS, Young BK.
Artif Cells Nanomed Biotechnol. 2014 Oct 23

OBJECTIVE:

The placenta of mid-gestation mice is a known rich source of hematopoietic stem cells. We hypothesized that it is also a source of other multipotent stem cells.

METHODS:

We isolated fetal cells from the murine placenta across the second half of gestation and characterized their expression of surface antigens known to be associated with mesenchymal stem cells (MSCs) on a subset of hematopoietic lineage-negative cells. Using real-time reverse-transcriptase

quantitative polymerase chain reaction, we also evaluated the expression of intracellular transcription factors (TFs) known to be associated with renal development and/or multipotent stem cells.

RESULTS:

Cell phenotypes with surface marker and TF expression consistent with multipotent stem cells of a mesenchymal lineage as well as renal cell progenitors were found in the placenta. The expression of MSC and renal progenitor surface markers varied throughout gestation, but was highest on E12-15 where such cells represented a small but significant percentage of the population. Of the studied TFs, 10 of 11 renal TFs were found at moderate to high levels, and all stem cell TFs were found.

CONCLUSION:

The mid-gestation murine placenta may serve as a source of multipotent stem cells and also contains cells which may be renal cell progenitors.

17. Novel isolation strategy to deliver pure fetal-origin and maternal-origin mesenchymal stem cell (MSC) populations from human term placenta.

Patel J, Shafiee A, Wang W, Fisk NM, Khosrotehrani K.
Placenta. 2014 Nov

The placenta is an abundant source of mesenchymal stem/stromal cells (MSC). Although presumed of translationally-advantageous fetal origin, the literature instead suggests a high incidence of either contaminating or pure maternal MSC. Despite definitional criteria that MSC are CD34-, increasing evidence suggests that fetal MSC may be CD34 positive in vivo. We flow sorted term placental digests based on CD34+ expression and exploited differential culture

media to isolate separately pure fetal and maternal MSC populations. This method has considerable translational implications, in particular to clinical trials underway with "placental" MSC of uncertain or decidual origin.

18. Current View on Osteogenic Differentiation Potential of Mesenchymal Stromal Cells Derived from Placental Tissues.

Kmiecik G[1], Spoldi V, Silini A, Parolini O.

Stem Cell Rev. 2015 Aug

Mesenchymal stromal cells (MSC) isolated from human term placental tissues possess unique characteristics, including their peculiar immunomodulatory properties and their multilineage differentiation potential. The osteogenic differentiation capacity of placental MSC has been widely disputed, and continues to be an issue of debate. This review will briefly discuss the different MSC populations which can be obtained from different regions of human term placenta, along with their unique properties, focusing

specifically on their osteogenic differentiation potential. We will present the strategies used to enhance osteogenic differentiation potential in vitro, such as through the selection of subpopulations more prone to differentiate, the modification of the components of osteo-inductive medium, and even mechanical stimulation. Accordingly, the applications of three-dimensional environments in vitro and in vivo, such as non-synthetic, polymer-based, and ceramic scaffolds, will also be discussed, along with results obtained from pre-clinical studies of placental MSC for the regeneration of bone defects and treatment of bone-related diseases.

19. Delivery of placenta-derived mesenchymal stem cells ameliorates ischemia induced limb injury by immunomodulation.

Zhang B, Adesanya TM, Zhang L, Xie N, Chen Z, Fu M, Zhang J, Zhang J, Tan T, Kilic A, Li Z, Zhu H, Xie X.
Cell Physiol Biochem. 2014

BACKGROUND:

Peripheral artery disease (PAD) is a major health burden in the world. Stem cell-based therapy has emerged as an attractive treatment option in regenerative medicine. In this study, we sought to test the hypothesis that stem cell-based therapy can ameliorate ischemia induced limb injury.

METHODS:

We isolated mesenchymal stem cells derived from human placentas (PMSCs) and intramuscularly transplanted them into injured hind limbs. Treatment with PMSCs reduced acute muscle fibers apoptosis induced by ischemia.

RESULTS:

PMSC treatment significantly enhanced regeneration of the injured hind limb by reducing fibrosis and enhancing running capacity when the animals were subjected to treadmill training. Mechanistically, injected PMSCs can modulate acute inflammatory responses by reducing neutrophil and macrophage infiltration following limb ischemia. ELISA assays further confirmed that PMSC treatment can also reduce pro-inflammatory cytokines, TNF-α and IL-6, and

enhance anti-inflammatory cytokine, IL-10 at the injury sites.

CONCLUSION:

Taken together, our results demonstrated that PMSCs can be a potential effective therapy for treatment of PAD via immunomodulation.

20. In utero therapy for congenital disorders using amniotic fluid stem cells.

Ramachandra DL, Shaw SS, Shangaris P, Loukogeorgakis S, Guillot PV, Coppi PD, David.

Front Pharmacol. 2014 Dec 19

Congenital diseases are responsible for over a third of all pediatric hospital admissions. Advances in prenatal screening and molecular diagnosis have allowed the detection of many life-threatening genetic diseases early in gestation. In utero transplantation (IUT) with stem cells could cure affected fetuses but so far in humans, successful IUT using allogeneic hematopoietic stem cells (HSCs), has been limited to fetuses with severe immunologic defects and more recently IUT with allogeneic mesenchymal stem cell

transplantation, has improved phenotype in osteogenesis imperfecta. The options of preemptive treatment of congenital diseases in utero by stem cell or gene therapy changes the perspective of congenital diseases since it may avoid the need for postnatal treatment and reduce future costs. Amniotic fluid stem (AFS) cells have been isolated and characterized in human, mice, rodents, rabbit, and sheep and are a potential source of cells for therapeutic applications in disorders for treatment prenatally or postnatally. Gene transfer to the cells with long-term transgenic protein expression is feasible. Recently, pre-clinical autologous transplantation of transduced cells has been achieved in fetal sheep using minimally invasive ultrasound guided injection techniques. Clinically relevant levels of transgenic protein were expressed in the blood of transplanted lambs

for at least 6 months. The cells have also demonstrated the potential of repair in a range of pre-clinical disease models such as neurological disorders, tracheal repair, bladder injury, and diaphragmatic hernia repair in neonates or adults. These results have been encouraging, and bring personalized tissue engineering for prenatal treatment of genetic disorders closer to the clinic.

21. Placental amniotic epithelial cells and their therapeutic potential in liver diseases.

Tahan AC, Tahan V.

Front Med (Lausanne). 2014 Dec 8

As a unique source of stem cells, there is a growing interest in amniotic epithelial (AE) cells. Placenta is readily available; in fact, it is often discarded following delivery. As such, it is without the ethical concerns of embryonic stem cells. Further advantages to AE include that AE cells do not demonstrate tumorigenicity upon transplantation, and are gifted with immunomodulatory and anti-inflammatory properties. Thus, AE cells have exceptional features for use as cell-based therapies for liver disease.

22. Transplantation of placenta-derived mesenchymal stem cell-induced neural stem cells to treat spinal cord injury.

Li Z, Zhao W, Liu W, Zhou Y, Jia J, Yang L.
Stem Cell Rev. 2015 Jun

Because of their strong proliferative capacity and multi-potency, placenta-derived mesenchymal stem cells have gained interest as a cell source in the field of nerve damage repair. In the present study, human placenta-derived mesenchymal stem cells were induced to differentiate into neural stem cells, which were then transplanted into the spinal cord after local spinal cord injury in rats. The motor functional recovery and pathological changes in the injured spinal cord were observed for 3 successive weeks. The results showed that human placenta-derived

mesenchymal stem cells can differentiate into neuron-like cells and that induced neural stem cells contribute to the restoration of injured spinal cord without causing transplant rejection. Thus, these cells promote the recovery of motor and sensory functions in a rat model of spinal cord injury. Therefore, human placenta-derived mesenchymal stem cells may be useful as seed cells during the repair of spinal cord injury.

23. Human Chorionic Villous Mesenchymal Stem Cells Modify the Functions of Human Dendritic Cells, and Induce an Anti-Inflammatory Phenotype in CD1+ Dendritic Cells.

Abomaray FM, Al Jumah MA, Kalionis B, AlAskar AS, Al Harthy S, Jawdat D, Al Khaldi A, Alkushi A, Knawy BA, Abumaree MH.

Stem Cell Rev. 2015 Jun

BACKGROUND:

Mesenchymal stem cells derived from the chorionic villi of human term placenta (pMSCs) have drawn considerable interest because of their multipotent differentiation potential and their immunomodulatory capacity. These properties are the foundation for their clinical application in the fields of stem cell transplantation and regenerative

medicine. Previously, we showed that pMSCs induce an anti-inflammatory phenotype in human macrophages. In this study, we determined whether pMSCs modify the differentiation and maturation of human monocytes into dendritic cells (DCs). The consequences on dendritic function and on T cell proliferation were also investigated.

METHODS:

Interleukin-4 (IL-4) and granulocyte-macrophage colony stimulating factor (GM-CSF) were used to stimulate the differentiation of monocytes into immature dendritic cells (iDCs), which were subsequently co-cultured with pMSCs. Lipopolysaccharide (LPS) was used to induce maturation of iDCs into mature dendritic cells (mDCs). Flow cytometry and enzyme-linked immunosorbent assays (ELISA) were used to

quantify the effect pMSC co-culturing on DC differentiation using CD1a, a distinctive marker of DCs, as well as other molecules important in the immune functions of DCs. The phagocytic activity of iDCs co-cultured with pMSCs, and the effects of iDCs and mDC stimulation on T cell proliferation, were also investigated.

RESULTS:

Monocyte differentiation into iDCs was inhibited when co-cultured with pMSCs and maturation of iDCs by LPS treatment was also prevented in the presence of pMSCs as demonstrated by reduced expression of CD1a and CD83, respectively. The inhibitory effect of pMSCs on iDC differentiation was dose dependent. In addition, pMSC co-culture with iDCs and mDCs resulted in both phenotypic and functional changes as shown by reduced

expression of costimulatory molecules (CD40, CD80, CD83 and CD86) and reduced capacity to stimulate CD4(+) T cell proliferation. In addition, pMSC co-culture increased the surface expression of major histocompatibility complex (MHC-II) molecules on iDCs but decreased MHC-II expression on mDCs. Moreover, pMSC co-culture with iDCs or mDCs increased the expression of immunosuppressive molecules [B7H3, B7H4, CD273, CD274 and indoleamine-pyrrole 2,3-dioxygenase (IDO). Additionally, the secretion of IL-12 and IL-23 by iDCs and mDCs co-cultured with pMSCs was decreased. Furthermore, pMSC co-culture with mDCs decreased the secretion of IL-12 and INF-γ whilst increasing the secretion of IL-10 in a T cell proliferation experiment. Finally, pMSC co-culture with iDCs induced the phagocytic activity of iDCs.

CONCLUSIONS:

We have shown that pMSCs have an inhibitory effect on the differentiation, maturation and function of DCs, as well as on the proliferation of T cells, suggesting that pMSCs can control the immune responses at multiple levels.

24. Human Wharton's jelly-derived mesenchymal stem cells express oocyte developmental genes during co-culture with placental cells.

Asgari HR, Akbari M, Abbasi M, Ai J, Korouji M, Aliakbari F, Babatunde KA, Aval FS, Joghataei MT.

Iran J Basic Med Sci. 2015 Jan

OBJECTIVES:

The present day challenge is how to obtain germ cells from stem cells to treat patients with cancer and infertility. Much more efforts have been made to develop a procedure for attaining germ cells in vitro. Recently, human umbilical cord-derived mesenchymal stem cells (HUMSCs) have been introduced with higher efficacy for differentiation. In this work, we tried to explore the efficacy of

HUMSCs and some effective products of placental cells such as transforming growth factors. This study is aimed to optimize a co-culture condition for HUMSCs with placental cells to obtain primordial germ cells (PGCs) and reach into oocyte-like cells in vitro.

MATERIALS AND METHODS:

In this experimental study, HUMSCs and placental cells were co-cultured for 14 days without any external inducer in vitro. Then HUMSCs were assessed for expression of PGC markers; Octamer-binding transcription factor 4(OCT4), Tyrosine-protein kinase Kit (CKIT), Stage specific embryonic antigen 4 (SSEA4), DEAD (Asp-Glu-Ala-Asp) box polypeptide 4(DDX4) and oocyte specific markers; Growth differentiation factor-9(GDF9), Zona pellucida glycoprotein

3(ZP3). The pertinent markers were assessed by immunocytochemistry and Q-PCR.

RESULTS:

Co-cultured HUMSCs with placental cells (including amniotic and chorionic cells) presented Oct4 and DDX4, primordial germ cells specific markers significantly, but increment in expression of oocyte-like cell specific markers, GDF9 and ZP3 did not reach to statistically significant threshold.

CONCLUSION:

Placental cell supplements Transforming growth factor (TGF α, β) and basic fibroblast growth factor (bFGF) in a co-culture model can provide proper environment for induction of HUMSCs into PGCs and expression of oocyte-like markers.

25. Early gestation chorionic villi-derived stromal cells for fetal tissue engineering.

Lankford L, Selby T, Becker J, Ryzhuk V, Long C, Farmer D, Wang A.

World J Stem Cells. 2015 Jan 26

AIM:

To investigate the potential for early gestation placenta-derived mesenchymal stromal cells (PMSCs) for fetal tissue engineering.

METHODS:

PMSCs were isolated from early gestation chorionic villus tissue by explant culture. Chorionic villus sampling (CVS)-size tissue samples (mean = 35.93 mg) were used to test the feasibility of obtaining large cell numbers from CVS within a clinically relevant timeframe. We characterized PMSCs isolated

from 6 donor placentas by flow cytometry immunophenotyping, multipotency assays, and through immunofluorescent staining. Protein secretion from PMSCs was examined using two cytokine array assays capable of probing for over 70 factors in total. Delivery vehicle compatibility of PMSCs was determined using three common scaffold systems: fibrin glue, collagen hydrogel, and biodegradable nanofibrous scaffolds made from a combination of polylactic acid (PLA) and poly(lactic-co-glycolic acid) (PLGA). Viral transduction of PMSCs was performed using a Luciferase-GFP-containing lentiviral vector and efficiency of transduction was tested by fluorescent microscopy and flow cytometry analysis.

RESULTS:

We determined that an average of 2.09 × 10(6) (SD ± 8.59 × 10(5)) PMSCs could be obtained from CVS-size tissue samples within 30 d (mean = 27 d, SD ± 2.28), indicating that therapeutic numbers of cells can be rapidly expanded from very limited masses of tissue. Immunophenotyping by flow cytometry demonstrated that PMSCs were positive for MSC markers CD105, CD90, CD73, CD44, and CD29, and were negative for hematopoietic and endothelial markers CD45, CD34, and CD31. PMSCs displayed trilineage differentiation capability, and were found to express developmental transcription factors Sox10 and Sox17 as well as neural-related structural proteins NFM, Nestin, and S100β. Cytokine arrays revealed a robust and extensive profile of PMSC-secreted cytokines and growth factors, and detected 34 factors

with spot density values exceeding 10(3). Detected factors had widely diverse functions that include modulation of angiogenesis and immune response, cell chemotaxis, cell proliferation, blood vessel maturation and homeostasis, modulation of insulin-like growth factor activity, neuroprotection, extracellular matrix degradation and even blood coagulation. Importantly, PMSCs were also determined to be compatible with both biological and synthetic material-based delivery vehicles such as collagen and fibrin hydrogels, and biodegradable nanofiber scaffolds made from a combination of PLA and PLGA. Finally, we demonstrated that PMSCs can be efficiently transduced (> 95%) with a Luciferase-GFP-containing lentiviral vector for future in vivo cell tracking after transplantation.

CONCLUSION:

Our findings indicate that PMSCs represent a unique source of cells that can be effectively utilized for in utero cell therapy and tissue engineering.

26. Construction of corneal epithelium with human amniotic epithelial cells and repair of limbal deficiency in rabbit models.

Zhou Q, Liu XY, Ruan YX, Wang L, Jiang MM, Wu J, Chen J.
Hum Cell. 2015 Jan

This study aims to evaluate the effect of a human amniotic epithelial cell (HAEC)-rabbit corneal stroma tissue-engineered cornea on ocular reconstruction in three different animal models. HAECs were isolated from human placenta, seeded onto rabbit corneal stroma. HAECs-rabbit corneal stroma tissue engineering cornea transplantation was examined in three distinct rabbit models: transplantation of cornea constructed (1) with lamellar corneal HAECs and rabbit corneal

stroma, (2) with central corneal HAECs and rabbit corneal stroma, or (3) with full-thickness corneal HAECs and rabbit corneal stroma. In the tissue engineering corneal transplantation groups in all three models, the mean number of days to corneal epithelial healing was significantly shorter than that in the control group and the mean number of days to corneal neovascularization was significantly greater than in the control group. In addition, in the tissue engineering corneal transplantation groups in the central lamellar cornea model and the full-thickness corneal transplantation model neovascularization, corneal turbidity, and epithelial fluorescence were significantly less than in the control groups. HAECs can be induced to differentiate into corneal epithelial cells, which may be suitable for the reconstruction

of the corneal epithelium in cases of limbal stem cell deficiency.

27. Placental mesenchymal stromal cells derived from blood vessels or avascular tissues: what is the better choice to support endothelial cell function?

König J, Weiss G, Rossi D, Wankhammer K, Reinisch A, Kinzer M, Huppertz B, Pfeiffer D, Parolini O, Lang I.
Stem Cells Dev. 2015 Jan 1

Mesenchymal stromal cells (MSCs) are promising tools for therapeutic revascularization of ischemic tissues and for support of vessel formation in engineered tissue constructs. Recently, we could show that avascular-derived MSCs from placental amnion release soluble factors that exhibit survival-enhancing effects on endothelial cells (ECs). We hypothesize that MSCs derived from placental blood vessels might

have even more potent angiogenic effects. Therefore, we isolated and characterized MSCs from placental chorionic blood vessels (bv-MSCs) and tested their angiogenic potential in comparison to amnion-derived avascular MSCs (av-MSCs). bv-MSCs express a very similar surface marker profile compared with av-MSCs and could be differentiated toward the adipogenic and osteogenic lineages. bv-MSCs exert immunosuppressive properties on peripheral blood mononuclear cells, suggesting that they are suitable for cell transplantation settings. Conditioned medium (Cdm) from av-MSCs and bv-MSCs significantly enhanced EC viability, whereas only Cdm from bv-MSCs significantly increased EC migration and network formation (Matrigel assay). Angiogenesis array analysis of av- and bv-MSC-Cdm revealed a similar secretion

pattern of angiogenic factors, including angiogenin, interleukins-6 and -8, and tissue inhibitors of matrix metalloproteinase-1 and 2. Enzyme-linked immunosorbent assay analysis showed that, in contrast to av-MSCs, bv-MSCs secreted vascular endothelial growth factor. In direct coculture with bv-MSCs, ECs showed a significantly increased formation of vessel-like structures compared with av-MSCs. With regard to therapeutic treatment, bv-MSCs and particularly their Cdm might be valuable to stimulate angiogenesis especially in ischemic tissues. av-MSCs and their Cdm could be beneficial in conditions when it is required to promote the survival and stabilization of blood vessels without the risk of unmeant angiogenesis.

28. Human Placenta-Derived Multipotent Cells (hPDMCs) Modulate Cardiac Injury: From Bench to Small & Large Animal Myocardial Ischemia Studies.

Liu YH, Peng KY, Chiu YW, Ho YL, Wang YH, Shun CT, Huang SY, Lin YS, de Vries AA, Pijnappels DA, Lee NT, Yen BL, Yen ML.
Cell Transplant. 2015 Jan 23

Cardiovascular disease is the leading cause of death globally, and stem cell therapy remains one of the most promising strategies for regeneration or repair of the damaged heart. We report that human placenta-derived multipotent cells (hPDMCs) can modulate cardiac injury in small and large animal models of myocardial ischemia (MI), and elucidate the mechanisms involved. We found that hPDMCs can undergo in vitro

cardiomyogenic differentiation when co-cultured with mouse neonatal cardiomyocytes. Moreover, hPDMCs exert strong proangiogenic responses in vitro towards human endothelial cells mediated by secretion of hepatocyte growth factor, growth-regulated oncogene-a, and interleukin-8. To test the in vivo relevance of these results, small and large animal models of acute MI was induced in mice and minipigs, respectively, by permanent left anterior descending (LAD) artery ligation, followed by hPDMCs or culture medium only implantation with follow-up for up to 8 weeks. Transplantation of hPDMCs into mouse heart post-acute MI induction improved left ventricular function, with significantly enhanced vascularity in the cell-treated group. Furthermore, in minipigs post-acute MI induction, hPDMC transplantation

significantly improved myocardial contractility compared to the control group ($p=0.016$) at 8 weeks post-injury. In addition, tissue analysis confirmed that hPDMC transplantation induced increased vascularity, cardiomyogenic differentiation, and anti-apoptotic effects. Our findings offer evidence that hPDMCs can modulate cardiac injury in both small and large animal models, possibly through proangiogenesis, cardiomyogenesis, and suppression of cardiomyocyte apoptosis. Our study offers mechanistic insights and preclinical evidence on using hPDMCs as a therapeutic strategy to treat severe cardiovascular diseases.

29. Human placenta-derived adherent cells improve cardiac performance in mice with chronic heart failure.

Chen HJ, Chen CH, Chang MY, Tsai DC, Baum EZ, Hariri R, Herzberg U, Hsieh PC. Stem Cells Transl Med. 2015 Mar

Human placenta-derived adherent cells (PDACs) are a culture-expanded, undifferentiated mesenchymal-like population derived from full-term placental tissue, with immunomodulatory, anti-inflammatory, angiogenic, and neuroprotective properties. PDA-001 (cenplacel-L), an intravenous formulation of PDAC cells, is in clinical development for the treatment of autoimmune and inflammatory diseases. We tested the therapeutic effects of PDA-001 in mice with chronic heart failure (CHF). Three weeks

after transaortic constriction surgery to induce CHF, the mice underwent direct intramyocardial (IM) or i.v. injection of PDA-001 at a high (0.5 × 10(6) cells per mouse), medium (0.5 × 10(5) cells per mouse), or low (0.5 × 10(4) cells per mouse) dose. The mice were sacrificed 4 weeks after treatment. Echocardiography and ventricular catheterization showed that IM injection of PDA-001 significantly improved left ventricular systolic and diastolic function compared with injection of vehicle or i.v. injection of PDA-001. IM injection of PDA-001 also decreased cardiac fibrosis, shown by trichrome staining in the vicinity of the injection sites. Low-dose treatment showed the best improvement in cardiac performance compared with the medium- and high-dose groups. In another independent study to determine the mechanism of action with

bromodeoxyuridine labeling, the proliferation rates of endothelial cells and cardiomyocytes were significantly increased by low or medium IM dose PDA-001. However, no surviving PDA-001 cells were detected in the heart 1 month after injection. In vivo real-time imaging consistently revealed that the PDA-001 cells were detectable only within 2 days after IM injection of luciferase-expressing PDA-001. Together, these results have demonstrated the cardiac therapeutic potential of PDA-001, likely through a paracrine effect.

30. Alteration of histone acetylation pattern during long-term serum-free culture conditions of human fetal placental mesenchymal stem cells.

Zhu Y, Song X, Han F, Li Y, Wei J, Liu X.
PLoS One. 2015 Feb 11

Increasing evidence suggests that the mesenchymal stem cells (MSCs) derived from placenta of fetal origin (fPMSCs) are superior to MSCs of other sources for cell therapy. Since the initial number of isolated MSCs is limited, in vitro propagation is often required to reach sufficient numbers of cells for therapeutic applications, during which MSCs may undergo genetic and/or epigenetic alterations that subsequently increase the probability of spontaneous malignant transformation. Thus, factors that influence

genomic and epigenetic stability of MSCs following long-term expansions need to be clarified before cultured MSCs are employed for clinical settings. To date, the genetic and epigenetic stability of fPMSCs after long-term in vitro expansion has not been fully investigated. In this report, alterations to histone acetylation and consequence on the expression pattern of fPMSCs following in vitro propagation under serum-free conditions were explored. The results show that fPMSCs maintain their MSC characteristics before they reached a senescent state. Furthermore, acetylation modification patterns were changed in fPMSCs along with gradually increased global histone deacetylase (HDAC) activity and expression of HDAC subtypes HDAC4, HDAC5 and HDAC6, as well as a down-regulated global histone H3/H4 acetylation during in vitro culturing. In line

with the acetylation alterations, the expression of oncogenes Oct4, Sox2 and TERT were significantly decreased over the propagation period. Of note, the down-regulation of Oct4 was strongly associated with changes in acetylation. Intriguingly, telomere length in fPMSCs did not significantly change during the propagating process. These findings suggest that human fPMSCs may be a safe and reliable resource of MSCs and can be propagated under serum-free conditions with less risk of spontaneous malignancy, and warrants further validation in clinical settings.

31. Transplantation of human placenta-derived multipotent stem cells reduces ischemic brain injury in adult rats.

Wu KJ, Yu SJ, Chiang CW, Cho KH, Lee YW, Yen BL, Kuo LW, Wang Y.
Cell Transplant. 2015 Feb 9

After the onset of stroke, a series of progressive and degenerative reactions, including inflammation, is activated, which leads to cell death. We recently reported that human placenta-derived multipotent stem cells (hPDMCs) process potent anti-inflammatory effects. In this study, we examined the protective effect of hPDMC transplants in a rodent model of stroke. Adult male Sprague-Dawley rats were anesthetized. hPDMCs labeled with a vital dye of fluorescing microparticles, DiI, or vehicle

were transplanted into three cortical areas adjacent to the right middle cerebral artery (MCA). Five minutes after grafting, the right MCA was transiently occluded for 60 min. Stroke animals receiving hPDMCs showed a significant behavioral improvement and reduction in lesion volume examined by T2-weighted images 4 days poststroke. Brain tissues were collected 1 day later. Human-specific marker HuNu immunoreactivity and DiI fluorescence were found at the hPDMC graft sites, suggesting the survival of hPDMCs in host brain. Grafting of hPDMCs suppressed IBA1 immunoreactivity and deramification of IBA1(+) cells in the perilesioned area, suggesting activation of microglia was attenuated by the transplants. Taken together, our data indicate that hPDMC transplantation reduced cortical lesions and behavioral deficits in adult stroke rats, and

these cells could serve as a unique anti-inflammatory reservoir for the treatment of ischemic brain injury.

32. Direct evaluation of myocardial viability and stem cell engraftment demonstrates salvage of the injured myocardium.

Kim PJ, Mahmoudi M, Ge X, Matsuura Y, Toma I, Metzler S[1], Kooreman NG, Ramunas J[1], Holbrook C[1], McConnell MV, Blau H, Harnish P, Rulifson E, Yang PC.

Circ Res. 2015 Mar 27

RATIONALE:

The mechanism of functional restoration by stem cell therapy remains poorly understood. Novel manganese-enhanced MRI and bioluminescence reporter gene imaging were applied to follow myocardial viability and cell engraftment, respectively. Human-placenta-derived amniotic mesenchymal stem

cells (AMCs) demonstrate unique immunoregulatory and precardiac properties. In this study, the restorative effects of 3 AMC-derived subpopulations were examined in a murine myocardial injury model: (1) unselected AMCs, (2) ckit(+)AMCs, and (3) AMC-derived induced pluripotent stem cells (MiPSCs).

OBJECTIVE:

To determine the differential restorative effects of the AMC-derived subpopulations in the murine myocardial injury model using multimodality imaging.

METHODS AND RESULTS:

SCID (severe combined immunodeficiency) mice underwent left anterior descending artery ligation and were divided into 4 treatment arms: (1) normal saline control (n=14), (2) unselected AMCs (n=10), (3)

ckit(+)AMCs (n=13), and (4) MiPSCs (n=11). Cardiac MRI assessed myocardial viability and left ventricular function, whereas bioluminescence imaging assessed stem cell engraftment during a 4-week period. Immunohistological labeling and reverse transcriptase polymerase chain reaction of the explanted myocardium were performed. The unselected AMC and ckit(+)AMC-treated mice demonstrated transient left ventricular functional improvement. However, the MiPSCs exhibited a significantly greater increase in left ventricular function compared with all the other groups during the entire 4-week period. Left ventricular functional improvement correlated with increased myocardial viability and sustained stem cell engraftment. The MiPSC-treated animals lacked any evidence of de novo cardiac differentiation.

CONCLUSION:

The functional restoration seen in MiPSCs was characterized by increased myocardial viability and sustained engraftment without de novo cardiac differentiation, indicating salvage of the injured myocardium.

33. Therapeutic effect of placenta-derived mesenchymal stem cells on hypoxic-ischemic brain damage in rats.

Ding HF, Zhang H, Ding HF, Li D, Yi XH, Gao XY, Mou WW, Ju XL.

World J Pediatr. 2015 Feb

BACKGROUND:

Oxidative stress is involved in the development of hypoxic-ischemic brain damage (HIBD). In this study, we investigated the therapeutic effects of placenta-derived mesenchymal stem cells (PD-MSCs) and explored the NF-E2-related factor-2/heme oxygenase-1 (Nrf2/HO-1) signaling pathway in treating HIBD.

METHODS:

P7 rats were subjected to hypoxic-ischemic brain injury and randomly divided into four groups (control, HIBD, HIBD+PD-MSCs, and HIBD+fibroblasts). Forty-eight hours after the induction of HIBD, 5×10(5) of PD-MSCs were injected into cerebral tissue in the HIBD+PD-MSCs group, while the same dose of fibroblasts were injected in the HIBD+fibroblasts group. Morris Water Maze, gross and pathological changes were tested at P28. The level of malondialdehyde (MDA) was detected in rats' hippocampus. RT-PCR and western blot analysis were used to evaluate the changes of Nrf2/HO-1.

RESULTS:

The HIBD group showed significantly longer escape latency and a lower frequency of original platform crossing in the Morris Water Maze compared with the control group.

Rats receiving PD-MSCs showed significant improvement of HIBD. The pathological changes were evident after HIBD, but ameliorated in the PD-MSCs group. Compared with the control group, HO-1 and Nrf2 were up-regulated at gene and protein levels in the HI brain, beginning at 6 hours and peaking at 48 hours ($P<0.05$). The expression of HO-1 and Nrf2 in the PD-MSCs treatment group was more pronounced than in the HIBD group ($P<0.01$). PD-MSCs also decreased MDA production in the brain tissue.

CONCLUSION:

These results demonstrate that PD-MSCs have neuroprotective effect during the treatment of HIBD and that the mechanism may be partly due to alleviating oxidative stress.

34. Human placental eXpanded (PLX) mesenchymal-like adherent stromal cells confer neuroprotection to nerve growth factor (NGF)-differentiated PC12 cells exposed to ischemia by secretion of IL-6 and VEGF.

Lahiani A, Zahavi E, Netzer N, Ofir R, Pinzur L, Raveh S, Arien-Zakay H, Yavin E, Lazarovici P.

Biochim Biophys Acta. 2015 Feb

Mesenchymal stem cells are potent candidates in stroke therapy due to their ability to secrete protective anti-inflammatory cytokines and growth factors. We investigated the neuroprotective effects of human placental mesenchymal-like adherent stromal cells (PLX) using an established ischemic model of nerve growth factor (NGF)-differentiated pheochromocytoma PC12 cells exposed to

oxygen and glucose deprivation (OGD) followed by reperfusion. Under optimal conditions, 2×10^5 PLX cells, added in a trans-well system, conferred 30-60% neuroprotection to PC12 cells subjected to ischemic insult. PC12 cell death, measured by LDH release, was reduced by PLX cells or by conditioned medium derived from PLX cells exposed to ischemia, suggesting the active release of factorial components. Since neuroprotection is a prominent function of the cytokine IL-6 and the angiogenic factor VEGF165, we measured their secretion using selective ELISA of the cells under ischemic or normoxic conditions. IL-6 and VEGF165 secretion by co-culture of PC12 and PLX cells was significantly higher under ischemic compared to normoxic conditions. Exogenous supplementation of 10 ng/ml each of IL-6 and VEGF165 to insulted PC12 cells conferred

neuroprotection, reminiscent of the neuroprotective effect of PLX cells or their conditioned medium. Growth factors as well as co-culture conditioned medium effects were reduced by 70% and 20% upon pretreatment with 240 ng/ml Semaxanib (anti VEGF165) and/or 400 ng/ml neutralizing anti IL-6 antibody, respectively. Therefore, PLX-induced neuroprotection in ischemic PC12 cells may be partially explained by IL-6 and VEGF165 secretion. These findings may also account for the therapeutic effects seen in clinical trials after treatment with these cells.

35. Stable X chromosome reactivation in female human induced pluripotent stem cells.

Barakat TS, Ghazvini M, de Hoon B, Li T, Eussen B, Douben H, van der Linden R, van der Stap N, Boter M, Laven JS, Galjaard RJ[4], Grootegoed JA, de Klein A, Gribnau J.
Stem Cell Reports. 2015 Feb 10

In placental mammals, balanced expression of X-linked genes is accomplished by X chromosome inactivation (XCI) in female cells. In humans, random XCI is initiated early during embryonic development. To investigate whether reprogramming of female human fibroblasts into induced pluripotent stem cells (iPSCs) leads to reactivation of the inactive X chromosome (Xi), we have generated iPSC lines from fibroblasts

heterozygous for large X-chromosomal deletions. These fibroblasts show completely skewed XCI of the mutated X chromosome, enabling monitoring of X chromosome reactivation (XCR) and XCI using allele-specific single-cell expression analysis. This approach revealed that XCR is robust under standard culture conditions, but does not prevent reinitiation of XCI, resulting in a mixed population of cells with either two active X chromosomes (Xas) or one Xa and one Xi. This mixed population of XaXa and XaXi cells is stabilized in naive human stem cell medium, allowing expansion of clones with two Xas.

36. Cell recruitment by amnion chorion grafts promotes neovascularization.

Maan ZN, Rennert RC[1], Koob TJ, Januszyk M[1], Li WW[3], Gurtner GC.

J Surg Res. 2015 Feb

BACKGROUND:

Nonhealing wounds are a significant health burden. Stem and progenitor cells can accelerate wound repair and regeneration. Human amniotic membrane has demonstrated efficacy in promoting wound healing, though the underlying mechanisms remain unknown. A dehydrated human amnion chorion membrane (dHACM) was tested for its ability to recruit hematopoietic progenitor cells to a surgically implanted graft in a murine model of cutaneous ischemia.

METHODS:

dHACM was subcutaneously implanted under elevated skin (ischemic stimulus) in either wild-type mice or mice surgically parabiosed to green fluorescent protein (GFP) + reporter mice. A control acellular dermal matrix, elevated skin without an implant, and normal unwounded skin were used as controls. Wound tissue was harvested and processed for histology and flow cytometric analysis.

RESULTS:

Implanted dHACMs recruited significantly more progenitor cells compared with controls (*P < 0.05) and displayed in vivo SDF-1 expression with incorporation of CD34 + progenitor cells within the matrix. Parabiosis modeling confirmed the circulatory origin of recruited cells, which coexpressed progenitor cell markers and were localized to foci of neovascularization within implanted matrices.

CONCLUSIONS:

In summary, dHACM effectively recruits circulating progenitor cells, likely because of stromal derived factor 1 (SDF-1) expression. The recruited cells express markers of "stemness" and localize to sites of neovascularization, providing a partial mechanism for the clinical efficacy of human amniotic membrane in the treatment of chronic wounds.

37. Comparative investigation of human amniotic epithelial cells and mesenchymal stem cells for application in bone tissue engineering.

Si J, Dai J[1], Zhang J, Liu S, Gu J, Shi J[1], Shen SG, Guo L.

Stem Cells Int. 2015 Mar 5

Emerging evidence suggests amniotic epithelial cells (AECs) as a promising source of progenitor cells in regenerative medicine and bone tissue engineering. However, investigations comparing the regenerative properties of AECs with other sources of stem cells are particularly needed before the feasibility of AECs in bone tissue engineering can be determined. This study aimed to compare human amniotic epithelial cells (hAECs), human bone marrow mesenchymal stem cells (hBMSCs), and human amniotic

fluid derived mesenchymal stem cells (hAFMSCs) in terms of their morphology, proliferation, immunophenotype profile, and osteogenic capacity in vitro and in vivo. Not only greatly distinguished by cell morphology and proliferation, hAECs, hAFMSCs, and hBMSCs exhibited remarkably different signature regarding immunophenotypical profile. Microarray analysis revealed a different expression profile of genes involved in ossification along the three cell sources, highlighting the impact of different anatomical origin and molecular response to osteogenic induction on the final tissue-forming potential. Furthermore, our data indicated a potential role of FOXC2 in early osteogenic commitment.

38. Conditioned medium from human amniotic mesenchymal stromal cells limits infarct size and enhances angiogenesis.

Danieli P, Malpasso G, Ciuffreda MC, Cervio E, Calvillo L, Copes F, Pisano F, Mura M, Kleijn L,de Boer RA, Viarengo G, Rosti V, Spinillo A, Roccio M, Gnecchi M.
Stem Cells Transl Med. 2015 May.

The paracrine properties of human amniotic membrane-derived mesenchymal stromal cells (hAMCs) have not been fully elucidated. The goal of the present study was to elucidate whether hAMCs can exert beneficial paracrine effects on infarcted rat hearts, in particular through cardioprotection and angiogenesis. Moreover, we aimed to identify the putative active paracrine mediators. hAMCs were isolated, expanded, and

characterized. In vitro, conditioned medium from hAMC (hAMC-CM) exhibited cytoprotective and proangiogenic properties. In vivo, injection of hAMC-CM into infarcted rat hearts limited the infarct size, reduced cardiomyocyte apoptosis and ventricular remodeling, and strongly promoted capillary formation at the infarct border zone. Gene array analysis led to the identification of 32 genes encoding for the secreted factors overexpressed by hAMCs. Among these, midkine and secreted protein acidic and rich in cysteine were also upregulated at the protein level. Furthermore, high amounts of several proangiogenic factors were detected in hAMC-CM by cytokine array. Our results strongly support the concept that the administration of hAMC-CM favors the repair process after acute myocardial infarction.

39. Investigating the effect of hypoxic culture on the endothelial differentiation of human amniotic fluid-derived stem cells.

Lloyd-Griffith C, Duffy GP, O'Brien FJ.

J Anat. 2015 Mar 31

Amniotic fluid-derived stem cells (AFSCs) are a unique stem cell source that may have great potential for use in tissue engineering (TE) due to their pluripotentiality. AFSCs have previously shown angiogenic potential and may present an alternative cell source for endothelial-like cells that could be used in range of applications, including the pre-vascularisation of TE constructs and the treatment of ischaemic diseases. This study investigated the ability of these cells to differentiate down an endothelial lineage with the aim of producing an endothelial-like cell suitable for use in pre-vascularisation. As

hypoxia and the associated HIF-1 pathway have been implicated in the induction of angiogenesis in a number of biological processes, it was hypothesised that culture in hypoxic conditions could enhance the endothelial differentiation of AFSCs. The cells were cultured in endothelial cell media supplemented with 50 ng mL^{-1} of VEGF, maintained in normoxia, intermittent hypoxia or continuous hypoxia and assessed for markers of endothelial differentiation at day 7 and 14. The results demonstrated that AFSCs subjected to these culture conditions display an endothelial gene expression profile and adopted functional endothelial cell characteristics indicative of early endothelial differentiation. Culture in continuous hypoxia enhanced endothelial gene expression but did not enhance functional endothelial cell characteristics. Overall, AFSCs subjected to

endothelial stimuli demonstrated a less mature endothelial gene expression profile and phenotype when compared with HUVECs, the endothelial cell control. However, this study is the first time that the positive effect of an extended period of continuous hypoxic culture on endothelial differentiation in AFSCs has been demonstrated.

40. Human Amnion-Derived Mesenchymal Stem Cell Transplantation Ameliorates Dextran Sulfate Sodium-Induced Severe Colitis in Rats.

Onishi R, Ohnishi S, Higashi R, Watari M, Yamahara K, Okubo N, Nakagawa K, Katsurada T, Suda G,Natsuizaka M, Takeda H, Sakamoto N.

Cell Transplant. 2015 Mar 25

Mesenchymal stem cells (MSCs) are a valuable cell source in regenerative medicine. Recently, several studies have shown that MSCs can be easily isolated from human amnion. In this study, we investigated the therapeutic effect of human amnion-derived MSCs (AMSCs) in rats with severe colitis. Colitis was induced by the administration of 8% dextran sulfate sodium (DSS) from Day 0

to Day 5, and AMSCs (1×10^6 cells) were transplanted intravenously on Day 1. Rats were sacrificed on Day 5, and the colon length and histological colitis score were evaluated. The extent of inflammation was evaluated using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemistry. The effect of AMSCs on the inflammatory signals was investigated in vitro. AMSC transplantation significantly ameliorated the disease activity index score, weight loss, colon shortening and the histological colitis score. mRNA expression levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and migration inhibitory factor (MIF) were significantly decreased in the rectums of AMSC-treated rats. In addition, the infiltration of monocytes/macrophages was

significantly decreased in AMSC-treated rats. In vitro experiments demonstrated that activation of pro-inflammatory signals induced by TNF-α or lipopolysaccharide (LPS) in immortalized murine macrophage cells (RAW264.7) was significantly attenuated by co-culturing with AMSCs or by culturing with a conditioned medium obtained from AMSCs. Although the phosphorylation of I?B induced by TNF-α or LPS was not inhibited by the conditioned medium, nuclear translocation of NF-κB was significantly inhibited by the conditioned medium. Taken together, AMSC transplantation provided significant improvement in rats with severe colitis, possibly through the inhibition of monocyte/macrophage activity and through inhibition of NF-κB activation. AMSC could be considered as a new cell source for the treatment of severe colitis.

41. Human mesenchymal stem cells - current trends and future prospective.

Ullah I, Subbarao RB, Rho GJ.

Biosci Rep. 2015 Apr 28

Stem cells are cells specialized cell, capable of renewing themselves through cell division and can differentiate into multi-lineage cells. These cells are categorized as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and adult stem cells. Mesenchymal stem cells (MSCs) are adult stem cells which can be isolated from human and animal sources. Human MSCs (hMSCs) are the non-haematopoietic, multipotent stem cells with the capacity to differentiate into mesodermal lineage such as osteocytes, adipocytes and chondrocytes as well ectodermal (neurocytes) and endodermal

lineages (hepatocytes). MSCs express cell surface markers like cluster of differentiation (CD)29, CD44, CD73, CD90, CD105 and lack the expression of CD14, CD34, CD45 and HLA (human leucocyte antigen)-DR. hMSCs for the first time were reported in the bone marrow and till now they have been isolated from various tissues, including adipose tissue, amniotic fluid, endometrium, dental tissues, umbilical cord and Wharton's jelly which harbours potential MSCs. hMSCs have been cultured long-term in specific media without any severe abnormalities. Furthermore, MSCs have immunomodulatory features, secrete cytokines and immune-receptors which regulate the microenvironment in the host tissue. Multilineage potential, immunomodulation and secretion of anti-inflammatory molecules makes MSCs an effective tool in the

treatment of chronic diseases. In the present review, we have highlighted recent research findings in the area of hMSCs sources, expression of cell surface markers, long-term in vitro culturing, in vitro differentiation potential, immunomodulatory features, its homing capacity, banking and cryopreservation, its application in the treatment of chronic diseases and its use in clinical trials.

42. Stem cells from human amniotic fluid exert immunoregulatory function via secreted indoleamine 2,3-dioxygenase1.

Romani R, Pirisinu I, Calvitti M, Pallotta MT, Gargaro M, Bistoni G, Vacca C, Di Michele A,Orabona C, Rosati J, Pirro M, Giovagnoli S, Matino D, Prontera P, Rosi G, Grohmann U, Talesa VN, Donti E, Puccetti P, Fallarino F.

J Cell Mol Med. 2015 Jul

Although human amniotic fluid does contain different populations of foetal-derived stem cells, scanty information is available on the stemness and the potential immunomodulatory activity of in vitro expanded, amniotic fluid stem cells. By means of a methodology unrequiring immune selection, we isolated and characterized

different stem cell types from second-trimester human amniotic fluid samples (human amniotic fluid stem cells, HASCs). Of those populations, one was characterized by a fast doubling time, and cells were thus designated as fHASCs. Cells maintained their original phenotype under prolonged in vitro passaging, and they were able to originate embryoid bodies. Moreover, fHASCs exhibited regulatory properties when treated with interferon (IFN)-γ, including induction of the immunomodulatory enzyme indoleamine 2,3-dioxygenase 1 (IDO1). On coculture with human peripheral blood mononuclear cells, IFN-γ-treated fHASCs caused significantly decreased T-cell proliferation and increased frequency in CD4(+) CD25(+) FOXP3(+) regulatory T cells. Both effects required an intact IDO1 function and were cell contact-independent.

An unprecedented finding in our study was that purified vesicles from IFN-γ-treated fHASCs abundantly expressed the functional IDO1 protein, and those vesicles were endowed with an fHASC-like regulatory function. In vivo, fHASCs were capable of immunoregulatory function, promoting allograft survival in a mouse model of allogeneic skin transplantation. This was concurrent with the expansion of CD4(+) CD25(+) Foxp3(+) T cells in graft-draining lymph nodes from recipient mice. Thus fHASCs, or vesicles thereof, may represent a novel opportunity for immunoregulatory maneuvers both in vitro and in vivo.

43. Gene therapy strategies using engineered stem cells for treating gynecologic and breast cancer patients (Review).

Kim YS, Hwang KA, Go RE, Kim CW, Choi KC.

Oncol Rep. 2015 May.

There are three types of stem cells: embryonic stem (ES) cells, adult stem (AS) cells and induced pluripotent stem (iPS) cells. These stem cells have many benefits including the potential ability to differentiate into various organs. In addition, engineered stem cells (GESTECs) designed for delivering therapeutic genes may be capable of treating human diseases including malignant cancers. Stem cells have been found to possess the potential for serving as novel delivery

vehicles for therapeutic or suicide genes to primary or metastatic cancer formation sites as a part of gene-directed enzyme/prodrug combination therapy (GEPT). Given the advantageous properties of stem cells, tissue-derived stem cells are emerging as a new tool for anticancer therapy combined with prodrugs. In this review, the effects of GESTECs with different origins, i.e., neural, amniotic membrane and amniotic fluid, introduced to treat patients with diverse types of gynecologic and breast cancers are discussed. Data from the literature indicate the therapeutic potential of these cells as a part of gene therapy strategies to selectively target malignancies in women at clinically terminal stages.

44. Human amniotic mesenchymal stem cells inhibit allogeneic lymphocyte proliferation and reduce the secretion of interferon γ.

Song J, Cong S, Li Y, Bai L, Cao G.

Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2015 Mar.

OBJECTIVE:

To investigate the effects of human amniotic mesenchymal stem cells (hAMSCs) on the function of lymphocytes in vitro.

METHODS:

Enzymatic digestion method was used to isolate and culture hAMSCs. Fluorophore-labeled mouse anti-human monoclonal antibodies were used to identify cell surface antigens with flow cytometry. The expressions of vimentin and stage specific

embryonic antigen-4 (SSEA-4) were detected by immunofluorescence staining. Isolated lymphocytes were stimulated by concanavalin (ConA), and then 1×10^4, 5×10^4, 1×10^5 hAMSCs were co-cultured with the ConA-treated lymphocytes. Lymphocyte proliferation was measured by CCK-8 assay and the supernatant level of IFN-γ was determined by ELISA.

RESULTS:

ConA (5 μg/mL) could cause lymphocyte proliferation. hAMSCs inhibited lymphocyte proliferation induced by ConA in co-culture conditions, and with the increasing number of hAMSCs, the suppressing effect was more obvious. When the cells were cultured for 72 hours, CCK-8 assay showed that the number of lymphocytes treated with ConA alone was significantly higher than that of ConA-treated lymphocytes co-cultured with hAMSCs. The

best inhibitory group 1 × 10(6) lymphocytes co-cultured with 1 × 10^5 hAMSCs was selected to measure supernatant IFN-γ secretion by ELISA after 72 hours. The level of IFN-γ was significantly lower than that in the simple ConA-stimulated group.

CONCLUSION:

HAMSCs could inhibit lymphocyte proliferation and reduce IFN-γ secretion induced by ConA in vitro.

45. Stem cell therapy in inflammatory bowel disease: A promising therapeutic strategy?

Flores AI, Gómez-Gómez GJ, Masedo-González Á, Martínez-Montiel MP.
World J Stem Cells. 2015 Mar 26

Inflammatory bowel diseases are inflammatory, chronic and progressive diseases of the intestinal tract for which no curative treatment is available. Research in other fields with stem cells of different sources and with immunoregulatory cells (regulatory T-lymphocytes and dendritic T-cells) opens up new expectations for their use in these diseases. The goal for stem cell-based therapy is to provide a permanent cure. To achieve this, it will be necessary to obtain a cellular product, original or genetically modified, that has a high migration capacity

and homes into the intestine, has high survival after transplantation, regulates the immune reaction while not being visible to the patient's immune system, and repairs the injured tissue.

46. Placenta-based therapies for the treatment of epidermolysis bullosa.

Nevala-Plagemann C, Lee C, Tolar J.

Cytotherapy. 2015 Jun

Recessive dystrophic epidermolysis bullosa (RDEB) is a severe blistering skin disease caused by mutations in the COL7A1 gene. These mutations lead to decreased or absent levels of collagen VII at the dermal-epidermal junction. Over the past decade, significant progress has been made in the treatment of RDEB, including the use of hematopoietic cell transplantation, but a cure has been elusive. Patients still experience life-limiting and life-threatening complications as a result of painful and debilitating wounds. The continued suffering of these patients drives the need to improve existing therapies and

develop new ones. In this Review, we will discuss how recent advances in placenta-based, umbilical cord blood-based and amniotic membrane-based therapies may play a role in the both the current and future treatment of RDEB.

47. Amniotic fluid-derived stem cells demonstrate limited cardiac differentiation following small molecule-based modulation of Wnt signaling pathway.

Connell JP[1], Ruano R, Jacot JG.

Biomed Mater. 2015 Mar 18

Amniotic fluid-derived stem cells (AFSC) are a promising cell source for regenerative medicine and cardiac tissue engineering. However, a non-xenotropic differentiation protocol has not been established for cardiac differentiation of AFSC. We tested a small molecule-based modulation of Wnt signaling for directed cardiac differentiation of AFSC. Cells were treated with inhibitors of glycogen synthase kinase 3 and Wnt production and secretion in a time-dependent and sequential manner, as has been demonstrated successful

for cardiac differentiation of embryonic and induced pluripotent stem cells. Cells were then analyzed for gene and protein expression of markers along the cardiac lineage at multiple days during the differentiation protocol. At the midpoint of the differentiation, an increase in the percentage of AFSC expressing Islet-1, a transcription factor found in cardiac progenitor cells, and Nkx-2.5, a cardiac transcription factor, was observed. After a 15 d differentiation, a subpopulation of AFSC upregulated protein expression of smooth muscle actin, myosin light chain-2, and troponin I, all indicative of progression down a cardiac lineage. AFSC at the end of the differentiation also demonstrated organization of connexin 43, a key component of gap junctions, to cell membranes. However, no organized sarcomeres or spontaneous contraction were

observed. These results demonstrate that small molecule-based modulation of Wnt signaling alone is not sufficient to generate functional cardiomyocytes from AFSC, though an upregulation of genes and proteins common to cardiac lineage cells was observed.

48. Fetal endothelial and mesenchymal progenitors from the human term placenta: potency and clinical potential.

Shafiee A, Fisk NM, Hutmacher DW[3], Khosrotehrani K, Patel J.

Stem Cells Transl Med. 2015 May

Since the isolation of fetal stem cell populations from perinatal tissues, such as umbilical cord blood and placenta, interest has been growing in understanding their greater plasticity compared with adult stem cells and exploring their potential in regenerative medicine. The phenomenon of fetal microchimerism (FMC) naturally occurring during pregnancy through the transfer of fetal stem/progenitor cells to maternal blood and tissues has been integral in developing this dogma. Specifically,

microchimeric mesenchymal stem cells and endothelial progenitors of fetal origin have now demonstrated a capacity for tissue repair in the maternal host. However, the use of similar fetal stem cells in therapy has been significantly hampered by the availability of clinically relevant cell numbers and/or contamination with cells of maternal origin, particularly when using the chorionic and decidual placenta. In the present prospective review, we highlight the importance of FMC to the field of fetal stem cell biology and issues of maternal contamination from perinatal tissues and discuss specific isolation strategies to overcome these translational obstacles.

49. Rat-derived amniotic epithelial cells differentiate into mature hepatocytes in vivo with no evidence of cell fusion.

Marongiu M, Serra MP, Contini A, Sini M, Strom SC, Laconi E[1], Marongiu F.

Stem Cells Dev. 2015 Jun 15

Amniotic epithelial cells (AEC) derived from human placenta represent a useful and noncontroversial source for liver-based regenerative medicine. Previous studies suggested that human- and rat-derived AEC differentiate into hepatocyte-like cells upon transplantation. In the retrorsine (RS) model of liver repopulation, clusters of donor-derived cells engrafted in the recipient liver and, importantly, showed characteristics of mature hepatocytes. The aim of the current study was to investigate the possible

involvement of cell fusion in the emergence of hepatocyte clusters displaying a donor-specific phenotype. To this end, 4-week-old GFP(+)/DPP-IV(-) rats were treated with RS and then transplanted with undifferentiated AEC isolated from the placenta of DPP-IV(+) pregnant rats at 16-19 days of gestational age. Results indicated that clusters of donor-derived cells were dipeptidyl peptidase type IV (DPP-IV) positive, but did not express the green fluorescent protein (GFP), suggesting that rat amniotic epithelial cells (rAEC) did not fuse within the host parenchyma, as no colocalization of the two tags was observed. Moreover, rAEC-derived clusters expressed markers of mature hepatocytes (eg, albumin, cytochrome P450), but were negative for the expression of biliary/progenitor markers (eg, epithelial cell adhesion molecule [EpCAM]) and did not express the marker of

preneoplastic hepatic nodules glutathione S-transferase P (GST-P). These results extend our previous findings on the potential of AEC to differentiate into mature hepatocytes and suggest that this process can occur in the absence of cell fusion with host-derived cells. These studies support the hypothesis that amnion-derived epithelial cells can be an effective cell source for the correction of liver disease.

50. Differentiation of adipocytes and osteocytes from human adipose and placental mesenchymal stem cells.

Mohammadi Z, Afshari JT, Keramati MR, Alamdari DH, Ganjibakhsh M, Zarmehri AM, Jangjoo A,Sadeghian MH, Ameri MA, Moinzadeh L.

Iran J Basic Med Sci. 2015 Mar

OBJECTIVES:

Mesenchymal stem cells (MSC) can be isolated from adult tissues such as adipose tissue and other sources. Among these sources, adipose tissue (because of easy access) and placenta (due to its immunomodulatory properties, in addition to other useful properties), have attracted more attention in terms of research. The isolation and comparison of MSC from these two

sources provides a proper source for clinical experimentation. The aim of this study was to compare the characteristics of MSC isolated from human adipose tissue and placenta.

MATERIALS AND METHODS:

Adipose and placental MSC were isolated from the subcutaneous adipose tissues of 10 healthy women (25 to 40 years) and from a fresh term placenta (n= 1), respectively. Stem cells were characterized and compared by flow cytometry using CD29, CD31, CD34, CD44, CD45, CD105, CD166 and HLA-DR markers. Osteocytes and adipocytes were differentiated from isolated human mesenchymal stem cells (HMSC).

RESULTS:

Adipose and placenta-derived MSC exhibited the same morphological features. ADSC differentiated faster than placenta; however,

both were differentiated, taking up to 21 days for osteocyte and 14 days for adipocyte differentiation. About 90% of PLC-MSC and ADSC were positive for CD29, CD44, CD105, and CD166; and negative for CD31, CD34, CD45, and HLA-DR.

CONCLUSION:

The two sources of stem cells showed similar surface markers, morphology and differentiation potential and because of their multipotency for differentiating to adipocytes and osteocytes, they can be applied as attractive sources of MSC for regenerative medicine.

51. Secretory factors of human chorion-derived stem cells enhance activation of human fibroblasts.

Kim MK, Seo BF, Kim KJ, Lee SJ, Ryu YH, Rhie JW.

Cytotherapy. 2015 Mar

BACKGROUND AIMS:

Wound healing remains a principal challenge in modern medical science. Chorion-dervied stem cells (CDSCs), isolated from human placenta, have largely been overlooked, and few studies on their potential in wound healing have been conducted. In this study, we investigated the functional characteristics of CDSCs compared with adipose-derived stem cells (ASCs) on human fibroblasts (HFs).

METHODS:

We analyzed CDSCs by means of flow cytometry to confirm their mesenchymal stromal cell characteristics. We then evaluated the paracrine effects of CDSCs on HFs in a co-culture system and focused on fibroblast proliferation, migration and collagen synthesis. To explore the potential of CDSCs in wound healing, CDSC- and ASC-secreted factors were compared by use of a cytokine antibody array.

RESULTS:

CDSCs had morphology similar to MSCs and expressed a mesenchymal stromal cell phenotype. HF proliferation and migration increased more than 5-fold when co-cultured with CDSCs. Furthermore, Western blot and reverse transcription-polymerase chain reaction analysis showed that expression of collagen (types I and III) in fibroblasts was

upregulated 2-fold when co-cultured with CDSCs. Cytokine array results of CDSC-conditioned medium and ASC-conditioned medium revealed the presence of growth factors known to influence wound healing, including interleukin -6, interleukin -8, monocyte chemotactic protein 1 and regulated on activation, normal T cells expressed and secreted.

CONCLUSIONS:

Our data demonstrated that CDSCs are functionally similar to ASCs, promote HF activation, and secrete growth factors that influence wound healing. Therefore, we suggest that CDSCs are potentially applicable in wound healing.

52. Mesenchymal stem cells reside in a vascular niche in the decidua basalis and are absent in remodelled spiral arterioles.

Kusuma GD, Manuelpillai U, Abumaree MH, Pertile MD, Brennecke SP, Kalionis B.

Placenta. 2015 Mar

INTRODUCTION:

Maternal decidua basalis tissue attached to the placenta following delivery is a source of decidual mesenchymal stem cells (DMSCs). The in vitro characteristics of DMSCs have been partly defined but their in vivo function(s) are poorly understood. The anatomic location, or niche, provides clues regarding potential in vivo function(s) of DMSCs, but the niche has not been described.

METHODS:

Cells were isolated from the decidua basalis and flow cytometric analyses showed the expected phenotypic profile for MSC cell surface markers. In vitro, the cells differentiated into adipocytes, osteocytes, and chondrocytes. DMSCs were then stained with antibodies by immunofluorescence detection.

RESULTS:

Immunocytochemistry revealed that DMSCs were positive for FZD-9, STRO-1, 3G5, and α-SMA as expected and lacked expression of vWF and Ck7. Fluorescence in situ hybridization analysis showed the cultured cells were of maternal origin. Immunofluorescence was carried out on placental bed biopsies using the FZD-9, STRO-1, 3G5, and α-SMA antibodies. DMSCs were located in the vascular niche in decidua basalis. Immunofluorescence with

antibodies to FZD-9, Ck7 and vWF revealed DMSCs in the vascular niche surrounding intact non-transformed spiral arterioles but DMSCs were absent in fully transformed spiral arterioles.

DISCUSSION:

Spiral arteriole remodelling is a critical feature of human pregnancy. The DMSC niche was investigated in fully transformed and non-transformed spiral arterioles. DMSCs have not been previously implicated in spiral arteriole remodelling. The absence of DMSCs around fully transformed spiral arterioles suggests they are a target for replacement or destruction by invading placental extravillous trophoblast cells, which carry out spiral arteriole remodelling.

53. Differentiation Potential of Human Chorion-Derived Mesenchymal Stem Cells into Motor Neuron-Like Cells in Two- and Three-Dimensional Culture Systems.

Faghihi F, Mirzaei E, Ai J, Lotfi A, Sayahpour FA, Barough SE, Joghataei MT.

Mol Neurobiol. 2015 Mar 20

Many people worldwide suffer from motor neuron-related disorders such as amyotrophic lateral sclerosis and spinal cord injuries. Recently, several attempts have been made to recruit stem cells to modulate disease progression in ALS and also regenerate spinal cord injuries. Chorion-derived mesenchymal stem cells (C-MSCs), used to be discarded as postpartum medically waste product, currently represent a class of cells with self

renewal property and immunomodulatory capacity. These cells are able to differentiate into mesodermal and nonmesodermal lineages such as neural cells. On the other hand, gelatin, as a simply denatured collagen, is a suitable substrate for cell adhesion and differentiation. It has been shown that electrospinning of scaffolds into fibrous structure better resembles the physiological microenvironment in comparison with two-dimensional (2D) culture system. Since there is no report on potential of human chorion-derived MSCs to differentiate into motor neuron cells in two- and three-dimensional (3D) culture systems, we set out to determine the effect of retinoic acid (RA) and sonic hedgehog (Shh) on differentiation of human C-MSCs into motor neuron-like cells cultured on tissue culture plates (2D) and electrospun nanofibrous gelatin scaffold (3D).

54. Human Placenta-Derived CD146-Positive Mesenchymal Stromal Cells Display a Distinct Osteogenic Differentiation Potential.

Ulrich C, Abruzzese T, Maerz JK, Ruh M[1], Amend B, Benz K, Rolauffs B, Abele H[5], Hart ML, Aicher WK.

Stem Cells Dev. 2015 Jul 1

Mesenchymal stromal cells (MSCs) are multipotent cells that can be differentiated in vitro into a variety of cell types, including adipocytes or osteoblasts. Our recent studies indicated that a high expression of CD146 on MSCs from bone marrow correlates with their robust osteogenic differentiation potential. We therefore investigated if expression of CD146 on MSCs from the placenta correlates with a similar osteogenic differentiation

potential. The MSCs were isolated specifically from the endometrial and fetal parts of human term placenta and expanded in separate cultures and compared with MSCs from bone marrow as controls. The expression of cell surface antigens was investigated by flow cytometry. Differentiation of MSCs was documented by cytochemistry and analysis of typical lineage marker genes. CD146-positive MSCs were separated from CD146-negative cells by magnet-assisted cell sorts (MACS). We report that the expression of CD146 is associated with a higher osteogenic differentiation potential in human placenta-derived MSCs (pMSCs) and the CD146(pos) pMSCs generated a mineralized extracellular matrix, whereas the CD146(neg) pMSCs failed to do so. In contrast, adipogenic and chondrogenic differentiation of pMSCs was not different in

CD146(pos) compared with CD146(neg) pMSCs. Upon enrichment of pMSCs by MACS, the CD146(neg) and CD146(pos) populations maintained their expression levels for this antigen for several passages in vitro. We conclude that CD146(pos) pMSCs either respond to osteogenic stimuli more vividly or, alternatively, CD146(pos) pMSCs present a pMSC subset that is predetermined to differentiate into osteoblasts.

55. GFP Labeling and Hepatic Differentiation Potential of Human Placenta-Derived Mesenchymal Stem Cells.

Yu J, Su X, Zhu C, Pan Q, Yang J, Ma J, Shen L, Cao H, Li L.

Cell Physiol Biochem. 2015 Apr.

BACKGROUND:

Stem cell-based therapy in liver diseases has received increasing interest over the past decade, but direct evidence of the homing and implantation of transplanted cells is conflicting. Reliable labeling and tracking techniques are essential but lacking. The purpose of this study was to establish human placenta-derived mesenchymal stem cells (hPMSCs) expressing green fluorescent

protein (GFP) and to assay their hepatic functional differentiation in vitro.

METHODS:

The GFP gene was transduced into hPMSCs using a lentivirus to establish GFP(+) hPMSCs. GFP(+) hPMSCs were analyzed for their phenotypic profile, viability and adipogenic, osteogenic and hepatic differentiation. The derived GFP(+) hepatocyte-like cells were evaluated for their metabolic, synthetic and secretory functions, respectively.

RESULTS:

GFP(+) hPMSCs expressed high levels of HLA I, CD13, CD105, CD73, CD90, CD44 and CD29, but were negative for HLA II, CD45, CD31, CD34, CD133, CD271 and CD79. They possessed adipogenic, osteogenic and hepatic differentiation

potential. Hepatocyte-like cells derived from GFP(+) hPMSCs showed typical hepatic phenotypes.

CONCLUSIONS:

GFP gene transduction has no adverse influences on the cellular or biochemical properties of hPMSCs or markers. GFP gene transduction using lentiviral vectors is a reliable labeling and tracking method. GFP(+) hPMSCs can therefore serve as a tool to investigate the mechanisms of MSC-based therapy, including hepatic disease therapy.

56. Pre-clinical efficacy and safety evaluation of human amniotic fluid-derived stem cell injection in a mouse model of urinary incontinence.

Choi JY, Chun SY, Kim BS, Kim HT, Yoo ES, Shon YH[2], Lim JO, Yun SJ, Song PH, Chung SK,Yoo JJ, Kwon TG.

Yonsei Med J. 2015 May

PURPOSE:

Stem cell-based therapies represent new promises for the treatment of urinary incontinence. This study was performed to assess optimized cell passage number, cell dose, therapeutic efficacy, feasibility, toxicity, and cell trafficking for the first step of the pre-clinical evaluation of human amniotic fluid stem cell (hAFSC) therapy in a urinary incontinence animal model.

MATERIALS AND METHODS:

The proper cell passage number was analyzed with hAFSCs at passages 4, 6, and 8 at week 2. The cell dose optimization included 1×10^4, 1×10^5, and 1×10^6 cells at week 2. The in vivo cell toxicity was performed with 0.25×10^6, 0.5×10^6, and 1×10^6 cells at weeks 2 and 4. Cell tracking was performed with 1×10^6 cells at weeks 2 and 4.

RESULTS:

The selected optimal cell passage number was smaller than 6, and the optimal cell dose was 1×10^6 for the mouse model. In our pre-clinical study, hAFSC-injected animals showed normal values for several parameters. Moreover, the injected cells were found to be non-toxic and non-tumorigenic. Furthermore, the injected hAFSCs were rarely identified by in vivo cell trafficking in the target organs at week 2.

CONCLUSION:

This study demonstrates for the first time the pre-clinical efficacy and safety of hAFSC injection in the urinary incontinence animal model and provides a basis for future clinical applications.

57. How far are we from the clinical use of placental-derived mesenchymal stem cells?

Fierabracci A, Lazzari L, Muraca M, Parolini O.

Expert Opin Biol Ther. 2015 May

In recent years, multiple studies have investigated the biology and clinical applications of mesenchymal stem cells (MSCs), trying to define their markers, and elucidate their effects in animal models. MSCs are available from different tissues, and the use of placental-derived MSCs (PMSCs) for treating a variety of disorders is on the forefront. Herein, we discuss the most recent findings regarding the standardization of their isolation procedure and phenotype, along with advantages and limitations of their use. We also discuss the safety of the

placental cell products, including the issue of senescence and mutagenesis of PMSCs, and efficacy from preclinical studies.

58. The expression of neurogenic markers after neuronal induction of chorion-derived mesenchymal stromal cells.

Manochantr S, Marupanthorn K, Tantrawatpan C, Kheolamai P.

Neurol Res. 2015 May

OBJECTIVES:

Chorion is a tissue of early embryologic period that is discarded after delivery. It might be the potential source of mesenchymal stromal cells (MSCs) that can be used for research and eventually for therapeutic studies. At present, the biological properties and the differentiation capacity of chorion-derived MSCs are still poorly characterised. The objective of this study is to characterise and explore the differentiating potential of

chorion-derived MSCs towards the neuronal lineages.

METHODS:

Chorionic membrane was digested with enzyme and cultured in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum. The expression of MSC markers was examined using flow cytometry. The adipogenic, osteogenic and neurogenic differentiation were examined by culturing in appropriate induction media. The expression of neuronal markers was determined by immunofluorescence and quantitative real time-PCR.

RESULTS:

Chorion-derived MSCs were easily expanded up to 20 passages. They were positive for MSC markers (CD73, CD90 and CD105), and negative for haematopoietic markers

(CD34 and CD45). Chorion-derived MSCs could differentiate into several mesodermal-lineages including adipocytes and osteoblasts. Moreover, chorion-derived MSCs could differentiate into neuronal-like cells as characterised by cell morphology and the presence of neural markers including MAP-2, glial fibrillary acidic protein (GFAP) and beta-tubulin III.

DISCUSSION:

Chorion-derived MSCs can be readily obtained and expanded in culture. These cells also have transdifferentiation capacity as evidenced by their neuronal differentiation potential. Therefore, chorion can be used as an alternative source of MSCs for stem cell therapy in nervous system disorders.

59. Placental-derived stem cells: Culture, differentiation and challenges.

Oliveira MS, Barreto-Filho JB.

World J Stem Cells. 2015 May 26

Stem cell therapy is a promising approach to clinical healing in several diseases. A great variety of tissues (bone marrow, adipose tissue, and placenta) are potentially sources of stem cells. Placenta-derived stem cells (p-SCs) are in between embryonic and mesenchymal stem cells, sharing characteristics with both, such as non-carcinogenic status and property to differentiate in all embryonic germ layers. Moreover, their use is not ethically restricted as fetal membranes are considered medical waste after birth. In this context, the present review will be focused on the biological

properties, culture and potential cell therapy uses of placental-derived stem cells. Immunophenotype characterization, mainly for surface marker expression, and basic principles of p-SC isolation and culture (mechanical separation or enzymatic digestion of the tissues, the most used culture media, cell plating conditions) will be presented. In addition, some preclinical studies that were performed in different medical areas will be cited, focusing on neurological, liver, pancreatic, heart, muscle, pulmonary, and bone diseases and also in tissue engineering field. Finally, some challenges for stem cell therapy applications will be highlighted. The understanding of the mechanisms involved in the p-SCs differentiation and the achievement of pure cell populations (after differentiation) are key points that must be clarified before bringing

the preclinical studies, performed at the bench, to the medical practice.

60. Comparative analysis of neural differentiation potential in human mesenchymal stem cells derived from chorion and adult bone marrow.

Ziadlou R, Shahhoseini M, Safari F, Sayahpour FA, Nemati S, Eslaminejad MB.

Cell Tissue Res. 2015 May 30

The finding of a reliable and abundant source of stem cells for the replacement of missing neurons in nervous system diseases requires extensive characterization of neural-differentiation-associated markers in stem cells from various sources. Chorion-derived stem cells from the human placenta have recently been described as an abundant, ethically acceptable, and easily accessible source of cells that are not limited in the same way as bone marrow (BM) mesenchymal

stem cells (MSCs). We have isolated and cultured chorion MSCs (C-MSCs) and compared their proliferative capacity, multipotency, and neural differentiation ability with BM-MSCs. C-MSCs showed a higher proliferative capacity compared with BM-MSCs. The expression and histone modification of Nestin, as a marker for neural stem/progenitor cells, was evaluated quantitatively between the two groups. The Nestin expression level in C-MSCs was significantly higher than that in BM-MSCs. Notably, modifications of lys9, lys4, and lys27 of histone H3 agreed with the remarkable higher expression of Nestin in C-MSCs than in BM-MSCs. Furthermore, after neural differentiation of MSCs upon retinoic acid induction, both immunocytochemical and flow cytometry analyses demonstrated that the expression of neural marker genes

was significantly higher in neural-induced C-MSCs compared with BM-MSCs. Mature neuron marker genes were also expressed at a significantly higher level in C-MSCs than in BM-MSCs. Thus, C-MSCs have a greater potential than BM-MSCs for differentiation to neural cell lineages and can be regarded as a promising source of stem cells for the cell therapy of neurological disorders.

61. Epigenetic Alterations of IL-6/STAT3 Signaling by Placental Stem Cells Promote Hepatic Regeneration in a Rat Model with CCl4-induced Liver Injury.

Jung J, Moon JW, Choi JH, Lee YW, Park SH[2], Kim GJ.

Int J Stem Cells. 2015 May

BACKGROUND:

Human chorionic plate-derived mesenchymal stem cells (CP-MSCs) isolated from the placenta have been reported to demonstrate therapeutic effects in animal models of liver injury; however, the underlying epigenetic mechanism of this effect has not been elucidated. Thus, we investigated whether CP-MSCs influence epigenetic processes during regeneration of the injured liver.

METHODS:

CP-MSCs were engrafted into a carbon tetrachloride (CCl4)-injured rat model through direct transplantation into the liver (DTX), intrasplenic transplantation (STX), and intravenous transplantation via the tail vein (TTX). Non-transplanted (NTX) rats were maintained as sham controls. Liver tissues were analyzed after transplantation using immunohistochemistry, western blot analysis, and quantitative methylation-specific polymerase chain reaction. Proliferation and human interleukin-6 (hIL-6) enzyme-linked immunosorbent assays were performed using CCl4-treated hepatic cells that were co-cultured with CP-MSCs.

RESULTS:

The Ki67 labeling index, cell cyclins, albumin, IL-6, and gp130 levels were elevated in the CP-MSC transplantation

groups. The concentration of hIL-6 in supernatants and the proliferation of CCl4-treated rat hepatic cells were enhanced by co-culturing with CP-MSCs ($p<0.05$), while the methylation of IL-6/IL-6R and STAT3 by CP-MSC transplantation decreased.

CONCLUSION:

These results suggest that administration of CP-MSCs promotes IL-6/STAT3 signaling by decreasing the methylation of the IL-6/SATA3 promoters and thus inducing the proliferation of hepatic cells in a CCl4-injured liver rat model. These data advance our understanding of the therapeutic mechanisms in injured livers, and can facilitate the development of cell-based therapies using placenta-derived stem cells.

62. Transplantation of human amnion mesenchymal cells attenuates the disease development in rats with collagen-induced arthritis.

Shu J, Pan L, Huang X, Wang P, Li H, He X, Cai Z.

Clin Exp Rheumatol. 2015 May 11

OBJECTIVES:

Human amnion mesenchymal cells (hAMCs), isolated from the amniotic membrane of human placenta, are a unique population of mesenchymal stem cells (MSCs). Recent studies indicated that hAMCs had immunosuppressive functions and might be used in treatment of some autoimmune diseases. The aim of this study is to explore the feasibility of using hAMCs for treatment rats with collagen-induced arthritis (CIA), a

classic animal model for human rheumatoid arthritis.

METHODS:

SD rats were immunised with type II collagen and Freund's incomplete adjuvant. hAMCs were injected intraperitoneal when arthritis had become established. The arthritis was evaluated macroscopically and microscopically. Serum levels of IFN-γ, TNF-α, SOD, MDA, GSH-Px and T-AOC were detected by commercially assay kits. CD4+/CD8+ T-cell ratio in peripheral blood was examined by flow cytometry. Proliferation of splenocytes was evaluated using MTT assay.

RESULTS:

The results demonstrated that application of hAMCs significantly ameliorated severity of arthritis and decreased the histopathological

changes in CIA rats. Consistently, production of proinflammatory cytokines such as IFN-γ and TNF-α was dramatically inhibited. Moreover, hAMCs exerted anti-oxidative capacity by significantly raising the levels of SOD, GSH-Px, T-AOC and lowering the level of MDA. In addition, hAMCs also remarkably restored CD4+/CD8+ T-cell ratio and induced hyporesponsiveness of T lymphocytes by inhibiting their active proliferation. Finally, hAMCs had no obvious side effect on CIA rats.

CONCLUSIONS:

In conclusion, our results indicated that hAMCs could attenuate the disease development in rats with CIA, which might be a promising cell source for therapy of rheumatoid arthritis.

63. The use of human amniotic fluid stem cells as an adjunct to promote pulmonary development in a rabbit model for congenital diaphragmatic hernia.

DeKoninck P, Toelen J, Roubliova X, Carter S, Pozzobon M, Russo FM, Richter J, Vandersloten PJ, Verbeken E, De Coppi P, Deprest J.

OBJECTIVE:

This study aimed to evaluate the potential benefit of intra-tracheal injection of human amniotic fluid stem cells (hAFSC) on pulmonary development combined with TO in a rabbit model for CDH.

METHODS:

In time-mated pregnant does a left diaphragmatic defect was created at d23

(term = 31). At d28, previously operated fetuses were assigned to either TO and injection with 70 μL of phosphate buffered saline (PBS) or 1.0×10^6 c-Kit positive hAFSC expressing LacZ or were left untouched (CDH). Harvesting was done at d31 to obtain their lung-to-body weight ratio (LBWR), airway and vascular lung morphometry, X-gal staining and immunohistochemistry for Ki67 and surfactant protein-B (SP-B).

RESULTS:

CDH-induced pulmonary hypoplasia is countered by TO + PBS, this reverses LBWR, mean terminal bronchiole density (MTBD) and medial thickness to normal. The additional injection of hAFSC decreases MTBD and results in a non-significant decrease in muscularization of intra-acinary vessels. There were no inflammatory changes

and LacZ positive hAFSC were dispersed throughout the lung parenchyma 4 days after injection.

CONCLUSION:

HAFSC exert an additional effect on TO leading to a decrease in MTBD, a measure of alveolar number surrounding the terminal bronchioles, without signs of toxicity.

64. Role of hepatocyte growth factor in the immunomodulation potential of amniotic fluid stem cells.

Maraldi T, Beretti F, Guida M, Zavatti M, De Pol A.

Stem Cells Transl Med. 2015 Jun

Human amniotic fluid stem cells (hAFSCs) may be useful for regenerative medicine because of their potential to differentiate into all three germ layers and to modulate immune response with different types of secretion molecules. This last issue has not been completely elucidated. The aim of this study was to investigate the secretome profile of the hAFSC, focusing on the role of hepatocyte growth factor (HGF) in immunoregulation through short and long cocultures with human peripheral blood mononuclear cells. We

found that HGF produced by hAFSCs exerts a cytoprotective role, inducing an increase in caspase-dependent apoptosis in human immune cells. This study provides evidence supporting the hypothesis that amniotic fluid is an ideal source of stem cells for expansion and banking properties for therapeutic use. hAFSCs not only are less immunogenic but also can secrete immunoregulatory factors that may be useful in autoimmune diseases or allogenic implants.

SIGNIFICANCE:

New information about the secretome pattern is reported in this paper. Human amniotic fluid stem cells (hAFSCs) possess immunomodulatory properties involving hepatocyte growth factor production. hAFSCs could be used in immunotherapies and might be able to avoid allogenic rejection.

65. Microenvironmental factors involved in human amnion mesenchymal stem cells fate decisions.

Syva SH, Ampon K, Lasimbang H, Fatimah SS.

J Tissue Eng Regen Med. 2015 Jun 15

Human amnion mesenchymal stem cells (HAMCs) show great differentiation and proliferation potential and also other remarkable features that could serve as an outstanding alternative source of stem cells in regenerative medicine. Recent reports have demonstrated various kinds of effective artificial niche that mimic the microenvironment of different types of stem cell to maintain and control their fate and function. The components of the stem cell microenvironment consist mainly of soluble

and insoluble factors responsible for regulating stem cell differentiation and self-renewal. Extensive studies have been made on regulating HAMCs differentiation into specific phenotypes; however, the understanding of relevant factors in directing stem cell fate decisions in HAMCs remain underexplored. In this review, we have therefore identified soluble and insoluble factors, including mechanical stimuli and cues from the other supporting cells that are involved in directing HAMCs fate decisions. In order to strengthen the significance of understanding on the relevant factors involved in stem cell fate decisions, recent technologies developed to specifically mimic the microenvironments of specific cell lineages are also reviewed.

66. Placental mesenchymal stromal cells rescue ambulation in ovine myelomeningocele.

Wang A, Brown EG, Lankford L, Keller BA, Pivetti CD, Sitkin NA, Beattie MS[2], Bresnahan JC,Farmer DL.

Stem Cells Transl Med. 2015 Jun

Myelomeningocele (MMC)-commonly known as spina bifida-is a congenital birth defect that causes lifelong paralysis, incontinence, musculoskeletal deformities, and severe cognitive disabilities. The recent landmark Management of Myelomeningocele Study (MOMS) demonstrated for the first time in humans that in utero surgical repair of the MMC defect improves lower limb motor function, suggesting a capacity for improved neurologic outcomes in this disorder.

However, functional recovery was incomplete, and 58% of the treated children were unable to walk independently at 30 months of age. In the present study, we demonstrate that using early gestation human placenta-derived mesenchymal stromal cells (PMSCs) to augment in utero repair of MMC results in significant and consistent improvement in neurologic function at birth in the rigorous fetal ovine model of MMC. In vitro, human PMSCs express characteristic MSC markers and trilineage differentiation potential. Protein array assays and enzyme-linked immunosorbent assay show that PMSCs secrete a variety of immunomodulatory and angiogenic cytokines. Compared with adult bone marrow MSCs, PMSCs secrete significantly higher levels of brain-derived neurotrophic factor and hepatocyte growth factor, both of which

have known neuroprotective capabilities. In vivo, functional and histopathologic analysis demonstrated that human PMSCs mediate a significant, clinically relevant improvement in motor function in MMC lambs and increase the preservation of large neurons within the spinal cord. These preclinical results in the well-established fetal ovine model of MMC provide promising early support for translating in utero stem cell therapy for MMC into clinical application for patients.

SIGNIFICANCE:

This study presents placenta-derived mesenchymal stromal cell (PMSC) treatment as a potential therapy for myelomeningocele (MMC). Application of PMSCs can augment current in utero surgical repair in the well-established and rigorously applied fetal lamb model of MMC. Treatment with human PMSCs significantly and dramatically

improved neurologic function and preserved spinal cord neuron density in experimental animals. Sixty-seven percent of the PMSC-treated lambs were able to ambulate independently, with two exhibiting no motor deficits whatsoever. In contrast, none of the lambs treated with the vehicle alone were capable of ambulation. The locomotor rescue demonstrated in PMSC-treated lambs indicates great promise for future clinical trials to improve paralysis in children afflicted with MMC.

67. Effect of chondrocyte-derived early extracellular matrix on chondrogenesis of placenta-derived mesenchymal stem cells.

Park YB, Seo S, Kim JA, Heo JC, Lim YC, Ha CW.

Biomed Mater. 2015 Jun 24

The extracellular matrix (ECM) surrounding cells contains a variety of proteins that provide structural support and regulate cellular functions. Previous studies have shown that decellularized ECM isolated from tissues or cultured cells can be used to improve cell differentiation in tissue engineering applications. In this study we evaluated the effect of decellularized chondrocyte-derived ECM (CDECM) on the chondrogenesis of human placenta-derived mesenchymal stem cells (hPDMSCs) in a pellet culture system. After incubation with or

without chondrocyte-derived ECM in chondrogenic medium for 1 or 3 weeks, the sizes and wet masses of the cell pellets were compared with untreated controls (hPDMSCs incubated in chondrogenic medium without chondrocyte-derived ECM). In addition, histologic analysis of the cell pellets (Safranin O and collagen type II staining) and quantitative reverse transcription-PCR analysis of chondrogenic markers (aggrecan, collagen type II, and SOX9) were carried out. Our results showed that the sizes and masses of hPDMSC pellets incubated with chondrocyte-derived ECM were significantly higher than those of untreated controls. Differentiation of hPDMSCs (both with and without chondrocyte-derived ECM) was confirmed by Safranin O and collagen type II staining. Chondrogenic marker expression and glycosaminoglycan (GAG) levels were

significantly higher in hPDMSC pellets incubated with chondrocyte-derived ECM compared with untreated controls, especially in cells precultured with chondrocyte-derived ECM for 7 d. Taken together, these results demonstrate that chondrocyte-derived ECM enhances the chondrogenesis of hPDMSCs, and this effect is further increased by preculture with chondrocyte-derived ECM. This preculture method for hPDMSC chondrogenesis represents a promising approach for cartilage tissue engineering.

68. In vivo tracking of human placenta derived mesenchymal stem cells in nude mice via (14)C-TdR labeling.

Wu CG, Zhang JC, Xie CQ, Parolini O, Silini A[5], Huang YZ, Lian B[7], Zhang M, Huang YC, Deng L.

BMC Biotechnol. 2015 Jun 13

BACKGROUND:

In order to shed light on the regenerative mechanism of mesenchymal stem cells (MSCs) in vivo, the bio-distribution profile of implanted cells using a stable and long-term tracking method is needed. We herein investigated the bio-distribution of human placental deciduas basalis derived MSCs (termed as PDB-MSCs) in nude mice after intravenous injection by carbon radioisotope labeling thymidine ((14)C-TdR), which is

able to incorporate into new DNA strands during cell replication.

RESULTS:

The proliferation rate and radioactive emission of human PDB-MSCs after labeled with different concentrations of (14)C-TdR were measured. PDB-MSCs labeled with 1 μCi possessed high radioactivity, and the biological characteristics (i.e. morphology, colony forming ability, differentiation capabilities, karyotype and cell cycle) showed no significant changes after labeling. Thus, 1 μCi was the optimal concentration in this experimental design. In nude mice, 1 × 10(6) (14)C-TdR-labeled PDB-MSCs were injected intravenously and the organs were collected at days 1, 2, 3, 5, 30 and 180 after injection, respectively. Radiolabeled PDB-MSCs were found mainly in the lung, liver, spleen,

stomach and left femur of the recipient nude mice at the whole observation period.

CONCLUSIONS:

This work provided solid evidence that (14)C-TdR labeling did not alter the biological characteristics of human placental MSCs, and that this labeling method has potential to decrease the signal from non-infused or dead cells for cell tracking. Therefore, this labeling technique can be utilized to quantify the infused cells after long-term follow-up in pre-clinical studies.

69. Amniotic fluid as a source of multipotent cells for clinical use.

Young BK, Chan MK, Liu L, Basch RS.

J Perinat Med. 2015 Jun 26

Amniotic fluid cells (AFC) from 2nd trimester amniocentesis have been found to be a source of multipotent stem cells which might overcome the limitations of expansion, histocompatibility, tumorigenesis, and ethical issues associated with using human embryonic cells, umbilical cord, cord blood, bone marrow, and induced pluripotent cells. Previous work by our group and others demonstrated multipotency and the ability to grow well in culture. However, all these studies were done in media containing fetal calf serum. We sought to observe the properties of AFC grown in serum-free media

as that would be required for clinical transplantation in humans. Fresh samples were obtained from three patients, and each sample divided into a culture whose cells were not exposed to fetal calf serum, and the other half into a standard culture medium containing fetal calf serum. Doubling time and stem cell marker expression by flow cytometry were assessed. Differentiation to neural, osteoid, and chondrogenic lineages was induced using appropriate media and confirmed by fluorescent microscopy, histology, and immunohistochemistry. There were no statistically significant differences between cells grown serum-free and in standard media in any of these parameters. The data supports the possibility of clinical use of AFC in stem cell transplantation.

70. Amniotic fluid stem cells provide considerable advantages in epidermal regeneration: B7H4 creates a moderate inflammation microenvironment to promote wound repair.

Sun Q, Li F, Li H, Chen RH, Gu YZ, Chen Y, Liang HS, You XR, Ding SS, Gao L, Wang YL, Qin MD, Zhang XG.

Sci Rep. 2015 Jun 23

The current treatments for severe skin injury all involve skin grafting. However, there is a worldwide shortage of donor skin tissue. In this study, we examined the advantages of using human amniotic fluid stem (hAFS) cells in skin wound healing. In vitro, hAFS cells differentiate into keratinocytes (termed hAFS-K). Like keratinocytes, hAFS-K cells express the markers K5, K14, K10 and

involucrin; display typical cellular structure, including a tonofibril-rich cytoplasm; and construct a completely pluristratified epithelium in 3D culture. In vivo, in a mouse excisional wound model, GFP-positive hAFS cells participate in wound repair. Co-localization of GFP/K14 and GFP/K10 in the repaired epidermis demonstrated that hAFS cells can differentiate into keratinocytes. Real-time PCR results confirmed that hAFS cells can initiate and promote early-stage repair of skin damage. During wound repair, hAFS cells did not directly secrete repair-related factors, such as bFGF, VEGF, CXCL12, TGF-β1 and KGF, and provided a moderate inflammation reaction with lower expression of IL-1β, IL-6, TNF-α, Cox2 and Mac3. In hAFS cells, the negative co-stimulatory molecule B7H4 regulates low immunogenicity, which can provide a modest

inflammatory reaction microenvironment for wound repair. Furthermore, with their uniquely high proliferation rate, hAFS cells offer a promising alternative for epidermal regeneration.

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