AMNIOTIC FLUID AND CHORIONIC VILLI STEM CELLS

SCIENTIFIC PAPER REVIEW

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Introduction

This review is a collection of the most important articles related to amniotic fluid and chorionic villi stem cells. It includes articles published from April 2013 to March 2014. 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 e da villi coriali. La rassegna contiene articoli pubblicati da Aprile 2013 a Marzo 2014; 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.*

SUMMARY

immunomodulatory properties. *CD106 è identificativo di una subpopolazione di cellule staminali mesenchimali con proprietà immunomodulatorie peculiari.* **(35)**

5. Placental mesenchymal stem cells: a unique source for cellular cardiomyoplasty. *Cellule staminali mesenchimali della placenta: una risorsa cellulare nella cardiomioplastica.* **(37)**

6. Cardiomyocyte differentiation of perinatally-derived mesenchymal stem cells. *Cellule staminali mesenchimali estratte da fonti perinatali: differenziazione in cardiomiociti* **(38)**

7. Transplantation-potential-related biological properties of decidua basalis mesenchymal stem cells from maternal human term placenta. *Proprietà biologiche delle cellule staminali mesenchimali della decidua basalis da placenta umana al parto ad uso trapianto.* **(41)**

8. Human amniotic fluid-derived and dental pulp-derived stem cells seeded into collagen scaffold repair critical-size bone defects promoting vascularization. *Cellule staminali da liquido amniotico umano e da polpa dentale umana, impiantate in un supporto di collagene, riparano difetti ossei attraverso l'induzione di vascolarizzazione.* **(43)**

9. Therapeutic effects of human amniotic fluid-derived stem cells on renal interstitial fibrosis in a murine model of unilateral ureteral obstruction. *Effetti terapeutici delle cellule staminali da liquido amniotico umano sulla fibrosi interstiziale renale in un modello murino di ostruzione unilaterale dell'uretere.* **(45)**

10. Formation of functional gap junctions in amniotic fluid-derived stem cells induced by transmembrane co-culture with neonatal rat cardiomyocytes. *Formazione di gap junctions funzionali nelle cellule staminali da liquido amniotico, in*

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11. Construction of a recombinant eukaryotic expression vector containing a leptin gene and its expression in HPMSCs. *Costruzione di un vettore ricombinante eucariotico di espressione, contenente il gene della leptina: sua espressione in cellule staminali mesenchimali umane da placenta (HPMSC).* **(49)**

12. Amniotic fluid derived stem cells give rise to neuron-like cells without a further differentiation potential into retina-like cells. *Cellule staminali da liquido amniotico inducono la differenziazione in cellule simili a neuroni senza un'ulteriore differenziazione in cellule simili a quelle della retina.* **(51)**

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14. Amniotic fluid stem cells in a bone microenvironment: driving host angiogenic response. *Cellule staminali del liquido amniotico in un micro-ambiente osseo inducono una risposta angiogenica nel paziente.* **(56)**

15. Immunomodulatory effects of human placental-derived mesenchymal stem cells on immune rejection in mouse allogeneic skin transplantation. *Effetti immunomodulatori delle cellule staminali mesenchimali da placenta umana sul rigetto nel trapianto allogenico di cute nei topi.* **(58)**

16. *In vitro* fabrication of autologous living tissue-engineered vascular grafts based on prenatally harvested ovine amniotic fluid-derived stem cells. *Produzione in vitro di innesti vascolari ingegnerizzati con tessuto autologo derivante*

da cellule staminali in coltura da liquido amniotico prenatale ovino. **(61)**

17. Towards an ideal source of mesenchymal stem cell isolation for possible therapeutic application in regenerative medicine. *Verso una fonte ideale d'isolamento di cellule staminali mesenchimali per possibili applicazioni terapeutiche in medicina rigenerativa.* **(64)**

18. Human placenta derived mesenchymal stem cells promote hepatic regeneration in CCl4 -injured rat liver model via increased autophagic mechanism. *Cellule staminali mesenchimali estratte da placenta umana inducono rigenerazione epatica attraverso l'aumentato meccanismo autofagico in un modello murino di danneggiamento del fegato da* CCl_4. **(66)**

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22. Placenta mesenchymal stem cell accelerates wound healing by enhancing angiogenesis in diabetic Goto-Kakizaki (GK) rats. *Cellule staminali mesenchimali della placenta accelerano la guarigione delle ferrite nei topi diabetici Goto-Kakizaki (GK) attraverso l'induzione dell'angiogenesi.* **(77)**

26. Human amniotic fluid stem cells have a potential to recover ovarian function in mice with chemotherapy-induced sterility. *Le cellule staminali umane del liquido amniotico sono in grado di recuperare la funzionalità ovarica in topi affetti da sterilità indotta da chemioterapia.* **(88)**

27. Calcium sensing receptor expression in ovine amniotic fluid mesenchymal stem cells and the potential role of R-568 during osteogenic differentiation. *Espressione di recettori sensori del calcio in cellule staminali mesenchimali del liquido amniotico ovino e potenziale ruolo di R-568 durante la differenziazione osteogenica.* **(91)**

28. Amniotic fluid stem cells from EGFP transgenic mice attenuate hyperoxia-induced acute lung injury. *Cellule staminali da liquido amniotico di topi transgenici EGFP attenuano il danno polmonare acuto indotto da iperossia.* **(94)**

29. Stem cell-like dog placenta cells afford neuroprotection against ischemic stroke model via heat shock protein upregulation. *Cellule di placenta canina simili a cellule staminali inducono neuroprotezione in modelli di ictus aumentando l'attività delle heat shock protein.* **(96)**

30. Amniotic fluid stem cells morph into a cardiovascular lineage: analysis of a chemically induced cardiac and vascular commitment. *Cellule staminali da liquido amniotico si differenziano in una linea cellulare cardiovascolare: analisi di una applicazione cardiaco e vascolare indotta chimicamente.* **(98)**

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69. Human second-trimester amniotic fluid cells are able to create embryoid body-like structures *in vitro* and to show typical expression profiles of embryonic and primordial germ cells. *Le cellule umane del liquido amniotico del secondo trimestre sono in grado di creare strutture simili ai corpi embrioidi in vitro e di mostrare un profilo di espressione tipico delle cellule embrionali e germinali primordiali.* **(192)**

70. Can we fix it? Evaluating the potential of placental stem cells for the treatment of pregnancy disorders. *Possiamo guarirlo? Valutazione del potenziale delle cellule staminali della placenta per il trattamento di patologie durante la gravidanza.* **(195)**

71. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. *Cellule staminali da liquido amniotico migliorano la sopravvivenza e promuovono la guarigione*

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75. Low Oxygen Tension and Insulin-like Growth Factor-I (IGF-I) Promote Proliferation and Multipotency of Placental Mesenchymal Stem Cells (PMSCs) from Different Gestations via Distinct Signaling Pathways. *Bassa tensione d'ossigeno e IGF-I (fattore di crescita-I insulino-simile) attraverso differenti pathway di signaling ,promuovono la proliferazione e la multipotenza delle cellule staminali mesenchimali estratte da placenta.* **(206)**

76. Enhanced *ex vivo* expansion of adult mesenchymal stem cells by fetal mesenchymal stem cell ECM. *Migliore espansione ex vivo delle cellule staminali mesenchimali adulte attraverso una matrice extracellulare ECM di cellule staminali mesenchimali fetali.* **(208)**

77. Stem cells from fetal membranes and amniotic fluid: markers for cell isolation and therapy. *Cellule staminali da*

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78. The subtype CD200-positive, chorionic mesenchymal stem cells from the placenta promote regeneration of human hepatocytes. *La famiglia CD200-positiva di cellule staminali mesenchimali corioniche estratte dalla placenta promuove la rigenerazione degli epatociti umani.* **(212)**

79. Human Amniotic Fluid Stem Cells Possess the Potential to Differentiate into Primordial Follicle Oocytes *In Vitro*. *Cellule staminali umane da liquido amniotico posseggono il potenziale per differenziarsi in oociti del follicolo primario in vitro.* **(214)**

80. Multilineage potential research of bovine amniotic fluid mesenchymal stem cells. *Potenziale ricerca ad ampio spettro sulle cellule staminali mesenchimali da liquido amniotico bovino.* **(216)**

The article below is based on a research project that was developed along with TOMA Advanced Biomedical Assays and the Structural and Functional Biology Department of the Università dell'Insubria of Varese. This article was presented in several international convenctions during the last year and the audience considered it to be extremely interesting for the topic.

L'articolo che segue è frutto della ricerca effettuata da Biocell Center in collaborazione con il gruppo TOMA Advanced Biomedical Assays e con il Dipartimento di Biologia Strutturale e Funzionale dell'Università dell'Insubria di Varese. E' stato presentato nel corso dell'anno in numerosi congressi internazionali, riscuotendo l'interesse e l'attenzione dei partecipanti.

1. Fetal mesenchymal stromal cells from cryopreserved human chorionic villi: cytogenetic and molecular analysis of genome stability in long-term cultures.

Roselli EA, Lazzati S, Iseppon F, Manganini M, Marcato L, Gariboldi MB, Maggi F, Grati FR, Simoni G.

Cytotherapy; 2013 Nov.

BACKGROUND/AIMS: First-trimester chorionic villi (CV) are an attractive source of human mesenchymal stromal cells (hMSC) for possible applications in cellular therapy and regenerative medicine. Human MSC from CV were monitored for genetic stability in long-term cultures.

METHODS: We set up a good manufacturing practice cryopreservation procedure for small amounts of native CV samples. After isolation, hMSC were in vitro cultured and analyzed for biological end points. Genome stability at different passages of expansion was explored by karyotype, genome-wide

array-comparative genomic hybridization and microsatellite genotyping.
RESULTS: Growth curve analysis revealed a high proliferative potential of CV-derived cells. Immunophenotyping showed expression of typical MSC markers and absence of hematopoietic markers. Analysis of multilineage potential demonstrated efficient differentiation into adipocytes, osteocytes, chondrocytes and induction of neuro-glial commitment. In angiogenic experiments, differentiation in endothelial cells was detected by in vitro Matrigel assay after vascular endothelial growth factor stimulation. Data obtained from karyotyping, array-comparative genomic hybridization and microsatellite genotyping comparing early with late DNA passages did not show any genomic variation at least up to passage 10. Aneuploid clones appeared in four of 14 cases at latest passages, immediately before culture growth arrest.
CONCLUSIONS: Our findings indicate that hCV-MSC are genetically stable in long-term

cultures at least up to passage 10 and that it is possible to achieve clinically relevant amounts of hCV-MSC even after few stages of expansion. Genome abnormalities at higher passages can occasionally occur and are always associated with spontaneous growth arrest. Under these circumstances, hCV-MSC could be suitable for therapeutic purposes.

2. Nodal, Nanog, DAZL and SMAD gene expression in human amniotic fluid stem cells.

Stefanidis K, Pergialiotis V, Christakis D, Loutradis D, Antsaklis A.

J Stem Cells; 2013.

The aim of this study was to investigate the expression of Nodal (NODAL Homolog (mouse), Nanog (Nanog Homeobox), DAZL (Deleted in Azoospermia Like) and SMAD (SMAD Family member) genes and their potential role in the regulation of self-renewal in human amniotic fluid-derived stem cells. In this experimental study human amniotic fluid-derived stem cells were analyzed for messenger RNA expression of Nodal. Immunocytochemistry also was performed to determine Nanog and DAZL. SMAD genes expression analysis was performed using cDNA Microarray analysis. Nodal mRNA was positively expressed in all samples of amniotic fluid derived stem cells. Amniotic fluid-derived stem cells showed strong

immunoreactivity for molecular markers of undifferentiated human embryonic stem cells including Nanog and DAZL. Among the 8 SMAD genes expressions analyzed SMAD1, SMAD2, SMAD3, SMAD4 and SMAD7 showed positive expression. In conclusion amniotic fluid-derived stem cells seem to express Nodal, Nanog and DAZL and it speculated that the regulation of self-renewal in AFSc could be similar as in human embryonic stem cells.

3. Isolation of c-Kit+ human amniotic fluid stem cells from second trimester.

Pozzobon M, Piccoli M, Schiavo AA, Atala A, De Coppi P.

Methods Mol Biol; 2013.

Amniotic fluid-derived stem (AFS) cells have been described as an appealing source of stem cells because of their (1) fetal, non-embryonic origin, (2) easy access during pregnancy overcoming the ethical issues related both to the use of human embryonic cells and to the postnatal tissue biopsy with donor site morbidity, and (3) their undemanding ability to be expanded. We and others have demonstrated the broad differentiation potential and here we describe the established protocol we developed to obtain c-Kit+ human AFS cells, starting from second trimester amniocentesis samples.

4. CD106 identifies a subpopulation of mesenchymal stem cells with unique immunomodulatory properties.

Yang ZX, Han ZB, Ji YR, Wang YW, Liang L, Chi Y, Yang SG, Li LN, Luo WF, Li JP, Chen DD, Du WJ, Cao XC, Zhuo GS, Wang T, Han ZC.

PLoS One; 2013 Mar 12.

Mesenchymal stem cells (MSCs) reside in almost all of the body tissues, where they undergo self-renewal and multi-lineage differentiation. MSCs derived from different tissues share many similarities but also show some differences in term of biological properties. We aim to search for significant differences among various sources of MSCs and to explore their implications in physiopathology and clinical translation. We compared the phenotype and biological properties among different MSCs isolated from human term placental chorionic villi (CV), umbilical cord (UC), adult bone marrow (BM) and adipose (AD). We found

that CD106 (VCAM-1) was expressed highest on the CV-MSCs, moderately on BM-MSCs, lightly on UC-MSCs and absent on AD-MSCs. CV-MSCs also showed unique immune-associated gene expression and immunomodulation. We thus separated CD106(+)cells and CD106(-)cells from CV-MSCs and compared their biological activities. Both two subpopulations were capable of osteogenic and adipogenic differentiation while CD106(+)CV-MSCs were more effective to modulate T helper subsets but possessed decreased colony formation capacity. In addition, CD106(+)CV-MSCs expressed more cytokines than CD106(-)CV-MSCs. These data demonstrate that CD106 identifies a subpopulation of CV-MSCs with unique immunoregulatory activity and reveal a previously unrecognized mechanism underlying immunomodulation of MSCs.

5. Placental mesenchymal stem cells: a unique source for cellular cardiomyoplasty.

Makhoul G, Chiu RC, Cecere R.

Ann Thorac Surg; 2013 May.

In coronary heart disease, the use of stem cells for regeneration purposes has been broadly studied. Whereas bone marrow mesenchymal stem cells remain the most extensively investigated, other cell sources have been reported. Here we discuss and compare the characteristics of placenta-derived mesenchymal stem cells as a novel alternative cell source for cellular cardiomyoplasty. These cells are isolated from the human term placenta, which is normally discarded post partum. With their lack of ethical conflicts and young age, the readily available placenta-derived mesenchymal stem cells could be more suitable for myocardial regenerative therapy.

6. Cardiomyocyte differentiation of perinatally-derived mesenchymal stem cells.

Nartprayut K, U-Pratya Y, Kheolamai P, Manochantr S, Chayosumrit M, Issaragrisil S, Supokawej A.

Mol Med Rep; 2013 May.

Coronary heart disease is major cause of mortality worldwide and several risk factors have been shown to play a role in its pathogenesis, including smoking, obesity, hypertension and hypercholesterolemia. A number of therapeutic methods have been developed to improve the quality of patients' lives, including stem cell therapy using mesenchymal stem cells (MSCs). Perinatal sources, including the placenta (PL) and umbilical cord (UC), are rich sources of MSCs and have been identified as a potential source of cells for therapeutic use. Their role in cardiogenic differentiation is also of contemporary medical interest. The present study demonstrated the induced

differentiation of MSCs obtained from the UC, PL and Wharton's jelly (WJ) into cardiomyocytes, using 10 µM 5-azacytidine. The characteristics of the MSCs from each source were studied and their morphology was compared. An immunofluorescence analysis for the cardiac-specific markers, GATA4 and Troponin T (TnT), was performed and tested positive in all sources. The expression of the cardiac-specific genes, Nkx2.5, α-cardiac actin and TnT, was analyzed by real-time RT-PCR and presented as fold change increases. The expression of each of the markers was observed to be higher in the 5-azacytidine-treated MSCs. The differences in expression among the sources of treated MSCs was as follows: TnT had the highest level of expression in the bone marrow (BM) MSCs; α-cardiac actin had the highest level of expression in the PLMSCs; and all the genes were expressed at significantly high levels in the WJMSCs compared with the control group. The present

study showed the ability of alternative perinatally-derived MSCs to differentiate into cardiomyocyte-like cells and how this affects the therapeutic use of these cells.

7. Transplantation-potential-related biological properties of decidua basalis mesenchymal stem cells from maternal human term placenta.

Lu G, Zhu S, Ke Y, Jiang X, Zhang S.
Cell Tissue Res; 2013 May.

Human placental decidua basalis originates from the maternal side of the placenta and has been described as a source of mesenchymal stem cells (MSCs). However, for its application in tissue regeneration and repair, the transplantation-potential-related biological properties of decidua-basalis-derived mesenchymal stem cells (DBMSCs) remain to be elucidated. We obtained DBMSCs through enzymatic digestion and density gradient centrifugation and confirmed their capacity to differentiate into cell types of the mesodermal lineage, such as osteoblasts, adipocytes and chondroblasts. Karyotype analysis showed that the isolated DBMSCs maintained chromosomal stability after long-term culture in vitro. Growth kinetics and

ultrastructural observation revealed a high level of DBMSC proliferative activity. In addition, DBMSCs showed immunosuppressive properties by suppressing both mitogen- and alloantigen-induced peripheral lymphocyte proliferation. All of these properties suggest that DBMSCs, which are abundant and easily accessible, are a novel potential source of seed cells for cell transplantation treatments.

8. Human amniotic fluid-derived and dental pulp-derived stem cells seeded into collagen scaffold repair critical-size bone defects promoting vascularization.

Maraldi T, Riccio M, Pisciotta A, Zavatti M, Carnevale G, Beretti F, La Sala GB, Motta A, De Pol A.

Stem Cell Res Ther; 2013 May 21.

INTRODUCTION: The main aim of this study is to evaluate potential human stem cells, such as dental pulp stem cells and amniotic fluid stem cells, combined with collagen scaffold to reconstruct critical-size cranial bone defects in an animal model.

METHODS: We performed two symmetric full-thickness cranial defects on each parietal region of rats and we replenished them with collagen scaffolds with or without stem cells already seeded into and addressed towards osteogenic lineage in vitro. After 4 and 8 weeks, cranial tissue samples were taken for histological and immunofluorescence analysis.

RESULTS: We observed a new bone formation in all of the samples but the most relevant differences in defect correction were shown by stem cell-collagen samples 4 weeks after implant, suggesting a faster regeneration ability of the combined constructs. The presence of human cells in the newly formed bone was confirmed by confocal analysis with an antibody directed to a human mitochondrial protein. Furthermore, human cells were found to be an essential part of new vessel formation in the scaffold.

CONCLUSION: These data confirmed the strong potential of bioengineered constructs of stem cell-collagen scaffold for correcting large cranial defects in an animal model and highlighting the role of stem cells in neovascularization during skeletal defect reconstruction.

9. Therapeutic effects of human amniotic fluid-derived stem cells on renal interstitial fibrosis in a murine model of unilateral ureteral obstruction.

Sun D1, Bu L, Liu C, Yin Z, Zhou X, Li X, Xiao A.

PLoS One; 2013 May 28.

Interstitial fibrosis is regarded as the main pathway for the progression of chronic kidney disease (CKD) and is often associated with severe renal dysfunction. Stem cell-based therapies may provide alternative approaches for the treatment of CKD. Human amniotic fluid-derived stem cells (hAFSCs) are a novel stem cell population, which exhibit both embryonic and mesenchymal stem cell characteristics. Herein, the present study investigated whether the transplantation of hAFSCs into renal tissues could improve renal interstitial fibrosis in a murine model of unilateral ureteral obstruction (UUO). We showed that hAFSCs provided a protective effect and alleviated interstitial fibrosis as

reflected by an increase in microvascular density; additionally, hAFSCs treatment beneficially modulated protein levels of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α) and transforming growth factor-β1 (TGF-β1). Therefore, we hypothesize that hAFSCs could represent an alternative, readily available source of stem cells that can be applied for the treatment of renal interstitial fibrosis.

10. Formation of functional gap junctions in amniotic fluid-derived stem cells induced by transmembrane co-culture with neonatal rat cardiomyocytes.

Connell JP, Augustini E, Moise KJ Jr, Johnson A, Jacot JG.

J Cell Mol Med; 2013 Jun.

Amniotic fluid-derived stem cells (AFSC) have been reported to differentiate into cardiomyocyte-like cells and form gap junctions when directly mixed and cultured with neonatal rat ventricular myocytes (NRVM). This study investigated whether or not culture of AFSC on the opposite side of a Transwell membrane from NRVM, allowing for contact and communication without confounding factors such as cell fusion, could direct cardiac differentiation and enhance gap junction formation. Results were compared to shared media (Transwell), conditioned media and monoculture media controls. After a 2-week culture period, AFSC did not express cardiac myosin heavy chain or troponin T in

any co-culture group. Protein expression of cardiac calsequestrin 2 was up-regulated in direct transmembrane co-cultures and media control cultures compared to the other experimental groups, but all groups were up-regulated compared with undifferentiated AFSC cultures. Gap junction communication, assessed with a scrape-loading dye transfer assay, was significantly increased in direct transmembrane co-cultures compared to all other conditions. Gap junction communication corresponded with increased connexin 43 gene expression and decreased phosphorylation of connexin 43. Our results suggest that direct transmembrane co-culture does not induce cardiomyocyte differentiation of AFSC, though calsequestrin expression is increased. However, direct transmembrane co-culture does enhance connexin-43-mediated gap junction communication between AFSC.

11. Construction of a recombinant eukaryotic expression vector containing a leptin gene and its expression in HPMSCs.
Jin J, Wang B, Zhu Z, Chen Y, Mao Z, Wang J, Du B, Wang S, Liu Z.
Cytotechnology; 2013 Jun 27.

Leptin gene fragments were amplified from human adipose tissue using reverse transcription polymerase chain reaction technology. The leptin gene was reconstructed in pIRES2-EGFP and transfected into human placenta-derived mesenchymal stem cells (HPMSCs) using a liposome-mediated method. Leptin mRNA and protein was detected in the transfected cells using RT-PCR and Western blot analysis, and the results showed that HPMSCs transfected with pIRES2-EGFP-leptin expressed significantly more leptin mRNA and protein than HPMSCs transfected with pIRES2-EGFP. EGFP expression was observed under a fluorescence microscope, and results showed the report gene to have

been successfully transferred into the target cells. The biological activity of leptin and the cell proliferation activity of HPMSCs transfected with pIRES2-EGFP-leptin was detected using an MTT assay, which showed that leptin can promote the proliferation of HPMSCs. However, leptin in HPMSCs transfected with pIRES2-EGFP-leptin showed significantly more activity than HPMSCs transfected with pIRES2-EGFP. Identification of multipotency showed that HPMSCs transfected with pIRES2-EGFP-leptin maintained their multipotency.

12. Amniotic fluid derived stem cells give rise to neuron-like cells without a further differentiation potential into retina-like cells.

Hartmann K, Raabe O, Wenisch S, Arnhold S.
Am J Stem Cells; 2013 Jun 30.

Amniotic fluid contains heterogeneous cell types and has become an interesting source for obtaining fetal stem cells. These stem cells have a high proliferative capacity and a good differentiation potential and may thus be suitable for regenerative medicine. As there is increasing evidence, that these stem cells are also able to be directed into the neural lineage, in our study we investigated the neuronal and glial differentiation potential of these cells, so that they may also be applied to cure degenerative diseases of the retina. Mesenchymal stem cells were isolated from routine prenatal amniocentesis at 15 to 18 weeks of pregnancy of human amniotic fluid and expanded in the cell culture. Cells were cultivated according to standard procedures

for mesenchymal stem cells and were differentiated along the neural lineage using various protocols. Furthermore, it was also tried to direct them into cell types of the retina as well as into endothelial cells. Cells of more than 72 amniotic fluid samples were collected and characterized. While after induction neural-like phenotypes could actually be detected, which was confirmed using neural marker proteins such as GFAP and ßIII tubulina further differentiation into retinal like cells could not reliably be shown. These data suggest that amniotic fluid derived cells are an interesting cell source, which may also give rise to neural-like cells. However, a more specific differentiation into neuronal and glial cells could not unequivocally be shown, so that further investigations have to becarried out.

13. Connective tissue growth factor induced differentiation of placenta mesenchymal stem cell into dermal fibroblast.

Yang WX, Ouyang X, Song YQ, Zhang XG, Zhang J.

Zhonghua Zheng Xing Wai Ke Za Zhi; 2013 Jul.

OBJECTIVE: To investigate the possibility of placenta mesenchymal stem cells (PMSCs) differentiation into dermal fibroblast, and the potency of PMSCs used in cutaneous wound healing and stored as seed cells.

METHODS: Enzyme digestion method was used to obtain PMSCs, and PMSCs were amplified after culture in vitro. Flow cytometry assay, osteogenic and adipogenic differentiation were done for MSCs identification. The induction medium composed of DMEM/F12 + 50 microg/ml VC + 100 ng/ml connective tissue growth factor (CTGF) was added into the 24-well plate for 16 days induction period. Pictures were taken

to record morphologic change. Immunofluorescence tests were performed to detect Vimentin, FSP-1, collagen I , collagen III, desmin and laminin expression before and after induction. At the same time osteogenic and adipogenic differentiation were used to assay the differentiation ability change after induction. The induced dermal fibroblasts were frozen in liquid nitrogenand and recovery and trypan blue was used to detect cell viability.

RESULTS: After CTGF induction, PMSCs got obvious fibroblasts morphology, the protein level of Vimentin, FSP-1, collagen I, collagen III and Laminin increased, PMSCs started to express Desmin, the dermal fibroblasts specific proteins, and osteogenic and adipogenic differentiation ability was diminished. PMSCs were successfully induced into dermal fibroblasts, and these induced cells could get a high cell viability (more than 90%) after recovery.

CONCLUSIONS: PMSCs could be induced into dermal fibroblasts by CTGF in vitro.

PMSCs have the potential application in skin wound healing, and can be used as seed cells of dermal fibroblasts.

14. Amniotic fluid stem cells in a bone microenvironment: driving host angiogenic response.

Mirabella T, Gentili C, Daga A, Cancedda R.
Stem Cell Res; 2013 Jul.

The repair of skeletal defects remains a substantial economic and biomedical burden. The extra-embryonic fetal stem cells derived from amniotic fluid (AFSCs) have been used for the treatment of large bone defects, but mechanisms of repair are not clear. Here we studied the potential contribution of human AFSCs to the modeling of an ectopic bone. We found that AFSCs are not osteogenic in vivo, and, compared to bone marrow-derived stromal cells, recruit more host CD31 and VEGF-R2 positive cells. Finally, when AFSCs were co-implanted with human-bone forming cells, a normo-osteosynthesis occurred, the engineered ossicle was hyper-vascularized, but AFSCs were not retrieved in the implant within 2weeks. We concluded that AFSCs do not contribute to the

deposition of new bone, but act as a powerful proinflammatory/proangiogenic boost, driving a host response, ending in AFSC clearance and vascularization of the bone environment. In our model, a source of osteocommitted cells, capable to engraft and proliferate in vivo, is needed in order to engineer bone. The angio-attractant properties of AFSCs could be exploited in strategies of endogenous cell homing to actively recruit host progenitors into a predefined anatomic location for in situ bone tissue regeneration.

15. Immunomodulatory effects of human placental-derived mesenchymal stem cells on immune rejection in mouse allogeneic skin transplantation.

Wang Q, Liu T, Zhang Y, Chen D, Wang L, Li Y, Wei J.

Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi; 2013 Jul.

OBJECTIVE: To investigate the effect of human placental-derived mesenchymal stem cells (PMSCs) on immunological rejection in mouse allogeneic skin transplantation.

METHODS: The placenta fetal tissues from voluntary donors were used to isolate and culture the PMSCs, and the 3rd passage PMSCs were used in the experiment. Thirty Vr: CD1 (ICR) mice at age of 1-2 days were used as skin donors for allogeneic skin transplantation. Thirty C57BL/6 mice at age of 6-8 weeks as recipients were made back skin defect of 12 mm in diameter and were randomly divided into 3 groups (n=10): group A, autograft; group B, allogeneic graft + PBS

tail vein injection; and group C, allogeneic graft + human PMSCs (1 x 10(5) cells/mouse) tail vein injection. The flap survival was observed. At 7 days after skin transplantation, blood leukocyte counting, abdominal fluid macrophage activation, and the expression levels of interleukin 4 (IL-4), interleukin 17 (IL-17), and interferon gamma (INF-gamma) in blood and spleen were detected by ELISA and RT-PCR, respectively.

RESULTS: The flap survival time was significantly longer in group A [(58.33 +/- 4.04) days] than in groups B and C [(3.80 +/- 0.92) days and (6.80 +/- 0.82) days] ($P < 0.05$), and in group C than in group B ($P < 0.05$). At 7 days after transplantation, the blood leukocyte number was (6.32 +/- 0.45) x 10(9)/L in group A, (7.45 +/- 0.52) x 10(9)/L in group B, and (6.35 +/- 0.39) x 10(9)/L in group C, and it was significantly more in group B than in groups A and C ($P < 0.05$). The macrophage activation rate of the abdominal fluid was 6.87% +/- 2.40% in group A, 7.84% +/- 0.44% in group B, and

15.98% +/- 2.87% in group C; group C was significantly higher than groups A and B ($P < 0.01$). ELISA results showed that there was no significant difference in the concentrations of IL-4 among 3 groups ($P > 0.05$). Compared with group B, the concentrations of IL-17 and IFN-gamma were significantly reduced in group C ($P < 0.05$), while the concentration of IFN-gamma was significantly increased in group B when compared with group A ($P < 0.05$). RT-PCR results showed that there were significant differences in the expressions of IL-4, IL-17, and IFN-gamma mRNA between groups B, C and group A ($P < 0.05$); the expressions of IL-4 and IFN-gamma mRNA were significantly lower in group C than in group B ($P < 0.05$).

CONCLUSION: Human PMSCs transplantation can suppress the acute immunological rejection in allogeneic skin transplantation. The possible mechanism may be partially related to the inhibitory effect on the secretion of IL-17 and IFN-gamma.

16. *In vitro* fabrication of autologous living tissue-engineered vascular grafts based on prenatally harvested ovine amniotic fluid-derived stem cells.

Weber B, Kehl D, Bleul U, Behr L, Sammut S, Frese L, Ksiazek A, Achermann J, Stranzinger G, Robert J, Sanders B, Sidler M, Brokopp CE, Proulx ST, Frauenfelder T, Schoenauer R, Emmert MY, Falk V, Hoerstrup SP.
J Tissue Eng Regen Med; 2013 Jul 24.

Amniotic fluid cells (AFCs) have been proposed as a valuable source for tissue engineering and regenerative medicine. However, before clinical implementation, rigorous evaluation of this cell source in clinically relevant animal models accepted by regulatory authorities is indispensable. Today, the ovine model represents one of the most accepted preclinical animal models, in particular for cardiovascular applications. Here, we investigate the isolation and use of autologous ovine AFCs as cell source for cardiovascular tissue engineering

applications. Fetal fluids were aspirated in vivo from pregnant ewes (n = 9) and from explanted uteri post mortem at different gestational ages (n = 91). Amniotic non-allantoic fluid nature was evaluated biochemically and in vivo samples were compared with post mortem reference samples. Isolated cells revealed an immunohistochemical phenotype similar to ovine bone marrow-derived mesenchymal stem cells (MSCs) and showed expression of stem cell factors described for embryonic stem cells, such as NANOG and STAT-3. Isolated ovine amniotic fluid-derived MSCs were screened for numeric chromosomal aberrations and successfully differentiated into several mesodermal phenotypes. Myofibroblastic ovine AFC lineages were then successfully used for the in vitro fabrication of small- and large-diameter tissue-engineered vascular grafts (n = 10) and cardiovascular patches (n = 34), laying the foundation for the use of this relevant pre-clinical in vivo assessment model for future

amniotic fluid cell-based therapeutic applications.

17. Towards an ideal source of mesenchymal stem cell isolation for possible therapeutic application in regenerative medicine.

Bellavia M, Altomare R, Cacciabaudo F, Santoro A, Allegra A, Concetta Gioviale M, Lo Monte AI.

Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub; 2013 Jul 29.

BACKGROUND: The possibility of obtaining mesenchymal stem cells (MSCs) from fetal tissue such as amniotic fluid, chorionic villi and placenta is well-known and a comparison between MSCs originating in different sources such as fetal tissue and those from bone marrow in terms of yield and function is a topical issue. The mesenchymal stem cells isolated from bone marrow are well-characterized. Unfortunately the low quantitative yield during isolation is a major problem. For this reason, other tissue sources for MSCs are of paramount importance.

CONCLUSION: In this review, starting from a description of the molecular and cellular biology of MSCs, we describe alternative sources of isolation other than bone marrow. Finally, we describe the potential therapeutic application of these cells.

18. Human placenta-derived mesenchymal stem cells promote hepatic regeneration in CCl4 -injured rat liver model via increased autophagic mechanism.

Jung J, Choi JH, Lee Y, Park JW, Oh IH, Hwang SG, Kim KS, Kim GJ.

Stem Cells; 2013 Aug.

Mesenchymal stem cells (MSCs) have great potential for cell therapy in regenerative medicine, including liver disease. Even though ongoing research is dedicated to the goal of bringing MSCs to clinical applications, further understanding of the complex underlying mechanisms is required. Autophagy, a type II programmed cell death, controls cellular recycling through the lysosomal system in damaged cells or tissues. However, it is still unknown whether MSCs can trigger autophagy to enhance regeneration and/or to provide a therapeutic effect as cellular survival promoters. We therefore investigated autophagy's activation in carbon tetrachloride (CCl4)-injured rat liver

following transplantation with chorionic plate-derived MSCs (CP-MSCs) isolated from placenta. The expression markers for apoptosis, autophagy, cell survival, and liver regeneration were analyzed. Whereas caspase 3/7 activities were reduced ($p < .05$), the expression levels of hypoxia-inducible factor-1α (HIF-1α) and factors for autophagy, survival, and regeneration were significantly increased by CP-MSCs transplantation. Decreased necrotic cells ($p < .05$) and increased autophagic signals ($p < .005$) were observed in CCl4 -treated primary rat hepatocytes during in vitro coculture with CP-MSCs. Furthermore, the upregulation of HIF-1α promotes the regeneration of damaged hepatic cells through an autophagic mechanism marked by increased levels of light chain 3 II (LC 3II). These results suggest that the administration of CP-MSCs promotes repair by systemically concomitant mechanisms involving HIF-1α and autophagy. These findings provide further understanding of the mechanisms involved in

these processes and will help develop new cell-based therapeutic strategies for regenerative medicine in liver disease.

19. *In vitro* differentiation into insulin-producing β-cells of stem cells isolated from human amniotic fluid and dental pulp.

Carnevale G, Riccio M, Pisciotta A, Beretti F, Maraldi T, Zavatti M, Cavallini GM, La Sala GB, Ferrari A, De Pol A.

Dig Liver Dis; 2013 Aug.

AIM: To investigate the ability of human amniotic fluid stem cells and human dental pulp stem cells to differentiate into insulin-producing cells.

METHODS: Human amniotic fluid stem cells and human dental pulp stem cells were induced to differentiate into pancreatic β-cells by a multistep protocol. Islet-like structures were assessed in differentiated human amniotic fluid stem cells and human dental pulp stem cells after 21 days of culture by dithizone staining. Pancreatic and duodenal homebox-1, insulin and Glut-2 expression were detected by immunofluorescence and confocal microscopy. Insulin secreted from

differentiated cells was tested with SELDI-TOF MS and by enzyme-linked immunosorbent assay.

RESULTS: Human amniotic fluid stem cells and human dental pulp stem cells, after 7 days of differentiation started to form islet-like structures that became evident after 14 days of induction. SELDI-TOF MS analysis, revealed the presence of insulin in the media of differentiated cells at day 14, further confirmed by enzyme-linked immunosorbent assay after 7, 14 and 21 days. Both stem cell types expressed, after differentiation, pancreatic and duodenal homebox-1, insulin and Glut-2 and were positively stained by dithizone. Either the cytosol to nucleus translocation of pancreatic and duodenal homebox-1, either the expression of insulin, are regulated by glucose concentration changes. Day 21 islet-like structures derived from both human amniotic fluid stem cells and human dental pulp stem cell release insulin in a glucose-dependent manner.

CONCLUSION: The present study demonstrates the ability of human amniotic fluid stem cells and human dental pulp stem cell to differentiate into insulin-producing cells, offering a non-pancreatic, low-invasive source of cells for islet regeneration.

20. Amniotic fluid-derived stem cells for cardiovascular tissue engineering applications.

Petsche Connell J, Camci-Unal G, Khademhosseini A, Jacot JG.

Tissue Eng Part B Rev; 2013 Aug.

Recent research has demonstrated that a population of stem cells can be isolated from amniotic fluid removed by amniocentesis that are broadly multipotent and nontumorogenic. These amniotic fluid-derived stem cells (AFSC) could potentially provide an autologous cell source for treatment of congenital defects identified during gestation, particularly cardiovascular defects. In this review, the various methods of isolating, sorting, and culturing AFSC are compared, along with techniques for inducing differentiation into cardiac myocytes and endothelial cells. Although research has not demonstrated complete and high-yield cardiac differentiation, AFSC have been shown to effectively differentiate into endothelial cells

and can effectively support cardiac tissue. Additionally, several tissue engineering and regenerative therapeutic approaches for the use of these cells in heart patches, injection after myocardial infarction, heart valves, vascularized scaffolds, and blood vessels are summarized. These applications show great promise in the treatment of congenital cardiovascular defects, and further studies of isolation, culture, and differentiation of AFSC will help to develop their use for tissue engineering, regenerative medicine, and cardiovascular therapies.

21. Amniotic fluid stem cells inhibit the progression of bleomycin-induced pulmonary fibrosis via CCL2 modulation in bronchoalveolar lavage.

Garcia O, Carraro G, Turcatel G, Hall M, Sedrakyan S, Roche T, Buckley S, Driscoll B, Perin L, Warburton D.

PLoS One; 2013 Aug 13.

The potential for amniotic fluid stem cell (AFSC) treatment to inhibit the progression of fibrotic lung injury has not been described. We have previously demonstrated that AFSC can attenuate both acute and chronic-fibrotic kidney injury through modification of the cytokine environment. Fibrotic lung injury, such as in Idiopathic Pulmonary Fibrosis (IPF), is mediated through pro-fibrotic and pro-inflammatory cytokine activity. Thus, we hypothesized that AFSC treatment might inhibit the progression of bleomycin-induced pulmonary fibrosis through cytokine modulation. In particular, we aimed to investigate the effect of AFSC treatment on

the modulation of the pro-fibrotic cytokine CCL2, which is increased in human IPF patients and is correlated with poor prognoses, advanced disease states and worse fibrotic outcomes. The impacts of intravenous murine AFSC given at acute (day 0) or chronic (day 14) intervention time-points after bleomycin injury were analyzed at either day 3 or day 28 post-injury. Murine AFSC treatment at either day 0 or day 14 post-bleomycin injury significantly inhibited collagen deposition and preserved pulmonary function. CCL2 expression increased in bleomycin-injured bronchoalveolar lavage (BAL), but significantly decreased following AFSC treatment at either day 0 or at day 14. AFSC were observed to localize within fibrotic lesions in the lung, showing preferential targeting of AFSC to the area of fibrosis. We also observed that MMP-2 was transiently increased in BAL following AFSC treatment. Increased MMP-2 activity was further associated with cleavage of CCL2, rendering it a putative antagonist for

CCL2/CCR2 signaling, which we surmise is a potential mechanism for CCL2 reduction in BAL following AFSC treatment. Based on this data, we concluded that AFSC have the potential to inhibit the development or progression of fibrosis in a bleomycin injury model during both acute and chronic remodeling events.

22. Placenta mesenchymal stem cell accelerates wound healing by enhancing angiogenesis in diabetic Goto-Kakizaki (GK) rats.

Kong P, Xie X, Li F, Liu Y, Lu Y.

Biochem Biophys Res Commun; 2013 Aug 23.

Multipotent mesenchymal stem cells have recently emerged as an attractive cell type for the treatment of diabetes-associated wounds. The purpose of this study was to examine the potential biological function of human placenta-derived mesenchymal stem cells (PMSCs) in wound healing in diabetic Goto-Kakizaki (GK) rats. PMSCs were isolated from human placenta tissue and characterized by flow cytometry. A full-thickness circular excisional wound was created on the dorsum of each rat. Red fluorescent CM-DiI-labeled PMSCs were injected intradermally around the wound in the treatment group. After complete wound healing, full-thickness skin samples were taken from the wound sites for

histological evaluation of the volume and density of vessels. Our data showed that the extent of wound closure was significantly enhanced in the PMSCs group compared with the no-graft controls. Microvessel density in wound bed biopsy sites was significantly higher in the PMSCs group compared with the no-graft controls. Most surprisingly, immunohistochemical studies confirmed that transplanted PMSCs localized to the wound tissue and were incorporated into recipient vasculature with improved angiogenesis. Notably, PMSCs secreted comparable amounts of proangiogenic molecules, such as VEGF, HGF, bFGF, TGF-β and IGF-1 at bioactive levels. This study demonstrated that PMSCs improved the wound healing rate in diabetic rats. It is speculated that this effect can be attributed to the PMSCs engraftment resulting in vascular regeneration via direct de novo differentiation and paracrine mechanisms. Thus, placenta-derived mesenchymal stem cells are implicated as a

potential angiogenesis cell therapy for repair-resistant chronic wounds in diabetic patients.

23. Effect of amniotic fluid stem cells and amniotic fluid cells on the wound healing process in a white rat model.

Yang JD, Choi DS, Cho YK, Kim TK, Lee JW, Choi KY, Chung HY, Cho BC, Byun JS.

Arch Plast Surg; 2013 Sep.

BACKGROUND: Amniotic-fluid-derived stem cells and amniocytes have recently been determined to have wound healing effects, but their mechanism is not yet clearly understood. In this study, the effects of amniotic fluid stem cells and amniocytes on wound healing were investigated through animal experiments.

METHODS: On the back of Sprague-Dawley rats, four circular full-thickness skin wounds 2 cm in diameter were created. The wounds were classified into the following four types: a control group using Tegaderm disc wound dressings and experimental groups using collagen discs, amniotic fluid stem cell discs, and amniocyte discs. The wounds were assessed through macroscopic histological

examination and immunohistochemistry over a period of time.
RESULTS: The amniotic fluid stem cell and amniocyte groups showed higher wound healing rates compared with the control group; histologically, the inflammatory cell invasion disappeared more quickly in these groups, and there was more significant angiogenesis. In particular, these groups had significant promotion of epithelial cell reproduction, collagen fiber formation, and angiogenesis during the initial 10 days of the wound healing process. The potency of transforming growth factor-β and fibronectin in the experimental group was much greater than that in the control group in the early stage of the wound healing process. In later stages, however, no significant difference was observed.
CONCLUSIONS: The amniotic fluid stem cells and amniocytes were confirmed to have accelerated the inflammatory stage to contribute to an enhanced cure rate and

shortened wound healing period. Therefore, they hold promise as wound treatment agents.

24. Role of amniotic fluid mesenchymal cells engineered on MgHA/collagen-based scaffold allotransplanted on an experimental animal study of sinus augmentation.

Berardinelli P, Valbonetti L, Muttini A, Martelli A, Peli R, Zizzari V, Nardinocchi D, Vulpiani MP, Tetè S, Barboni B, Piattelli A, Mattioli M.

Clin Oral Investig; 2013 Sep.

OBJECTIVES:

The present research has been performed to evaluate whether a commercial magnesium-enriched hydroxyapatite (MgHA)/collagen-based scaffold engineered with ovine amniotic fluid mesenchymal cells (oAFMC) could improve bone regeneration process in vivo.

MATERIALS AND METHODS:

Bilateral sinus augmentation was performed on eight adult sheep in order to compare the tissue regeneration process at 45 and 90 days after implantation of the oAFMC-engineered

scaffold (Test Group) or of the scaffold alone (Ctr Group). The process of tissue remodeling was analyzed through histological, immunohistochemical, and morphometric analyses by calculating the proliferation index (PI) of oAFMC loaded on the scaffold, the total vascular area (VA), and vascular endothelial growth factor (VEGF) expression levels within the grafted area.

RESULTS:

MgHA/collagen-based scaffold showed high biocompatibility preserving the survival of oAFMC for 90 days in grafted sinuses. The use of oAFMC increased bone deposition and stimulated a more rapid angiogenic reaction, thus probably supporting the higher cell PI recorded in cell-treated sinuses. A significantly higher VEGF expression (Test vs. Ctr Group; $p = 0.0004$) and a larger total VA ($p = 0.0006$) were detected in the Test Group at 45 days after surgery. The PI was significantly higher ($p = 0.027$) at 45 days and became significantly lower at 90 days ($p = 0.0007$) in the Test Group sinuses, while

the PI recorded in the Ctr Group continued to increase resulting to a significantly higher PI at day 90 (CTR day 45 vs. CTR day 90; $p = 0.022$).

CONCLUSIONS:

The osteoinductive effect of a biomimetic commercial scaffold may be significantly improved by the presence of oAFMC.

CLINICAL RELEVANCE:

The amniotic fluid mesenchymal cell (AFMC) may represent a novel, largely and easily accessible source of mesenchymal stem cells to develop cell-based therapy for maxillofacial surgery.

25. MePR: a novel human mesenchymal progenitor model with characteristics of pluripotency.

Miceli M, Franci G, Dell'Aversana C, Ricciardiello F, Petraglia F, Carissimo A, Perone L, Maruotti GM, Savarese M, Martinelli P, Cancemi M, Altucci L.

Stem Cells Dev; 2013 Sep 1.

Human embryo stem cells or adult tissues are excellent models for discovery and characterization of differentiation processes. The aims of regenerative medicine are to define the molecular and physiological mechanisms that govern stem cells and differentiation. Human mesenchymal stem cells (hMSCs) are multipotent adult stem cells that are able to differentiate into a variety of cell types under controlled conditions both in vivo and in vitro, and they have the remarkable ability of self-renewal. hMSCs derived from amniotic fluid and characterized by the expression of Oct-4 and Nanog, typical markers of pluripotent cells,

represent an excellent model for studies on stemness. Unfortunately, the limited amount of cells available from each donation and, above all, the limited number of replications do not allow for detailed studies. Here, we report on the immortalization and characterization of novel mesenchymal progenitor (MePR) cell lines from amniotic fluid-derived hMSCs, whose biological properties are similar to primary amniocytes. Our data indicate that MePR cells display the multipotency potential and differentiation rates of hMSCs, thus representing a useful model to study both mechanisms of differentiation and pharmacological approaches to induce selective differentiation. In particular, MePR-2B cells, which carry a bona fide normal karyotype, might be used in basic stem cell research, leading to the development of new approaches for stem cell therapy and tissue engineering.

26. Human amniotic fluid stem cells have a potential to recover ovarian function in mice with chemotherapy-induced sterility.

Lai D, Wang F, Chen Y, Wang L, Wang Y, Cheng W.

BMC Dev Biol; 2013 Sep 4.

BACKGROUND: Human amniotic fluid cells (hAFCs) may differentiate into multiple cell lineages and thus have a great potential to become a donor cell source for regenerative medicine. The ability of hAFCs to differentiate into germ cell and oocyte-like cells has been previously documented. Herein we report the potential use of hAFCs to help restore follicles in clinical condition involving premature ovarian failure.

RESULTS: Human amniotic fluid was obtained via amniocentesis, yielding a subpopulation of cloned hAFCs that was able to form embryoid bodies (EBs) and differentiate into three embryonic germ layers. Moreover, culture of EBs in medium containing human follicular fluid (HFF) or a

germ cell maturation factor cocktail (FAC), expressed germ cells markers such as BLIMP1, STELLA, DAZL, VASA, STRA8, SCP3, SCP1, and GDF9. Furthermore, one cell line was grown from clone cells transfected with lentivirus-GFP and displaying morphological characteristics of mesenchymal cells, had the ability to restore ovarian morphology following cell injection into the ovaries of mice sterilized by intraperitoneal injection of cyclophosphamide and busulphan. Restored ovaries displayed many follicle-enclosed oocytes at all stages of development, but no oocytes or follicles were observed in sterilized mice whose ovaries had been injected with medium only (control). Notably, identification of GFP-labeled cells and immunostaining with anti-human antigen-specific antibodies demonstrated that grafted hAFCs survived and differentiated into granulosa cells which directed oocyte maturation. Furthermore, labeling of ovarian tissue for anti-Müllerian hormone expression, a functional marker of folliculogenesis, was

strong in hAFCs-transplanted ovaries but inexistent in negative controls.
CONCLUSION: These findings highlight the possibility of using human amniotic fluid-derived stem cells in regenerative medicine, in particular in the area of reproductive health.

27. Calcium sensing receptor expression in ovine amniotic fluid mesenchymal stem cells and the potential role of R-568 during osteogenic differentiation.

Di Tomo P, Pipino C, Lanuti P, Morabito C, Pierdomenico L, Sirolli V, Bonomini M, Miscia S, Mariggiò MA, Marchisio M, Barboni B, Pandolfi A.

PLoS One; 2013 Sep 9.

Amniotic fluid-derived stem (AFS) cells have been identified as a promising source for cell therapy applications in bone traumatic and degenerative damage. Calcium Sensing Receptor (CaSR), a G protein-coupled receptor able to bind calcium ions, plays a physiological role in regulating bone metabolism. It is expressed in different kinds of cells, as well as in some stem cells. The bone CaSR could potentially be targeted by allosteric modulators, in particular by agonists such as calcimimetic R-568, which may potentially be helpful for the treatment of bone disease. The aim of our study was first

to investigate the presence of CaSR in ovine Amniotic Fluid Mesenchymal Stem Cells (oAFMSCs) and then the potential role of calcimimetics in in vitro osteogenesis. oAFMSCs were isolated, characterized and analyzed to examine the possible presence of CaSR by western blotting and flow cytometry analysis. Once we had demonstrated CaSR expression, we worked out that 1 µM R-568 was the optimal and effective concentration by cell viability test (MTT), cell number, Alkaline Phosphatase (ALP) and Alizarin Red S (ARS) assays. Interestingly, we observed that basal diffuse CaSR expression in oAFMSCs increased at the membrane when cells were treated with R-568 (1 µM), potentially resulting in activation of the receptor. This was associated with significantly increased cell mineralization (ALP and ARS staining) and augmented intracellular calcium and Inositol trisphosphate (IP3) levels, thus demonstrating a potential role for calcimimetics during osteogenic differentiation. Calhex-231, a

CaSR allosteric inhibitor, totally reversed R-568 induced mineralization. Taken together, our results demonstrate for the first time that CaSR is expressed in oAFMSCs and that calcimimetic R-568, possibly through CaSR activation, can significantly improve the osteogenic process. Hence, our study may provide useful information on the mechanisms regulating osteogenesis in oAFMSCs, perhaps prompting the use of calcimimetics in bone regenerative medicine.

28. Amniotic fluid stem cells from EGFP transgenic mice attenuate hyperoxia-induced acute lung injury.

Wen ST, Chen W, Chen HL, Lai CW, Yen CC, Lee KH, Wu SC, Chen CM.

PLoS One; 2013 Sep 11.

High concentrations of oxygen aggravate the severity of lung injury in patients requiring mechanical ventilation. Although mesenchymal stem cells have been shown to effectively attenuate various injured tissues, there is limited information regarding a role for amniotic fluid stem cells (AFSCs) in treating acute lung injury. We hypothesized that intravenous delivery of AFSCs would attenuate lung injury in an experimental model of hyperoxia-induced lung injury. AFSCs were isolated from EGFP transgenic mice. The in vitro differentiation, surface markers, and migration of the AFSCs were assessed by specific staining, flow cytometry, and a co-culture system, respectively. The in vivo therapeutic potential of AFSCs was

evaluated in a model of acute hyperoxia-induced lung injury in mice. The administration of AFSCs significantly reduced the hyperoxia-induced pulmonary inflammation, as reflected by significant reductions in lung wet/dry ratio, neutrophil counts, and the level of apoptosis, as well as reducing the levels of inflammatory cytokine (IL-1β, IL-6, and TNF-α) and early-stage fibrosis in lung tissues. Moreover, EGFP-expressing AFSCs were detected and engrafted into a peripheral lung epithelial cell lineage by fluorescence microscopy and DAPI stain. Intravenous administration of AFSCs may offer a new therapeutic strategy for acute lung injury (ALI), for which efficient treatments are currently unavailable.

29. Stem cell-like dog placenta cells afford neuroprotection against ischemic stroke model via heat shock protein upregulation.
Yu S, Tajiri N, Franzese N, Franzblau M, Bae E, Platt S, Kaneko Y, Borlongan CV.
PLoS One; 2013 Sep 25.

In this study, we investigated the dog placenta as a viable source of stem cells for stroke therapy. Immunocytochemical evaluation of phenotypic markers of dog placenta cells (DPCs) cultured in proliferation and differentiation medium revealed that DPCs expressed both stem cell and neural cell markers, respectively. Co-culture with DPCs afforded neuroprotection of rat primary neural cells in a dose-dependent manner against oxygen-glucose deprivation. Subsequent in vivo experiments showed that transplantation of DPCs, in particular intravenous and intracerebral cell delivery, produced significant behavioral recovery and reduced histological deficits in ischemic stroke animals compared to those that received intra-

arterial delivery of DPCs or control stroke animals. Furthermore, both in vitro and in vivo studies implicated elevated expression of heat shock protein 27 (Hsp27) as a potential mechanism of action underlying the observed therapeutic benefits of DPCs in stroke. This study supports the use of stem cells for stroke therapy and implicates a key role of Hsp27 signaling pathway in neuroprotection.

30. Amniotic fluid stem cells morph into a cardiovascular lineage: analysis of a chemically induced cardiac and vascular commitment.

Maioli M, Contini G, Santaniello S, Bandiera P, Pigliaru G, Sanna R, Rinaldi S, Delitala AP, Montella A, Bagella L, Ventura C.

Drug Des Devel Ther; 2013 Sep 27.

Mouse embryonic stem cells were previously observed along with mesenchymal stem cells from different sources, after being treated with a mixed ester of hyaluronan with butyric and retinoic acids, to show a significant increase in the yield of cardiogenic and vascular differentiated elements. The aim of the present study was to determine if stem cells derived from primitive fetal cells present in human amniotic fluid (hAFSCs) and cultured in the presence of a mixture of hyaluronic (HA), butyric (BU), and retinoic (RA) acids show a higher yield of differentiation toward the cardiovascular phenotype as compared with untreated cells.

During the differentiation process elicited by exposure to HA + BU + RA, genes controlling pluripotency and plasticity of stem cells, such as Sox2, Nanog, and Oct4, were significantly downregulated at the transcriptional level. At this point, a significant increase in expression of genes controlling the appearance of cardiogenic and vascular lineages in HA + BU + RA-treated cells was observed. The protein expression levels typical of cardiac and vascular phenotypes, evaluated by Western blotting, immunofluorescence, and flow cytometry, were higher in hAFSCs cultured in the presence of HA + BU + RA, as compared with untreated control cells. Appearance of the cardiac phenotype was further inferred by ultrastructural analysis using transmission and scanning electron microscopy. These results demonstrate that a mixture of HA + BU + RA significantly increased the yield of elements committed toward cardiac and vascular phenotypes, confirming what we have previously observed in other cellular types.

31. Amniotic fluid stem cells with low γ-interferon response showed behavioral improvement in parkinsonism rat model.

Chang YJ, Ho TY, Wu ML, Hwang SM, Chiou TW, Tsai MS.

PLoS One; 2013 Sep 30.

Amniotic fluid stem cells (AFSCs) are multipotent stem cells that may be used in transplantation medicine. In this study, AFSCs established from amniocentesis were characterized on the basis of surface marker expression and differentiation potential. To further investigate the properties of AFSCs for translational applications, we examined the cell surface expression of human leukocyte antigens (HLA) of these cells and estimated the therapeutic effect of AFSCs in parkinsonian rats. The expression profiles of HLA-II and transcription factors were compared between AFSCs and bone marrow-derived mesenchymal stem cells (BMMSCs) following treatment with γ-IFN. We found that stimulation of AFSCs with γ-IFN

prompted only a slight increase in the expression of HLA-Ia and HLA-E, and the rare HLA-II expression could also be observed in most AFSCs samples. Consequently, the expression of CIITA and RFX5 was weakly induced by γ-IFN stimulation of AFSCs compared to that of BMMSCs. In the transplantation test, Sprague Dawley rats with 6-hydroxydopamine lesioning of the substantia nigra were used as a parkinsonian-animal model. Following the negative γ-IFN response AFSCs injection, apomorphine-induced rotation was reduced by 75% in AFSCs engrafted parkinsonian rats but was increased by 53% in the control group after 12-weeks post-transplantation. The implanted AFSCs were viable, and were able to migrate into the brain's circuitry and express specific proteins of dopamine neurons, such as tyrosine hydroxylase and dopamine transporter. In conclusion, the relative insensitivity AFSCs to γ-IFN implies that AFSCs might have immune-tolerance in γ-IFN inflammatory conditions. Furthermore,

the effective improvement of AFSCs transplantation for apomorphine-induced rotation paves the way for the clinical application in parkinsonian therapy.

32. Therapeutic potential of amniotic fluid-derived cells for treating the injured nervous system.

Rennie K, Haukenfrers J, Ribecco-Lutkiewicz M, Ly D, Jezierski A, Smith B, Zurakowski B, Martina M, Gruslin A, Bani-Yaghoub M.
Biochem Cell Biol; 2013 Oct.

There is a need for improved therapy for acquired brain injury, which has proven resistant to treatment by numerous drugs in clinical trials and continues to represent one of the leading causes of disability worldwide. Research into cell-based therapies for the treatment of brain injury is growing rapidly, but the ideal cell source has yet to be determined. Subpopulations of cells found in amniotic fluid, which is readily obtained during routine amniocentesis, can be easily expanded in culture, have multipotent differentiation capacity, are non-tumourigenic, and avoid the ethical complications associated with embryonic stem cells, making them a promising cell

source for therapeutic purposes. Beneficial effects of amniotic fluid cell transplantation have been reported in various models of nervous system injury. However, evidence that amniotic fluid cells can differentiate into mature, functional neurons in vivo and incorporate into the existing circuitry to replace lost or damaged neurons is lacking. The mechanisms by which amniotic fluid cells improve outcomes after experimental nervous system injury remain unclear. However, studies reporting the expression and release of neurotrophic, angiogenic, and immunomodulatory factors by amniotic fluid cells suggest they may provide neuroprotection and (or) stimulate endogenous repair and remodelling processes in the injured nervous system. In this paper, we address recent research related to the neuronal differentiation of amniotic fluid-derived cells, the therapeutic efficacy of these cells in animal models of nervous system injury, and the possible mechanisms

mediating the positive outcomes achieved by amniotic fluid cell transplantation.

33. Routine clonal expansion of mesenchymal stem cells derived from amniotic fluid for perinatal applications.

Zia S, Toelen J, Mori da Cunha M, Dekoninck P, de Coppi P, Deprest J.

Prenat Diagn; 2013 Oct.

INTRODUCTION: Stem cells (SCs) isolated from amniotic fluid (AF) are a promising source for autologous perinatal cell therapy. The aim of this study was to develop a routine isolation, selection, and expansion protocol of clonal SC lines from redundant clinical amniocentesis samples.

MATERIALS AND METHODS: Amniotic fluids were collected between 15 and 22 weeks of gestation, and SCs were isolated by CD117-based and mechanical selection protocols. SCs were characterized by mesenchymal SC marker expression and differentiation protocols. Cells were manipulated with a lentiviral vector system expressing the β-galactosidase reporter gene and were injected into immunodeficient

newborn mouse pups. Qualitative assessment was performed to detect the infused cells after 1 week.

RESULTS: A total of 78 clonal AF SC populations were successfully isolated by mechanical selection from 21 consecutive amniocentesis samples. They were positive for mesenchymal SC cluster of differentiation markers and could be differentiated into the different lineages. SCs were stably labeled using β-galactosidase and were detected in the lungs and hearts of the neonatal mice.

CONCLUSION: We demonstrate that mesenchymal SCs can be routinely isolated and clonally expanded from mid-gestation human AF using mechanical isolation. They can easily be transduced and be tested for perinatal treatment in animal models.

34. Wnt signaling behaves as a "master regulator" in the osteogenic and adipogenic commitment of human amniotic fluid mesenchymal stem cells.

D'Alimonte I, Lannutti A, Pipino C, Di Tomo P, Pierdomenico L, Cianci E, Antonucci I, Marchisio M, Romano M, Stuppia L, Caciagli F, Pandolfi A, Ciccarelli R.

Stem Cell Rev; 2013 Oct.

Human amniotic fluid mesenchymal stem cells (huAFMSCs) are emerging as a promising therapeutic option in regenerative medicine. Here, we characterized huAFMSC phenotype and multipotentiality. When cultured in osteogenic medium, huAFMSC displayed a significant increase in: Alkaline Phosphatase (ALP) activity and mRNA expression, Alizarin Red S staining and Runx2 mRNA expression; whereas maintaining these cells in an adipogenic culture medium gave a time-dependent increase in PPARγ and FABP4 mRNA expression, glycerol-3-phosphate

dehydrogenase (GPDH) activity and positivity to Oil Red Oil staining. These results confirm that huAFMSCs can differentiate toward osteogenic and adipogenic phenotypes. The canonical Wnt/ßcatenin signaling pathway appears to trigger huAFMSC osteoblastogenesis, since during early phases of osteogenic differentiation, the expression of Dishevelled-2 (Dvl-2), of the non-phosphorylated form of ß-catenin, and the phosphorylation of glycogen synthase kinase-3ß (GSK3ß) at serine 9 were upregulated. On the contrary, during adipogenic differentiation Dvl-2 expression decreased, whereas that of ß-catenin remained unchanged. This was associated with a late increase in GSK3ß phosphorylation. Consistent with this scenario, huAFMSCs exposure to Dickkopf-1, a selective inhibitor of the Wnt signaling, abolished Runx2 and ALP mRNA upregulation during huAFMSC osteogenic differentiation, whereas it enhanced FABP4 expression in adipocyte-differentiating cells.

Taken together, these results unravel novel molecular determinants of huAFMSC commitment towards osteoblastogenesis, which may represent potential targets for directing the differentiation of these cells and improving their use in regenerative medicine.

35. Research development of amniotic fluid-derived stem cells in regenerative medicine.

Li C, Li W, Liu H, Wang J.

Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi; 2013 Oct.

OBJECTIVE: To review the latest development of amniotic fluid-derived stem cells (AFSCs) in regenerative medicine, and to discuss issues related to the studies in the field of AFSCs.

METHODS: The recent articles about AFSCs were extensively reviewed. The important knowledge of AFSCs was introduced in the field of regenerative medicine, and the basic and clinical researches of AFSCs were summarized and discussed.

RESULTS: Currently, it is confirmed that AFSCs have a multi-directional differentiation capacity, therefore, they have a wide application prospect in regenerative medicine, anti-tumor, and other fields.

CONCLUSION: AFSCs will become one of the ideal seed cells in the field of regenerative medicine with extensive research value because of the advantages of easy amniotic fluid sampling, little maternal and child trauma, no tumorigenesis, and no ethical restrictions.

36. Gene expression of stem cells at different stages of ontological human development.

Allegra A, Altomare R, Curcio P, Santoro A, Lo Monte AI, Mazzola S, Marino A.

Eur J Obstet Gynecol Reprod Biol; 2013 Oct.

OBJECTIVES: To compare multipotent mesenchymal stem cells (MSCs) obtained from chorionic villi (CV), amniotic fluid (AF) and placenta, with regard to their phenotype and gene expression, in order to understand if MSCs derived from different extra-embryonic tissues, at different stages of human ontological development, present distinct stemness characteristics.

STUDY DESIGN: MSCs obtained from 30 samples of CV, 30 of AF and 10 placentas (obtained from elective caesarean sections) were compared. MSCs at second confluence cultures were characterized by immunophenotypic analysis with flow cytometry using FACS CANTO II. The expression of the genes Oct-4 (Octamer-

binding transcription factor 4, also known as POU5F1), Sox-2 (SRY box-containing factor 2), Nanog, Rex-1 (Zfp-42) and Pax-6 (Paired Box Protein-6), was analyzed. Real-time quantitative PCR was performed by ABI Prism 7700, after RNA isolation and retro-transcription in cDNA. Statistical analysis was performed using non-parametric test Kruskal-Wallis (XLSTAT 2011) and confirmed by REST software, to estimate fold changes between samples. Each gene was defined differentially expressed if p-value was <0.05.

RESULTS: Cells from all samples were negative for haematopoietic antigens CD45, CD34, CD117 and CD33 and positive for the typical MSCs antigens CD13, CD73 and CD90. Nevertheless, MSCs from AF and placentas showed different fluorescence intensity, reflecting the heterogeneity of these tissues. The gene expression of OCT-4, SOX-2, NANOG was not significantly different among the three groups. In AF, REX-1 and

PAX-6 showed a higher expression in comparison to CV.

CONCLUSIONS: MSCs of different extra-embryonic tissues showed no differences in immunophenotype when collected from second confluence cultures. The expression of OCT-4, NANOG and SOX-2 was not significantly different, demonstrating that all fetal sources are suitable for obtaining MSCs. These results open new possibilities for the clinical use of MSCs derived from easily accessible sources, in order to develop new protocols for clinical and experimental research.

37. Human placental multipotent mesenchymal stromal cells modulate trophoblast migration via Rap1 activation.

Chen CP, Huang JP, Chu TY, Aplin JD, Chen CY, Wu YH.

Placenta; 2013 Oct.

INTRODUCTION:

Little is known about the interaction between human placental multipotent mesenchymal stromal cell (hPMSC) and trophoblast. We hypothesize that hPMSCs produce hepatocyte growth factor (HGF) which may interact with trophoblasts and regulate their migration during placentation.

METHODS:

hPMSCs were isolated from term placentas and conditioned medium was collected after 2 days of culture in hypoxic (<1% O2) or control (20% O2) conditions. Selective agonist and inhibitor or siRNA for protein kinase A (PKA) or Rap1 were combined with Rap1-GTP pull down assays, flow cytometry, integrin β1 activation assays and adhesion

and migration studies to investigate HGF signaling effects in trophoblasts. The hPMSC abundance and HGF level in preeclamptic placentas were compared with gestational age-matched controls.

RESULTS:

HGF was expressed by hPMSCs and was decreased in hypoxia. HGF induced cAMP production and Rap1 activation in trophoblasts, which in turn activated integrin β1. The HGF and PKA activator 6-Bnz-cAMP induced Rap1 activation with increased trophoblast adhesion and migration. The alterations were inhibited by PKA inhibitor H89 or Rap1 siRNA. HGF and cAMP expression were reduced in preeclamptic placentas. hPMSC number was decreased in preeclamptic placenta compared to controls (0.68 ± 0.1% vs. 1.32 ± 0.5%; P = 0.026). hPMSC conditioned medium enhanced trophoblast migration which was inhibited by c-Met blocking antibody, but migration was reduced by conditioned medium from hPMSC cultured in hypoxia.

CONCLUSIONS:

hPMSCs secrete HGF and increase trophoblast cAMP production. The cAMP effector PKA modulates adhesion and migration of trophoblast via signaling to Rap1 and integrin β1.

38. The contribution of stem cell therapy to skeletal muscle remodeling in heart failure.

Castellani C, Vescovo G, Ravara B, Franzin C, Pozzobon M, Tavano R, Gorza L, Papini E, Vettor R, De Coppi P, Thiene G, Angelini A.

Int J Cardiol; 2013 Oct 3.

BACKGROUND:

The aim of our study was to investigate whether stem cell (SC) therapy with human amniotic fluid stem cells (hAFS, fetal stem cells) and rat adipose tissue stromal vascular fraction cells-GFP positive cells (rSVC-GFP) was able to produce favorable effects on skeletal muscle (SM) remodeling in a well-established rat model of right heart failure (RHF).

METHODS:

RHF was induced by monocrotaline (MCT) in Sprague-Dawley rats. Three weeks later, four millions of hAFS or rSVC-GFP cells were injected via tail vein. SM remodeling was assessed by Soleus muscle fiber cross

sectional area (CSA), myocyte apoptosis, myosin heavy chain (MHC) composition, satellite cells pattern, and SC immunohistochemistry.

RESULTS:

hAFS and rSVC-GFP injection produced significant SC homing in Soleus (0.68 ± 1.0 and 0.67 ± 0.75% respectively), with a 50% differentiation toward smooth muscle and endothelial cells. Pro-inflammatory cytokines were down regulated to levels similar to those of controls. SC-treated (SCT) rats showed increased CSA ($p<0.004$ vs MCT) similarly to controls with a reshift toward the slow MHC1 isoform. Apoptosis was significantly decreased (11.12.± 8.8 cells/mm(3) hAFS and 13.1+7.6 rSVC-GFP) ($p<0.001$ vs MCT) and similar to controls (5.38 ± 3.0 cells/mm(3)). RHF rats showed a dramatic reduction of satellite cells(MCT 0.2 ± 0.06% Pax7 native vs controls 2.60 ± 2.46%, $p<0.001$), while SCT induced a repopulation of both native and SC derived satellite cells ($p<0.005$).

CONCLUSIONS:

SC treatment led to SM remodeling with satellite cell repopulation, decreased atrophy and apoptosis. Modulation of the cytokine milieu might play a crucial pathophysiological role with a possible scenario for autologous transplantation of SC in pts with CHF myopathy.

39. Stem cell therapy to protect and repair the developing brain: a review of mechanisms of action of cord blood and amnion epithelial derived cells.

Castillo-Melendez M, Yawno T, Jenkin G, Miller SL.

Front Neurosci; 2013 Oct 24.

In the research, clinical, and wider community there is great interest in the use of stem cells to reduce the progression, or indeed repair brain injury. Perinatal brain injury may result from acute or chronic insults sustained during fetal development, during the process of birth, or in the newborn period. The most readily identifiable outcome of perinatal brain injury is cerebral palsy, however, this is just one consequence in a spectrum of mild to severe neurological deficits. As we review, there are now clinical trials taking place worldwide targeting cerebral palsy with stem cell therapies. It will likely be many years before strong evidence-based results emerge from these trials. With

such trials underway, it is both appropriate and timely to address the physiological basis for the efficacy of stem-like cells in preventing damage to, or regenerating, the newborn brain. Appropriate experimental animal models are best placed to deliver this information. Cell availability, the potential for immunological rejection, ethical, and logistical considerations, together with the propensity for native cells to form teratomas, make it unlikely that embryonic or fetal stem cells will be practical. Fortunately, these issues do not pertain to the use of human amnion epithelial cells (hAECs), or umbilical cord blood (UCB) stem cells that are readily and economically obtained from the placenta and umbilical cord discarded at birth. These cells have the potential for transplantation to the newborn where brain injury is diagnosed or even suspected. We will explore the novel characteristics of hAECs and undifferentiated UCB cells, as well as UCB-derived endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs), and how

immunomodulation and anti-inflammatory properties are principal mechanisms of action that are common to these cells, and which in turn may ameliorate the cerebral hypoxia and inflammation that are final pathways in the pathogenesis of perinatal brain injury.

40. Neuronal cell differentiation of mesenchymal stem cells originating from canine amniotic fluid.

Kim EY, Lee KB, Yu J, Lee JH, Kim KJ, Han KW, Park KS, Lee DS, Kim MK.

Hum Cell; 2013 Oct 29.

The amniotic fluid contains mesenchymal stem cells (MSCs) and can be readily available for tissue engineering. Regenerative treatments such as tissue engineering, cell therapy, and transplantation show potential in clinical trials of degenerative diseases. Disease presentation and clinical responses in the Canis familiaris not only are physiologically similar to human compared with other traditional mammalian models but is also a suitable model for human diseases. The aim of this study was to investigate whether canine amniotic-fluid-derived mesenchymal stem cells (cAF-MSCs) can differentiate into neural precursor cells in vitro when exposed to neural induction reagent. During neural differentiation, cAF-

MSCs progressively acquire neuron-like morphology. Messenger RNA (mRNA) expression levels of neural-specific genes, such as NEFL, NSE, and TUBB3 (βIII-tubulin) dramatically increased in the differentiated cAF-MSCs after induction. In addition, protein expression levels of nestin, βIII-tubulin, and tyrosine hydroxylase remarkably increased in differentiated cAF-MSCs. This study demonstrates that cAF-MSCs have great potential for neural precursor differentiation in vitro. Therefore, amniotic fluid may be a suitable alternative source of stem cells and can be applied to cell therapy in neurodegenerative diseases.

41. Modulation of physical environment makes placental mesenchymal stromal cells suitable for therapy.

Mathew SA, Rajendran S, Gupta PK, Bhonde R.

Cell Biol Int; 2013 Nov.

Low level of oxygen at the site of injury is likely to affect the viability and proliferation of the transplanted mesenchymal stromal cells (MSCs). Hence there is a need to understand the effect of the physical environment on transplanted stromal cells. Therefore, we have studied the effect of the duration of hypoxic exposure alone or in combination with normoxia on placenta derived mesenchymal stem cell (PDMSCs). PDMSCs and bone marrow MSCs (BMMSCs) were analysed under four different culture conditions, exposure to direct normoxia (N), direct hypoxia (H) and intermittent normoxia followed by hypoxia (NH) and intermittent hypoxia followed by normoxia (HN). The effect on morphology, proliferation,

metabolic activity by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) and viability by 7AAD (7-amino-actinomycin D) were assayed, along with markers for MSCs and HLADR. No change in morphology, marker expression or HLADR was detected in N, H, NH or HN. An increase in proliferation rate, decrease in population doubling-time (PDT) and a relative increase in metabolic activity was strongly noted in the order: NH, N/HN and H. No significant difference was observed in the viability between N, H, NH or HN. A similar pattern was also observed in BMMSCS, indicating comparable suitability of PDMSCs in therapeutic applications. Thus we conclude that intermittent exposure to normoxia prior to hypoxic exposure is a better option than direct exposure to hypoxia. This may have clinical relevance in that they probably mirror the in vivo scenario of systemic delivery (NH) of cells as opposed to local delivery (H), thereby suggesting that systemic delivery is better than local delivery.

42. Immune tolerance of amniotic fluid stem cell-induced rat kidney graft and influences on oxidative stress.

Feng J, Zhao L, Deng H, Wei M, Li J, Xu K.

Transplant Proc; 2013 Nov.

OBJECTIVE: This study aimed to use amniotic fluid stem cells of donors to induce immune tolerance of heterogenous rat kidney graft for investigating the formation mechanism of immune tolerance.

METHODS: With Wistar rats as donors and Sprague-Dawley (SD) rats as receptors, the heterogenous kidney graft animal model was established, and amniotic fluid stem cells of Wistar rats were isolated and cultured. Moreover, 40 SD rats were randomly divided into 4 groups. Creatinine (Cr), blood urea nitrogen (BUN), interleukin (IL) 2, interferon (IFN) γ, and oxidative stress levels in serum were detected, flow cytometry was used to detect changes of CD4 and CD8 cells, and quantitative changes of urinary protein and

pathologic changes of transplanted kidney were observed.
RESULTS: BUN, Cr, IL-2, IFN-γ, and oxidative stress levels and urinary protein quantity in rat serum of the test group were significantly lower than those of the control group, creatinine clearance rate was significantly higher than that of the control group, and renal pathologic injury extent was significantly milder than that of the control group.
CONCLUSIONS: Amniotic fluid stem cells can induce immune tolerance of rat kidney graft and inhibit oxidative stress level, improve kidney function, and alleviate kidney injury.

43. Isolation, culture, and identification of amniotic fluid-derived mesenchymal stem cells.

Fei X, Jiang S, Zhang S, Li Y, Ge J, He B, Goldstein S, Ruiz G.

Cell Biochem Biophys; 2013 Nov.

Amniotic fluid-derived mesenchymal stem cells (AF-MSC) are newly described, excellent seed cells that have good differentiation capability and are convenient to obtain. However, it is important to develop a method to isolate and culture AF-MSC efficiently. Amniotic fluid samples were obtained from rabbits and the adherence method was used for AF-MSC culture. Flow cytometry, western blot, and immunofluorescence studies were used to analyze the phenotypic characteristics of the cultured AF-MSC. Amniotic fluid-derived mesenchymal stem cells were successfully isolated and cultured from amniotic fluid. Flow cytometric analysis demonstrated that these cells expressed CD29 and CD44, while

they did not express CD34. The expression of transcription factor Oct-4 was confirmed by western blot and immunofluorescence analysis. Using the adherence method, we developed a successful, reproducible protocol for the isolation of AF-MSC from amniotic fluid. The results of our phenotypic analysis revealed that the AF-MSC isolated in the present study were multipotent cells.

44. Treatment with placenta-derived mesenchymal stem cells mitigates development of bronchiolitis obliterans in a murine model.

Zhao Y, Gillen JR, Harris DA, Kron IL, Murphy MP, Lau CL.

J Thorac Cardiovasc Surg; 2013 Nov 4.

OBJECTIVE: Bone marrow-derived mesenchymal stem cells (MSCs) have shown therapeutic potential in acute lung injury. Recently, placenta-derived human mesenchymal stem cells (PMSCs) have shown similarities with bone marrow-derived MSCs in terms of regenerative capabilities and immunogenicity. This study investigates the hypothesis that treatment with PMSCs reduces the development of bronchiolitis obliterans in a murine heterotopic tracheal transplant model.

METHODS: A murine heterotopic tracheal transplant model was used to study the continuum from acute to chronic rejection. In the treatment groups, PMSCs or PMSC-

conditioned medium (PMSCCM) were injected either locally or intratracheally into the allograft. Phosphate-buffered saline (PBS) or blank medium was injected in the control groups. Tracheal luminal obliteration was assessed on sections stained with hematoxylin and eosin. Infiltration of inflammatory and immune cells and epithelial progenitor cells was assessed using immunohistochemistry and densitometric analysis.

RESULTS: Compared with injection of PBS, local injection of PMSCs significantly reduced luminal obliteration at 28 days after transplantation (P = .015). Intratracheal injection of PMSCs showed similar results to local injection of PMSCs compared with injection of PBS and blank medium (P = .022). Tracheas treated with PMSC/PMSCCM showed protection against the loss of epithelium on day 14, with an increase in P63+CK14+ epithelial progenitor cells and Foxp3+ regulatory T cells. In addition, injection of PMSCs and PMSCCM

significantly reduced the number of neutrophils and CD3+ T cells on day 14.
CONCLUSIONS: This study demonstrates that treatment with PMSCs is protective against the development of bronchiolitis obliterans in an heterotopic tracheal transplant model. These results indicate that PMSCs could provide a novel therapeutic option to reduce chronic rejection after lung transplant.

45. Dose dependent side effect of superparamagnetic iron oxide nanoparticle labeling on cell motility in two fetal stem cell populations.

Diana V, Bossolasco P, Moscatelli D, Silani V, Cova L.

PLoS One; 2013 Nov 7.

Multipotent stem cells (SCs) could substitute damaged cells and also rescue degeneration through the secretion of trophic factors able to activate the endogenous SC compartment. Therefore, fetal SCs, characterized by high proliferation rate and devoid of ethical concern, appear promising candidate, particularly for the treatment of neurodegenerative diseases. Super Paramagnetic Iron Oxide nanoparticles (SPIOn), routinely used for pre-clinical cell imaging and already approved for clinical practice, allow tracking of transplanted SCs and characterization of their fate within the host tissue, when combined with Magnetic Resonance Imaging (MRI). In this work we

investigated how SPIOn could influence cell migration after internalization in two fetal SC populations: human amniotic fluid and chorial villi SCs were labeled with SPIOn and their motility was evaluated. We found that SPIOn loading significantly reduced SC movements without increasing production of Reactive Oxygen Species (ROS). Moreover, motility impairment was directly proportional to the amount of loaded SPIOn while a chemoattractant-induced recovery was obtained by increasing serum levels. Interestingly, the migration rate of SPIOn labeled cells was also significantly influenced by a degenerative surrounding. In conclusion, this work highlights how SPIOn labeling affects SC motility in vitro in a dose-dependent manner, shedding the light on an important parameter for the creation of clinical protocols. Establishment of an optimal SPIOn dose that enables both a good visualization of grafted cells by MRI and the physiological migration rate is a main step in order to maximize the effects of SC therapy

in both animal models of neurodegeneration and clinical studies.

46. Stem cells as a potential therapy for necrotizing enterocolitis.

Eaton S, Zani A, Pierro A, De Coppi P.

Expert Opin Biol Ther; 2013 Dec.

INTRODUCTION: Necrotizing enterocolitis (NEC) is a severe gastrointestinal disease of neonates, especially those born prematurely, that remains an important cause of morbidity and mortality. Although current treatments such as inotropes, antibiotics and ventilation are supportive, there is an urgent need for novel therapies that specifically target the affected intestine.

AREAS COVERED: We briefly introduce the disease and the effects on intestinal epithelia. We provide a brief description of amniotic fluid stem (AFS) cells, and then describe some recent data in which AFS cells were beneficial in an animal model of NEC and a potential mechanism is described. The effects of AFS cells are compared with data on bone marrow mesenchymal stem cells.

The potential implications of these findings for therapy are discussed.
EXPERT OPINION: The current data are promising and demonstrate that stem cells do have an effect in rodent models of NEC. However, the short timescale, limited ability for longitudinal evaluation and uncertain clinical relevance of these models means that there are considerable challenges to be overcome before attempting stem cell therapy in clinical trials. Nevertheless, these data open up novel areas of research into a prevention or therapy for this devastating disease.

47. Human amniotic fluid stem cell differentiation along smooth muscle lineage.

Ghionzoli M, Repele A, Sartiani L, Costanzi G, Parenti A, Spinelli V, David AL, Garriboli M, Totonelli G, Tian J, Andreadis ST, Cerbai E, Mugelli A, Messineo A, Pierro A, Eaton S, De Coppi P.

FASEB J; 2013 Dec.

Functional smooth muscle engineering requires isolation and expansion of smooth muscle cells (SMCs), and this process is particularly challenging for visceral smooth muscle tissue where progenitor cells have not been clearly identified. Herein we showed for the first time that efficient SMCs can be obtained from human amniotic fluid stem cells (hAFSCs). Clonal lines were generated from c-kit(+) hAFSCs. Differentiation toward SM lineage (SMhAFSCs) was obtained using a medium conditioned by PDGF-BB and TGF-β1. Molecular assays revealed higher level of α smooth muscle actin (α-SMA),

desmin, calponin, and smoothelin in SMhAFSCs when compared to hAFSCs. Ultrastructural analysis demonstrated that SMhAFSCs also presented in the cytoplasm increased intermediate filaments, dense bodies, and glycogen deposits like SMCs. SMhAFSC metabolism evaluated via mass spectrometry showed higher glucose oxidation and an enhanced response to mitogenic stimuli in comparison to hAFSCs. Patch clamp of transduced hAFSCs with lentiviral vectors encoding ZsGreen under the control of the α-SMA promoter was performed demonstrating that SMhAFSCs retained a smooth muscle cell-like electrophysiological fingerprint. Eventually SMhAFSCs contractility was evident both at single cell level and on a collagen gel. In conclusion, we showed here that hAFSCs under selective culture conditions are able to give rise to functional SMCs.

48. *Ex vivo* expansion of hematopoietic stem- and progenitor cells from cord blood in coculture with mesenchymal stroma cells from amnion, chorion, Wharton's jelly, amniotic fluid, cord blood, and bone marrow.

Klein C, Strobel J, Zingsem J, Richter RH, Goecke TW, Beckmann MW, Eckstein R, Weisbach V.

Tissue Eng Part A; 2013 Dec.

In most cases, the amount of hematopoietic stem and progenitor cells (HSPCs) in a single cord blood (CB) unit is not sufficient for allogenic transplantation of adults. Therefore, two CB units are usually required. The ex vivo expansion of HSPCs from CB in coculture with mesenchymal stroma cells (MSCs) might be an alternative. It was investigated, whether bone marrow-derived MSCs, which have to be obtained in an invasive procedure, introduce a further donor and increases the risk of transmissible infectious diseases for the patient can be

replaced by MSCs from amnion, chorion, Wharton's jelly, amniotic fluid, and CB, which can be isolated from placental tissue which is readily available when CB is sampled. In a two-step ex vivo coculture mononuclear cells from cryopreserved CB were cultured with different MSC-feederlayers in a medium supplemented with cytokines (stem cell factor, thrombopoietin [TPO], and granulocyte colony-stimulating factor). Expansion rates were analyzed as well, by long-term culture-initiating cell (LTC-IC) and colony-forming unit (CFU) assays, as by measuring CD34(+)- and CD45(+)-cells. Due to the comparably low number of 5×10(2) to 1×10(4) CD34(+)-cells per cm(2) MSC-monolayer, we observed comparably high expansion rates from 80 to 391,000 for CFU, 70 to 313,000 for CD34(+)-, and 200 to 352,000 for CD45(+)-cells. Expansion of LTC-IC was partly observed. Compared to the literature, we found a better expansion rate of CD34(+)-cells with MSCs from all different sources. This is probably

due to the comparably low number of 5×10(2) to 1×10 CD34(+)-cells per cm(2) MSC-monolayer we used. Comparably, high expansion rates were observed from 80 to 391,000 for CFUs, 70 to 313,000 for CD34(+)-, and 200 to 352,000 for CD45(+)-cells. However, the expansion of CD34(+)-cells was significantly more effective with MSCs from bone marrow compared to MSCs from amnion, chorion, and Wharton's jelly. The comparison of MSCs from bone marrow with MSCs from CB and amniotic fluid showed no significant difference. We conclude that MSCs from placental tissues might be useful in the expansion of HSPCs, at least if low numbers of CD34(+)-cells per cm(2) MSC-monolayer and a high TPO concentration are implemented in the expansion culture.

49. Expression and co-expression of surface markers of pluripotency on human amniotic cells cultured in different growth media.

Bryzek A, Czekaj P, Plewka D, Komarska H, Tomsia M, Lesiak M, Sieroń AL, Sikora J, Kopaczka K.

Ginekol Pol; 2013 Dec.

OBJECTIVES: Despite constant advances in the field of biology and medical application of human embryonic stem cells, the molecular mechanism of pluripotency remains largely unknown. So far, definitions of pluripotent stem cells (SC) have been based on a limited number of antigenic markers and have not allowed for unambiguous determination of the homogeneity of each subpopulation. Moreover, the use of some crucial pluripotency markers such as SSEA-3 and SSEA-4 has recently been questioned due to the possibility that the pattern of surface glycans may be changed depending on the content of the cell culture medium.

AIM: Quantitative analysis of amniotic SC subpopulations cultured in different media, based on the following pluripotency surface markers: SSEA-3, SSEA-4, TRA- 1-60 and TRA- 1-81 expression and co-expression.

MATERIAL AND METHODS: Immunofluorescence and fluorescence microscopy were used to identify and localize SC within a normal human placenta at term. The number of SSEA-4+, SSEA-3+, TRA-1-60+ and TRA-1-81+ cells and cells with co-expression of the above mentioned markers, cultured in media containing different protein supplements of animal origin, was counted by flow cytometry

RESULTS AND CONCLUSIONS: Cells with characteristics of embryonic SC were identified in the amniotic epithelium and the chorion, but not in the decidua basalis. Amniotic epithelium contained various types of SC, with SSEA-4+ as the most numerous. Disproportion in the number of SSEA-4+, SSEA-3+, TRA-1-60+ and TRA-1-81+ cells and cells characterized by co-expression of

these antigens, as well as lack of quantitative differences between SC subpopulations cultured in different media, was observed. In conclusion, the amniotic epithelium is composed of SC at different stages of the development but human amnion might become an alternative source of SSEA-4+ embryonic-like SC. The composition of the evaluated media, characterized by different content of animal-derived proteins, does not influence the number of cells identified within the SC subpopulations.

50. Bladder cancer cell in co-culture induces human stem cell differentiation to urothelial cells through paracrine FGF10 signaling.

Chung SS, Koh CJ.

In Vitro Cell Dev Biol Anim; 2013 Dec.

Fibroblast growth factor 10 (FGF10) is required for embryonic epidermal morphogenesis including brain development, lung morphogenesis, and initiation of limb bud formation. In this study, we investigated the role of FGF10 as a lead induction factor for stem cell differentiation toward urothelial cell. To this end, human multipotent stem cell in vitro system was employed. Human amniotic fluid stem cells were co-cultured with immortalized bladder cancer lines to induce directed differentiation into urothelial cells. Urothelial markers, uroplakin II, III, and cytokeratin 8, were monitored by RT-PCR, immunocytochemistry, and Western blot analysis. Co-cultured stem cells began to express uroplakin II, III, and cytokeratin 8.

Targeted FGF10 gene knockdown from bladder cancer cells abolished the directed differentiation. In addition, when FGF10 downstream signaling was blocked with the Mek inhibitor, the co-culture system lost the capacity to induce urothelial differentiation. Exogenous addition of recombinant FGF10 protein promoted stem cell differentiation into urothelium cell lineage. Together, this report suggests that paracrine FGF10 signaling stimulates the differentiation of human stem cell into urothelial cells. Current study provides insight into the potential role of FGF10 as a lead growth factor for bladder regeneration and its therapeutic application for bladder transplantation.

51. MYOD mediates skeletal myogenic differentiation of human amniotic fluid stem cells and regeneration of muscle injury.

Kim JA, Shon YH, Lim JO, Yoo JJ, Shin HI, Park EK1.

Stem Cell Res Ther; 2013 Dec 11.

INTRODUCTION: Human amniotic fluid stem (hAFS) cells have been shown to differentiate into multiple lineages, including myoblasts. However, molecular mechanisms underlying the myogenic differentiation of hAFS cells and their regenerative potential for muscle injury remain to be elucidated.

METHODS: In order to induce myogenic differentiation of hAFS cells, lentiviruses for MYOD were constructed and transduced into hAFS cells. Formation of myotube-like cells was analyzed by immunocytochemistry, and expression of molecular markers for myoblasts was analyzed by reverse transcription polymerase chain reaction and Western blotting. For in vivo muscle

regeneration, MYOD transduced hAFS cells were injected into left tibialis anterior (TA) muscles injured with cardiotoxin, and muscle regeneration was analyzed using hematoxylin and eosin, immunocytochemistry and formation of neuro-muscular junction.

RESULTS: MYOD expression in hAFS cells successfully induced differentiation into multinucleated myotube-like cells. Consistently, significant expression of myogenic marker genes, such as MYOG, DES, DMD and MYH, was induced by MYOD. Analysis of pre-myogenic factors showed that expression of PAX3, MEOX1 and EYA2 was significantly increased by MYOD. MYOD was phosphorylated and localized in the nucleus. These results suggest that in hAFS cells, MYOD is phosphorylated and localized in the nucleus, thus inducing expression of myogenic factors, resulting in myogenic differentiation of hAFS cells. To test regenerative potential of MYOD-transduced hAFS cells, we transplanted them into injured muscles of immunodeficient

BALB/cSlc-nu mice. The results showed a substantial increase in the volume of TA muscle injected with MYOD-hAFS cells. In addition, TA muscle tissue injected with MYOD-hAFS cells has more numbers of neuro-muscular junctions compared to controls, indicating functional restoration of muscle injury by MYOD-hAFS cells.

CONCLUSIONS: Collectively, our data suggest that transduction of hAFS cells with MYOD lentiviruses induces skeletal myogenic differentiation in vitro and morphological and functional regeneration of injured muscle in vivo.

52. Placenta-derived mesenchymal stem cells improve memory dysfunction in an Aβ1-42-infused mouse model of Alzheimer's disease.

Yun HM, Kim HS, Park KR, Shin JM, Kang AR, il Lee K, Song S, Kim YB, Han SB, Chung HM, Hong JT.

Cell Death Dis; 2013 Dec 12.

Mesenchymal stem cells (MSCs) promote functional recoveries in pathological experimental models of central nervous system (CNS) and are currently being tested in clinical trials for neurological disorders, but preventive mechanisms of placenta-derived MSCs (PD-MSCs) for Alzheimer's disease are poorly understood. Herein, we investigated the inhibitory effect of PD-MSCs on neuronal cell death and memory impairment in Aβ1-42-infused mice. After intracerebroventrical (ICV) infusion of Aβ1-42 for 14 days, the cognitive function was assessed by the Morris water maze test and passive avoidance test. Our results showed

that the transplantation of PD-MSCs into Aβ1-42-infused mice significantly improved cognitive impairment, and behavioral changes attenuated the expression of APP, BACE1, and Aβ, as well as the activity of β-secretase and γ-secretase. In addition, the activation of glia cells and the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were inhibited by the transplantation of PD-MSCs. Furthermore, we also found that PD-MSCs downregulated the release of inflammatory cytokines as well as prevented neuronal cell death and promoted neuronal cell differentiation from neuronal progenitor cells in Aβ1-42-infused mice. These data indicate that PD-MSC mediates neuroprotection by regulating neuronal death, neurogenesis, glia cell activation in hippocampus, and altering cytokine expression, suggesting a close link between the therapeutic effects of MSCs and the damaged CNS in Alzheimer's disease.

53. A novel source of cultured podocytes.
Da Sacco S, Lemley KV, Sedrakyan S, Zanusso I, Petrosyan A, Peti-Peterdi J, Burford J, De Filippo RE, Perin L.
PLoS One; 2013 Dec 12.

Amniotic fluid is in continuity with multiple developing organ systems, including the kidney. Committed, but still stem-like cells from these organs may thus appear in amniotic fluid. We report having established for the first time a stem-like cell population derived from human amniotic fluid and possessing characteristics of podocyte precursors. Using a method of triple positive selection we obtained a population of cells (hAKPC-P) that can be propagated in vitro for many passages without immortalization or genetic manipulation. Under specific culture conditions, these cells can be differentiated to mature podocytes. In this work we compared these cells with conditionally immortalized podocytes, the current gold standard for in vitro studies. After in vitro differentiation,

both cell lines have similar expression of the major podocyte proteins, such as nephrin and type IV collagen, that are characteristic of mature functional podocytes. In addition, differentiated hAKPC-P respond to angiotensin II and the podocyte toxin, puromycin aminonucleoside, in a way typical of podocytes. In contrast to immortalized cells, hAKPC-P have a more nearly normal cell cycle regulation and a pronounced developmental pattern of specific protein expression, suggesting their suitability for studies of podocyte development for the first time in vitro. These novel progenitor cells appear to have several distinct advantages for studies of podocyte cell biology and potentially for translational therapies.

54. Urothelial differentiation of human amniotic fluid stem cells by urothelium specific conditioned medium.

Chung SS, Kang H, Kang HG.

Cell Biol Int; 2013 Dec 23.

Human amniotic fluid stem cells (HAFSCs) have a high proliferative capacity and a good differentiation potential, and may thus be suitable for regenerative medicine. To date, urothelial differentiation mechanisms of HAFSCs are poorly understood. We have investigated the urothelial differentiation potential of HAFSCs so that they can be therapeutically applied to cure defective diseases of bladder. To induce the stem cell differentiation, HAFSCs were cultured in a bladder cancer-derived conditioned medium. After 2 weeks of culture, HAFSCs began to express the urothelial lineage-specific markers (UPII, CK8 and FGF10). Meanwhile, pluripotency markers (Oct-4, Sox-2 and Nanog) were downregulated at both RNA and protein levels in the differentiated HAFSCs.

Immunocytochemistry data revealed that differentiated HAFSCs expressed urothelial markers of UPII and CK8. We have screened the receptor tyrosine kinase arrays with the differentiated HAFSCs. The screening showed that MuSK, Tie-1 and EphA4 receptor tyrosine kinases were upregulated, whereas EphA7 and FGF R1 kinases were downregulated in HAFSCs. The data suggest that HAFSCs can be an important urothelium cell source, which can be use for urinary tract engineering.

55. Inhibition of nuclear nox4 activity by plumbagin: effect on proliferative capacity in human amniotic stem cells.

Guida M1, Maraldi T, Resca E, Beretti F, Zavatti M, Bertoni L, La Sala GB, De Pol A.

Oxid Med Cell Longev; 2013.

Human amniotic fluid stem cells (AFSC) with multilineage differentiation potential are novel source for cell therapy. However, in vitro expansion leads to senescence affecting differentiation and proliferative capacities. Reactive oxygen species (ROS) have been involved in the regulation of stem cell pluripotency, proliferation, and differentiation. Redox-regulated signal transduction is coordinated by spatially controlled production of ROS within subcellular compartments. NAD(P)H oxidase family, in particular Nox4, has been known to produce ROS in the nucleus; however, the mechanisms and the meaning of this function remain largely unknown. In the present study, we show that Nox4 nuclear expression

(nNox4) increases during culture passages up to cell cycle arrest and the serum starvation causes the same effect. With the decrease of Nox4 activity, obtained with plumbagin, a decline of nuclear ROS production and of DNA damage occurs. Moreover, plumbagin exposure reduces the binding between nNox4 and nucleoskeleton components, as Matrin 3. The same effect was observed also for the binding with phospho-ERK, although nuclear ERK and P-ERK are unchanged. Taken together, we suggest that nNox4 regulation may have important pathophysiologic effects in stem cell proliferation through modulation of nuclear signaling and DNA damage.

56. Making surrogate β-cells from mesenchymal stromal cells: perspectives and future endeavors.

Bhonde RR, Sheshadri P, Sharma S, Kumar A.

Int J Biochem Cell Biol; 2014 Jan.

Generation of surrogate β-cells is the need of the day to compensate the short supply of islets for transplantation to diabetic patients requiring daily shots of insulin. Over the years several sources of stem cells have been claimed to cater to the need of insulin producing cells. These include human embryonic stem cells, induced pluripotent stem cells, human perinatal tissues such as amnion, placenta, umbilical cord and postnatal tissues involving adipose tissue, bone marrow, blood monocytes, cord blood, dental pulp, endometrium, liver, labia minora dermis-derived fibroblasts and pancreas. Despite the availability of such heterogonous sources, there is no substantial breakthrough in selecting and implementing an ideal source

for generating large number of stable insulin producing cells. Although the progress in derivation of β-cell like cells from embryonic stem cells has taken a greater leap, their application is limited due to controversy surrounding the destruction of human embryo and immune rejection. Since multipotent mesenchymal stromal cells are free of ethical and immunological complications, they could provide unprecedented opportunity as starting material to derive insulin secreting cells. The main focus of this review is to discuss the merits and demerits of MSCs obtained from human peri- and post-natal tissue sources to yield abundant glucose responsive insulin producing cells as ideal candidates for prospective stem cell therapy to treat diabetes.

57. Amniotic fluid stem cells prevent β-cell injury.

Villani V, Milanesi A, Sedrakyan S, Da Sacco S, Angelow S, Conconi MT, Di Liddo R, De Filippo R, Perin L.

Cytotherapy; 2014 Jan.

BACKGROUND/AIMS: The contribution of amniotic fluid stem cells (AFSC) to tissue protection and regeneration in models of acute and chronic kidney injuries and lung failure has been shown in recent years. In the present study, we used a chemically induced mouse model of type 1 diabetes to determine whether AFSC could play a role in modulating β-cell injury and restoring β-cell function.

METHODS: Streptozotocin-induced diabetic mice were given intracardial injection of AFSC; morphological and physiological parameters and gene expression profile for the insulin pathway were evaluated after cell transplantation.

RESULTS: AFSC injection resulted in protection from β-cell damage and increased β-cell regeneration in a subset of mice as indicated by glucose and insulin levels, increased islet mass and preservation of islet structure. Moreover, β-cell preservation/regeneration correlated with activation of the insulin receptor/Pi3K/Akt signaling pathway and vascular endothelial growth factor-A expression involved in maintaining β-cell mass and function.

CONCLUSIONS: Our results suggest a therapeutic role for AFSC in preserving and promoting endogenous β-cell functionality and proliferation. The protective role of AFSC is evident when stem cell transplantation is performed before severe hyperglycemia occurs, which suggests the importance of early intervention. The present study demonstrates the possible benefits of the application of a non-genetically engineered stem cell population derived from amniotic fluid for the treatment of type 1 diabetes mellitus and gives new insight on the

mechanism by which the beneficial effect is achieved.

58. Sox2 Suppression by miR-21 Governs Human Mesenchymal Stem Cell Properties.

Trohatou O, Zagoura D, Bitsika V, Pappa KI, Antsaklis A, Anagnou NP, Roubelakis MG.

Stem Cells Transl Med; 2014 Jan.

MicroRNAs (miRNAs) have recently been shown to act as regulatory signals for maintaining stemness and for determining the fate of adult and fetal stem cells, such as human mesenchymal stem cells (hMSCs). hMSCs constitute a population of multipotent stem cells that can be expanded easily in culture and are able to differentiate into many lineages. We have isolated two subpopulations of fetal mesenchymal stem cells (MSCs) from amniotic fluid (AF) known as spindle-shaped (SS) and round-shaped (RS) cells and characterized them on the basis of their phenotypes, pluripotency, proliferation rates, and differentiation potentials. In this study, we analyzed the miRNA profile of MSCs derived from AF,

bone marrow (BM), and umbilical cord blood (UCB). We initially identified 67 different miRNAs that were expressed in all three types of MSCs but at different levels, depending on the source. A more detailed analysis revealed that miR-21 was expressed at higher levels in RS-AF-MSCs and BM-MSCs compared with SS-AF-MSCs. We further demonstrated for the first time a direct interaction between miR-21 and the pluripotency marker Sox2. The induction of miR-21 strongly inhibited Sox2 expression in SS-AF-MSCs, resulting in reduced clonogenic and proliferative potential and cell cycle arrest. Strikingly, the opposite effect was observed upon miR-21 inhibition in RS-AF-MSCs and BM-MSCs, which led to an enhanced proliferation rate. Finally, miR-21 induction accelerated osteogenesis and impaired adipogenesis and chondrogenesis in SS-AF-MSCs. Therefore, these findings suggest that miR-21 might specifically function by regulating Sox2 expression in human MSCs and might also act as a key

molecule determining MSC proliferation and differentiation.

59. Hypoxic conditioned medium from human amniotic fluid-derived mesenchymal stem cells accelerates skin wound healing through TGF-β/SMAD2 and PI3K/Akt pathways.

Jun EK, Zhang Q, Yoon BS, Moon JH, Lee G, Park G, Kang PJ, Lee JH, Kim A, You S.
Int J Mol Sci; 2014 Jan 6.

In a previous study, we isolated human amniotic fluid (AF)-derived mesenchymal stem cells (AF-MSCs) and utilized normoxic conditioned medium (AF-MSC-norCM) which has been shown to accelerate cutaneous wound healing. Because hypoxia enhances the wound healing function of mesenchymal stem cell-conditioned medium (MSC-CM), it is interesting to explore the mechanism responsible for the enhancement of wound healing function. In this work, hypoxia not only increased the proliferation of AF-MSCs but also maintained their constitutive characteristics (surface marker expression and differentiation potentials).

Notably, more paracrine factors, VEGF and TGF-β1, were secreted into hypoxic conditioned medium from AF-MSCs (AF-MSC-hypoCM) compared to AF-MSC-norCM. Moreover, AF-MSC-hypoCM enhanced the proliferation and migration of human dermal fibroblasts in vitro, and wound closure in a skin injury model, as compared to AF-MSC-norCM. However, the enhancement of migration of fibroblasts accelerated by AF-MSC-hypoCM was inhibited by SB505124 and LY294002, inhibitors of TGF-β/SMAD2 and PI3K/AKT, suggesting that AF-MSC-hypoCM-enhanced wound healing is mediated by the activation of TGF-β/SMAD2 and PI3K/AKT. Therefore, AF-MSC-hypoCM enhances wound healing through the increase of hypoxia-induced paracrine factors via activation of TGF-β/SMAD2 and PI3K/AKT pathways.

60. Neuroprotective Effects of GDNF-expressing Human Amniotic Fluid Cells.
Jezierski A, Rennie K, Zurakowski B, Ribecco-Lutkiewicz M, Haukenfrers J, Ajji A, Gruslin A, Sikorska M, Bani-Yaghoub M.
Stem Cell Rev; 2014 Jan 11.

Brain injury continues to be one of the leading causes of disability worldwide. Despite decades of research, there is currently no pharmacologically effective treatment for preventing neuronal loss and repairing the brain. As a result, novel therapeutic approaches, such as cell-based therapies, are being actively pursued to repair tissue damage and restore neurological function after injury. In this study, we examined the neuroprotective potential of amniotic fluid (AF) single cell clones, engineered to secrete glial cell derived neurotrophic factor (AF-GDNF), both in vitro and in a surgically induced model of brain injury. Our results show that pre-treatment with GDNF significantly increases cell survival in cultures

of AF cells or cortical neurons exposed to hydrogen peroxide. Since improving the efficacy of cell transplantation depends on enhanced graft cell survival, we investigated whether AF-GDNF cells seeded on polyglycolic acid (PGA) scaffolds could enhance graft survival following implantation into the lesion cavity. Encouragingly, the AF-GDNF cells survived longer than control AF cells in serum-free conditions and continued to secrete GDNF both in vitro and following implantation into the injured motor cortex. AF-GDNF implantation in the acute period following injury was sufficient to activate the MAPK/ERK signaling pathway in host neural cells in the peri-lesion area, potentially boosting endogenous neuroprotective pathways. These results were complemented with promising trends in beam walk tasks in AF-GDNF/PGA animals during the 7 day timeframe. Further investigation is required to determine whether significant behavioural improvement can be achieved at a longer timeframe.

61. Curculigoside improves osteogenesis of human amniotic fluid-derived stem cells.

Liu M, Li Y, Yang ST.

Stem Cells Dev; 2014 Jan 15.

Curculigoside, a phenolic glycoside, is the main active compound of Curculigo orchioides (Amaryllidaceae, rhizome). C. orchioides is a traditional Chinese herbal medicine and has been commonly used to treat orthopedic disorders and bone healing in Asia. This study evaluated the effect of curculigoside on osteogenic differentiation of human amniotic fluid-derived stem cells (hAFSCs). The results showed that curculigoside stimulated alkaline phosphatase activity and calcium deposition of hAFSCs during osteogenic differentiation in a dose-dependent manner (1-100 μg/mL), while the effects were reduced at the higher concentration of 200 μg/mL. From reverse transcriptase-polymerase chain reaction analysis, the osteogenic genes osteopontin (OPN) and Collagen I were upregulated with

curculigoside treatment (1-100 μg/mL). Concurrently, the ratio of osteoprotegerin (OPG) to receptor activator of nuclear factor kappa-B ligand (RANKL) was increased, indicating the inhibition of osteoclastogenesis by curculigoside. Moreover, the role of Wnt/β-catenin signaling during curculigoside treatment was revealed by the upregulation of β-catenin and Cyclin D1. In summary, curculigoside improved osteogenesis and inhibited osteoclastogenesis of hAFSCs, suggesting its potential use to regulate hAFSC osteogenic differentiation for treating bone disorders.

62. Effect of mesenchymal stem cells and extracts derived from the placenta on trophoblast invasion and immune responses.

Choi JH, Jung J, Na KH, Cho KJ, Yoon TK, Kim GJ.

Stem Cells Dev; 2014 Jan 15.

Tightly regulated trophoblast invasion and immunomodulation at the feto-maternal interface is important during implantation and fetal development. Although trophoblasts as a pregnancy-specific cell has been reported to be a key factor capable of regulating certain events during implantation, however, its regulatory mechanisms are still unclear. In this study, we analyzed the effects of chorionic plate-derived mesenchymal stem cells (CP-MSCs) and human placenta extract (hPE) isolated from human normal placentas on trophoblasts invasion and immune responses. We investigated the effects of CP-MSCs, hPE treatment, and their combination on trophoblasts invasion and on T-cells

suppression through human leukocyte antigen-G (HLA-G) expression. Trophoblasts invasion was significantly increased by co-culture of CP-MSCs or by hPE treatment ($P<0.05$), and enhanced by the combination of CP-MSCs and hPE treatment ($P<0.05$). The proliferation of T-cells was decreased by co-culture of CP-MSCs and hPE treatment, whereas the population of regulatory T-cells was increased ($P<0.05$). Also, the dynamics alterations of multiple cytokines were observed in the culture supernatants of trophoblasts and T-cells depending on CP-MSCs co-culture and hPE treatment. Interestingly, the concentration of soluble HLA-G was increased by CP-MSCs co-culture, by hPE treatment and by combination of them on trophoblasts and activated T-cells ($P<0.05$). These findings suggested that CP-MSCs and hPE can regulate trophoblasts invasion and T-cell by alteration of HLA-G expression. These results will provide understandings of trophoblasts invasion and the immunological network at the feto-

maternal interface during pregnancy and contribute to the foundation of a new treatment strategy for pregnancy disorders.

63. Mesenchymal stem cell - based tissue engineering strategies for repair of articular cartilage.

Ahmed TA, Hincke MT.

Histol Histopathol; 2014 Jan 23.

Restoration of articular cartilage function and structure following pathological or traumatic damage is still considered a challenging problem in the orthopaedic field. Currently, tissue engineering-based reconstruction of articular cartilage is a feasible and continuously developing strategy to restore structure and function. Successful articular cartilage tissue engineering strategy relies largely on several essential components including cellular component, supporting 3D carrier scaffolding matrix, bioactive agents, proper physical stimulants, and safe gene delivery. Designing the right formulations from these components remain the main concern of the orthopaedic community. Utilization of mesenchymal stem cells (MSCs) for articular cartilage tissue

engineering is continuously increasing compared to use of chondrocytes. Various sources of MSCs have been investigated including adipose tissue, amniotic fluid, blood, bone marrow, dermis, embryonic stem cells, infrapatellar fat pad, muscle, periosteum, placenta, synovium, trabecular bone, and umbilical cord. MSCs derived from bone marrow and umbilical cord are currently in different phases of clinical trials. A wide range of matrices have been investigated to develop tissue engineering - based strategies including carbohydrate-based scaffolds (agarose, alginate, chitosan/chitin, and hyaluronate), protein-based scaffolds (collagen, fibrin, and gelatin), and artificial polymers (polyglycolic acid, polylactic acid, poly(lactic-co-glycolic acid), polyethylene glycol, and polycaprolactone). Collagen - based scaffolds and photopolymerizable PEG - based scaffolds are currently in different phases of clinical trials. TGF-β1, TGF-β3, BMP-2, and hypoxic environment are the recommended bioactive agents to induce

optimum chondrogenesis of MSCs, while TGF-β1, TGF-β3, SOX-9, BMP-2, and BMP-7 genes are the best candidate for gene delivery to MSCs. Electromagnetic field and the combination of shear forces/dynamic compression are the best maturation-promoting physical stimulants.

64. Neocartilage formation from mesenchymal stem cells grown in type II collagen-hyaluronan composite scaffolds.

Yeh HY, Lin TY, Lin CH, Yen BL, Tsai CL, Hsu SH.

Differentiation; 2014 Jan 23.

Three-dimensional (3D) collagen type II-hyaluronan (HA) composite scaffolds (CII-HA) which mimics the extracellular environment of natural cartilage were fabricated in this study. Rheological measurements demonstrated that the incorporation of HA increased the compression modulus of the scaffolds. An initial in vitro evaluation showed that scaffolds seeded with porcine chondrocytes formed cartilaginous-like tissue after 8 weeks, and HA functioned to promote the growth of chondrocytes into scaffolds. Placenta-derived multipotent cells (PDMC) and gingival fibroblasts (GF) were seeded on tissue culture polystyrene (TCPS), CII-HA films, and small intestinal submucosa (SIS) sheets for

comparing their chondrogenesis differentiation potentials with those of adipose-derived adult stem cells (ADAS) and bone marrow-derived mesenchymal stem cells (BMSC). Among different cells, PDMC showed the greatest chondrogenic differentiation potential on both CII-HA films and SIS sheets upon TGF-β3 induction, followed by GF. This was evidenced by the up-regulation of chondrogenic genes (Sox9, aggrecan, and collagen type II), which was not observed for cells grown on TCPS. This finding suggested the essential role of substrate materials in the chondrogenic differentiation of PDMC and GF. Neocartilage formation was more obvious in both PDMC and GF cells plated on CII-HA composite scaffolds vs. 8-layer SIS at 28 days in vitro. Finally, implantation of PDMC/CII-HA constructs into NOD-SCID mice confirmed the formation of tissue-engineered cartilage in vivo.

65. Cell fusion phenomena detected after in utero transplantation of Ds-red-harboring porcine amniotic fluid stem cells into EGFP transgenic mice.

Peng SY, Chen YS, Chou CJ, Wang YH, Lee HM, Cheng WT, Shaw SW, Wu SC.

Prenat Diagn; 2014 Jan 26.

OBJECTIVES:

Amniotic fluid stem cells (AFSCs) are derived from the amniotic fluid of the developing fetus and can give rise to diverse differentiated cells of ectoderm, mesoderm, and endoderm lineages. Intrauterine transplantation is an approach used to cure inherited genetic fetal defects during the gestation period of pregnant dams. Certain disease such as osteogenesis imperfecta was successfully treated in affected fetal mice using this method. However, the donor cell destiny remains uncertain.

METHODS:

The purpose of this study was to investigate the biodistribution and cell fate of Ds-red-

harboring porcine AFSCs (Ds-red pAFSCs) after intrauterine transplantation into enhanced green fluorescent protein-harboring fetuses of pregnant mice. Pregnant mice (12.5 days) underwent open laparotomy with intrauterine pAFSC transplantation (5*104 cells per pup) into fetal peritoneal cavity.

RESULTS:

Three weeks after birth, the mice were sacrificed. Several samples from different organs were obtained for histological examination and flow cytometric analysis. Ds-red pAFSCs migrated most frequently into the intestines. Furthermore, enhanced green fluorescent protein and red fluorescent protein signals were co-expressed in the intestine and liver cells via immunohistochemistry studies.

CONCLUSION:

In utero xenotransplantation of pAFSCs fused with recipient intestinal cells instead of differentiating or maintaining the undifferentiated status in the tissue.

66. Hypoxia enhances protective effect of placental-derived mesenchymal stem cells on damaged intestinal epithelial cells by promoting secretion of insulin-like growth factor-1.

Du L, Yu Y, Ma H, Lu X, Ma L, Jin Y, Zhang H.

Int J Mol Sci; 2014 Jan 27.

Apoptosis and necrosis of intestinal epithelial cells (IECs), induced by ischemia-reperfusion (I/R) injury, can lead to dysfunction of the intestinal barrier, which could cause multiple organ dysfunction syndromes. Mesenchymal stem cells (MSCs) have the potential of providing protective effects on damaged IECs via paracrine action. This study investigated whether hypoxia can enhance the protective effect of placental-derived MSCs (pMSCs) on H2O2-treated-caco2 cells, and explored the possible mechanism. The pMSCs isolated by tissue culture were fibroblast-like, positive for CD73, CD90 and CD105 and can differentiate into chondrocytes and

endothelial cells. Five days after treatment with H2O2, the numbers of living caco2 cells significantly decreased. More live H2O2-treated-caco2 cells were observed in pMSCs hypoxia culture medium (pMSCs-HCM) than pMSCs normoxia culture medium (pMSCs-NCM), and the application of a specific antibody that blocked insulin-like growth factor-1 (IGF-1) leads to a significant decrease of the protective effect of pMSCs-HCM. Hypoxia can promote IGF-1 expression of pMSCs at mRNA and protein levels, and caco2 stably expressed IGF-1 receptor. Knocking down IGF-1 expression in pMSCs by siRNA resulted in a significant attenuation of the increase in apoptosis of H2O2-treated-caco2 cultured in pMSCs-HCM. In conclusion, hypoxia can increase the protective effect of pMSCs on H2O2-treated-caco2 cells via a promotion of their paracrine actions, and the key cytokine involved is IGF-1.

67. Proteomic profiling of human placenta-derived mesenchymal stem cells upon transforming LIM mineralization protein-1 stimulation.

Zhu Z, Liu Z, Liu J, Bi M, Yang T, Wang J.
Cytotechnology; 2014 Jan 28.

Human placenta-derived mesenchymal stem cells (hPDMSCs) can differentiate into different types of cells and thus have tremendous potential for cell therapy and tissue engineering. LIM mineralization protein-1 (LMP-1) plays an important role in osteoblast differentiation, maturation and bone formation. To determine a global effect of LMP-1 on hPDMSCs, we designed a study using a proteomic approach combined with adenovirus-mediated gene transfer of LMP-1 to identify LMP-1-induced changes in hPDMSCs on proteome level. We have generated proteome maps of undifferentiated hPDMSCs and LMP-1 induced hPDMSCs. Two dimensional gel electrophoresis revealed 22 spots with at least 2.0-fold changes in

expression and 15 differently expressed proteins were successfully identified by MALDI-TOF-MS. The proteins regulated by LMP-1 included cytoskeletal proteins, cadmium-binding proteins, and metabolic proteins, etc. The expression of some identified proteins was confirmed by further Western blot analyses. Our results will play an important role in better elucidating the underlying molecular mechanism in LMP-1 included hPDMSCs differentiation into osteoblasts.

68. Third trimester NG2-positive amniotic fluid cells are effective in improving repair in spinal cord injury.

Bottai D, Scesa G, Cigognini D, Adami R, Nicora E, Abrignani S, Di Giulio AM, Gorio A.

Exp Neurol; 2014 Jan 29.

Spinal cord injury presents a significant therapeutic challenge since the treatments available are mostly vain. The use of stem cells to treat this condition represents a promising new therapeutic strategy; therefore, a variety of stem cell treatments have been recently examined in animal models of CNS trauma. In this work, we analyzed the effects of third trimester amniotic fluid cells in a mouse model of spinal cord injury. Among the different cultures used for transplantation, some were able to induce a significant improvement in motor recovery (cultures #3.5, #3.6 and #7.30), evaluated by means of open field free locomotion. All effective cell cultures expressed the surface marker

nerve/glial antigen 2, ortholog of the human chondroitin sulfate proteoglycan 4, which is present on several types of immature progenitor cells. The improved motor functional recovery was correlated with higher myelin preservation in the ventral horn white matter and an increased vascularization in the peri-lesion area. Real-Time PCR analysis showed higher expression levels of vascular endothelial growth factor and hypoxia-inducible factor-1α mRNA two days after cells transplantation compared to PBS-treated animals, indicating that an angiogenic pathway might have been activated by these cells, possibly through the production of hepatocyte growth factor. This cytokine appears to be produced mostly in filtering organs, such as the lung, of the transplanted animals and is likely released in the blood suggesting an endocrine role of hepatocyte growth factor in targeting the injury site.

69. Human second-trimester amniotic fluid cells are able to create embryoid body-like structures *in vitro* and to show typical expression profiles of embryonic and primordial germ cells.

Antonucci I, Pietro RD, Alfonsi M, Centurione MA, Centurione L, Sancilio S, Pelagatti F, D Amico MA, Baldassarre AD, Piattelli A, Tetè S, Palka G, Borlongan CV, Stuppia L.

Cell Transplant; 2014 Jan 29.

Human Amniotic Fluid-derived Stem Cells (AFSCs) represent a novel class of broadly multipotent stem cellssharing characteristics of both embryonic and adult stem cells. However, both the origin of these cells and their actual properties interms of pluripotent differentiation potential are still debated. In order to verify the presence of features of pluripotency in human second-trimester AFSCs, wehave investigated the ability of these cells to form in vitrothree-dimensional aggregates, known as embryoid bodies (EBs),

and to express specific genes of embryonic stem cells (ESCs) and primordial germ cells (PGCs). EBs were obtained after 5 daysof AFSCs culture in suspension, and showed positivity for alkaline phosphatase (AP) staining and for specific markers of pluripotency (OCT4 and SOX2). Moreover, EBs derived cells showed the expression of specific transcripts of the three germ layers. RT-PCR analysis, carried out atdifferent culture times (2nd, 3th, 4th, 5th and 8th passage), evidenced the presence of specific markers of ESCs (such as FGF4 and DAPPA4), as well as of markers typical of PGCs, and in particular genes involved in early stages of germ cell development (Fragilis, Stella, Vasa, c-Kit, Rnf17). Finally, the expression of genes related to the control of DNA methylation (DNMT3A, DNMT3b1, DNMT1, DNMT3L, MBD1, MBD2, MBD3, MDB4, MeCP2) as well as the lack of inactivation of the X-chromosome in female samples was also evidenced. Taken together, these data provide further evidence for the

presence of common features among human AFSCs, PGC and ESC cells.

70. Can we fix it? Evaluating the potential of placental stem cells for the treatment of pregnancy disorders.

James JL, Srinivasan S, Alexander M, Chamley LW.

Placenta; 2014 Feb.

In pregnancy disorders such as pre-eclampsia, intrauterine growth restriction (IUGR) and recurrent miscarriage a poorly functioning placenta is thought to be a major component of the disease process. However, despite their prevalence, we currently have no way to fix dysfunctional placentae or directly treat these disorders. Over the past two decades our understanding of the role that stem cells play in organ development and regeneration has expanded rapidly, and over the past 5 years the therapeutic use of stem cells to both regenerate damaged tissues, and act as potent modulators of diseased microenvironments, has become a reality in many organs including the heart, kidney, liver, skin and eye. Over its short lifespan the placenta

undergoes rapid and continuous growth and differentiation, meaning that placental 'organogenesis' only truly ends at delivery, and thus stem cells are likely to play important roles in placental function for the duration of pregnancy. Two populations of stem cells exist in the placenta that contribute to this on-going growth and differentiation: trophoblast stem cells and mesenchymal stem cells. This review will address our current understanding of how each of these stem cell populations contributes to successful placental function, how epithelial and mesenchymal stem cell populations are being translated to the clinic in other fields, and whether these advances can teach us anything about how placental stem cells could be used to fix faulty placentae in the future.

71. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism.

Zani A, Cananzi M, Fascetti-Leon F, Lauriti G, Smith VV, Bollini S, Ghionzoli M, D'Arrigo A, Pozzobon M, Piccoli M, Hicks A, Wells J, Siow B, Sebire NJ, Bishop C, Leon A, Atala A, Lythgoe MF, Pierro A, Eaton S, De Coppi P.

Gut; 2014 Feb.

OBJECTIVE:

Necrotising enterocolitis (NEC) remains one of the primary causes of morbidity and mortality in neonates and alternative strategies are needed. Stem cells have become a therapeutic option for other intestinal diseases, which share some features with NEC. We tested the hypothesis that amniotic fluid stem (AFS) cells exerted a beneficial effect in a neonatal rat model of NEC.

DESIGN:

Rats intraperitoneally injected with AFS cells and their controls (bone marrow mesenchymal stem cells, myoblast) were analysed for survival, behaviour, bowel imaging (MRI scan), histology, bowel absorption and motility, immunofluorescence for AFS cell detection, degree of gut inflammation (myeloperoxidase and malondialdehyde), and enterocyte apoptosis and proliferation.

RESULTS:

AFS cells integrated in the bowel wall and improved rat survival and clinical conditions, decreased NEC incidence and macroscopic gut damage, improved intestinal function, decreased bowel inflammation, increased enterocyte proliferation and reduced apoptosis. The beneficial effect was achieved via modulation of stromal cells expressing cyclooxygenase 2 in the lamina propria, as shown by survival studies using selective and non-selective cyclooxygenase 2 inhibitors. Interestingly, AFS cells differentially

expressed genes of the Wnt/β-catenin pathway, which regulate intestinal epithelial stem cell function and cell migration and growth factors known to maintain gut epithelial integrity and reduce mucosal injury.

CONCLUSIONS:

We demonstrated here for the first time that AFS cells injected in an established model of NEC improve survival, clinical status, gut structure and function. Understanding the mechanism of this effect may help us to develop new cellular or pharmacological therapies for infants with NEC.

72. Potential of human fetal chorionic stem cells for the treatment of osteogenesis imperfecta.

Jones GN, Moschidou D, Abdulrazzak H, Kalirai BS, Vanleene M, Osatis S, Shefelbine SJ, Horwood NJ, Marenzana M, De Coppi P, Bassett JH, Williams GR, Fisk NM, Guillot PV.

Stem Cells Dev; 2014 Feb 1.

Osteogenesis imperfecta (OI) is a genetic bone pathology with prenatal onset, characterized by brittle bones in response to abnormal collagen composition. There is presently no cure for OI. We previously showed that human first trimester fetal blood mesenchymal stem cells (MSCs) transplanted into a murine OI model (oim mice) improved the phenotype. However, the clinical use of fetal MSC is constrained by their limited number and low availability. In contrast, human fetal early chorionic stem cells (e-CSC) can be used without ethical restrictions and isolated in high numbers from the

placenta during ongoing pregnancy. Here, we show that intraperitoneal injection of e-CSC in oim neonates reduced fractures, increased bone ductility and bone volume (BV), increased the numbers of hypertrophic chondrocytes, and upregulated endogenous genes involved in endochondral and intramembranous ossification. Exogenous cells preferentially homed to long bone epiphyses, expressed osteoblast genes, and produced collagen COL1A2. Together, our data suggest that exogenous cells decrease bone brittleness and BV by directly differentiating to osteoblasts and indirectly stimulating host chondrogenesis and osteogenesis. In conclusion, the placenta is a practical source of stem cells for the treatment of OI.

73. The Potential of Mesenchymal Stem Cells Derived from Amniotic Membrane and Amniotic Fluid for Neuronal Regenerative Therapy.

Kim EY, Lee KB, Kim MK.

BMB Rep; 2014 Feb 6.

The mesenchymal stem (MSCs) derived from mesoderm, considered readily available source for tissue engineering. They have multipotent differentiation capacity and can be differentiated into various cell types. Many studies demonstrated that the MSCs identified from amniotic membrane (AM-MSCs) and amniotic fluid (AF-MSCs) are possible non-invasive isolation, has multipotency, self-renewal, low-immunogenicity, anti-inflammation, non-tumorigenicity and little ethical problem. The AF-MSCs and AM-MSCs may be appropriate sources of mesenchymal stem cells for regenerative medicine alternative to embryonic stem cells (ESCs). Recently, regenerative treatments such as tissue engineering and cell

transplantation have potential in clinical application for degenerative diseases. Therefore, amnion and mesenchymal stem cells derived from it can be applied to cell therapy in neuro-degeneration diseases. In this review, we will describe potential of the AM-MSCs and AF-MSCs focus on cure of neuronal degenerative diseases.

74. Platelet-Rich Plasma (PRP) Promotes Fetal Mesenchymal Stem/Stromal Cell Migration and Wound Healing Process.

Roubelakis MG, Trohatou O, Roubelakis A, Mili E, Kalaitzopoulos I, Papazoglou G, Pappa KI, Anagnou NP.

Stem Cell Rev; 2014 Feb 6.

Numerous studies have shown the presence of high levels of growth factors during the process of healing. Growth factors act by binding to the cell surface receptors and contribute to the subsequent activation of signal transduction mechanisms. Wound healing requires a complex of biological and molecular events that includes attraction and proliferation of different type of cells to the wound site, differentiation and angiogenesis. More specifically, migration of various cell types, such as endothelial cells and their precursors, mesenchymal stem/stromal cells (MSCs) or skin fibroblasts (DFs) plays an important role in the healing process. In recent years, the application of platelet rich

plasma (PRP) to surgical wounds and skin ulcerations is becoming more frequent, as it is believed to accelerate the healing process. The local enrichment of growth factors at the wound after PRP application causes a stimulation of tissue regeneration. Herein, we studied: (i) the effect of autologous PRP in skin ulcers of patients of different aetiology, (ii) the proteomic profile of PRP, (iii) the migration potential of amniotic fluid MSCs and DFs in the presence of PRP extract in vitro, (iv) the use of the PRP extract as a substitute for serum in cultivating AF-MSCs. Considering its easy access, PRP may provide a valuable tool in multiple therapeutic approaches.

75. Low Oxygen Tension and Insulin-like Growth Factor-I (IGF-I) Promote Proliferation and Multipotency of Placental Mesenchymal Stem Cells (PMSCs) from Different Gestations via Distinct Signaling Pathways.

Youssef A, Iosef C, Han VK.

Endocrinology; 2014 Feb 7.

The microenvironment of placental mesenchymal stem cells (PMSCs) is dynamic throughout gestation and determines changes in cell fate. In vivo, PMSCs initially develop in low oxygen tension and low IGF-I concentrations, and both increase gradually with gestation. The impact of varying concentrations of IGF-I and changing oxygen tension on PMSC signaling and multipotency was investigated in PMSCs from early (Preterm) and late (Term) gestation human placentae. Preterm PMSCs had greater proliferative response to IGF-I, which was further enhanced by low oxygen tension. Low oxygen tension alone was sufficient to induce

ERK1/2 phosphorylation, whereas IGF-I was required for AKT phosphorylation. Low oxygen tension prolonged ERK1/2 and AKT phosphorylation with a slowed phosphorylation decay even in presence of IGF-I. Low oxygen tension maintained higher levels of IGF-IR and IRS-1 that were otherwise decreased by exposure to IGF-I, and induced a differential phosphorylation pattern on IGF-IRβ and IRS-1. Phosphorylation of ERK1/2 and AKT were different between the Preterm and Term PMSCs, and p-AKT, and not p-ERK1/2 was the major determinant of PMSC proliferation and OCT4 levels. These studies demonstrate that low oxygen tension regulates the fate of PMSCs from early and late gestations in response to IGF-I, both independently and dependently, via specific signal transduction mechanisms.

76. Enhanced *ex vivo* expansion of adult mesenchymal stem cells by fetal mesenchymal stem cell ECM.

Ng CP, Mohamed Sharif AR, Heath DE, Chow JW, Zhang CB, Chan-Park MB, Hammond PT, Chan JK, Griffith LG.

Biomaterials; 2014 Feb 20.

Large-scale expansion of highly functional adult human mesenchymal stem cells (aMSCs) remains technologically challenging as aMSCs lose self renewal capacity and multipotency during traditional long-term culture and their quality/quantity declines with donor age and disease. Identification of culture conditions enabling prolonged expansion and rejuvenation would have dramatic impact in regenerative medicine. aMSC-derived decellularized extracellular matrix (ECM) has been shown to provide such microenvironment which promotes MSC self renewal and "stemness". Since previous studies have demonstrated superior proliferation and osteogenic potential of

human fetal MSCs (fMSCs), we hypothesize that their ECM may promote expansion of clinically relevant aMSCs. We demonstrated that aMSCs were more proliferative (~1.6×) on fMSC-derived ECM than aMSC-derived ECMs and traditional tissue culture wares (TCPS). These aMSCs were smaller and more uniform in size (median ± interquartile range: 15.5 ± 4.1 μm versus 17.2 ± 5.0 μm and 15.5 ± 4.1 μm for aMSC ECM and TCPS respectively), exhibited the necessary biomarker signatures, and stained positive for osteogenic, adipogenic and chondrogenic expressions; indications that they maintained multipotency during culture. Furthermore, fMSC ECM improved the proliferation (~2.2×), size (19.6 ± 11.9 μm vs 30.2 ± 14.5 μm) and differentiation potential in late-passaged aMSCs compared to TCPS. In conclusion, we have established fMSC ECM as a promising cell culture platform for ex vivo expansion of aMSCs.

77. Stem cells from fetal membranes and amniotic fluid: markers for cell isolation and therapy.

Pozzobon M, Piccoli M, De Coppi P.

Cell Tissue Bank; 2014 Feb 20.

Stem cell therapy is in constant need of new cell sources to conceive regenerative medicine approaches for diseases that are still without therapy. Scientists drew the attention toward amniotic membrane and amniotic fluid stem cells, since these sources possess many advantages: first of all as cells can be extracted from discarded foetal material it is inexpensive, secondly abundant stem cells can be obtained and finally, these stem cell sources are free from ethical considerations. Many studies have demonstrated the differentiation potential in vitro and in vivo toward mesenchymal and non-mesenchymal cell types; in addition the immune-modulatory properties make these cells a good candidate for allo- and xenotransplantation. This review offers an

overview on markers characterisation and on the latest findings in pre-clinical or clinical setting of the stem cell populations isolated from these sources.

78. The subtype CD200-positive, chorionic mesenchymal stem cells from the placenta promote regeneration of human hepatocytes.

Wang J, Zhu Z, Huang Y, Wang P, Luo Y, Gao Y, Du Z.

Biotechnol Lett; 2014 Feb 23.

Human placental mesenchymal stem cells (hPMSCs), for the treatment of fulminant hepatic failure, have been widely studied. Only a few studies have investigated the effect of the subtype CD200+hPMSCs on regeneration of human hepatocytes. CD200+hPMSCs can down-regulate activity of several immunocytes and suppress TNF-α secretion from macrophages via the CD200-CD200R axis. We have investigated the influence of CD200-positive human placenta chorionic mesenchymal stem cells (CD200+hPCMSCs) on metabolism, proliferation and apoptosis of human hepatocytes in vitro. CD200+hPCMSCs promote urea synthesis, albumin secretion

and hepatocytes proliferation at co-culture ratios of 1:1 and 3:1. Additionally, CD200+hPCMSCs inhibit hepatocyte apoptosis via up-regulation of an anti-apoptotic protein, Bcl-xL. Thus, CD200+hPCMSCs can provide supportive benefit for the regeneration of human hepatocytes and also have immunosuppressive properties. Therefore, CD200+hPCMSCs may be an ideal candidate for stem cell-based therapy in hepatic failure.

79. Human Amniotic Fluid Stem Cells Possess the Potential to Differentiate into Primordial Follicle Oocytes *In Vitro*.

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

Biol Reprod; 2014 Feb 26.

Previous reports demonstrated that embryonic stem (ES) 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 follicle fluid (pFF) 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 re-plated in oocyte culture medium for an additional 7 to 10 days. OLCs ranging from 50 to 120 μm presenting zona pellucida were observed in cumulus oocyte complexes (COCs); 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.

80. Multilineage potential research of bovine amniotic fluid mesenchymal stem cells.

Gao Y, Zhu Z, Zhao Y, Hua J, Ma Y, Guan W. Int J Mol Sci; 2014 Feb 28.

The use of amnion and amniotic fluid (AF) are abundant sources of mesenchymal stem cells (MSCs) that can be harvested at low cost and do not pose ethical conflicts. In human and veterinary research, stem cells derived from these tissues are promising candidates for disease treatment, specifically for their plasticity, their reduced immunogenicity, and high anti-inflammatory potential. This work aimed to obtain and characterize bovine amniotic fluid mesenchymal stem cells (AFMSC). The bovine AF from the amniotic cavity of pregnant gilts in the early stages of gestation (3- and 4-m-old bovine embryos) was collected. AFMSCs exhibit a fibroblastic-like morphology only starting from the fourth passage, being heterogeneous during the primary culture.

Immunofluorescence results showed that AFMSCs were positive for β -integrin, CD44, CD73 and CD166, but negative for CD34, CD45. Meanwhile, AFMSCs expressed ES cell markers, such as Oct4, and when appropriately induced, are capable of differentiating into ectodermal and mesodermal lineages. This study reinforces the emerging importance of these cells as ideal tools in veterinary medicine; future studies aimed at a deeper evaluation of their immunological properties will allow a better understanding of their role in cellular therapy.

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