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AMNIOTIC STEM CELLS

SCIENTIFIC PAPER REVIEW

MAY 2012

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Introduction

This issue represents a collection of the most important articles related to amniotic fluid stem cells, mesenchymal stem cells and clinical trials. The review includes articles published in 2012 and 2011. The summary reports titles, and finally, for each article are listed scientific paper, publication date, authors and abstract.

Introduzione

In questo fascicolo sono stati raccolti gli articoli più significativi relativi alle cellule staminali da liquido amniotico. La rassegna copre i primi mesi del 2012 e l'anno 2011; l'indice generale riporta i titoli delle ricerche. Nel dettaglio vengono riportati la rivista scientifica sulla quale è stato pubblicato, la data di pubblicazione, gli autori e l'abstract.

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1. In vitro and in vivo cardiomyogenic differentiation of amniotic fluid stem cells

Sveva Bollini, Michela Pozzobon, Muriel Nobles, Johannes Riegler, Xuebin Dong, Martina Piccoli, Angela Chiavegato, Anthony N. Price, Marco Ghionzoli and King K. Cheung, et al

Stem Cell Reviews and Report, 2011 - Springer

Abstract

Cell therapy has developed as a complementary treatment for myocardial regeneration. While both autologous and allogeneic uses have been advocated, the ideal candidate has not been identified yet. Amniotic fluid-derived stem (AFS) cells are potentially a promising resource for cell therapy and tissue engineering of myocardial injuries. However, no information is available regarding their use in an allogeneic context. c-kit-sorted, GFP-positive rat AFS (GFP-rAFS) cells and neonatal rat cardiomyocytes (rCMs) were characterized by cyto centrifugation and flow cytometry for the expression of mesenchymal,

embryonic and cell lineage-specific antigens. The activation of the myocardial gene program in GFP-rAFS cells was induced by co-culture with rCMs. The stem cell differentiation was evaluated using immunofluorescence, RT-PCR and single cell electrophysiology. The *in vivo* potential of Endorem-labeled GFP-rAFS cells for myocardial repair was studied by transplantation in the heart of animals with ischemia/reperfusion injury (I/R), monitored by magnetic resonance imaging (MRI). Three weeks after injection a small number of GFP-rAFS cells acquired an endothelial or smooth muscle phenotype and to a lesser extent CMs. Despite the low GFP-rAFS cells count in the heart, there was still an improvement of ejection fraction as measured by MRI. rAFS cells have the *in vitro* propensity to acquire a cardiomyogenic phenotype and to preserve cardiac function, even if their potential may be limited by poor survival in an allogeneic setting.

2. Amniotic fluid as a rich source of mesenchymal stromal cells for transplantation therapy

Antonucci, Ivana; Stuppia, Liborio; Kaneko, Yuji; Yu, Seongjin; Tajiri, Naoki; Bae, Eunkyung C.; Chheda, Sonia H.; Weinbren, Nathan L.; Borlongan, Cesar V.

Cell Transplantation, Volume 20, Number 6, 2011

Abstract

Stem cells isolated from amniotic fluid are known to be able to differentiate into different cells types, thus being considered as a powerful tool for cellular therapy of different human diseases. In the last 4 years, amniotic fluid-derived stem (AFS) cells have been shown to express embryonic and adult stem cell markers. These cells can be considered an intermediate stage between embryonic stem cells and adult stem cells. AFS cells can give rise to adipogenic, osteogenic, myogenic,

endothelial, neurogenic, and hepatic lineages, inclusive of all embryonic germ layers. AFS cells have a high renewal capacity and can be expanded for over 250 doublings without any detectable loss of chromosomal telomere length. Taken together, all these data provide evidence that amniotic fluid represents a new and very promising source of stem cells for research, as well as clinical applications. Certainly stem cells from amniotic fluid will be useful both for a customized cell supply for newly born children and for banking cells to be used for therapeutic cell transplantation in immunologically matched recipients. Further investigations are also warranted to fully explore the amniotic cells' potential for adult human disorders.

3. Amniotic fluid stem cells are cardioprotective following acute myocardial infarction

S Bollini, KK Cheung, J Riegler...

Stem cells and ..., 2011

Abstract

In recent years, various types of stem cells have been characterized and their potential for cardiac regeneration has been investigated. We have previously described the isolation of broadly multipotent cells from amniotic fluid, defined as amniotic fluid stem (AFS) cells. The aim of this study was to investigate the therapeutic potential of human AFS cells (hAFS) in a model of acute myocardial infarction. Wistar rats underwent 30 min of ischemia by ligation of the left anterior descending coronary artery, followed by administration of hAFS cells and 2 h of reperfusion. Infarct size was assessed by 2,3,5-triphenyltetrazolium chloride staining and planimetry. hAFS cells were also analyzed by enzyme-linked immunosorbent assay to detect secretion of putative paracrine factors, such as the

actin monomer-binding protein thymosin β 4 (T β 4). The systemic injection of hAFS cells and their conditioned medium (hAFS-CM) was cardioprotective, improving myocardial cell survival and decreasing the infarct size from 53.9% \pm 2.3% (control animals receiving phosphate-buffered saline injection) to 40.0% \pm 3.0% (hAFS cells) and 39.7% \pm 2.5% (hAFS-CM, $P < 0.01$). In addition, hAFS cells were demonstrated to secrete T β 4, previously shown to be both cardioprotective and proangiogenic. Our results suggest that AFS cells have therapeutic potential in the setting of acute myocardial infarction, which may be mediated through paracrine effectors such as T β 4. Therefore, AFS cells might represent a novel source for cell therapy and cell transplantation strategies in repair following ischemic heart disease, with a possible paracrine mechanism of action and a potential molecular candidate for acute cardioprotection.

4. Neurogenic potential of mesenchymal-like stem cells from human amniotic fluid: the influence of extracellular growth factors.

Sveva Bollini, King K. Cheung, Johannes Riegler, Xuebin Dong, Nicola Smart, Marco Ghionzoli, S.P. Loukogeorgakis, Panagiotis Maghsoudlou, Karina N. Dubé, Paul R. Riley, Mark F. Lythgoe, and Paolo De Coppi.

Stem Cells and Development. November 2011

Abstract

Amniotic fluids contain human stem cells, among which mesenchymal stem cells could be isolated. These cells have multipotent differentiation ability and no tumorigenic potential after transplantation in mice. These features make them good candidates for in vitro studies and for therapeutic purposes. The aim of this study was to isolate mesenchymal stem cell-like cultures from different amniotic fluids in order to study in vitro their neurogenic potential and assess if this

process could be reproducible and standardized. We focused attention on the possible differential effects of soluble growth factors. Immunophenotypical and molecular characterization showed that the 31 amniotic fluid-derived cultures expressed mesenchymal markers as well as some stemness properties. These cells also appeared to be responsive to purines or acetylcholine showing an intracellular calcium increase, also reported for mesenchymal stem cells derived from other sources. Interestingly, in the presence of retinoic acid, these cells assumed a neuronal-like morphology. In addition, functional and molecular analyses revealed that retinoic acid-treated cells showed immature electric functional properties, the expression of neuronal markers and stemness genes. In conclusion, even if further investigations are required, the results presented here contribute to support the finding that amniotic fluid contains cells able to differentiate in vitro towards neural-like lineage in the presence of retinoic acid. The ability of retinoic acid to induce a possible neuronal progenitor culture makes the model useful to study a possible in vivo transplantation of these cells and to contribute to define the protocols for cell therapy.

5. *In vitro* cardiomyogenic potential of human amniotic fluid stem cells

Xuan Guan, Dawn M. Delo, Anthony Atala, Shay Soker

Journal of Tissue Engineering and Regenerative Medicine - Volume 5, Issue 3, pages 220–228, March 2011

Abstract

Stem cell therapy for damaged cardiac tissue is currently limited by a number of factors, including inability to obtain sufficient cell numbers, the potential tumorigenicity of certain types of stem cells and the possible link between stem cell therapy and the development of malignant arrhythmias. In this study, we investigated whether human amniotic fluid-derived stem (hAFS) cells could be a potential source of cells for cardiac cell therapy, by testing the *in vitro* differentiation capabilities. Undifferentiated hAFS cells express several cardiac genes, including the transcription factor *mef2*, the gap junction *connexin43*, and *H-* and *N-cadherin*. A 24 h incubation with 5-aza-2'-deoxycytidine (5-AZA-

dC) induced hAFS cell differentiation along the cardiac lineage. Evidence for this differentiation included morphological changes, upregulation of cardiac-specific genes (*cardiac troponin I* and *cardiac troponin T*) and redistribution of *connexin43*, as well as downregulation of the stem cell marker SRY-box 2 (*sox2*). When co-cultured with neonatal rat cardiomyocytes (NRCs), hAFS cells formed both mechanical and electrical connections with the NRCs. Dye transfer experiments showed that calcein dye could be transferred from NRCs to hAFS cells through cellular connections. The gap junction *connexin43* likely involved in the communication between the two cell types, because 12-*O*-tetradecanoylphorbol 13-acetate (TPA) could partially block cellular crosstalk. We conclude that hAFS cells can be differentiated into a cardiomyocyte-like phenotype and can establish functional communication with NRCs. Thus, hAFS cells may potentially be used for cardiac cell therapy.

6. Endometrial stem cell transplantation restores dopamine production in a Parkinson's disease model

Erin F. Wolff, Xiao-Bing Gao, Katherine V. Yao, Zane B. Andrews, Hongling Du, John D. Elsworth, Hugh S. Taylor

Journal of Cellular and Molecular Medicine -
Volume 15, Issue 4, pages 747–755, April
2011

Abstract

Parkinson's disease (PD) is a neurodegenerative disorder caused by the loss of dopaminergic neurons. Adult human endometrial derived stem cells (HEDSC), a readily obtainable type of mesenchymal stem-like cell, were used to generate dopaminergic cells and for transplantation. Cells expressing CD90, platelet derived growth factor (PDGF)-R β and CD146 but not CD45 or CD31 were differentiated *in vitro* into dopaminergic neurons that exhibited axon

projections, pyramidal cell bodies and dendritic projections that recapitulate synapse formation; these cells also expressed the neural marker nestin and tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. Whole cell patch clamp recording identified G-protein coupled inwardly rectifying potassium current 2 channels characteristic of central neurons. A 1-methyl 4-phenyl 1,2,3,6-tetrahydro pyridine induced animal model of PD was used to demonstrate the ability of labelled HEDSC to engraft, migrate to the site of lesion, differentiate *in vivo* and significantly increase striatal dopamine and dopamine metabolite concentrations. HEDSC are a highly inducible source of allogenic stem cells that rescue dopamine concentrations in an immunocompetent PD mouse model.

7. Neurogenic differentiation of amniotic fluid stem cells

M. Rosner, M. Mikula, A. Preitschopf, M. Feichtinger, K. Schipany and M. Hengstschläger

Amino Acids, 2011, Springer

Abstract

In 2003, human amniotic fluid has been shown to contain stem cells expressing Oct-4, a marker for pluripotency. This finding initiated a rapidly growing and very promising new stem cell research field. Since then, amniotic fluid stem (AFS) cells have been demonstrated to harbour the potential to differentiate into any of the three germ layers and to form three-dimensional aggregates, so-called embryoid bodies, known as the principal step in the differentiation of pluripotent stem cells. Marker selection and minimal dilution approaches allow the establishment of monoclonal AFS cell lineages with high proliferation potential.

AFS cells have a lower risk for tumour development and do not raise the ethical issues of embryonic stem cells. Compared to induced pluripotent stem cells, AFS cells do not need exogenic treatment to induce pluripotency, are chromosomal stable and do not harbour the epigenetic memory and accumulated somatic mutations of specific differentiated source cells. Compared to adult stem cells, AFS can be grown in larger quantities and show higher differentiation potential. Accordingly, in the recent past, AFS became increasingly accepted as an optimal tool for basic research and probably also for specific cell-based therapies. Here, we review the current knowledge on the neurogenic differentiation potential of AFS cells.

8. Proliferation potential of human amniotic fluid stem cells differently responds to mercury and lead exposure

C. Gundacker, M. Scheinast, L. Damjanovic, C. Fuchs, M. Rosner and M. Hengstschläger
Amino Acids, November 2011

Abstract

There are considerable gaps in our knowledge on cell biological effects induced by the heavy metals mercury (Hg) and lead (Pb). In the present study we aimed to explore the effects of these toxicants on proliferation and cell size of primary human amniotic fluid stem (AFS) cells. Monoclonal human AFS cells were incubated with three dosages of Hg and Pb (single and combined treatment; ranging from physiological to cytotoxic concentrations) and the intracellular Hg and Pb concentrations were analyzed, respectively. At different days of incubation the effects of Hg and Pb on proliferation, cell

size, apoptosis, and expression of cyclins and the cyclin-dependent kinase inhibitor p27 were investigated. Whereas we found Hg to trigger pronounced effects on proliferation of human AFS cells already at low concentrations, anti-proliferative effects of Pb could only be detected at high concentrations. Exposure to high dose of Hg induced pronounced downregulation of cyclin A confirming the anti-proliferative effects observed for Hg. Co-exposure to Hg and Pb did not cause additive effects on proliferation and size of AFS cells, and on cyclin A expression. Our here presented data provide evidence that the different toxicological effects of Pb and Hg on primary human stem cells are due to different intracellular accumulation levels of these two toxicants. These findings allow new insights into the functional consequences of Pb and Hg for mammalian stem cells and into the cell biological behavior of AFS cells in response to toxicants.

9. Amniotic-Fluid Stem Cells: Growth Dynamics and Differentiation Potential after a CD-117-Based Selection Procedure

S. Arnhold, S. Glüer, K. Hartmann, O. Raabe, K. Addicks, S. Wenisch, and M. Hoopmann

Stem cells International, 2011

Abstract

Amniotic fluid (AF) has become an interesting source of fetal stem cells. However, AF contains heterogeneous and multiple, partially differentiated cell types. After isolation from the amniotic fluid, cells were characterized regarding their morphology and growth dynamics. They were sorted by magnetic associated cell sorting using the surface marker CD 117. In order to show stem cell characteristics such as pluripotency and to evaluate a possible therapeutic application of these cells, AF fluid-derived stem cells were differentiated

along the adipogenic, osteogenic, and chondrogenic as well as the neuronal lineage under hypoxic conditions. Our findings reveal that magnetic associated cell sorting (MACS) does not markedly influence growth characteristics as demonstrated by the generation doubling time. There was, however, an effect regarding an altered adipogenic, osteogenic, and chondrogenic differentiation capacity in the selected cell fraction. In contrast, in the unselected cell population neuronal differentiation is enhanced.

10. Calcification after myocardial infarction is independent of amniotic fluid stem cell injection

Dawn M. Delo, Xuan Guan, Zhan Wang, Leanne Groban, Michael Callahan, Tom Smith, David C. Sane, R. Mark Payne, Anthony Atala, Shay Soker

Cardiovascular Pathology, Volume 20, 2011

Abstract

Ischemic heart disease remains one of the most common causes of mortality in developed countries. Recently, stem cell therapy is being considered for treating ischemic heart diseases. On the other hand, there has been evidence of chondro-osteogenic mass formation after stem cell injection in the heart. In a recent publication, Chiavegato et al. (J Mol Cell Cardiol. 42 (2007) 746–759) has suggested that amniotic fluid-derived stem (AFS) cells cause chondro-osteogenic masses in the infarcted heart. The goal of the current study was to further examine the formation of such masses, specifically, the role of AFS cells in this process. Our results confirm the

presence of similar bone-like masses in the left ventricular wall of infarcted rats; however, this phenomenon occurred independent of AFS cell injection into the myocardium. Moreover, AFS cell injection did not increase the presence of chondro-osteogenic masses. Echocardiographic analysis of large infarctions in rats frequently revealed the presence of echogenic structures in the left ventricular wall. We further demonstrated a significant relationship between the infarction size and chondro-osteogenic formation and subsequent decrease in cardiac function. Collectively, our study indicates that chondro-osteogenic differentiation can take place in infarcted rat heart independent of cell injection. These results have significant implications for future design and testing of stem cell therapy for treatment of cardiac muscle diseases

11. Cell sourcing for bone tissue engineering: Amniotic fluid stemcells have a delayed, robust differentiation compared to mesenchymal stemcells

Alexandra Peister, Maria A. Woodruff, Jarod J. Prince, Derwin P. Gray, Dietmar W. Hutmacher, Robert E. Guldberg

Stem Cell Research, Volume 7, Issue 1, July 2011

Abstract

Cell based therapies for bone regeneration are an exciting emerging technology, but the availability of osteogenic cells is limited and an ideal cell source has not been identified. Amniotic fluid-derived stemcells (AFS) and bone-marrow derived mesenchymal stemcells (MSCs) were compared to determine their osteogenic differentiation capacity in both 2D and 3D environments. In 2D culture, the AFS cells produced more mineralized matrix but

delayed peaks in osteogenic markers. Cells were also cultured on 3D scaffolds constructed of poly- ϵ -caprolactone for 15 weeks. MSCs differentiated more quickly than AFS cells on 3D scaffolds, but mineralized matrix production slowed considerably after 5 weeks. In contrast, the rate of AFS cell mineralization continued to increase out to 15 weeks, at which time AFS constructs contained 5-fold more mineralized matrix than MSC constructs. Therefore, cell source should be taken into consideration when used for cell therapy, as the MSCs would be a good choice for immediate matrix production, but the AFS cells would continue robust mineralization for an extended period of time. This study demonstrates that stemcell source can dramatically influence the magnitude and rate of osteogenic differentiation in vitro.

12. Transplanted human amniotic membrane-derived mesenchymal stem cells ameliorate carbon tetrachloride-induced liver cirrhosis in mouse

DingGuo Zhang, MinYue Jiang, DengShun Miao

PloS one, 2011

Abstract

Background: Human amniotic membrane-derived mesenchymal stem cells (hAMCs) have the potential to reduce heart and lung fibrosis, but whether could reduce liver fibrosis remains largely unknown.

Methodology/Principal Findings: Hepatic cirrhosis model was established by infusion of CCl₄ (1 ml/kg body weight twice a week for 8 weeks) in immunocompetent C57Bl/6J mice. hAMCs, isolated from term delivered placenta, were infused into the spleen at 4 weeks after mice were challenged with CCl₄. Control mice received only saline infusion. Animals were sacrificed at 4 weeks post-transplantation. Blood analysis was performed to evaluate alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Histological analysis of the livers for fibrosis, hepatic stellate cells activation, hepatocyte apoptosis, proliferation and senescence were performed. The donor

cell engraftment was assessed using immunofluorescence and polymerase chain reaction. The areas of hepatic fibrosis were reduced ($6.2\% \pm 2.1$ vs. control $9.6\% \pm 1.7$, $p < 0.05$) and liver function parameters (ALT 539.6 ± 545.1 U/dl, AST 589.7 ± 342.8 U/dl, vs. control ALT 139.1 ± 138.3 U/dl, $p < 0.05$ and AST 212.3 ± 110.7 U/dl, $p < 0.01$) were markedly ameliorated in the hAMCs group compared to control group. The transplantation of hAMCs into liver-fibrotic mice suppressed activation of hepatic stellate cells, decreased hepatocyte apoptosis and promoted liver regeneration. More interesting, hepatocyte senescence was depressed significantly in hAMCs group compared to control group. Immunofluorescence and polymerase chain reaction revealed that hAMCs engraftment into host livers and expressed the hepatocyte-specific markers, human albumin and α -fetoprotein.

Conclusions/Significance: The transplantation of hAMCs significantly decreased the fibrosis formation and progression of CCl₄-induced cirrhosis, providing a new approach for the treatment of fibrotic liver disease.

13. Enhancement of cell retention and functional benefits in myocardial infarction using human amniotic-fluid stem-cell bodies enriched with endogenous ECM

WY Lee, HJ Wei, WW Lin, YC Yeh, SM Hwang...

Biomaterials, 2011

Abstract

Stem cell transplantation may repair the infarcted heart. Despite the encouraging preliminary results, an optimal cell type used and low retention of the transplanted cells remain to be overcome. In this study, a multiwelled methylcellulose hydrogel system was used to cultivate human amniotic-fluid stem cells (hAFSCs) to form spherically symmetric cell bodies for cellular cardiomyoplasty. The grown hAFSC bodies enriched with extracellular matrices (ECM) were xenogenically transplanted in the peri-infarct area of an immune-suppressed rat, via direct intramyocardial injection. Results of bioluminescence imaging and real-time PCR

revealed that hAFSC bodies could considerably enhance cell retention and engraftment in short-term and long-term observations, when compared with dissociated hAFSCs. Echocardiography and magnetic resonance imaging showed that the enhanced cell engraftment in the hAFSC-body group could significantly attenuate the progression of heart failure, improve the global function, and increase the regional wall motion. At the infarct, expressions of HGF, bFGF and VEGF were significantly up-regulated, an indication of the significantly increased vessel densities in the hearts treated with hAFSC bodies. The injected hAFSC bodies could undergo differentiation into angiogenic and cardiomyogenic lineages and contribute to functional benefits by direct regeneration. The aforementioned results demonstrate that hAFSC bodies can attenuate cell loss after intramuscular injection by providing an adequate physical size and offering an enriched ECM environment to retain the transplanted cells in the myocardium, thus improving heart function.

14. Amniotic liquid derived stem cells as reservoir of secreted angiogenic factors capable of stimulating neo-arteriogenesis in an ischemic model

M Teodelinda, C Michele, C Sebastiano, C Ranieri...
Biomaterials, 2011

Abstract

Most urgent health problems are related to a blood vessel formation failure. The use of stem cells from different sources or species for both in vitro and in vivo engineering of endothelium does not necessarily imply their direct commitment towards a vascular phenotype. In the present study, we used human amniotic fluid stem cells (AFSC) to evoke a strong angiogenic response in murine recipients, in terms of host guided-regeneration of new vessels, and we demonstrated that the AFSC secretome is responsible for the vascularising properties of

these cells. We indentified in AFSC conditioned media (ACM) pro-angiogenic soluble factors, such as MCP-1, IL-8, SDF-1, VEGF. Our in vitro results suggest that ACM are cytoprotective, pro-differentiative and chemoattractive for endothelial cells. We also tested ACM on a pre-clinical model of hind-limb ischemic mouse, concluding that ACM contain mediators that promote the neo-arteriogenesis, as remodelling of pre-existing collateral arteries to conductance vessels, thus preventing the capillary loss and the tissue necrosis of distal muscles. In line with the current regenerative medicine trend, in the present study we assert the concept that stem cell-secreted mediators can guide the tissue repair by stimulating or recruiting host reparative cells.

15. Isolation and characterization of equine amniotic fluid-derived multipotent stem cells

SB Park, MS Seo, JG Kang, JS Chae...
Cytotherapy, 2011

Abstract

Background aims. Amniotic fluid (AF) is a well-known source of stem cells. However, there have been no reports regarding equine AF stem cells. We have isolated equine AF-derived multipotent stem cells (MSC) (eAF-MSC) and show that these cells exhibit self-renewal ability and multilineage differentiation. *Methods.* AF was obtained from thoroughbred mares and mononuclear cells (MNC) were isolated by Ficoll–Paque density gradient. We measured the cumulative population doubling level (CPDL) and characterized the immunophenotype by flow cytometry. To investigate differentiation ability, a trilineage differentiation assay was

conducted. *Results.* eAF-MSC could be isolated and the proliferation level was high. eAF-MSC presented typical MSC phenotypic markers, as determined by flow cytometry. Moreover, eAF-MSC showed a trilineage differentiation capability. *Conclusions.* Equine AF is a good source of MSC. Furthermore, eAF-MSC may be useful as a cell therapy application for horses.

16. Clinical applications of prenatal and postnatal therapy using stem cells retrieved from amniotic fluid

Shaw, Sheng Wen S; David, Anna L; De Coppi, Paolo

Current Opinion in Obstetrics & Gynecology
- April 2011 - Volume 23 - Issue 2 - p 109–116

Abstract

Purpose of review: To review the potential of stem cells derived from amniotic fluid and applications in prenatal and postnatal therapy. Recent findings: We have recently described that pluripotent stem cells can be isolated from amniotic fluid defined as amniotic fluid stem (AFS) cells by selection for expression of the membrane stem cell factor receptor c-Kit. AFS cells maintained for over 250 population doublings retained long telomeres and normal karyotype. Clonal human lines verified by retroviral marking were induced

to differentiate into cell types representing each embryonic germ layer, including adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages. Rat AFS cells have been able to improve the repair of damaged smooth muscle in cryoinjury bladders. Furthermore, AFS cells could be differentiated toward cardiomyogenic lineages, when co-cultured with neonatal cardiomyocytes and have potential to generate hematopoietic lineages both *in vitro* and *in vivo*. These cells have been applied into fetal therapy, and widely used for tissue repair in animal models. Finally, we demonstrated a feasible way to do in-utero autologous AFS transplantation in sheep.

Summary: Stem cells derived from amniotic fluid are a relatively new source of cells that could have a therapeutic value in various diseases prenatally and/or postnatally.

17. Autologous Transplantation of Amniotic Fluid-Derived Mesenchymal Stem Cells Into Sheep Fetuses

Shaw, S. W. Steven; Bollini, Sveva; Nader, Khalil Abi; Gastadello, Annalisa; Mehta, Vedanta; Filppi, Elisa; Cananzi, Mara; Gaspar, H. Bobby; Qasim, Waseem; De Coppi, Paolo; David, Anna L.
Cell Transplantation, Volume 20, Number 7, 2011 , pp. 1015-1031

Abstract

Long-term engraftment and phenotype correction has been difficult to achieve in humans after in utero stem cell transplantation mainly because of allogeneic rejection. Autologous cells could be obtained during gestation from the amniotic fluid with minimal risk for the fetus and the mother. Using a sheep model, we explored the possibility of using amniotic fluid mesenchymal stem cells (AFMSCs) for autologous in utero stem cell/gene therapy. We collected amniotic fluid (AF) under ultrasound-guided amniocentesis in early gestation

pregnant sheep ($n = 9$, 58 days of gestation, term = 145 days). AFMSCs were isolated and expanded in all sampled fetal sheep. Those cells were transduced using an HIV vector encoding enhanced green fluorescent protein (GFP) with 63.2% (range 38.3-96.2%) transduction efficiency rate. After expansion, transduced AFMSCs were injected into the peritoneal cavity of each donor fetal sheep at 76 days under ultrasound guidance. One ewe miscarried twin fetuses after amniocentesis. Intraperitoneal injection was successful in the remaining 7 fetal sheep giving a 78% survival for the full procedure. Tissues were sampled at postmortem examination 2 weeks later. PCR analysis detected GFP-positive cells in fetal tissues including liver, heart, placenta, membrane, umbilical cord, adrenal gland, and muscle. GFP protein was detected in these tissues by Western blotting and further confirmed by cytofluorimetric and immunofluorescence analyses. This is the first demonstration of autologous stem cell transplantation in the fetus using AFMSCs. Autologous cells derived from AF showed widespread organ migration and could offer an alternative way to ameliorate prenatal congenital disease.

18. Recruitment of host's progenitor cells to sites of human amniotic fluid stem cells implantation

M Teodelinda, P Alessandro, S Monica, M Massimo...

Biomaterials, 2011

Abstract

The amniotic fluid is a new source of multipotent stem cells with a therapeutic potential for human diseases. Cultured at low cell density, human amniotic fluid stem cells (hAFSCs) were still able to generate colony-forming unit-fibroblast (CFU-F) after 60 doublings, thus confirming their staminal nature. Moreover, after extensive in vitro cell expansion hAFSCs maintained a stable karyotype. The expression of genes, such as SSEA-4, SOX2 and OCT3/4 was confirmed at early and later culture stage. Also, hAFSCs showed bright expression of mesenchymal lineage markers and immunoregulatory

properties. hAFSCs, seeded onto hydroxyapatite scaffolds and subcutaneously implanted in nude mice, played a pivotal role in mounting a response resulting in the recruitment of host's progenitor cells forming tissues of mesodermal origin such as fat, muscle, fibrous tissue and immature bone. Implanted hAFSCs migrated from the scaffold to the skin overlying implant site but not to other organs. Given their in vivo: (i) recruitment of host progenitor cells, (ii) homing towards injured sites and (iii) multipotentiality in tissue repair, hAFSCs are a very appealing reserve of stem cells potentially useful for clinical application in regenerative medicine.

19. Cell delivery with fixed amniotic membrane reconstructs corneal epithelium in rabbits with limbal stem cell deficiency

P Wan, X Wang, P Ma, N Gao, J Ge, Y

Mou...

Investigative ophthalmology & visual Science, 2011

Abstract

Purpose. To explore the feasibility and efficacy of a cell delivery system using amniotic membrane (AM) fixed by a novel biomembrane-fixing device (BMFD) for corneal epithelium reconstruction in rabbits with limbal stem cell deficiency (LSCD).

Methods. Sixty female rabbits with LSCD were created and randomly assigned to three groups of 20 each: LSCD rabbits without treatment (the control), LSCD rabbits treated with BMFD-fixed AM (BMFD-AM), and rabbits treated with male human limbal epithelial cells delivered with BMFD-fixed AM (BMFD-AM+cells). They were followed up with slit lamp observation and corneal fluorescein staining for 14 days. Cytokeratin K3 and K4 and mucin 5AC were used to evaluate

corneal conjunctivalization. Sry gene detection was used to trace the delivered cells.

Results. The corneal re-epithelialization time was 5.60 ± 0.46 days in the BMFD-AM+cell group, significantly shorter ($P < 0.05$) than in the LSCD (12.45 ± 0.65 days) and the BMFD-AM (9.25 ± 0.51 days) groups. Conjunctivalization and neovascularization were observed to be severe in the LSCD group and moderate in the BMFD-AM group. The prevention of conjunctivalization in the BMFD-AM+cell group was evidenced by positive K3/K12 and negative MUC5AC and K4 observed on re-epithelialized corneal epithelium. The histologic sections at different time points and positive Sry gene expression indicated that the delivered cells adhered to the wounded corneal surface and proliferated well.

Conclusions. These findings demonstrate that the BMFD with fixed AM served well as a cell delivery system for the ocular surface. The delivered limbal epithelial cells promoted corneal re-epithelialization and prevented corneas from conjunctivalization and neovascularization in rabbits with experimental LSCD

20. Human mesenchymal stem cells from chorionic villi and amniotic fluid are not susceptible to transformation after extensive in vitro expansion

Poloni, Antonella; Maurizi, Giulia; Babini, Lucia; Serrani, Federica; Berardinelli, Eleonora; Mancini, Stefania; Costantini, Benedetta; Discepoli, Giancarlo; Leoni, Pietro

Cell Transplantation, Volume 20, Number 5, 2011 , pp. 643-654

Abstract

Mesenchymal stem cells (MSCs) are promising candidates for cell therapy and tissue engineering. Increasing evidence suggests that MSCs isolated from fetal tissues are more plastic and grow faster than adult MSCs. In this study, we characterized human mesenchymal progenitor cells from chorionic villi (CV) and amniotic fluid (AF) isolated during the first and second trimesters, respectively, and compared them with adult bone marrow-derived MSCs (BM). We evaluated 10 CV, 10 AF, and 6 BM samples expanded until the MSCs reached senescence. We used discarded cells from prenatal analyses for all the experiments. To

evaluate the replicative stability of these cells, we studied the telomerase activity, hTERT gene transcription, and telomere length in these cells. Spontaneous chromosomal alterations were excluded by cytogenetic analysis. We studied the expression of c-myc and p53, tumor-associated genes, at different passage in culture and the capacity of these cells to grow in an anchorage-independent manner by using soft agar assay. We isolated homogeneous populations of spindle-shaped CV, AF, and BM cells expressing mesenchymal immunophenotypic markers throughout the period of expansion. CV cells achieved 14 ± 0.9 logs of expansion in 118 days and AF cells achieved 21 ± 0.9 logs in 118 days, while BM cells achieved 11×0.4 logs in 84 days. Despite their high proliferation capacity, fetal MSCs showed no telomerase activity, no hTERT and c-myc transcriptions, and maintained long, stable telomeres. A constant expression level of p53 and a normal karyotype were preserved throughout long-term expansion, suggesting the safety of fetal MSCs. In conclusion, our results indicate that fetal MSCs could be an alternative, more accessible resource for cell therapy and regenerative medicine

21. Mesenchymal stem cells and progenitor cells in connective tissue engineering and regenerative medicine: is there a future for transplantation?

A Hilfiker, C Kasper, R Hass...

Langenbeck's Archives of Surgery, 2011 - Springer

Abstract

Purpose

Transplantation surgery suffers from a shortage of donor organs worldwide. Cell injection and tissue engineering (TE), thus emerge as alternative therapy options. The purpose of this article is to review the progress of TE technology, focusing on mesenchymal stem cells (MSC) as a cell source for artificial functional tissue.

Results

MSC from many different sources can be minimally invasively harvested: peripheral blood, fat tissue, bone marrow, amniotic fluid, cord blood. In comparison to embryonic stem cells (ESC), there are no ethical concerns; MSC can be extracted from autologous or allogenic tissue and

cause an immune modulatory effect by suppressing the graft-versus-host reaction (GvHD). Furthermore, MSC do not develop into teratomas when transplanted, a consequence observed with ESC and iPS cells.

Conclusion

MSC as multipotent cells are capable of differentiating into mesodermal and non-mesodermal lineages. However, further studies must be performed to elucidate the differentiation capacity of MSC from different sources, and to understand the involved pathways and processes. Already, MSC have been successfully applied in clinical trials, e.g., to heal large bone defects, cartilage lesions, spinal cord injuries, cardiovascular diseases, hematological pathologies, osteogenesis imperfecta, and GvHD. A detailed understanding of the behavior and homing of MSC is desirable to enlarge the clinical application spectrum of MSC towards the in vitro generation of functional tissue for implantation, for example, resilient cartilage, contractile myocardial replacement tissue, and bioartificial heart valves.

22. Wharton's Jelly Mesenchymal stem cells as candidates for beta cells regeneration: extending the differentiative and immunomodulatory benefits of adult mesenchymal stem cells for the treatment of Type 1 Diabetes.

R Anzalone, M Lo Iacono, T Loria...

Stem Cell Reviews and Reports, 2011

Abstract

Mesenchymal stem cells (MSC) are uniquely capable of crossing germinative layers borders (i.e. are able to differentiate towards ectoderm-, mesoderm- and endoderm-derived cytotypes) and are viewed as promising cells for regenerative medicine approaches in several diseases. Type I diabetes therapy should potentially benefit from such differentiated cells: the search for alternatives to organ/islet transplantation strategies via stem cells differentiation is an ongoing task, significant goals having been achieved in most experimental settings (e.g. insulin production and euglycaemia restoration), though caution is still needed to ensure safe and durable effects in vivo. MSC are

obtainable in high numbers via ex vivo culture and can be differentiated towards insulin-producing cells (IPC). Moreover, recent reports evidenced that MSC possess immunomodulatory activities (acting on both innate and acquired immunity effectors) which should result in a reduction of the immunogenicity of transplanted cells, thus limiting rejection. Moreover it has been proposed that MSC administration should be used to attenuate the autoimmune processes which lead to the destruction of beta cells. This review illustrates the recent advances made in differentiating human MSC to IPC. In particular, we compare the effectiveness of the differentiation protocols applied, the markers and functional assays used to characterize differentiated progeny, and the in vivo controls. We further speculate on how MSC derived from Wharton's jelly of human umbilical cord may represent a more promising regenerative medicine tool, as recently demonstrated for endoderm-derived organs (as liver) in human subjects, also considering their peculiar immunomodulatory features compared to other MSC populations.

23. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC

R Hass, C Kasper, S Böhm...

Cell Communication and ..., 2011

Abstract

The mesenchymal stroma harbors an important population of cells that possess stem cell-like characteristics including self renewal and differentiation capacities and can be derived from a variety of different sources. These multipotent mesenchymal stem cells (MSC) can be found in nearly all tissues and are mostly located in perivascular niches. MSC have migratory abilities and can secrete protective factors and act as a primary matrix for tissue regeneration during inflammation, tissue injuries and certain cancers. These functions underlie the important physiological

roles of MSC and underscore a significant potential for the clinical use of distinct populations from the various tissues. MSC derived from different adult (adipose tissue, peripheral blood, bone marrow) and neonatal tissues (particular parts of the placenta and umbilical cord) are therefore compared in this mini-review with respect to their cell biological properties, surface marker expression and proliferative capacities. In addition, several MSC functions including in vitro and in vivo differentiation capacities within a variety of lineages and immunomodulatory properties are highlighted. Differences in the extracellular milieu such as the presence of interacting neighbouring cell populations, exposure to proteases or a hypoxic microenvironment contribute to functional developments within MSC populations originating from different tissues, and intracellular conditions such as the expression levels of certain micro RNAs can additionally balance MSC function and fate.

24. Malignant Tumor Formation After Transplantation of Short-Term Cultured Bone Marrow Mesenchymal Stem Cells in Experimental Myocardial Infarction and Diabetic Neuropathy

JO Jeong, JW Han, JM Kim, HJ Cho...
Circulation research, 2011

Abstract

Rationale: Bone marrow (BM)-derived mesenchymal stem cells (MSCs) hold great promise for cardiovascular cell therapy owing to their multipotency and culture expandability.

Objective: The aim of the study was to investigate whether MSCs can treat experimental acute myocardial infarction (MI) and diabetic neuropathy.

Methods and Results: We isolated mononuclear cells from mouse BM and cultured MSCs in a conventional manner. Flow cytometry analyses of these cultured cells at passage 4 showed expression of typical MSC markers such as CD44 and CD29, but not hematopoietic markers such as

c-kit, flk1, and CD34. To determine the therapeutic effects of MSCs, we injected MSCs into the peri-infarct area after ligation of the left anterior descending coronary arteries of mice and, as separate experiments, injected the same batch of MSCs into hindlimb muscles of mice with diabetic neuropathy. During the follow-up at 4 to 8 weeks after cell transplantation, growing tumors were observed in 30% of hearts in the MI model, and in 46% of hindlimbs in the diabetic neuropathy model. Histological examination of the tumors revealed hypercellularity, pleomorphic nucleoli, cytological atypia and necrosis, and positive staining for α -smooth muscle actin, indicative of malignant sarcoma with myogenic differentiation. Chromosomal analysis of these MSCs showed multiple chromosomal aberrations including fusion, fragmentation, and ring formation.

Conclusions: Genetically unmodified MSCs can undergo chromosomal abnormalities even at early passages and form malignant tumors when transplanted *in vivo*. These results suggest that careful monitoring of chromosomal status is warranted when *in vitro* expanded MSCs are used for cell therapy such as for MI.

25. Amniotic fluid stem cell-based models to study the effects of gene mutations and toxicants on male germ cell formation

Claudia Gundacker, Helmut Dolznig, Mario Mikula, Margit Rosner, Oliver Brandau and Markus Hengstschläger

Asian Journal of Andrology 14, 247-250
(March 2012)

Abstract

Male infertility is a major public health issue predominantly caused by defects in germ cell development. In the past, studies on the genetic regulation of spermatogenesis as well as on negative environmental impacts have been hampered by the fact that human germ cell development is intractable to direct analysis in vivo. Compared with model organisms including mice, there are fundamental differences in the molecular processes of human germ cell development. Therefore, an in vitro model mimicking human sperm formation would be an extremely valuable research tool. In the recent past, both human embryonic stem (ES) cells and

induced pluripotent stem (iPS) cells have been reported to harbour the potential to differentiate into primordial germ cells and gametes. We here discuss the possibility to use human amniotic fluid stem (AFS) cells as a biological model. Since their discovery in 2003, AFS cells have been characterized to differentiate into cells of all three germ layers, to be genomically stable, to have a high proliferative potential and to be non-tumourigenic. In addition, AFS cells are not subject of ethical concerns. In contrast to iPS cells, AFSs cells do not need ectopic induction of pluripotency, which is often associated with only imperfectly cleared epigenetic memory of the source cells. Since AFS cells can be derived from amniocentesis with disease-causing mutations and can be transfected with high efficiency, they could be used in probing gene functions for spermatogenesis and in screening for male reproductive toxicity.

26. Injection of Amniotic Fluid Stem Cells Delays Progression of Renal Fibrosis.

Sedrakyan S, Da Sacco S, Milanesi A, Shiri L, Petrosyan A, Varimezova R, Warburton D, Lemley KV, De Filippo RE, Perin L.

J Am Soc Nephrol. 2012 Apr; 661-73.

Abstract

Injection of amniotic fluid stem cells ameliorates the acute phase of acute tubular necrosis in animals by promoting proliferation of injured tubular cells and decreasing apoptosis, but whether these stem cells could be of benefit in CKD is unknown. Here, we used a mouse model of Alport syndrome, Col4a5(-/-) mice, to determine whether amniotic fluid stem cells could modify the course of progressive renal fibrosis. Intracardiac administration of amniotic fluid stem cells before the onset of proteinuria delayed interstitial fibrosis and progression of glomerular sclerosis, prolonged animal survival, and ameliorated the decline in kidney function. Treated animals exhibited decreased recruitment and activation of M1-type macrophages and a

higher proportion of M2-type macrophages, which promote tissue remodeling. Amniotic fluid stem cells did not differentiate into podocyte-like cells and did not stimulate production of the collagen IVa5 needed for normal formation and function of the glomerular basement membrane. Instead, the mechanism of renal protection was probably the paracrine/endocrine modulation of both profibrotic cytokine expression and recruitment of macrophages to the interstitial space. Furthermore, injected mice retained a normal number of podocytes and had better integrity of the glomerular basement membrane compared with untreated Col4a5(-/-) mice. Inhibition of the renin-angiotensin system by amniotic fluid stem cells may contribute to these beneficial effects. In conclusion, treatment with amniotic fluid stem cells may be beneficial in kidney diseases characterized by progressive renal fibrosis

27. Pro-angiogenic soluble factors from Amniotic Fluid Stem Cells mediate the recruitment of endothelial progenitors in a model of ischemic fasciocutaneous flap.

Teodelinda Mirabella, Joachim Hartinger, Christian Lorandi, Chiara Gentili, Martijn van Griensven, and Ranieri Cancedda

Stem Cell and Development, 2012

Abstract

Skin flaps are routinely used in surgery for the functional and cosmetic repair of wounds or disfiguring scars. The recent concept of therapeutic angiogenesis has emerged as an attractive approach to overcome the problem of blood supply deficiency, often resulting in the flap grafting failure. In the present study, we embedded a gelatin membrane with amniotic fluid stem cells (AFSC) derived conditioned media (ACM) to topically deliver angiogenic growth factors and cytokines into a rat model of ischemic full-thickness skin

flap elevated in the epigastric region. AFSC secretome triggered the endogenous repair by the recruitment of endothelial progenitor cells. We studied the vascular perfusion rate, the vessel distribution, and the survival of ACM-treated flaps. In detail, the ischemic sectors of ACM-treated flaps showed at day 7 a perfusion level 50% higher than the preoperation baseline. The ensuing necrosis development was delayed and the histology analysis showed a normal arrangement of epidermal and dermal structures and a high density of vessels in subcutaneous tissues. Further, we found that ACM recruited CD31+/VEGFR2+ and CD31+/CD34+ cells into the ischemic subcutaneous tissues and that the isolated progenitors were capable to form clusters of von Willebrand factor-positive cells in culture. We propose ACM as a cell-free cocktail of chemokines and growth factors to be adopted for clinical applications

28. Prenatally engineered autologous amniotic fluid stem cell-based heart valves in the fetal circulation

Benedikt Weber, Maximilian Y. Emmert, Luc Behr, Roman Schoenauer, Chad Brokopp, Cord Drögemüller, Peter Modregger, Marco Stampanoni, Divya Vats, Markus Rudin, Wilfried Bürzle, Marc Farine, Edoardo Mazza, Thomas Frauenfelder, Andrew C. Zannettino, Gregor Zünd, Oliver Kretschmar, Volkmar Falk, Simon P. Hoerstrup
Biomaterials, 2012

Abstract

Prenatal heart valve interventions aiming at the early and systematic correction of congenital cardiac malformations represent a promising treatment option in maternal-fetal care. However, definite fetal valve replacements require growing implants adaptive to fetal and postnatal development. The presented study investigates the fetal implantation of prenatally engineered living

autologous cell-based heart valves. Autologous amniotic fluid cells (AFCs) were isolated from pregnant sheep between 122 and 128 days of gestation via transuterine sonographic sampling. Stented trileaflet heart valves were fabricated from biodegradable PGA-P4HB composite matrices (n = 9) and seeded with AFCs in vitro. Within the same intervention, tissue engineered heart valves (TEHVs) and unseeded controls were implanted orthotopically into the pulmonary position using an in-utero closed-heart hybrid approach. The transapical valve deployments were successful in all animals with acute survival of 77.8% of fetuses. TEHV in-vivo functionality was assessed using echocardiography as well as angiography. Fetuses were harvested up to 1 week after implantation representing a birth-relevant gestational age. TEHVs showed in vivo functionality with intact valvular integrity and absence of thrombus formation. The presented approach may serve as an experimental basis for future human prenatal cardiac interventions using fully biodegradable autologous cell-based living materials.

29. Tuberin and PRAS40 are anti-apoptotic gatekeepers during early human amniotic fluid stem-cell differentiation

Christiane Fuchs, Margit Rosner, Helmut Dolznig, Mario Mikula, Nina Kramer and Markus Hengstschläger

Human Molecular Genetics, 2012

Abstract

Embryoid bodies (EBs) are three-dimensional multicellular aggregates allowing the in vitro investigation of stem-cell differentiation processes mimicking early embryogenesis. Human amniotic fluid stem (AFS) cells harbor high proliferation potential, do not raise the ethical issues of embryonic stem cells, have a lower risk for tumor development, do not need exogenic induction of pluripotency and are chromosomal stable. Starting from a single human AFS cell, EBs can be formed accompanied by the differentiation into cells of all three embryonic germ layers. Here, we report that

siRNA-mediated knockdown of the endogenous tuberous sclerosis complex-2 (TSC2) gene product tuberin or of proline-rich Akt substrate of 40 kDa (PRAS40), the two major negative regulators of mammalian target of rapamycin (mTOR), leads to massive apoptotic cell death during EB development of human AFS cells without affecting the endodermal, mesodermal and ectodermal cell differentiation spectrum. Co-knockdown of endogenous mTOR demonstrated these effects to be mTOR-dependent. Our findings prove this enzyme cascade to be an essential anti-apoptotic gatekeeper of stem-cell differentiation during EB formation. These data allow new insights into the regulation of early stem-cell maintenance and differentiation and identify a new role of the tumor suppressor tuberin and the oncogenic protein PRAS40 with the relevance for a more detailed understanding of the pathogenesis of diseases associated with altered activities of these gene products.

30. Amniotic Fluid Stem Cells: a Promising Therapeutic Resource for Cell-Based Regenerative Therapy.

Antonucci I, Pantalone A, Tete S, Salini V, Borlongan CV, Hess D, Stuppia L.

Curr Pharm Des. 2012

Abstract

Stem cells have been proposed as a powerful tool in the treatment of several human diseases, both for their ability to represent a source of new cells to replace those lost due to tissue injuries or degenerative diseases, and for the ability of produce trophic molecules able to minimize damage and promote recovery in the injured tissue. Different cell types, such as embryonic, fetal or adult stem cells, human fetal tissues and genetically engineered cell lines, have been tested for their ability to replace damaged cells and to restore the tissue function after transplantation. Amniotic fluid -derived Stem

cells (AFS) are considered a novel resource for cell transplantation therapy, due to their high renewal capacity, the "in vitro" expression of embryonic cell lineage markers, and the ability to differentiate in tissues derived from all the three embryonic layers. Moreover, AFS do not produce teratomas when transplanted into animals and are characterized by a low antigenicity, which could represent an advantage for cell transplantation or cell replacement therapy. The present review focuses on the biological features of AFS, and on their potential use in the treatment of pathological conditions such as ischemic brain injury and bone damages.

31. Nucleofection of Ovine Amniotic Fluid-Derived Mesenchymal Stem Cells

V Curini, A Colosimo, A Mauro, M Turriani, A. Gloria, M. Mattioli, B. Barboni
Veterinary Science, 2012

Abstract

Amniotic fluid has attracted increasing attention in recent years as a possible source of stem cells. Amniotic stem cells have high differentiation ability and low immunogenicity, and are thus an ideal candidate for stem cell-based regenerative therapy. To assess their potential applicability, preclinical studies have been initiated. In this context, the availability of GFP-expressing cells could be extremely useful as a protein marker to visualize transferred stem cells within damaged tissue. In the present study, nucleofection, a recent electroporation-based technique, was used to transfect GFP-expressing plasmids into ovine

amniotic fluid-derived stem cells. The study shows that this transfection method can be used to generate stable transgene expression in amniotic stem cells without altering their differentiation potential.

32. Haematopoietic stem cells derived from sheep and human amniotic fluid engraft after transplantation

SW Shaw, AL David, M Blundell, S Howe, C Pipino, P Maghsoudlou, KH Lee, A Atala, CD Porada, A Thrasher, P De Coppi
. ADC Fetal and Neonatal, 2012

Abstract

Introduction Mouse amniotic fluid c-Kit(+)/Lin(-) stem (AFS) cells display hematopoietic potential. We explored the haematopoietic potential of sheep and human AFS cells after in utero stem cell transplantation.

Methods Human AFS cells (hAFSC) were isolated from women undergoing 3rd trimester amniodrainage. Sheep AFS cells (sAFSC) were collected under ultrasound guidance (59.5±4.5days, term=145days), and isolated using a sheep-specific CD34 antibody.

hAFSC were transplanted into the peritoneal cavity of fetal mice from CD1 mothers (14dpc, n=6). The peripheral blood of recipient mice was analysed 4 weeks postnatal for engraftment by

flow-cytometry using anti-human beta2-microglobulin antibody. Neonatal tissues collected at 6 weeks were analysed by PCR and immunostaining for anti-human mitochondrial antibody; bone marrow (BM) was assayed for colony-forming cells. sAFSC were transduced overnight using a lentivirus vector (HIV-SFFV-eGFP, MOI=50) and injected either intravenously into NOD-SCID-gamma (NSG) mice (3×10^5 , N=4 per group) or by ultrasound-guided peritoneal injection back into donor sheep fetuses (n=7; 2×10^4 sAFSC).

Results hAFS cells were detectable in the peripheral blood, liver, spleen and BM of neonatal mice at 6 weeks postnatal; harvested BM generated colonies of human origin. GFP+ve cells were detected in the peripheral blood, spleen, liver, and BM of NSG mice 3 months after transplantation of transduced sAFSC. Five lambs injected with autologous transduced GFP+CD34+ cells survived to birth (71.4%); peripheral blood of all lambs contained GFP+ cells (1.9-3.8%) maintained at 6 months postnatal. GFP+ cells were detected in the liver and BM of lambs.

Conclusion AFSC have haematopoietic potential and be useful for autologous transplantation

33. Fibroin Scaffold Repairs Critical-Size Bone Defects In Vivo Supported by Human Amniotic Fluid and Dental Pulp Stem Cells

Massimo Riccio, Tullia Maraldi, Alessandra Pisciotta, Giovanni B. La Sala, Adriano Ferrari, Giacomo Bruzzesi, Antonella Motta, Claudio Migliaresi, and A. De Pol

Tissue Engineering Part A. May 2012

Abstract

The main aim of this study was the comparative evaluation of fibroin scaffolds combined with human stem cells, such as dental pulp stem cells (hDPSCs) and amniotic fluid stem cells (hAFSCs), used to repair critical-size cranial bone defects in immunocompromised rats. Two symmetric full-thickness cranial defects on each parietal region of rats have been replenished with silk fibroin scaffolds with or without preseeded stem cells addressed toward osteogenic lineage *in vitro*. Animals were euthanized

after 4 weeks postoperatively and cranial tissue samples were taken for histological analysis. The presence of human cells in the new-formed bone was confirmed by confocal analysis with an antibody directed to a human mitochondrial protein. Fibroin scaffolds induced mature bone formation and defect correction, with higher bone amount produced by hAFSC-seeded scaffolds. Our findings demonstrated the strong potential of stem cells/fibroin bioengineered constructs for correcting large cranial defects in animal model and is likely a promising approach for the reconstruction of human large skeletal defects in craniofacial surgery.

34. Characterization, GFP gene nucleofection and allotransplantation in injured tendons of ovine amniotic fluid-derived stem cells.

Colosimo A, Curini V, Russo V, Mauro A, Bernabò N, Marchisio M, Alfonsi M, Muttini A, Mattioli M, Barboni B.

Cell Transplant, 2012 April 10

Abstract

Amniotic fluid has drawn increasing attention in the recent past as a cost-effective and accessible source of fetal stem cells. Amniotic fluid-derived mesenchymal stem cells (AFMSCs) that display high proliferation rate, large spectrum of differentiation potential and immunosuppressive features are considered optimal candidates for allogeneic repair of mesenchymal damaged tissues. In this study, ovine AFMSCs (oAFMSCs) isolated from 3 months-old sheep fetuses were characterized for their proliferation rate, specific surface antigen and pluripotency marker expression, genomic stability and mesenchymal lineage differentiation during their in vitro expansion (12 passages) and after nucleofection. The high proliferation rate of oAFMSCs gradually decreased during the first 6

subculture passages while the expression of surface molecules (CD29, CD58, CD166) and of pluripotency-associated markers (OCT4, TERT, NANOG, SOX2), the *in vitro* osteogenic differentiation potential and a normal karyotype were maintained. Afterwards, oAFMSCs were nucleofected with a selectable plasmid coding for green fluorescent protein (GFP) using two different programs, U23 and C17, previously optimized for human mesenchymal stem cells. Transfection efficiencies were ~63% and ~37% while cell recoveries were ~10% and ~22%, respectively. Nucleofected oAFMSCs expressing the GFP transgene conserved their pluripotency marker profile, retained a normal karyotype and the osteogenic differentiation ability. Seven single clones with a GFP expression ranging from 80% to 97% were then isolated and expanded over one month, thus providing stably transfected cells with long-term therapeutic potential. The *in vivo* behavior of GFP-labeled oAFMSCs was tested on a previously validated preclinical model of experimentally induced Achille's tendon defect. The allotransplanted oAFMSCs were able to survive within the host tissue for one month enhancing the early phase of tendon healing as indicated by morphological and biomechanical results. Altogether these data suggest that genetically modified oAFMSCs might represent a valuable tool for *in vivo* preclinical studies in a highly valid translational model

35. Bilayered constructs aimed at osteochondral strategies: The influence of medium supplements in the osteogenic and chondrogenic differentiation of amnioticfluid-derived stemcells

Márcia T. Rodrigues, Sang Jin Lee, Manuela E. Gomes, Rui L. Reis, Anthony Atala, James J. Yoo

Acta Biomaterialia, 2012

Abstract

The development of osteochondral tissue engineered interfaces would be a novel treatment for traumatic injuries and aging associated diseases that affect joints. This study reports the development of a bilayered scaffold, which consists of both bone and cartilage regions. On the other hand, amnioticfluid-derived stemcells (AFSCs) could be differentiated into either osteogenic or chondrogenic cells, respectively. In this study we have developed a bilayered scaffolding system, which includes a starch/polycaprolactone (SPCL) scaffold for osteogenesis and an agarose hydrogel for chondrogenesis. AFSC-seeded scaffolds were cultured for 1 or 2 weeks in an osteochondral-defined culture medium containing both osteogenic and

chondrogenic differentiation factors. Additionally, the effect of the presence or absence of insulin-like growth factor-1 (IGF-1) in the culture medium was assessed. Cell viability and phenotypic expression were assessed within the constructs in order to determine the influence of the osteochondral differentiation medium. The results indicated that, after osteogenic differentiation, AFSCs that had been seeded onto SPCL scaffolds did not require osteochondral medium to maintain their phenotype, and they produced a protein-rich, mineralized extracellular matrix (ECM) for up to 2 weeks. However, AFSCs differentiated into chondrocyte-like cells appeared to require osteochondral medium, but not IGF-1, to synthesize ECM proteins and maintain the chondrogenic phenotype. Thus, although IGF-1 was not essential for creating osteochondral constructs with AFSCs in this study, the osteochondral supplements used appear to be important to generate cartilage in long-term tissue engineering approaches for osteochondral interfaces. In addition, constructs generated from agarose-SPCL bilayered scaffolds containing pre-differentiated AFSCs may be useful for potential applications in regeneration strategies for damaged or diseased joints.

36. Epigenetic Stability of Single-Cell Clones of Human Amniotic Fluid Mesenchymal Stem Cell

TH Wang, SM Hwan, HH Peng

5TH EUROPEAN CONFERENCE OF THE INTERNATIONAL FEDERATION FOR MEDICAL AND BIOLOGICAL ENGINEERING - IFMBE Proceedings, 2012, Volume 37, Part 1, Part 14

Abstract

Epigenetic regulation is critical for tissue differentiation. To further understand the epigenetic stability of a potentially useful cell source, we investigated the methylation characteristics of CpG islands of imprinted genes in human amniotic fluid mesenchymal stem cells (AFMSC), focusing on the differences among various single-cell clones and the effect of in vitro culture on the epigenetic changes of imprinted genes. Two amniotic fluid specimens were collected by amniocentesis, and a total of six single-cell clones AFMSC were derived using the in vitro cell culture method. We tested the differentially methylated regions (DMRs) of

imprinted genes that are known to be important in cell biological functions or to be related to imprinting disorders, including H19, SNRPN, and KCNQ1OT1. DNA methylation statuses at DMRs of imprinted genes were analyzed by bisulfite genomic sequencing. Our results show that human AFMSC contain a unique epigenetic signature. The hypermethylation status of imprinting gene (H19 and KCNQ1OT1) in most single-cell clones of AFMSC was different from those of normal human blood cells. Nevertheless, H19 and KCNQ1OT1 possessed a substantial degree of epigenetic stability, despite differences in genetic background. Epigenetic instability of the imprinting gene (SNRPN) was observed during in vitro cell culture of human AFMSC. Our results urge further understanding of epigenetic status and epigenetic stability of AFMSC before it is applied in cell-replacement therapy.

37. Specific Labeling of Neurogenic, Endothelial, and Myogenic Differentiated Cells Derived from Human Amniotic Fluid Stem Cells with Silica-Coated Magnetic Nanoparticles

Lee JK, Chun SY, Im JY, Jin HK, Kwon TG, Bae JS.

J Vet Med Sci. 2012 Mar 14

Abstract

Stem cell based cell therapies offer significant potential for the field of regenerative medicine. Human amniotic fluid stem cells (hAFSCs) are an attractive source for lineage-specific differentiated stem cell therapy since they have properties that are able to differentiate into cells representing all three germ layers. To better understand the fate and location of implanted hAFSCs, a means to monitor cells in living subjects is essential. Here, we showed that differentiated cells, such as neurogenic, endothelial, and

myogenic cells, derived from hAFSCs can be effectively labeled by the FITC-incorporated silica-coated nanoparticles, MNPs@SiO₂ (FITC), although the labeling efficacy and cytotoxicity were distinct depending on the differentiated cell type. In addition, we observed that MNPs@SiO₂-labeled cells provided sufficient signals for detection by optical and confocal microscope imaging when transplanted into the mice. These results suggest that the fluorescent dye incorporated MNPs@SiO₂ are a useful tool for the cell labeling and in vivo tracking of differentiated cells derived from hAFSCs

38. Dual regeneration of muscle and nerve by intravenous administration of human amniotic fluid–derived mesenchymal stem cells regulated by stromal cell–derived factor-1 α in a sciatic nerve injury model

Dar-Yu Yang et al.

Journal of Neosurgery, 2012

Abstract - Human amniotic fluid–derived mesenchymal stem cells (AFMSCs) have been shown to promote peripheral nerve regeneration. The expression of stromal cell–derived factor-1 α (SDF-1 α) in the injured nerve exerts a trophic effect by recruiting progenitor cells that promote nerve regeneration. In this study, the authors investigated the feasibility of intravenous administration of AFMSCs according to SDF-1 α expression time profiles to facilitate neural regeneration in a sciatic nerve crush injury model. METHODS - Peripheral nerve injury was induced in 63 Sprague-Dawley rats by crushing the left sciatic nerve using a vessel clamp. The animals were randomized into 1 of 3 groups: Group I, crush injury as the control; Group II, crush injury and intravenous administration of AFMSCs (5×10^6 cells for 3 days) immediately after injury (early administration); and Group III, crush injury and intravenous administration of AFMSCs (5×10^6 cells for 3 days) 7 days after injury (late administration).

Evaluation of neurobehavior, electrophysiological study, and assessment of regeneration markers were conducted every week after injury. The expression of SDF-1 α and neurotrophic factors and the distribution of AFMSCs in various time profiles were also assessed. Stromal cell-derived factor-1 α increased the migration and wound healing of AFMSCs in vitro, and the migration ability was dose dependent. Crush injury induced the expression of SDF-1 α at a peak of 10–14 days either in nerve or muscle, and this increased expression paralleled the expression of its receptor, chemokine receptor type-4 (CXCR-4). Most AFMSCs were distributed to the lung during early or late administration. Significant deposition of AFMSCs in nerve and muscle only occurred in the late administration group. Significantly enhanced neurobehavior, electrophysiological function, nerve myelination, and expression of neurotrophic factors and acetylcholine receptor were demonstrated in the late administration group. Amniotic fluid-derived mesenchymal stem cells can be recruited by expression of SDF-1 α in muscle and nerve after nerve crush injury. The increased deposition of AFMSCs paralleled the expression profiles of SDF-1 α and its receptor CXCR-4 in either muscle or nerve. Administration of AFMSCs led to improvements in neurobehavior and expression of regeneration markers. Intravenous administration of AFMSCs may be a promising alternative treatment strategy in peripheral nerve disorder.

39. Clone-derived human AF-amniotic fluid stem cells are capable of skeletal myogenic differentiation in vitro and in vivo

Xiaorong Ma, Shengli Zhang, Junmei Zhou, Baisong Chen, Yafeng Shang, Tongbing Gao, Xue Wang, Hua Xie, Fang Chen

Journal of Tissue Engineering and Regenerative Medicine, 2012

Abstract

Stem cell-based therapy may be the most promising method to cure skeletal muscle degenerative diseases such as Duchenne muscular dystrophy (DMD) and trauma in the future. Human amniotic fluid is enriched with early-stage stem cells from developing fetuses and these cells have cardiomyogenic potential both *in vitro* and *in vivo*. In the present study, we investigated the characteristics of human amniotic fluid-derived AF-type stem (HAF-AFS) cells by flow cytometry, immunofluorescence staining, reverse-transcription polymerase chain reaction, and osteogenic and adipogenic differentiation analysis.

After confirming the stemness of HAF-AFS cells, we tested whether HAF-AFS cells could differentiate into skeletal myogenic cells *in vitro* and incorporate into regenerating skeletal muscle *in vivo*. By temporary exposure to the DNA demethylation agent 5-aza-2'-deoxycytidine (5-Aza dC) or co-cultured with C2C12 myoblasts, HAF-AFS cells differentiated into skeletal myogenic cells, expressing skeletal myogenic cell-specific markers such as Desmin, Troponin I (Tn I) and α -Actinin. Four weeks after transplantation into cardiotoxin-injured and X-ray-irradiated tibialis anterior (TA) muscles of NOD/SCID mice, HAF-AFS cells survived, differentiated into myogenic precursor cells and fused with host myofibres. The findings that HAF-AFS cells differentiate into myogenic cells *in vitro* and incorporate in skeletal muscle regeneration *in vivo* hold the promise of HAF-AFS cell-based therapy for skeletal muscle degenerative diseases.

40. Evaluation of Endothelial Cells Differentiated from Amniotic Fluid-Derived Stem Cells

Omar M. Benavides, B.S., Jennifer J. Petsche, B.S., Kenneth J. Moise Jr., Anthony Johnson and Jeffrey G. Jacot
Tissue Engineering, 2012

Abstract

Amniotic fluid holds great promise as a stem cell source, especially in neonatal applications where autologous cells can be isolated and used. This study examined chemical-mediated differentiation of amniotic fluid-derived stem cells (AFSC) into endothelial cells and verified the function of AFSC-derived endothelial cells (AFSC-EC). AFSC were isolated from amniotic fluid obtained from second trimester amnioreduction as part of therapeutic intervention from pregnancies affected with twin-twin transfusion syndrome. Undifferentiated AFSC were of normal karyotype with a subpopulation of cells positive for the embryonic stem cell marker SSEA4, hematopoietic stem cell marker c-kit, and mesenchymal stem cell markers CD29, CD44, CD73, CD90, and CD105. Additionally, these cells were negative for the endothelial marker CD31 and hematopoietic differentiation marker CD45. AFSC were

cultured in endothelial growth media with concentrations of vascular endothelial growth factor (VEGF) ranging from 1 to 100 ng/mL. After 2 weeks, AFSC-EC expressed von Willebrand factor, endothelial nitric oxide synthase, CD31, VE-cadherin, and VEGF receptor 2. Additionally, the percentage of cells expressing CD31 was positively correlated with VEGF concentration up to 50 ng/mL, with no increase at higher concentrations. AFSC-EC showed a decrease in stem cells markers c-kit and SSEA4 and were morphologically similar to human umbilical vein endothelial cells (HUVEC). In functional assays, AFSC-EC formed networks and metabolized acetylated low-density lipoprotein, also characteristic of HUVEC. Nitrate levels for AFSC-EC, an indirect measure of nitric oxide synthesis, were significantly higher than undifferentiated controls and significantly lower than HUVEC. These results indicate that AFSC can differentiate into functional endothelial-like cells and may have the potential to provide vascularization for constructs used in regenerative medicine strategies.

41. Generation of Human β -thalassemia Induced Pluripotent Stem Cells from Amniotic Fluid Cells Using a Single Excisable Lentiviral Stem Cell Cassette.

Fan Y, Luo Y, Chen X, Li Q, Sun X.

J Reprod Dev. 2012 Apr 13

Abstract

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients represent a powerful tool for biomedical research and may have a wide range of applications in cell and gene therapy. However, the safety issues and the low efficiency associated with generating human iPSCs have limited their usage in clinical settings. The cell type used to create iPSCs can significantly influence the reprogramming efficiency and kinetics. Here, we show that amniotic fluid cells from the prenatal diagnosis of a β -thalassemia patient can be efficiently reprogrammed using a doxycycline (DOX)-inducible humanized version of the single lentiviral "stem cell cassette" vector flanked by loxP sites, which can be excised with Cre recombinase. We also demonstrated that the

patient-derived iPSCs can be characterized based on the expression of pluripotency markers, and they can be differentiated into various somatic cell types *in vitro* and *in vivo*. Moreover, microarray analysis demonstrates a high correlation coefficient between human β -thalassemia iPSC cells and human embryonic stem (hES) cells but a low correlation coefficient between human β -thalassemia amniotic fluid cells and human β -thalassemia iPSC cells. Our data suggest that amniotic fluid cells may be an ideal human somatic cell resource for rapid and efficient generation of patient-specific iPSC cells

The Journal of reproduction ..., 2012 -
ncbi.nlm.nih.gov

Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients represent a powerful tool for biomedical research and may have a wide range of applications in cell and gene therapy. However, the safety issues and the low efficiency associated with ...

42. Amniotic fluid-derived stem cells in regenerative medicine research

S Joo, IK Ko, A Atala, JJ Yoo, SJ Lee
Archives of Pharmacal Research, 2012

Abstract

The stem cells isolated from amniotic fluid present an exciting possible contribution to the field of regenerative medicine and amniotic fluid-derived stem (AFS) cells have significant potential for research and therapeutic applications. AFS cells are multipotent, showing the ability to differentiate into cell types from all three embryonic germ layers. They express both embryonic and adult stem cell markers, expand extensively without feeder cells, double in 36 h, and are not tumorigenic. The AFS cells can be maintained for over 250 population doublings and preserve their telomere length and a normal karyotype. They differentiate easily into specific cell lineages

and do not require human embryo tissue for their isolation, thus avoiding the current controversies associated with the use of human embryonic stem (ES) cells. The discovery of the AFS cells has been recent, and a great deal of work remains to be performed on the characterization and use of these cells. This review describes the various differentiated lineages that AFS cells can form and the future of these promising new stem cells in regenerative medicine research

43. Human amniotic fluid-derived stem cells expressing cytosine deaminase and thymidine kinase inhibits the growth of breast cancer cells in cellular and xenograft mouse model

N-H Kang, K-A Hwang, B-R Yi, H J Lee, E-B Jeung, S U Kim and K-C Choi

Cancer Gene Therapy , (13 April 2012)

Abstract

As human amniotic fluid-derived stem cells (hAFSCs) are capable of multiple lineage differentiation, extensive self-renewal and tumor targeting, they may be valuable for clinical anticancer therapies. In this study, we used hAFSCs as vehicles for targeted delivery of therapeutic suicide genes to breast cancer cells. hAFSCs were engineered to produce AF2.CD-TK cells in order to express two suicide genes encoding bacterial cytosine deaminase (CD) and herpes simplex virus thymidine kinase (HSV-TK) that convert non-toxic prodrugs, 5-fluorocytosine (5-FC) and mono-phosphorylate ganciclovir (GCV-MP), into cytotoxic metabolites, 5-fluorouracil (5-FU) and triphosphate ganciclovir (GCV-TP), respectively. In cell viability test in vitro, AF2.CD-TK cells inhibited the growth of MDA-MB-231 human

breast cancer cells in the presence of the 5-FC or GCV prodrugs, or a combination of these two reagents. When the mixture of 5-FC and GCV was treated together, an additive cytotoxic effect was observed in the cell viability. In animal experiments using female BALB/c nude mouse xenografts, which developed by injecting MDA-MB-231 cells, treatment with AF2.CD-TK cells in the presence of 5-FC and GCV significantly reduced tumor volume and weight to the same extent seen in the mice treated with 5-FU. Histopathological and fluorescent staining assays further showed that AF2.CD-TK cells were located exactly at the site of tumor formation. Furthermore, breast tissues treated with AF2.CD-TK cells and two prodrugs maintained their normal structures (for example, the epidermis and reticular layers) while breast tissue structures in 5-FU-treated mice were almost destroyed by the potent cytotoxicity of the drug. Taken together, these results indicate that AF2.CD-TK cells can serve as excellent vehicles in a novel therapeutic cell-based gene-directed prodrug system to selectively target breast malignancies.

44. BMP15 Gene Is Activated During Human Amniotic Fluid Stem Cell Differentiation into Oocyte-Like Cells

Shuai Chen, Xiaoli Yu, Pengsheng Zheng and Huayan Wang

Department of The Journal of DNA and Cell Biology, 2012

Abstract

The generation of oocyte-like cells (OLCs) from stem cell differentiation *in vitro* provides an optimal approach for studying the mechanism of oocyte development and maturation. The aim of this study was to investigate the activation of bone morphogenetic protein 15 gene (*BMP15*) during the differentiation of human amniotic fluid stem cells (hAFSCs) into OLCs. After 15 days of differentiation, OLCs with a diameter of 50–60 μm and zona pellucida (ZP)-like morphology were observed. Reverse transcription-polymerase chain

reaction (RT-PCR) analysis showed the *BMP15* was activated from approximately day 10 of differentiating hAFSCs and thereafter. The reporter construct pBMP15-enhanced green fluorescent protein (EGFP) was transiently transfected into the differentiated hAFSCs and the EGFP expression driven by the *BMP15* promoter was positive in the OLCs. Moreover, RT-PCR analysis showed that the oocyte-specific markers including *ZP1*, *ZP2*, *ZP3*, and *c-kit* were expressed in the differentiated hAFSCs, and the immunofluorescence assay confirmed that the *ZP2* was detected in the OLCs. Quantitative RT-PCR revealed that *ZP2* and *ZP3* were significantly elevated in the differentiated hAFSCs. Further, in the OLCs derived from hAFSCs, the *BMP15* promoter directing the EGFP reporter was colocalized with *ZP2*. Together, these results illustrated that the *BMP15* could be used as an oogenesis marker to track hAFSCs differentiation into the OLCs

45. Cryopreservation does not alter karyotype, multipotency, or NANOG/SOX2 gene expression of amniotic fluid mesenchymal stem cells

P.C. Ângelo, A.C.S. Ferreira, V.D. Fonseca, S.P. Frade, C.S. Ferreira, F.S.V. Malta, A.K. Pereira, H.V. Leite, A.P. Brum, V.C. Pardini, K.B. Gomes and A.C.V. Cabral
Genetics and Molecular Research, 2012

Abstract

Cryopreservation of mesenchymal stem cells from amniotic fluid is of clinical importance, as these cells can be harvested during the prenatal period and stored for use in treatments. We examined the behavior of mesenchymal stem cells from human amniotic fluid in culture that had been subjected to cryopreservation. We assessed chromosomal stability through karyotype analysis, determined whether multipotent capacity (differentiation into adipogenic,

chondrogenic, and osteogenic cells) is maintained, and analyzed SOX2 and NANOG expression after thawing. Five amniotic fluid samples were cryopreserved for 150 days. No chromosomal aberrations were observed. The expression levels of NANOG and SOX2 also were quite similar before and after cryopreservation. Capacity for differentiation into adipogenic, chondrogenic, and osteogenic tissues also remained the same. We conclude that cryopreservation of amniotic fluid does

46. Evaluation of a low cost cryopreservation system on the biology of human amniotic fluid-derived mesenchymal stromal cells

Jose Maria Miranda-Sayago, Nieves Fernandez-Arcas, Carmen Benito, Armando Reyes-Engel, Jose Ramon Herrero, Antonio Alonso

Cryobiology, 2012

Abstract

Background

Human amniotic-derived mesenchymal stromal cells (hAMSC) are a novel population of multipotent stemcells that have been shown to have great potential for use in regenerative medicine. However, procedures to store and preserve hAMSC for future clinical applications have not been explored extensively.

Methods

In this study, we analyzed the influence of

cryopreservation, using a protocol based on freezing rate of 1 °C/min, 10% dimethyl sulfoxide as cryoprotectant and a thawing rate >100 °C/min, on hAMSC morphology, proliferation rates, viability, cell cycle, karyotype, immune phenotype and multilineage differentiation potential.

Results

This study found that this cryopreservation protocol does not affect the biological properties of hAMSC.

Discussion

This shows that this protocol is a viable system for banking hAMSC, with the associated advantages that has a low cost in terms of expense, time and personnel involved and is easy to implement

47. Stem Cell Therapy Ameliorates Bladder Dysfunction in an Animal Model of Parkinson Disease

Claudius Füllhase, Ariel Hanson, Lysanne Campeau, Cesar Santos, Karl-Erik Andersson
The Journal of Urology, 2012 - Elsevier

Abstract

Purpose - Different cell based therapies have been tested, focusing on motor function. We evaluated the effect of human amnioticfluidstemcells and bone marrow derived mesenchymal stemcells (ALLCELLS, Emeryville, California) on bladder dysfunction in a rat model of Parkinson disease.

Material and Methods - A nigrostriatal lesion was induced by 6-hydroxydopamine in 96 athymic nude female rats divided into 3 treatment groups. After 2 weeks the groups were injected with human amnioticfluidstemcells, bone marrow derived mesenchymal stemcells and vehicle for sham treatment, respectively. At 3, 7, 14 and 28 days the bladder function of 8 rats per group was analyzed by conscious cystometry. Brains were extracted for immunostaining.

Results - The nigrostriatal lesion caused bladder dysfunction, which was consistent in sham treated animals throughout the study. Several cystometric parameters improved 14 days after human amnioticfluidstemcell or bone marrow derived mesenchymal stemcell injection, concomitant with the presence of human stemcells in the brain. At 14 days only a few cells were observed in a more caudal and lateral position. At 28 days the functional improvement subsided and human stemcells were no longer seen. Human stemcell injection improved the survival of dopaminergic neurons until 14 days. Human stemcells expressed superoxide dismutase-2 and seemed to modulate the expression of interleukin-6 and glial cell-derived neurotrophic factor by host cells.

Conclusions - Cell therapy with human amnioticfluidstemcells and bone marrow derived mesenchymal stemcells temporarily ameliorated bladder dysfunction in a Parkinson disease model. In contrast to integration, cells may act on the injured environment via cell signaling.

48. Third trimester amniotic fluid cells with the capacity to develop neural phenotypes and with heterogeneity among sub-populations

Daniele Bottai, Daniela Cigognini, Emanuela Nicora, Monica Moro, Maria Grazia Grimoldi, Raffaella Adami, Sergio Abrignani, Anna Maria Marconi, Anna Maria Di Giulio, Alfredo Gorio

Restorative neurology and Neuroscience, 2012

Abstract

Purpose: Our aim was the search for new sources of cells potentially useful for central nervous system regenerative medicine. Extra-embryonic tissues are promising sources of pluripotent stem cells. Among these, human second-trimester amniotic fluid (AF) contains cell populations exhibiting self-renewal capacity, multipotency and the expression of embryonic cell markers. **Methods:** Here we report the properties of the easily available third-trimester AF cells (AFCs).

Different cell types from 6 of 9 AF samples were separated, expanded, and characterized by assessing their morphological, proliferative, and differentiative properties. Results: All isolated cultures presented CD105, CD90 and CD73 mesenchymal markers, whereas they differed among themselves in CD117, CD146, CD31, NG2 and CD133 expression. Their doubling time and telomere length were conserved throughout many passages. Importantly, immunofluorescence and Real-time PCR showed that, during their proliferative state and differentiation, several cultures expressed neuronal and glial markers such as nestin, GFAP, β -tubulin III and neurofilament H indicating their potential attitude towards a neural fate. Indeed, these cells showed a rather poor capacity to differentiate in adipogenic and osteogenic lineages. Conclusions: In this work we report that cells with neural differentiation capability can be isolated from third-trimester AF, such properties could be useful for neuro-regenerative purposes.

49. Amniotic Mesenchymal Stem Cells: A New Source for Hepatocyte-Like Cells and Induction of CFTR Expression by Coculture with Cystic Fibrosis Airway Epithelial Cells

Valentina Paracchini, Annalucia Carbone, Federico Colombo, Stefano Castellani, Silvia Mazzucchelli, Sante Di Gioia, Dario Degiorgio, Manuela Seia, Laura Porretti, Carla Colombo, and Massimo Conese

Journal of Biomedicine and Biotechnology,
2012

Abstract

Cystic fibrosis (CF) is a monogenic disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, with lung and liver manifestations. Because of pitfalls of gene therapy, novel approaches for reconstitution of the airway epithelium and CFTR expression should be explored. In the present study, human amniotic mesenchymal stem cells (hAMSCs) were

isolated from term placentas and characterized for expression of phenotypic and pluripotency markers, and for differentiation potential towards mesoderm (osteogenic and adipogenic) lineages. Moreover, hAMSCs were induced to differentiate into hepatocyte-like cells, as demonstrated by mixed function oxidase activity and expression of albumin, alpha1-antitrypsin, and CK19. We also investigated the CFTR expression in hAMSCs upon isolation and in coculture with CF airway epithelial cells. Freshly isolated hAMSCs displayed low levels of CFTR mRNA, which even decreased with culture passages. Following staining with the vital dye CM-DiI, hAMSCs were mixed with CFBE41o- respiratory epithelial cells and seeded onto permeable filters. Flow cytometry demonstrated that 33–50% of hAMSCs acquired a detectable CFTR expression on the apical membrane, a result confirmed by confocal microscopy. Our data show that amniotic MSCs have the potential to differentiate into epithelial cells of organs relevant in CF pathogenesis and may contribute to partial correction of the CF phenotype.

50. Enhanced generation of retinal progenitor cells from human retinal pigment epithelial cells induced by amniotic fluid

Fatemeh Sanie-Jahromi, Hamid Ahmadieh, Zahra-Soheila Soheili, Maliheh Davari, Shima Ghaderi, Mozghan Rezaei Kanavi, Shahram Samiei, Abdolkhalegh Deezagi, Jalil Pakravesht, Abouzar Bagheri
BMC Research Notes, 2012

Abstract

Background - Retinal progenitor cells are a convenient source of cell replacement therapy in retinal degenerative disorders. The purpose of this study was to evaluate the expression patterns of the homeobox genes PAX6 and CHX10 (retinal progenitor markers) during treatment of human retinal pigment epithelium (RPE) cells with amniotic fluid (AF), RPE cells harvested from neonatal cadaver globes were cultured in a mixture of DMEM and Ham's F12 supplemented with 10% FBS. At different passages, cells were trypsinized and co-cultured with 30% AF obtained

from normal fetuses of 14–16 weeks gestational age.

Results - Compared to FBS-treated controls, AF-treated cultures exhibited special morphological changes in culture, including appearance of spheroid colonies, improved initial cell adhesion and ordered cell alignment. Cell proliferation assays indicated a remarkable increase in the proliferation rate of RPE cells cultivated in 30% AF-supplemented medium, compared with those grown in the absence of AF. Immunocytochemical analyses exhibited nuclear localization of retinal progenitor markers at a ratio of 33% and 27% for CHX10 and PAX6, respectively. This indicated a 3-fold increase in retinal progenitor markers in AF-treated cultures compared to FBS-treated controls. Real-time PCR data of retinal progenitor genes (PAX6, CHX10 and VSX-1) confirmed these results and demonstrated AF's capacity for promoting retinal progenitor cell generation.

Conclusion - Taken together, the results suggest that AF significantly promotes the rate of retinal progenitor cell generation, indicating that AF can be used as an enriched supplement for serum-free media used for the in vitro propagation of human progenitor cells.

51. Stem Cell Banking: Ethical and Policy Issues

N King

QScience Proceedings, 2012

Abstract

Much stem cell research and many stem cell-based interventions depend on the availability of numerous viable cell lines. Multipotent and pluripotent stem cell lines may be created from embryonic stem cells, but also from stem cells found in amniotic fluid, umbilical cord blood, and other non-embryonic sources. Stem cell banks could collect, store and share enough cell lines to make good HLA matches with the vast majority of the inhabitants of a country or region, and thus might be easier and less expensive to use widely than individually matched induced pluripotent stem cell lines.

The collection, storage and sharing of stem cells in and through biobanks raises a well-

known set of interesting ethical and policy issues for stakeholders: the individuals who provide their stem cells for banking, the biobanks that collect and store them, the investigators who use them for many types of research, and the patient-subjects who receive them in research studies or innovative interventions.

This presentation explores these ethical and policy issues, which include: informed consent, confidentiality and recontact, ownership and benefit-sharing, scope and control of future uses, innovation and the therapeutic misconception, and considerations of justice.

52. Mesenchymal Stem Cell Isolation and Expansion Methodology

Mario Ricciardi, Luciano Pacelli, Giulio Bassi, Francesco Bifari, Federico Mosna and Mauro Krampera

Stem cells and cancer stem cells, 2012

Abstract

Mesenchymal stem cells (MSCs) are adult non-hematopoietic stem cells originally isolated from bone marrow (BM) (Prockop, 1997), but they are virtually present and can be isolated from almost every tissue of the body (Da Silva et al., 2006), including peripheral blood (Roufosse et al., 2004). This evidence suggests that MSCs could be part of a mesenchymal-stromal cell system diffused throughout the body. The real in vivo counterpart of culture-expanded MSCs is still unknown; however, different Authors suggested that MSCs are a subgroup of vessel-lining pericytes that may contribute to vessel homeostasis by reacting to tissue damage with regenerative processes, locally modulating the inflammatory reaction, and entering systemic circulation to migrate according

to cytokine gradients (Crisan et al., 2008). The International Society of Cellular Therapy (ISCT) stated the following three criteria for the definition of MSCs after *in vitro* expansion (Dominici et al., 2006): (1) the adherence to plastic under standard tissue culture conditions; (2) the expression of a specific combination of cell surface markers; (3) the capability of multilineage differentiation under appropriate *in vitro* conditions. These criteria are necessary to overcome the problems due to the absence of MSC-specific cell surface markers, the high heterogeneity in terms of differentiation potential, and the similarities to fibroblasts displayed by isolated and expanded MSCs. Consequently, ISTC suggested to define MSCs as “Multipotent Mesenchymal Stromal Cells” instead of “Mesenchymal Stem Cells”. In this Chapter, MSC isolation, expansion and functional characterization will be discussed in details.

53. Autologous Stem Cells For Personalised Medicine

Weerapong Prasongchean, Patrizia Ferretti
New Biotechnology, 2012

Abstract

Increasing understanding of stemcell biology, the ability to reprogramme differentiated cells to a pluripotent state and evidence of multipotency in certain adult somatic stemcells has opened the door to exciting therapeutic advances as well as a great deal of regulatory and ethical issues. Benefits will come from the possibility of modelling human diseases and develop individualised therapies, and from their use in transplantation and bioengineering. The use of autologous stemcells is highly desirable, as it avoids the problem of tissue rejection, and also reduces ethical and regulatory issues. Identification of the most appropriate cell sources for different potential applications,

development of appropriate clinical grade methodologies and large scale well controlled clinical trials will be essential to assess safety and value of cell based therapies, which have been generating much hope, but are by and large not yet close to becoming standard clinical practice. We briefly discuss stemcells in the context of tissue repair and regenerative medicine, with a focus on individualised clinical approaches, and give examples of sources of autologous cells with potential for clinical intervention.

54. Human AB serum for generation of mesenchymal stem cells from human chorionic villi: comparison with other source and other media including platelet lysate

A. Poloni, G. Maurizi, F. Serrani, S. Mancini, G. Discepoli, A. L. Tranquilli, R. Bencivenga, P. Leoni

Cell Proliferation, Volume 45, Issue 1, pages 66–75, February 2012

Abstract

Objectives: We have investigated foetal mesenchymal stem cells (MSCs) obtained from first-trimester chorionic villi (CV) and second-trimester amniotic fluid (AF), comparing them to adult bone marrow-derived MSCs.

Materials and methods: We report on cell population growth in human allogeneic serum (HS) and platelet lysate (PL), immunophenotype, cytokine expression profile and immunoregulatory activity, of these foetal MSCs on stimulated peripheral blood mononuclear and lymphocyte

subpopulations.

Results: Chorionic villi cells grow rapidly in HS, with 20 populations doublings (PDs) after 59 days (six passages), and also in animal serum, with 27 PDs after 65 days (seven passages). PL allowed for expansion in 60% of the samples tested, although it was lower than in HS. HS supported an average of 40 PDs of expansion in 20% of AF cells after 90 days, whereas animal serum supported 28.5 PDs in 66 days. CV and AF cells inhibited proliferation of stimulated T lymphocytes, suppressing population growth of both CD4⁺ and CD8⁺ T subpopulations and sometimes also, CD19⁺ cells.

Conclusions: Our results indicate that CV would be an optimal source of MSCs with high expansion potential in a HS propagation system and immunoregulatory capacity of T and B lymphocytes. More than 90% of CV samples achieved large-scale expansion in HS, which is encouraging for potential clinical applications of these cells.

55. Amnion Epithelial Cells as a Candidate Therapy for Acute and Chronic Lung Injury

Ryan J. Hodges, Rebecca Lim, Graham Jenkin, and Euan M. Wallace

Stem Cells International, 2012

Abstract

Acute and chronic lung injury represents a major and growing global burden of disease. For many of these lung diseases, the damage is irreparable, exhausting the host's ability to regenerate new lung, and current therapies are simply supportive rather than restorative. Cell-based therapies offer the promise of tissue regeneration for many organs. In this paper, we examine the potential application of amnion epithelial cells, derived from the term placenta, to lung regeneration. We discuss their unique properties of plasticity and immunomodulation, reviewing the experimental evidence that amnion epithelial

cells can prevent and repair lung injury, offering the potential to be applied to both neonatal, childhood, and adult lung disease. It is amazing to suggest that the placenta may offer renewed life after birth as well as securing new life before.

56. Renal differentiation of amniotic fluid stem cells: perspectives for clinical application and for studies on specific human genetic diseases

Margit Rosner, Katharina Schipany, Claudia Gundacker, Bharanidharan Shanmugasundaram, Kongzhao Li, Christiane Fuchs, Gert Lubec, Markus Hengstschläger

European Journal of Clinical Investigation, 2011

Abstract

Background Owing to growing rates of diabetes, hypertension and the ageing population, the prevalence of end-stage renal disease, developed from earlier stages of chronic kidney disease, and of acute renal failure is dramatically increasing. Dialysis and preferable renal transplantation are widely applied therapies for this incurable condition. However these options are limited because of morbidity, shortage of compatible organs and costs. Therefore, stem cell-based

approaches are becoming increasingly accepted as an alternative therapeutic strategy.

Design This review summarizes the current findings on the nephrogenic potential of amniotic fluid stem (AFS) cells and their putative implications for clinical applications and for studies on specific human genetic diseases.

Results Since their discovery in 2003, AFS cells have been shown to be pluripotent with the potential to form embryoid bodies. Compared to adult stem cells, induced pluripotent stem cells or embryonic stem cells, AFS cells harbour a variety of advantages, such as their high differentiation and proliferative potential, no need for ectopic induction of pluripotency and no somatic mutations and epigenetic memory of source cells, and no tumourigenic potential and associated ethical controversies, respectively.

Conclusions Recently, the results of different independent studies provided evidence that AFS cells could indeed be a powerful tool for renal regenerative medicine.

57. Human amniotic fluid stem cell preconditioning improves their regenerative potential

Cinzia Rota, Barbara Imberti, Michela Pozzobon, Martina Piccoli, Paolo De Coppi, Anthony Atala, Elena Gagliardini, Christodoulos Xinaris, Valentina Benedetti, Aline S.C. Fabricio, Elisa Squarcina, Mauro Abbate, Ariela Benigni, Giuseppe Remuzzi, and Marina Morigi.

Stem Cells and Development, 2011

Abstract

Human amniotic fluid stem (hAFS) cells, a novel class of broadly multipotent stem cells that share characteristics of both embryonic and adult stem cells, have been regarded as promising candidate for cell therapy. Taking advantage by the well-established murine model of acute kidney injury (AKI), we studied the proregenerative effect of hAFS cells in immunodeficient mice injected with the nephrotoxic drug cisplatin. Infusion of hAFS cells in cisplatin mice improved renal function and

limited tubular damage, although not to control level, and prolonged animal survival. Human AFS cells engrafted injured kidney predominantly in peritubular region without acquiring tubular epithelial markers. Human AFS cells exerted antiapoptotic effect, activated Akt, and stimulated proliferation of tubular cells possibly via local release of factors, including interleukin-6, vascular endothelial growth factor, and stromal cell-derived factor-1, which we documented in vitro to be produced by hAFS cells. The therapeutic potential of hAFS cells was enhanced by cell pretreatment with glial cell line-derived neurotrophic factor (GDNF), which markedly ameliorated renal function and tubular injury by increasing stem cell homing to the tubulointerstitial compartment. By in vitro studies, GDNF increased hAFS cell production of growth factors, motility, and expression of receptors involved in cell homing and survival. These findings indicate that hAFS cells can promote functional recovery and contribute to renal regeneration in AKI mice via local production of mitogenic and prosurvival factors. The effects of hAFS cells can be remarkably enhanced by GDNF preconditioning

58. Human Amniotic Fluid-Derived Mesenchymal Stem Cells As Therapeutic Vehicles: A Novel Approach For the Treatment of Bladder Cancer

Vasiliki Bitsika, Maria G. Roubelakis, Dimitra Zagoura, Ourania Trohatou, Manousos Makridakis, Kalliopi I. Pappa, Frank C. Marini, Antonia Vlahou, and Nicholas P. Anagnostou.

Stem Cells and Development. May 1, 2012

Abstract

Recent studies support cell-based therapies for cancer treatment. An advantageous cell type for such therapeutic schemes are the mesenchymal stem cells (MSCs) that can be easily propagated in culture, genetically modified to express therapeutic proteins, and exhibit an innate tropism to solid tumors in vivo. Recently, we successfully isolated and expanded MSCs from second-trimester amniotic fluid (AF-MSCs). The main characteristic of AF-MSCs is their efficient and rapid expansion in vitro. Herein, we investigated the AF-MSCs tropism and capability to transport

interferon beta (IFN β) to the region of neoplasia in a bladder tumor model. To this end, we used the T24M bladder cancer cell line, previously generated from our studies, and developed a disease progression model in immunosuppressed mice, that can recapitulate the molecular events of bladder carcinogenesis. Our results documented that AF-MSCs exhibited high motility, when migrated either to T24M cells or to T24M-conditioned medium, and we further identified and studied the secreted factors which may trigger these enhanced migratory properties. Further, lentivirus-transduced AF-MSCs, expressing green fluorescent protein (GFP) or IFN β , were intravenously administered to T24M tumor-bearing animals at multiple doses to examine their therapeutic effect. GFP- and IFN β -AF-MSCs successfully migrated and colonized at the tumor site. Notably, significant inhibition of tumor growth as well as prolonged survival of mice were observed in the presence of IFN β -AF-MSCs. Collectively, these results document the great potential of AF-MSCs as anti-cancer vehicles, implemented by the targeting of the tumor site and further facilitated by their high proliferation rate and expansion efficiency in culture.

59. The potential use of stem cells derived from human amniotic fluid in renal diseases

Irene L Noronha, Rita C Cavaglieri, Felipe L Janz, Sergio A Duarte, Marco A B Lopes, Marcelo Zugaib and Sergio P Bydlowski
Kidney International Supplements 1, 77-82
(September 2011)

Abstract

Amniotic fluid (AF) contains a variety of cell types derived from fetal tissues that can easily grow in culture. These cells can be obtained during amniocentesis for prenatal screening of fetal genetic diseases, usually performed during the second trimester of pregnancy. Of particular interest, some expanded sub-populations derived from AF cells are capable of extensive self-renewal and maintain prolonged undifferentiated proliferation, which are defining properties of stem cells. These human AF stem cells (hAFSCs) exhibit multilineage potential and can differentiate into the three germ layers. They have high proliferation rates and express mesenchymal and

embryonic markers, but do not induce tumor formation. In this study, hAFSCs derived from amniocentesis performed at 16–20 weeks of pregnancy were isolated, grown in culture, and characterized by flow cytometry and by their potential ability to differentiate into osteogenic, adipogenic, and chondrogenic lineages. After 4–7 passages, 5×10^5 hAFSCs were inoculated under the kidney capsule of Wistar rats that were subjected to an experimental model of chronic renal disease, the 5/6 nephrectomy model (Nx). After 30 days, Nx rats treated with hAFSCs displayed significant reductions in blood pressure, proteinuria, macrophages, and α -smooth muscle actin expression compared with Nx animals. These preliminary results suggest that hAFSCs isolated and expanded from AF obtained by routine amniocentesis can promote renoprotection in the Nx model. Considering that the AF cells not used for fetal karyotyping are usually discarded, and that their use does not raise ethical issues, they may represent an alternative source of stem cells for cell therapy and regenerative medicine.

60. Clinical applications of prenatal and postnatal therapy using stem cells retrieved from amniotic fluid

SWS Shaw, Sheng Wen S, David, Anna L; De Coppi, Paolo

Current opinion in Obstetrics and Gynecology, 2011

Abstract

Purpose of review: To review the potential of stem cells derived from amniotic fluid and applications in prenatal and postnatal therapy. Recent findings: We have recently described that pluripotent stem cells can be isolated from amniotic fluid defined as amniotic fluid stem (AFS) cells by selection for expression of the membrane stem cell factor receptor c-Kit. AFS cells maintained for over 250 population doublings retained long telomeres and normal karyotype. Clonal human lines verified by retroviral marking were induced to differentiate into cell types representing

each embryonic germ layer, including adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages. Rat AFS cells have been able to improve the repair of damaged smooth muscle in cryoinjury bladders. Furthermore, AFS cells could be differentiated toward cardiomyogenic lineages, when co-cultured with neonatal cardiomyocytes and have potential to generate hematopoietic lineages both *in vitro* and *in vivo*. These cells have been applied into fetal therapy, and widely used for tissue repair in animal models. Finally, we demonstrated a feasible way to do in-utero autologous AFS transplantation in sheep.

Summary: Stem cells derived from amniotic fluid are a relatively new source of cells that could have a therapeutic value in various diseases prenatally and/or postnatally.

61. Effects of mesenchymal stem cells isolated from amniotic fluid and platelet-rich plasma gel on severe decubitus ulcers in a septic neonatal foal

E. Iacono, B. Merlo, A. Pirrone, C. Antonelli, L. Brunori, N. Romagnoli, C. Castagnetti
Research in Veterinary Science, 2012 - Elsevier

Abstract

This paper documents the treatment of severe decubitus ulcers with amniotic fluid mesenchymal stem cells and platelet rich plasma (PRP) gel in a septic neonatal foal. The colt needed 25 days of hospitalization: during this period ulcers were treated for 15 days with mesenchymal stem cells (MSCs) plus PRP, PRP gel alone, or aloe gel. Healing was faster using MSCs + PRP, and at 7 months an ulcer treated with aloe gel was still not completely healed.

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Edited by Biocell Center group

Thanks to:

Prof. Giuseppe Simoni

Dr. Fabio Frattini

Dr. Emanuele Lanteri

Dr. Federico Maggi

Ing. Marco Reguzzoni

Dr. Edoardo Borgo

Dr. Massimiliano Manganini

Dr.a Federica Favaretto

Dr.a Maria Elena Colombo