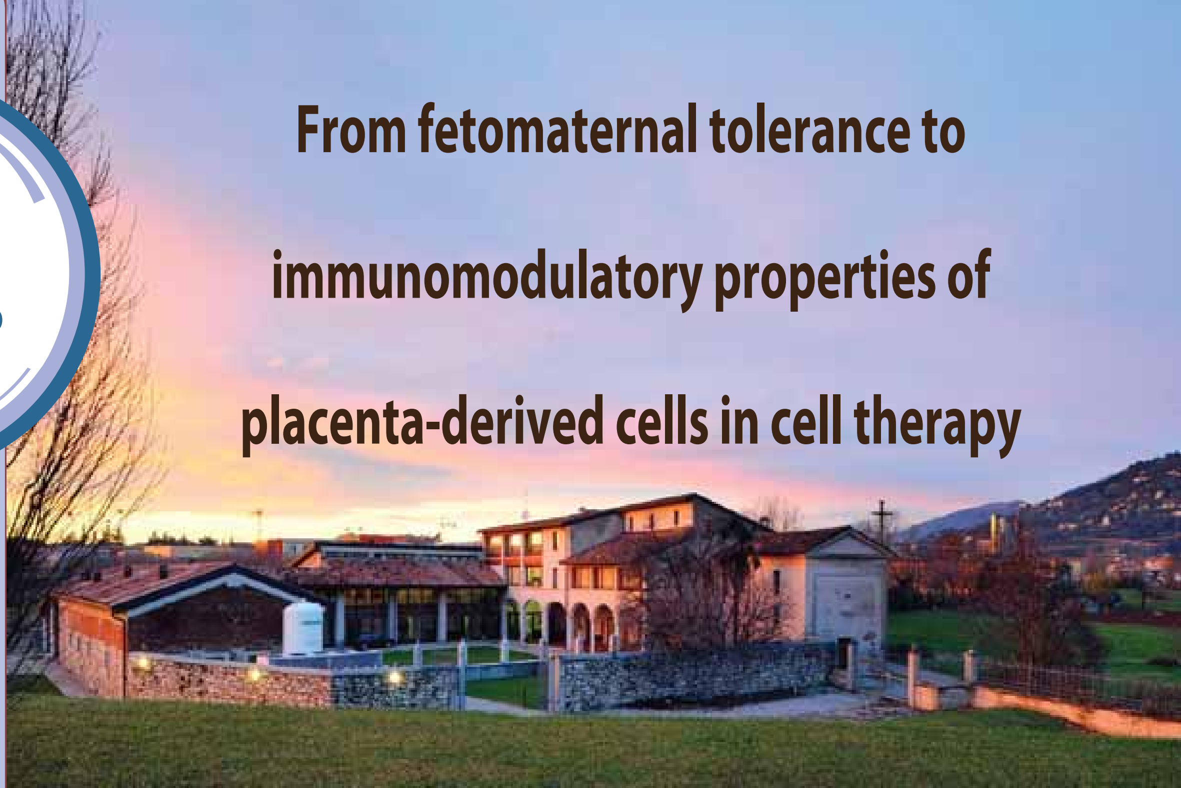
EMBO Workshop

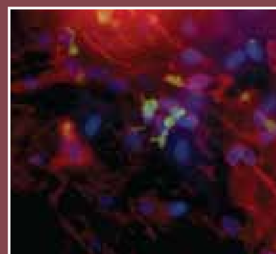
From fetomaternal tolerance to immunomodulatory properties of placenta-derived cells in cell therapy

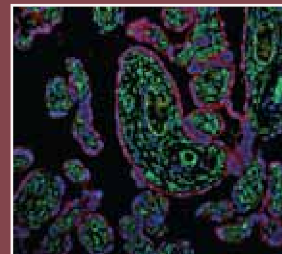


3 - 6 October | 2010 | Brescia | Italy

First meeting of the International Placenta Stem Cell Society (IPLASS)

PROGRAMME & ABSTRACT BOOK





http://cwp.embo.org/w10-33

EMBO European Molecular Biology Organization

Welcome from the Scientific Organizer

Firstly, I would like to dedicate a few words in extending a very warm welcome to all those who have made the journey to Brescia, Italy, for this important event.

As the President of the International Placenta Stem Cell Society (IPLASS), I am very pleased to announce that this meeting also marks the first official appointment of IPLASS, which was recently formed by a group of international scientists who were driven by a shared desire to consolidate placental stem cell research at the international level. Indeed, this meeting follows on in many ways from two previous meetings on placenta-derived cells, held in 2007 and 2009, which also took place here at Centro di Ricerca E. Menni.

This meeting will allow us to begin with a basic view of the placenta as an immunoprivileged organ essential for maintaining fetomaternal tolerance, and to then extend on this by exploring the characteristics of the specific cell types contained within this organ that would make them applicable for cell therapy approaches. This will be fostered by the presence of both scientists who study basic immunology as well as those who specialize in placenta-derived cells, which I believe will form the basis of a unique opportunity for valuable scientific cross-exchange.

The abstracts which are published in this booklet will also be displayed on the IPLASS website at www.iplass.org. I am very pleased to announce that we will also have the opportunity to publish materials from this meeting in a special dedicated issue of the journal Placenta, and I believe that this serves as testament to the growing interest and recognition from the broader scientific community regarding the importance of this field of research.

As the President of IPLASS, I hope that as many of you as possible may join this new Society and provide your own input on how we may work together to consolidate research into placenta-derived cells. Meanwhile, I also invite the participation of companies with expertise in translation of basic research to clinical application in order to help us achieve our ultimate goal of assisting patients through therapeutic approaches based on the use of placenta-derived cells.

Sincerely,

Ornella Parolini

Brescia, October 3rd 2010

INFORMATION

Venue

Main Meeting Venue

Sala Congressi
Fondazione Poliambulanza – Istituto Ospedaliero
Via Bissolati, 57
25124 Brescia, Italy

Poster Session Venue

Main Corridor
Centro di Ricerca E. Menni
Via Romiglia, 4
25124 Brescia, Italy

Scientific Organization

Meeting Chair

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Ursula Manuelpillai (Australia)
Heinz Redl (Austria)
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SPONSORS

Platinum contributions:

Silver contributions:

Bronze contributions:

LIST OF SPEAKERS — *Abstract*

Francesco Alviano — *pag. 46*
University of Bologna
Bologna, Italy

Alexander G. Betz — *pag. 19*
Medical Research Council
Cambridge, UK

Diana W. Bianchi — *pag. 12*
Tufts University School of Medicine
Floating Hospital for Children at Tufts Medical Center
Boston, USA

Cesar V. Borlongan — *pag. 49*
University of South Florida College of Medicine
Tampa, USA

Cecilia Götherström — *pag. 16*
Karolinska University Hospital Huddinge
Stockholm, Sweden

Ursula Manuelpillai — *pag. 38*
Monash University
Clayton, Australia

Andrew L. Mellor — *pag. 21*
Medical College of Georgia
Augusta, USA

Racheli Ofir — *pag. 34*
Pluristem Therapeutics Inc.
Haifa, Israel

Ornella Parolini — *pag. 26*
Centro di Ricerca E. Menni
Brescia, Italy

Peter Ponsaerts — *pag. 43*
University of Antwerp
Wilrijk, Belgium

Sicco Scherjon *pag. 23*
Leiden University Medical Center,
Leiden, The Netherlands

Stephen Strom *pag. 36*
University of Pittsburgh
Pittsburgh, USA

Mark L. Weiss *pag. 29*
Kansas State University
Manhattan, USA

Susanne Wolbank *pag. 32*
Ludwig Boltzmann Institute for clinical and experimental Traumatology
AUVA research center
Austrian Cluster for Tissue Regeneration
Vienna, Austria

Kathryn J. Wood *pag. 14*
University of Oxford
Oxford, UK

WORKSHOP PROGRAMME

Sunday, October 3rd 2010

10:00	***Registration and poster setup at Poster Session Venue***
12:00	*Panini & Pizza*
14:30 - 16:30	***Meet the experts***
	Concurrently:
14:30 - 16:30	***IPLASS Board Meeting*** *(closed to the IPLASS Board of Directors)*
16:30	*Shift to Main Meeting Venue*
17:00	***Opening and Welcome Remarks of the Meeting Chair*** IPLASS President, **Ornella Parolini**
17:30 - 18:30	**Diana W. Bianchi** (USA) OPENING KEYNOTE LECTURE: Fetal cells in the adult female following pregnancy: an under-appreciated source of progenitor cells.
19:30	*Welcome cocktail with cheese and wine*

Monday, October 4th 2010

Session I - Immunology of transplantation
Chairs: *Alexander Betz and Andrew Mellor*

9:00 - 9:45	**Kathryn J. Wood** (UK) Translating transplantation tolerance in the clinic: where are we, where do we go?
9:45 - 10:30	**Cecilia Götherström** (Sweden) Immunomodulation by mesenchymal stem cells and clinical experience.
10:30 – 11:00	*Coffee break*

Session II - Natural/physiological mechanisms to escape graft rejection: fetomaternal tolerance
Chairs: *Kathryn Wood and Cecilia Götherström*

11:00 - 11:45	**Alexander G. Betz** (UK) Regulatory T cells in pregnancy.
11:45 - 12:30	**Andrew L. Mellor** (USA) Indoleamine 2,3 dioxygenase (IDO): a pivotal counter-regulatory switch at sites of inflammation.
12:30 - 13:00	**Sicco Scherjon** (The Netherlands) MSC and the possible role in fetomaternal tolerance: a paradigm for transplantation tolerance.
13:00 – 14:00	*Lunch at Poster Session Venue*
14:00 – 15:00	*Coffee with Poster Session and Stand Displays by Sponsor Companies involved in banking and cell therapy*
15:00	*Return to Main Meeting Venue.*

Session III - Potential and immunological characteristics of cells from different placental region

Chairs: *Heinz Redl and Stephen Strom*

15:30 - 16:15 **Ornella Parolini** (Italy)
Placenta generalities: structure and immunomodulatory properties - *in vitro* and *in vivo* studies.

16:15 - 17:00 **Mark L. Weiss** (USA)
Wharton's jelly mesenchymal stromal cells (WJCs) as immunoregulators in allogeneic transplantation.

Tuesday, October 5th 2010

Chairs: *Cesar Borlongan and Ursula Manuelpillai*

9:00 - 9:30 **Susanne Wolbank** (Austria)
Suitability of amniotic membrane and cells thereof for tissue regeneration approaches.

9:30 - 10:00 **Racheli Ofir** (Israel)
Placenta derived adherent stromal cells for the treatment of Critical Limb Ischemia (CLI)-Lessons from first clinical trial.

10:00 - 11:00 **Selected Oral Presentations:**
Eissner G. *(pag. 68)*
Gramignoli R. *(pag. 72)*
Heazlewood C. *(pag. 74)*
König J. *(pag. 76)*

11:00 – 11:30 *Coffee break*

11:30 - 13:00 **Selected Oral Presentations:**
La Rocca G. *(pag. 82)*
Lee Y. *(pag. 84)*
Pozzobon M. *(pag. 96)*
Weber B. *(pag. 114)*

13:00 – 14:30 *Lunch with Poster Session and Stand Displays by Sponsor Companies involved in banking and cell therapy*

15:00 Afternoon social programme followed by networking dinner

Wednesday, October 6th 2010

Session IV - Cell therapy approaches: where do we stand?
Chairs: *Mark Weiss and Daniel Surbek*

9:00 - 9:30	**Stephen Strom** (USA) Amnion-derived cells to treat liver disease.
9:30 - 10:00	**Ursula Manuelpillai** (Australia) Human amnion epithelial cells: a cellular therapy for inflammatory diseases?
10:00 - 10:30	**Peter Ponsaerts** (Belgium) Physiological comparison of autologous and allogeneic cell implantation in the central nervous system: defining and regulating immune cell activity against mesenchymal and neural stem cell grafts.
10:30 - 11:00	**Selected Oral Presentations:** Olivares E. *(pag. 90)* Taghizadeh R. *(pag. 110)*
11:00 - 11:30	*Coffee break*
11:30 - 12:00	**Francesco Alviano** (Italy) Amniotic membrane-derived stem cells and pancreatic islet-cell differentiation.
12:00 - 12:30	**Cesar V. Borlongan** (USA) Cell therapy for stroke: towards clinical application of Celgene human placenta-derived cells.
12:30 – 14:00	*Lunch with panini and pizza*
14:00	**ROUND TABLE: *Discussion on applications of placenta-derived cells with young researchers as panellists*.** **Looking toward the next meeting, message on behalf of the IPLASS board.**
16:00	**Closing address** and farewell drinks with **Prizes for best oral and poster presentations** to be presented by representatives of the awarding Company or Institution.

ABSTRACTS

-Lectures-

Fetal cells in the adult female following pregnancy: an under-appreciated source of progenitor cells

Bianchi DW.

Tufts University School of Medicine, Boston, MA, USA

As a result of pregnancy, all human females acquire progenitor cells from their fetuses. These cells and their progeny survive for decades post-partum in maternal blood and organs (Bianchi et al. PNAS 1996; 93: 705-8). Significant knowledge gaps exist as to whether these fetal cells have characteristics of embryonic or adult stem cells, and whether they have po sitive, negative, or neutral effects on the health of the mother. This is important, because these fetal cells may have unique therapeutic properties in maternal organs. Furthermore, the long-term presence of fetal cells in women may have broader significance, in that they may be an important factor in diseases that manifest gender-related differences. The long-range goal of my laboratory is to understand the consequences of feto-maternal cell trafficking to determine if pregnancy results in long-term beneficial health effects for the mother. Our central hypothesis is that fetal cells have phenotypically and functionally unique properties that can be developed and applied for novel therapeutic purposes. We are specifically testing this hypothesis by examining whether fetal cells contribute to repair of or recovery from tissue injury in the maternal lung in a murine model system. This organ was selected on the basis of our significant and reproducible murine data that demonstrate that the lung is the maternal organ that contains the most fetal cells. We are currently in the process of determining key factors that distinguish fetal cells from adult cells in maternal organs using immunohistochemistry and gene expression studies.

Notes

Translating transplantation tolerance in the clinic: where are we, where do we go?

Wood K.

Transplantation Research Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK.

Strategies for the induction of specific unresponsiveness to donor alloantigens currently under investigation in the clinic take advantage of two of the major mechanisms for the induction of tolerance to self antigens – deletion and immunoregulation/suppression. Deletion of donor antigen reactive cells from the immune repertoire of the recipient will potentially prevent or reduce the risk of the transplanted organ or cells from being attacked and rejected. Whereas, active control of immune responsiveness to donor antigens after transplantation by regulatory/suppressor T cells will enable tight control of the response directed against the transplant throughout the post-transplant course.

After exposure to alloantigen in vivo and in vitro, alloantigen reactive immunoregulatory activity is enriched in a population of CD4+ T cells that express high levels of CD25, the α chain of the interleukin-2 receptor, and the transcription factor Foxp3. In vivo, common mechanisms underpin the activity of CD25+CD4+ Treg in both naive and manipulated adult hosts. We have identified a unique role for IFNγ in the functional activity of CD25+CD4+ alloantigen reactive Treg during the development of operational tolerance to donor alloantigens in vivo that is consistent with observations showing that tolerance to alloantigens cannot be induced in the absence of IFNγ.

The identification and characterisation of Treg that can control immune responsiveness to alloantigens has opened up exciting opportunities for new therapies in transplantation. These observations may have important implications for the design of clinical protocols to induce allograft tolerance in adult recipients.

Notes

Immunomodulation by mesenchymal stem cells and clinical experience

Götherström C.

Karolinska Institutet, CLINTEC, Karolinska University Hospital Huddinge, Stockholm, Sweden

Finding a suitable cell source is one of the main challenges in tissue engineering. Besides fulfilling the function of the reconstructed tissue, low immunogenicity is advantageous. Promising candidates are multipotent mesenchymal stromal cells, also known as mesenchymal stem cell (MSC), because of their low immunogenicity, differentiation potential and their capacity to be extensively expanded in culture in combination with a low oncogeneic risk *in vivo*. They differentiate into several mesodermal cell types, such as bone, cartilage and fat, but also into non-conventional mesenchymal lineages such as myogenic, endothelic, hepatic and neurogenic. In immunocompetent allogeneic/xenogeneic animal models, MSC engraft widely and demonstrate site-specific differentiation.
MSC also produce important cytokines, growth factors and extra cellular components. *In vitro*, MSC induce little, if any, proliferation of allogeneic lymphocytes. MSC also inhibit T-cell proliferation to alloantigens and mitogens and prevent the development of cytotoxic T-cells. *In vivo*, they prolong skin allograft survival and have several immunomodulatory effects. MSC preferentially seem to home to damaged tissue and therefore have therapeutic potential.

Stem cells are present at various stages of development, from the inner cell mass through fetal and finally, adult sources. MSC from the adult bone marrow are widely used in therapy today, as for example in the treatment of therapy-resistant severe acute graft-versus-host disease, where they have much potential.

The identification of MSC in the human fetus raises the possibility of using a more primitive MSC population for transplantation purposes. Fetal MSC, like adult MSC, escape recognition by the immune system in vitro suggesting that they are not inherently immunogenic. Fetal MSC differ from adult MSC in some aspects, which may be advantageous for transplantation. They demonstrate marked expansive capacity, and cycle faster than adult MSC, having a doubling time of 30 hours over 20 passages (50 population doublings) without expressing a differentiated phenotype. Like their adult counterparts, fetal MSC possess the ability to differentiate into at least 3 different mesenchymal tissues: bone, cartilage, fat. More recent work suggests that they are

more primitive than adult MSC, having longer telomeres and expressing embryonic pluripotency markers such as nanog and Oct4, differentiating more readily into bone and outside conventional lineage boundaries into skeletal muscle, and oligodendrocytes. Finally, it has been shown that there is an engraftment advantage of fetal liver compared to adult bone marrow cells in fetal recipients. *In utero* transplantation of human fetal MSC has shown promising results in mouse models of osteogenesis imperfect and Duchenne muscular dystrophy.

This characteristic of MSC is the basis for their extensive potential for tissue repair and in other indications like therapy-resistant severe acute graft-versus-host disease, treatment of rejection of organ allografts and in autoimmune disorders.

Notes

Regulatory T cells in pregnancy

Betz AG.

Medical Research Council, Cambridge, UK.

Regulatory T cells play a crucial role in maternal fetal tolerance. Immediately after conception, their number in the blood, spleen and uterus-draining lymph nodes substantially increases. Whilst this initial expansion is independent of exposure to paternal alloantigen, their accumulation in the gravid uterus appears to be constrained to alloantigen specific regulatory T cells. Clearly, the role of this shift in the composition and distribution of the T cell populations is the suppression of an extremely aggressive allogeneic response directed against the fetus. However, in women suffering from certain autoimmune diseases, such as arthritis, this also correlates with a marked improvement in symptoms. Unfortunately, the remission only lasts until delivery when the disease invariably returns with a vengeance. Observations of regulatory T cell behavior during pregnancy provide valuable hints regarding the use of regulatory T cells for therapeutic purposes and might explain why the transfer polyclonal regulatory T cells is likely to be insufficient to stop autoimmune diseases.

Notes

Indoleamine 2,3 dioxygenase (IDO): a pivotal counter-regulatory switch at sites of inflammation

Mellor AL.

Immunotherapy Center, Medical College of Georgia, Augusta GA. USA.

Indoleamine 2,3 dioxygenase (IDO) is a conserved, intracellular enzyme that catabolizes the amino acid tryptophan. IDO is induced in settings of inflammation associated with disease syndromes including cancer, autoimmune, and infectious diseases. IDO activity attenuates immune responses to create immune privilege. IDO-mediated counter-regulation may have evolved to suppress immunity to self-antigens and innocuous agents and commensal pathogens encountered at mucosal surfaces. Thus IDO is a promising therapeutic target to manipulate immunity in a range of inflammatory disease syndromes.

Pharmacologic inhibition of IDO activity during pregnancy in mice led to fetal rejection by maternal T cells. These findings identified IDO as a pivotal regulator of T cell immunity. Genetic ablation of IDO did not drive fetal rejection. Thus IDO-mediated suppression is the dominant mechanism that protects fetal allografts, but other mechanisms can substitute for developmental loss of IDO. The identity of IDO-expressing cells that inhibit T cell responses during pregnancy is unknown. Several cell types can express IDO such as tumor cells, stromal cells, and some plasmacytoid/myeloid cell types. In humans and mice small subsets of dendritic cells (DCs) with distinctive phenotypes are competent to express IDO in response to inflammatory stimuli such as TLR and NK cell ligands. When induced to express IDO DCs suppress T cell responses and promote differentiation and functions of regulatory T cells. In mice IDO-competent DCs exhibit a unique set of phenotypic attributes that overlap with those of B cells and plasmacytoid DCs. Tumor growth and tumor promoters stimulate DCs in local lymph nodes to express IDO and dominantly suppress natural and vaccine induced anti-tumor immunity. Some pathogens also stimulate DCs to express IDO and attenuate pathogen-specific immunity. IDO ablation - especially when combined with immunization strategies - increases pro-inflammatory cytokine expression and enhances T cell immunity to tumors and pathogens. Thus IDO is a pivotal regulator of T cell immunity to tumors and pathogens that promote cancer and chronic infections. Current developments in understanding the role of IDO during pregnancy, and in the context of manipulating IDO to improve therapy will be discussed.

Notes

MSC and the possible role in fetomaternal tolerance: a paradigm for transplantation tolerance

Scherjon S.

Department of Obstetrics, Leiden University Medical Center, Leiden, The Netherlands

MSCs are multipotent, non-hematopoietic cells, capable of differentiating into cells of the connective lineage such as adipose tissue, marrow stroma, cartilage, smooth muscle, cardiomyocytes, tendon and bone. In fetal tissues, MSC have been derived from many different sources: first trimester (bone marrow, blood and liver) and second trimester. Also term UCB contains MSCs. Growth characteristics of maternal (mMSCs) and fetal MSC (fMSCs) do not differ. Recently, it was shown that from both first trimester and term placenta MSC can be. There is no unique phenotype for MSC.
When MSC were shown to engraft even in immunocompetent sheep fetuses, it was also realized that they might have immunomodulatory properties. This is partly explained because MSC do not express HLA class II and co-stimulatory molecules (B7-1 and B7-2). In co-culture experiments, human MSC do not induce proliferation of allogenic lymphocytes, neither after the addition of CD28-stimulating antibodies nor after transfection of MSC with the co-stimulatory molecules B7-1 or B7-2. This possibility of reducing immunological rejection was shown in vitro, whereby both autologous and allogenic MSC inhibited the mixed lymphocyte reaction (MLR). Suppression of the proliferation in MLR occurred after the addition of both fMSCs and mMSCs (in a dose-dependent fashion, more profound inhibition by fMSCs). This suppression is independent from the sources (rodent, baboon or human) used.

The mechanism for suppression is also MHC independent and is most probably mediated by a soluble factor, at least in some systems, cell–cell contact is not needed. MLR with the addition of maternal or fMSCs was performed in transwell cultures, whereby the inhibitory effect of maternal and fMSCs was only partially abrogated. Suppression might rely on the secretion of certain cytokines. Neutralization of one of the potentially inhibitory cytokines IL-10, by the addition of anti-IL-10 could not abrogate the inhibitory effect of mMSCs and fMSCs in normal MLR cultures. In the transwell cultures, the inhibitory effect of fMSCs was abrogated by the addition of anti-IL-10. Recently, the possibility was raised that MSC induce regulatory antigen presenting cells (APCs; deletional APCs) or regulatory T cells The modulatory effect of

MSC could also rely on an inhibition of monocyte derived myeloid dendritic cells (DC) differentiation, while plasmacytoid DCs increased their production of IL-10. Also the production of 2,3-dioxygenase (IDO) by MSC could have an inhibiting effect on T cells.

The possibility of modifying the immune response because of an antiproliferative, immunomodulatory effect might make these cells of potential clinical relevance in GvHD and in the prevention of solid organ rejection.

Notes

Placenta generalities: structure and immunomodulatory properties - *in vitro* and *in vivo* studies

Parolini O.

Centro di Ricerca E. Menni, Fondazione Poliambulanza – Istituto Ospedaliero, Brescia, Italy.

At a glance, the placenta is generally recognised for the important functions which it carries out during the beginnings of life, such as nutrition, respiration and excretion for the developing embryo, as well as maintenance of fetomaternal tolerance. Closer consideration reveals the different regions which make up this organ - the fetal membranes, umbilical cord and trophoblast. However, scientists are now becoming more and more fascinated by the cell types which can be isolated from these different regions. Indeed, in recent years, a growing wealth of knowledge on the biology and properties of these cells has resulted in ever-increasing expectations regarding their possible use for cell-based therapeutic purposes.

It is now well-established that cell-based strategies are conceived mainly for two therapeutic approaches: for “regeneration”, i.e. treatments in which transplanted cells engraft into host tissues and differentiate into specific cell type(s) required to replace defective, necrotic or apoptotic cells; and for “repair”, i.e. treatments in which transplanted cells produce bioactive soluble molecules that modulate the local host environment and induce endogenous cells, or trigger a cascade of endogenous events, which lead to restoration of damaged tissues.

Through *in vitro* studies, we have previously shown that Amniotic Membrane (AM)-derived cells fail to induce an allogeneic T-cell response and actively suppress T-cell proliferation induced by alloantigens or by a mitogenic stimulus. Furthermore, we demonstrated that AM-derived cells can block differentiation and maturation of monocytes into dendritic cells both at the phenotypic and at functional levels, by impairing the allostimulatory ability of these cells on allogeneic T cells.

Through *in vivo* studies, we have demonstrated that cells isolated from the human amnion and chorion can successfully engraft long-term in newborn swine and rats, with human microchimerism detected in several organs, therefore indicating active tolerance of these cells.

Taken together, these results encouraged us to test the potential therapeutic effects of placenta-derived cells in pre-clinical animal models of different diseases.

First, we observed that intra-tracheal and intra-peritoneal transplantation of either allogeneic or xenogeneic fetal membrane-derived cells reduces bleomycin-induced lung fibrosis in mice. We also demonstrated that the application of a human AM fragment as a patch onto ischemic rat hearts significantly reduces post-ischemic cardiac injury. Indeed, the AM-treated rats showed higher preservation of cardiac dimensions and improved cardiac contractile function in terms of higher left ventricle ejection fraction, fractional shortening, and wall thickening, over a two month follow-up period. Finally, we recently provided evidence that human AM, when applied as a patch onto the liver surface, significantly reduces the severity and slows the progression of liver fibrosis in a rat model of bile duct ligation.

In all of these pre-clinical settings, the beneficial effects were observed despite a rare or absent level of detection of placenta-derived cells in host tissues. Therefore, it is conceivable that the effects exerted by the placenta-derived cells might not be due to a “regeneration” process, in which transplanted cells differentiate toward specific host cell types, but more likely, to paracrine actions exerted on host tissues by bioactive molecules secreted by these cells, therefore promoting a “repair” process.

Although identification of the specific paracrine bioactive molecules produced by placenta-derived cells and the target cells of these molecules remain to be explored, our results collectively reinforce the hypothesis that placenta constitutes a cell source with clinical potential. Furthermore, these studies also suggest that a cell-free treatment based on the use of culture medium conditioned by placenta-derived cells may represent a novel therapeutic strategy, which could potentially replace cell transplantation.

Notes

Wharton's jelly mesenchymal stromal cells (WJCs) as immunoregulators in allogeneic transplantation

Lopez-Rodriguez Y., Trevino E., Weiss ML.

Dept of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, USA

Mesenchymal stromal cells (MSCs) derived from bone marrow, adipose tissue or amnionic membrane have generated much interest for their therapeutic potential in cellular therapy and tissue engineering. MSCs have the properties of plastic adherence, self renewal and the ability to be differentiated into bone, fat and cartilage in vitro and by characteristic surface marker staining pattern. MSCs are in clinical trials and have been tested in myocardial infarction, tissue engineering, and immune disorders. Since it appears that bone marrow derived MSCs may lose therapeutic potency due to the natural ageing processes or due to disease, it would be advantageous to have an alternative source of MSCs. Several groups, including my lab, have examined components of the umbilical cord as such a source of MSCs. Here, we will discuss the factors which may affect MSCs for therapeutic use and focus on MSC derived from umbilical cord. The umbilical cord and amnion are fetal structures which have interesting properties in terms of their expansion potential and immune properties. The loose connective tissue that supports and cushions the umbilical vessels is called Wharton's jelly. Wharton's jelly contains an extracellular matrix rich in collagen, hyaluronic acid and growth factors, and contains a population of MSCs. The MSCs derived from Wharton's jelly (called WJCs, below) have properties of primitive stromal cells with robust expansion potential in vitro and applications in tissue engineering. Since WJCs are collected from a discarded tissue, WJCs can be collected safely and painlessly. Both WJCs and bone marrow-derived MSCs have similar immune properties. For example, allogeneic WJCs do not stimulate immune cell proliferation in one way mixed lymphocyte proliferations and WJCs suppress splenocyte proliferation in two way mixed lymphocyte proliferation assays, similar to bone marrow-derived MSCs. For this reason, MSCs have been investigated for their ability to modulate immune function in graft vs host disease and in other immune disorders. We speculate that WJCs will have similar benefits to MSCs for these disorders. To move into clinical investigation, we have evaluated the conditions required to produce GMP WJCs. First, we determined that expansion in serum-free

conditions did not affect expansion of WJCs or their surface phenotype. Second, we found that lowered oxygen concentration and lower plating density were important for rapid expansion of WJCs and for maintaining the stemmy population, based upon colony forming unit-fibroblast number. Third, we found that exposure to the cytokine interferon gamma markedly affected WJC physiology which may impact clinical effectiveness. For example, we found an increased induction of MHC by interferon gamma (IFN-gamma) after freezing, but not in unfrozen cells. Fourth, we found that exposure of INF-gamma did not affect the expression of the costimulatory molecules CD40, CD80 and CD86 which play a role in eliciting an effective immune response. By understanding and controlling these variables, we can produce a more uniform and potent clinical product. Our next step is to obtain FDA input in order to move into phase I clinical trial for chronic GVHD found after allogeneic hematopoietic stem cell transplant.

Notes

Suitability of amniotic membrane and cells thereof for tissue regeneration approaches

Wolbank S.[1,3], *Lindenmair A.*[1,3], *Stadler G.*[1,3], *Meinl A.*[1,3,4], *Peterbauer-Scherb A.*[2,3], *Eibl J.*[5], *Polin H.*[2], *Gabriel C.*[2,3], *van Griensven M.*[1,3], *Redl H.* [1,3]

[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingenstrasse 13, A-1200 Vienna, Austria; [2]Red Cross Blood Transfusion Service of Upper Austria, Scharitzerstrasse 6-8, A-4020 Linz, Austria; [3]Austrian Cluster for Tissue Regeneration, Austria; [4]Bernhard Gottlieb University School of Dentistry, Waehringerstrasse 25a, A-1090 Vienna, Austria; [5]Bio-Products & Bio-Engineering AG, Schottenring 10, A-1010 Vienna, Austria.

Tissue engineering strategies usually require cell isolation and combination with a suitable biomaterial. Human amniotic membrane (AM) represents a natural two-layered sheet comprising cells with proven stem cell characteristics. In our approach, we evaluated the differentiation potential of AM in toto with its sessile stem cells as alternative to conventional approaches requiring cell isolation and combination with biomaterials. For this, AM-biopsies were differentiated in vitro using two osteogenic media compared with control medium (CM) for 28 days. Mineralization and osteocalcin expression was demonstrated by (immuno)histochemistry. Alkaline phosphatase (AP) activity, calcium contents and mRNA expression of RUNX2, AP, osteopontin, osteocalcin, BMP-2 (bone morphogenetic protein), and BMP-4 were quantified and AM-viability was evaluated. Under osteogenic conditions, AM-biopsies mineralized successfully and by day 28 the majority of cells expressed osteocalcin. This was confirmed by a significant rise in calcium contents (up to 27.4±6.8mg/dl d28), increased AP-activity, and induction of RUNX2, AP, BMP-2 and BMP-4 mRNA expression. Relatively high levels of viability were retained, especially in osteogenic media (up to 78.3±19.0% d14; 62.9±22.3% d28) compared to CM (42.2±15.2% d14; 35.1±8.6% d28). By this strategy, stem cells within human AM can successfully be driven along the osteogenic pathways while residing within their natural environment. This novel approach may hence be a suitable alternative to current bone tissue engineering protocol.

Notes

Placenta derived adherent stromal cells for the treatment of Critical Limb Ischemia (CLI)-lessons from first clinical trial

Ofir R.
Pluristem Therapeutics Inc., Haifa, Israel.

Pluristem focuses on three dimensional (3D) expansion of Placenta derived Adherent Stromal Cells. Pluristem's first product, PLX-PAD, is designated for the treatment of Critical Limb Ischemia (CLI), the end-stage of Peripheral Artery Disease (PAD).

PLX-PAD cells are derived from the human decidua and are expanded using the company's 3D proprietary technology. The cells are characterized as CD73, CD90 CD29, CD105 positive and CD34, CD45, CD19 and CD14 negative. Analyses of the expression of stimulatory and co-stimulatory molecules on the surface of the PLX-PAD cells demonstrate the absence of HLA class II (HLA-DR), CD80, CD86 and CD40, supporting our findings that these cells are immunoprivileged. Accumulating data strongly suggest that PLX-PAD anti inflammatory and angiogenic properties are largely mediated via paracrine effects.

PLX-PAD based cell therapy for CLI offers an opportunity for patients that have exhausted all currently available interventions. Two phase I, open-label, dose-escalation studies, intended for the treatment of CLI were initiated in parallel in the EU and U.S. Enrollment was completed in Germany, and is ongoing in the US. Cells were administered in 30-50 intramuscularly injections in the effected limb.

Both trials have currently met their primary safety endpoints defined as absence of adverse events, safety laboratory values, ECG findings and lack of immunological reactions following three month follow-up. Immunological data, presents no evidence for PLX-specific humoral or T-cell allosensitization, confirming the low immunogenicity of HLA unmatched allogeneic PLX cells.

Efficacy parameters include: Ankle-Brachial Index (ABI), Toe-Brachial Index (TBI) and Transcutaneous Oxygen Tension (TcPO2) as well as quality of life, pain and wound assessment. Analysis related to twenty one patients who have completed their three-month follow-up is available and will be presented.

Our data offers extensive immunological evaluation of mesenchymal-*like* cell therapy in CLI clinical trials. Moreover, such data may have broad implications on allogeneic cell therapy in other disorders.

Notes

Amnion-derived cells to treat liver disease

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Abstract not provided.

Notes

Human amnion epithelial cells: a cellular therapy for inflammatory diseases?

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Chronic inflammation in organs such as lung and liver arising from diverse stimuli including pathogens, environmental, lifestyle and genetic factors induce cell death, reduced function and lead to fibrosis. Liver cirrhosis and lung fibrosis are amongst the leading causes of morbidity and mortality and are major burdens on healthcare systems worldwide. Due to drawbacks in conventional therapies, stem cell based approaches targeting cell replacement, tissue inflammation and fibrosis are being tested in animal models mimicking these diseases. Using bleomycin (lung) and carbon tetrachloride (CCl_4; liver) injured mice and *in vitro* approaches we are studying the effects of human amnion epithelial cells (hAEC) transplantation and potential mechanisms involved. In bleomycin injured mice, hAEC engrafted in higher numbers for longer periods and produced surfactant proteins A-D following transplantation suggesting transdifferentiation into type II alveolar epithelial cells in the mouse lung whereas MSC from Wharton's jelly MSC from the umbilical cord did not show long term engraftment or evidence of transdifferentiation. Mice treated with hAEC showed reduced apoptosis, inflammation and fibrosis and induced a pro-fibrolytic environment with significant remodelling to damaged lungs and livers. In hepatic fibrosis, exploring whether reduced inflammation could be due in part to effects exerted by hAEC on resident immune cells, we found IMM^+/$HLA\text{-}G^+$ hAEC in close proximity to $CD3^+$ T-cells without evidence of systemic or localized host immune response in C57/Bl6 mice exposed chronically to CCl_4. T-cell numbers in livers remained unaltered between mice given CCl_4+hAEC and CCl_4 alone, however $FoxP3^+$ regulatory T-cells were elevated in mice receiving cells. hAEC may escape detection due to lack of HLA Class II, CD80/86 and low HLA Class IA and CD40 expression. Secretion of immunosuppressive factors TGFβ, HLA-G, HGF and IDO activity by hAEC could modulate T-cell and also dendritic and NK cell activity. $F4/80^+$ resident liver macrophages, the Kupffer cells (KC), were significantly lower in CCl_4+hAEC treated mice compared to CCl_4 controls. MIF-1 from

hAEC may inhibit KC recruitment; however MCP-1 was also secreted by hAEC. Factors released by KC have been shown to activate hepatic stellate cells (HSC) into a pro-fibrogenic collagen depositing myofibroblast phenotype. Activated HSC are also a major source of inflammatory cytokines and MCP-1 that serve to perpetuate inflammation leading to fibrosis. *In vitro* studies showed that treatment with hAEC conditioned media did not stimulate HSC proliferation but enhanced apoptosis and altered cytokines secreted by these cells. Collectively, these findings suggest that hAEC transplantation may be useful for targeting tissue inflammation and that in some organs such as lung may also contribute to cell replacement.

Notes

Fetal stem cells for heart valve tissue engineering

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Tissue engineering is a highly interdisciplinary field of research, aiming at the development of next generation therapeutic strategies based on living, autologous replacements with the capacity of repair, regeneration and growth. With regard to cardiovascular applications of the tissue engineering concept, several groups have demonstrated the principal feasibility to create functional living heart valves, blood vessels, and myocardial structures using autologous cell systems and rapidly degrading scaffold materials. In recent long-term in vivo studies, we have been able to demonstrate for the first time functional growth of living, autologous tissue engineered structures (main pulmonary arteries) in a large animal model covering the full biological growth cycle.

In today's cardiovascular clinical scenario, the highest medical need for a tissue engineering solution is in the field of pediatric applications treating congenital heart disease (approx. 1% of all newborns). In this context, the introduction of a living, growing replacement such as e.g. tissue engineered heart valves made of the babies own cells would substantially reduce today's severe therapeutic limitations, which are mainly due to the need for repeat reoperations to adapt the current artificial prostheses to the somatic growth of the young patients. These reoperations are associated with substantial morbidity and mortality and beyond that represent a significant trauma to the young patients and their families.

Ideally, the cells to be used for babies with congenital heart disease (normally detected by ultrasound at week 20) can be obtained already during pregnancy to provide the time for the tissue engineering process prior to birth. In recent investigations we have demonstrated the feasibility to use various human fetal stem cells for tissue engineering of heart valves.

In particular, amniotic fluid-derived cells have shown promising potential for clinical realization of the congenital tissue engineering approach. Future studies have been initiated that will focus on safety profiles as well as the preclinical evaluation these cell sources with regard to their routine clinical implementation.

Notes

Physiological comparison of autologous and allogeneic cell implantation in the central nervous system: defining and regulating immune cell activity against mesenchymal and neural stem cell grafts

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Initial cell therapy studies by our group focussed on the direct intra-spinal implantation of human mesenchymal stem cells (MSC) genetically engineered to secrete neurotrophic factors as a therapeutic tool to treat spinal cord injury in rats. Although in these studies we were unable to demonstrate any therapeutic benefit for the chosen therapy, it became clear to us that – before further cell therapy studies could be initiated – several intermediate steps needed to be optimised, among them: (i) in vitro culture, characterisation and genetic modification of different stem cell types, (ii) pre-mortem bioluminescence (BLI) and magnetic resonance imaging techniques for real-time monitoring of cell graft localisation and survival in vivo, and (iii) post-mortem multi-colour histological analysis for unambiguous characterisation of grafted cells and surrounding tissue.

Having optimised the above described cell culture and imaging techniques, studies performed during the past 3 years mainly focussed on determining the survival, differentiation and immunogenicity of autologous and allogeneic cellular implants in the CNS of immune-competent mice.

Murine bone marrow-derived MSC and embryonic (E14) brain-derived neural stem cells (NSC) were cultured from FVB-Luciferase transgenic mice and further genetically engineered with the eGFP reporter gene. Following transplantation in non-injured CNS of syngeneic FVB mice and allogeneic BALB/c or C57BL/6 mice, cell survival was monitored pre-mortem using in vivo BLI (luciferase reporter gene) and post-mortem by histological analysis (eGFP reporter gene). Additional histological analysis: (i) for MSC and NSC-specific markers (respectively Sca1 and GFAP in combination with eGFP) confirmed cell graft identity, (ii) for different immune cell markers confirmed the presence or absence of Iba1+CD11b+/- microglia, CD4+ or CD8+ T-cells and NK-cells surrounding or infiltrating cell grafts, and (iii) for GFAP+ astrocytes confirmed the presence or absence of endogenous astrocytic scar tissue surrounding or infiltrating

cell grafts. Finally, ex vivo ELISPOT assay was performed to identify allograft-specific T-cell responses in the CNS. Furthermore, similar studies were performed following grafting of C57BL/6-eGFP transgenic mice derived MSC and NSC in the corpus callosum (CC) of healthy mice and in the demyelinated CC of mice treated for 4 weeks with a Cuprizone-supplemented diet.

Autologous transplantation of FVB-derived MSC resulted in graft survival of at least 4 weeks as demonstrated by in vivo BLI and histological analysis. However, MSC grafts became highly infiltrated by Iba1+CD11b- microglia and encapsulated by GFAP+ astrocytic scar tissue. Allogeneic transplantation of FVB-derived MSC in BALB/c or C57BL/6 mice resulted in graft rejection from on 2 weeks post-transplantation as demonstrated by in vivo BLI and histological analysis. The immune-based rejection of MSC allografts was solely mediated by highly activated Iba1+CD11b+ microglia without involvement of T-cells and NK-cells, as demonstrated by histological analysis and ex vivo ELISPOT analysis. Autologous transplantation of FVB-derived NSC resulted in graft survival of 2 weeks followed by a progressive decrease in graft survival as monitored by in vivo BLI. Histological analysis further confirmed that NSC grafts became highly infiltrated by endogenous Iba1+CD11b- microglia and GFAP+ astrocytes. Additionally, similar results were obtained following autologous grafting of C57BL/6-eGFP transgenic mouse derived MSC and NSC in the CC of healthy and demyelinated CNS tissue.

In conclusion, although the CNS has historically been considered to be immune-privileged, our data demonstrate that the CNS is not immune-ignorant to both autologous and allogeneic cellular implants. Therefore, we here suggest that a profound study of the interaction between cellular grafts and the brain's innate immune system will be inevitable before clinical cell transplantation in the CNS can be performed safely and successfully.

Notes

Amniotic membrane-derived stem cells and pancreatic islet-cell differentiation

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Amniotic Membrane is an attractive high throughput source of stem cells with features that encompass broad differentiation potential, important immunomodulatory properties and paracrine activities. Considering their characteristics, these cells represent prime candidates for possible application in regenerative/reparative medicine for treatment of diabetes.

Diabetes mellitus (DM) is a chronic and devastating disease characterized by hyperglycaemia, and arises due to defects in either insulin secretion or insulin action. Diabetic patients require lifelong insulin treatment for survival. However, this treatment does not mimic the tight control of glycaemia granted by insulin-secreting beta cells in healthy individuals, resulting in the appearance or the progression of a number of severe secondary complications.

Encouraging results in the field of diabetes treatment have been obtained by transplantation of whole pancreas or isolated islets, however current strategies now focus on identifying new sources of insulin-producing cells.

In this context, the use of stem cells represents an appealing, practical approach. The amniotic membrane harbours two main cell types: amniotic epithelial cells (AECs), capable of expressing beta-cell markers and functions after differentiation induction; amniotic mesenchymal stromal cells (AM-MSCs), which due to their angiogenic potential and trophic and immunomodulatory abilities, are excellent candidates for use in building composite pancreatic islets.

We have compared the *in vitro* pancreatic differentiation potential of AECs with that of a population of human pancreatic islet-derived stem cells displaying mesenchymal-like characteristics and endocrine/endothelial features.

These experiments confirmed the pancreatic differentiation ability of AECs: in particular, through flow cytometry analysis, we showed an increase in glucagon and insulin expression by these cells after specific induction. Meanwhile, *in vivo* studies using streptozotocin-diabetic rats yielded preliminary results regarding the actions of AECs and pancreatic-derived MSCs after infusion into the portal vein. In particular, we

observed that diabetic rats treated with AECs and pancreatic-derived MSCs underwent a partial and transient correction of the altered phenotypes of “water consumption per day” and glycemic control.

Finally, this study provided preliminary knowledge regarding the differentiation potential of amniotic membrane-derived stem cells, in order to allow significant improvement in the efficiency of pancreatic cell transplantation in the future.

Notes

Cell therapy for stroke: towards clinical application of Celgene human placenta-derived cells

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Stroke treatment remains a significant unmet clinical need worldwide. Cell therapy offers a neuorestorative treatment for stroke. Here, we explored the safety and efficacy of human placenta-derived cell therapy products (PDACs®) manufactured by Celgene Cellular Therapeutics (Warren, NJ) in adult rat models of stroke. Initially, we demonstrated that intravenous transplantation of PDACs® at day 2 after the transient occlusion of middle cerebral artery (MCA) resulted in significant behavioral recovery characterized by dose-dependent amelioration of stroke-induced motor and neurological deficits as early as 7 days post-stroke and stable up to 84 days post-stroke. Immunofluorescent microscopy of brains harvested at 84 days post-stroke revealed a dose-dependent level of immunoreactivity in neural phenotypes characterized by reduced GFAP immunoreactivity, while O4 and MAP2 immunoreactivity increased as the cell dose increased. Using DAPI as parameter of host cell survival within the ischemic striatum, a dose-dependent rescue of the host ischemic penumbra was also recognized. Next, we showed that intravenous transplantation of PDACs® exerts a similar degree of functional improvement in permanent MCA ligation stroke model. Compared to those that received non-viable PDACs® or vehicle, those that received viable cells displayed significant improvement in stroke-induced behavioral deficits and significant rescue of host cells in the ischemic penumbra. The behavioral recovery produced by transplantation of PDACs® is robust and stable starting at 1 month post-stroke and up to at least 6 months post-stroke. We also observed a general absence of human specific vimentin staining, indicating the graft survival per se was not essential for functional effects of PDACs®. The safety of PDACs® for transplantation is demonstrated in both studies in that none of the transplanted animals show any exacerbation of stroke-induced behavioral abnormalities. Moreover, no tumors or ectopic tissue formation was detected in any of the transplanted animals. In summary, we show that intravenous transplantation of Celgene PDACs® in stroke animals that received either transient occlusion or permanent ligation of the MCA exerted behavioral and histological benefits over long-term in the absence of immunosuppression.

Notes

Human amniotic epithelial cells express melatonin receptor type 1A (MT1), but not melatonin receptor type 1B (MT2): a new perspective to neuroprotection

Kaneko Y.[1], Hayashi T.[1], Yu S[1], Tajiri N.[1], Bae EC.[1],. Solomita MA. [1], Chheda SH.[1], Weinbren NL.[1], Parolini O.[2], Borlongan CV.[1]

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Recent studies have demonstrated that the human placenta is a sophisticated source of stem cells. We have provided laboratory evidence that transplantation of these human placenta-derived cells in vitro and in vivo stroke models promotes functional recovery. However, the mechanisms underlying these observed therapeutic benefits of human placenta-derived cells unfortunately remain poorly understood. Here, we examined the expression of two discrete types of melatonin receptors and their roles in proliferation and differentiation of cultured human amniotic epithelial cells (AECs). Cultured AECs express melatonin receptor type 1A (MT1), but not melatonin receptor type 1B (MT2). The proliferation of cultured AECs was increased in the melatonin-treated group in a dose-dependent manner, and the viability of cultured AECs could be further enhanced by melatonin. Moreover, the viability of AECs significantly decreased with H2O2 exposure, which was reversed by pre-treatment with melatonin, resulting in increased cell survival rate and cell proliferation. Immunocytochemically, administration of melatonin significantly suppressed nestin proliferation, but enhanced TUJ1 differentiation of MT1-expressing AECs. Additional experiments incorporating antibody blocking and synergistic AEC-melatonin treatments further showed AEC therapeutic benefits via MT1 modulation. Finally, analysis of trophic factors revealed cultured AECs secreted VEGF in the presence of melatonin. These data indicate that melatonin by stimulating MT1 increased cell proliferation and survival rate while enhancing neuronal differentiation of cultured AECs, which together with VEGF upregulation, rendered neuroprotection against experimental in vitro models of ischemic and oxidative stress injury.

Notes

ABSTRACTS

Abstract 1

Extravillous trophoblast cells (EVT) from human term placenta may not be good stem cells for use in cell therapy protocols

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The stem cells from human placenta has attracted great interest as a source of stem cells for regenerative medicine due to phenotypic plasticity of some of the various cell types isolated from this tissue. In this work we focused in Extravillous Trophoblast cells (EVT) from human term placenta. The EVT cell culture was made from cotyledon fragments of human term placenta (38-40 weeks of gestation), using enzymatic digestion and a gradient of density (Percoll) after. The method of placental cell culture adapted by our group was capable of isolate EVT cells with 90% of cells viability. Characterization of EVT cells showed positive staining for cytokeratin 7 and PIGF confirming the trophoblastic origin. However, cultivation of these cells showed that the EVT cells have low rate of cell proliferation, surviving for approximately 11 days under cell culture conditions, a characteristic that limits their use in cell therapy protocols. Taking into account our findings, we conclude that EVT cells are difficult to culture and low proliferation. Therefore, we believe it would be interesting to reprogramming (iPS) of these cells to check that they will be able to increase their proliferative capacity and still maintain their profile immunomodulation.

Notes

Abstract 2

Isolation, immunophenotyping and in vitro functional characterization of murine placenta-derived cells

Bergwerf I. [1], *Magatti M.*[2], *De Munari S.*[2], *Acali S.*[2], *Rossi D.*[2], *Ressel L.*[2], *Cargnoni A.*[2], *Ponsaerts P.*[1], *Parolini O.* [2]

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Due to their role in maintaining fetomaternal tolerance during pregnancy, the immune-modulatory properties of placenta-derived cells are gaining increasing interest in current cell therapy research. A growing number of scientific reports already described several immune-suppressive properties of placenta-derived cells in in vitro co-culture studies with different types of immune cells. While many of these studies focus on placenta-derived cells of human origin, we describe the isolation, characterization and in vitro immune-modulatory properties of murine placenta-derived cells in this study.

Placenta-derived cells were isolated from pregnant inbred Balb/c mice and characterized for the expression of several membrane markers. At passage 1, the majority of cultured cells express CD44 and Sca-1, indicating a mesenchymal stromal cell identity, while a the presence of a haematopoietic MHCII+/c-kit+/CD45+ subpopulation decreases during the following 2 passages. In order to investigate potential immune-modulatory properties of these cells, co-culture experiments were performed with PHA (phytohaemagglutinin)- and LPS (lypopolysaccharide)-stimulated allogeneic splenocytes at various ratios. Results indicate a gradual suppression of PHA-stimulated, and in lower extent of LPS-stimulated, splenocyte proliferation as measured by radioactive thymidine incorporation. Additionally, co-culture of PHA-stimulated splenocytes with cells cultured from maternal uterus tissue suppressed splenocyte activation to a much lower extent, indicating the existence of certain immune-modulatory properties of fetal placenta-derived cells.

In this study, we optimized the isolation and culture of murine placenta-derived cells and confirmed the existence of immune-modulatory properties. Further experiments, including autologous and allogeneic grafting of these cells, will have to determine their immune-suppressive properties in vivo.

Notes

Abstract 3

Use of amniotic membrane, amniotic fluid, and placental dressing in advanced burn patients

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The present study attempts to examine the effectiveness of the composite and judicious use of different stem cell rich pregnancy specific biological substances (PSBS) in extensive burn wound. PSBS includes freshly collected amniotic membrane, amniotic fluid and the placenta.

64 patients (male 24, age 2 to 96 years, mean 36 years + 5.4 years, and female 40, age 7 years–68 years, mean 32 years + 5.7 years), who had 26 to76 percent burn, were enrolled for the present study which was carried out first in Bijoygarh Hospital (1999-2006), and then in Vidyasagar Hospital (2006-2010).

The treatment is mainly a three step procedure. In Step I, the burn site is washed with normal saline and then rubbed with a freshly collected placenta's maternal attachment site as a dressing material. In Step II, the burn site is washed with freshly collected amniotic fluid and in Step III, amniotic membrane is applied on the affected site as a temporary biological wound cover.

Step I may have a positive cytokine impact on the process of healing. In Step II, amniotic fluid may act as a cell therapy source because it has a rich content of epithelial and mesenchymal stem cells. Its antibacterial propensity also makes it a helpful adjuvant. In step III, amniotic membrane expedites healing and reduces exudation, pain and infections. It has to be applied judiciously, i.e., the chorionic side augments vasculogenesis in early wound and the amniotic side promotes epithelialization later. This is an effective step to augment the cell therapy component of the amniotic fluid.

Currently used skin substitutes are expensive and not universally available. PSBS is easily procured and the procedure is simple. PSBS regimen has a profound regenerative impact in burns of any degree due to the transdifferentiation capacity of stem cells.

Notes

Abstract 4

Amniotic membrane reduces bile duct ligation-induced liver fibrosis

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Recently, stem/progenitor cell transplantation has been proposed as potential treatment for liver chronic disorders in alternative to organ/hepatocyte transplantation. In this study, basing on the fact that amniotic membrane (AM) has long been used for its anti-inflammatory and anti-scarring properties and that AM-derived cells display multipotent differentiation potential and immunomodulatory features, we investigated on the potential application of human AM as innovative therapeutic approach.

We evaluated the effects of hAM on biliary-type liver fibrosis induced in rats through bile duct ligation (BDL). A fragment of hAM was applied onto the liver surface after BDL and the rats were euthanised after 2, 4 and 6 weeks. The severity of liver fibrosis was assessed both semi-quantitatively by Knodell scoring system and quantitatively, by digital image analysis, evaluating the liver areas occupied by the ductular reaction (CK 19-positive cells), the activated myofibroblasts (a-SMA-positive cells) and collagen deposition (Masson's staining).

AM-treated rats presented a significantly lower liver fibrosis with respect to control rats. Indeed, in contrast with BDL rats which showed a progression of fibrosis to cirrhosis from week 4 to week 6, BDL+AM rats showed a fibrosis confined at the portal/periportal area and a collagen deposition at about 50% of that observed in control rats. Concomitantly, AM application slowed the progression of the ductular reaction and significantly reduced the area occupied by myofibroblasts.

These findings suggest that human AM patching might counteract the fibrosis progression in BDL-injured livers and could represent a new strategy to limit hepatic damage associated with fibrotic degeneration.

Notes

Abstract 5

Androgen reduction and mesenchymal stem cell therapies improve kinetics of thymic epithelial cell recovery following chemotherapy

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There is a widely accepted decline in immune system function with age. Clinically, this deterioration manifests as an increased susceptibility to opportunistic infections, poor responsiveness to vaccines and delayed immune recovery following anti-neoplastic cytoablative treatments and immunosuppressive drug therapy. Whilst young children recover their immune defences quickly, adults are at significant risk post-treatment due to a poorly functioning thymus with low T cell output, severely delaying recovery. This creates a clear, unmet clinical need to enhance thymic function and hence regeneration of the circulating T cell pool.

Age related involution and disorganization of the stromal microenvironment in the thymus is at least in part associated with the increase in sex steroid production from puberty. We have published widely that sex steroid ablation (SSA) either by surgical or reversible chemical castration leads to immune rejuvenation in the aged, and enhances hematopoietic stem cell engraftment and immune recovery following BMT and chemotherapy. This involves the restoration of both the thymic microenvironment and bone marrow stem cell niches. Preliminary experiments suggest that bone marrow derived MSC administration can improve the kinetics of thymic epithelial cell recovery. We are planning to compare these MSCs with those from the umbilical cord for their relative effectiveness. Mechanisms may involve reducing the inflammatory damage in the thymus and/or bone marrow and provision of mesenchyme-derived growth factors for thymic epithelial cell recovery. Since the thymus is an epithelial organ, we are also investigating the efficacy of amnion epithelial stem cells for their ability to enhance thymic recovery.

Notes

Abstract 6

Mesenchymal stem cells induce a time-dependent recruitment of microglia and astrocytes following autologous grafting in brain tissue

De Vocht N.[1]*, Bergwerf I.*[1]*, Daans J.*[1]*, Pauwels P.*[2]*, Berneman Z.*[1]*, Van der Linden A.*[3]*, Ponsaerts P.*[1]

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The use of stem cell transplantation as a therapeutic tool to treat neurodegenerative disorders has gained increasing interest over the last decade. However, a profound knowledge of cell implant behaviour, survival and differentiation will be necessary to understand potential therapeutic effects of stem cell transplantation.

In this study we aimed to follow up the survival of grafted bone marrow-derived mesenchymal stem cells (MSC) in the central nervous system (CNS) of mice by non-invasive bioluminescence imaging (BLI) combined with a post-mortem histological study of cell differentiation and recruitment of inflammatory cells towards the implant site.

BLI analysis shows stable survival of MSC-Luc/eGFP in vivo. These results were further validated by histology demonstrating the presence of Sca1+ and eGFP+ cells at every time point investigated (day 1 to day 14). At a very early time point (day 1), histological analysis did not show recruitment of microglia and/or astrocytes. However, starting from day 3, MSC grafts are invaded by Iba1+/CD11b+ microglia (activated microglia) and surrounded by a glial scar of astrocytes. From day 10 on, activated Iba1+/CD11b+ microglia were found in the surrounding of the implant, while Iba1+/CD11b- microglia remain within the MSC graft, which suggests that MSC might have certain immune-suppressive characteristics to modulate the activation status of microglia.

Although the CNS has historically been considered to be immune-privileged, our data demonstrate that the CNS is not immune-ignorant to autologous cellular implants. Further research should be undertaken to understand the in vivo interaction between MSC, microglia and astrocytes.

Notes

Abstract 7

Preclinical evaluation of mesenchymal-like stem cells derived from umbilical cord stroma as a possible therapeutic tool in multiple sclerosis

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Extraembryonic tissues such as umbilical cord are considered a promising source of (mesenchymal-like) stem cells. These cells comprise a potential tool for regenerative cell-based therapy. However, a large inconsistency among reports concerning stem cell isolation and expansion, marker expression, immunomodulatory properties and differentiation capacity highlights the need for a better characterization of the different stem cell populations derived from the umbilical cord.

The present work describes a comparison between both the enzymatic and mechanic isolation method for obtaining MSC from the UC matrix (UCMS). In addition we test 4 different culture media which are described in literature as potent growth media for the UCMS cells. In this setting we will verify the presence of different populations in the heterogeneous cell isolates, both at early and late passage, based on different expression patterns of stem cell related genes and early lineage or development makers (e.g. mesenchymal, immunological and pluripotency markers). In addition, the functionality of the present molecules is also tested when appropriate (e.g. differentiation, chemokine receptor functionality in transwell systems, immunomodulatory capacity). This preclinical evaluation will be a first step to further evaluate UCMS potency in the setting of MS.

Notes

Abstract 8

Mesenchymal stem cells from umbilical cord tissue differentiate into cardiomyocytes and constitute an alternative candidate for cardiac tissue engineering

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Mesenchymal stem cells (MSC) are adherent fibroblast-like cells with multipotent properties. In addition to bone marrow and fat tissue, they can easily be isolated from the Wharton´s jelly region of umbilical cords (UCMSC). This study investigated cardiovascular differentiation of UCMSC and seeding feasibility on synthetic scaffolds as well as their ability to grow under GMP-compliant culture conditions.

UCMSC were isolated as previously described (Seshareddy K et al. 2008). Endothelial and cardiomyocyte differentiation was driven by incubating the cells in vascular growth factors and 5-Azacytidine, respectively. Cells were also seeded on Titanium-coated expanded polytetrafluorethylene (Ti-ePTFE) with two alternative porous structures (Dual Mesh® and cardiovascular patch) and analyzed for viability, proliferation, and morphology.

The cardiomyocyte phenotype of differentiated UCMSC was verified morphologically and with a whole set of cardio markers. UCMSC can effectively be seeded on Ti-ePTFE scaffolds with a clear advantage for the cardiovascular patch. In addition, GMP-compliant growth media, especially the xeno- AND serum-free MesenCult® qualify for long-term cultures and expansion of UCMSC.

The umbilical cord tissue is an easily accessible source for mesenchymal stem cells. Due to their potential to give rise to cardiovascular cells and due to their immunosuppressive properties UCMSC constitute an attractive tool in regenerative and transplant medicine. UCMSC on Ti-ePTFE patches will now be used in a rodent model of myocardial infarction.

Selected for oral presentation

Notes

Abstract 9

Effect of the oxygen tension on the expression and function of Galβ1-3GalNAc disaccharide in the first trimester trophoblast cells

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Presence of carbohydrates in different cell types in human placenta suggests their major role in maternal-fetal exchanges, intercellular adhesion, cellular metabolism and villous vessels branching. During development, tissues differentiation are accompanied by changes in glycosylation that is largely sensitive to developmental stimuli. Physiological hypoxia is the powerful developmental stimulus for normal placentation. We investigated sugar chain expression, role and oxygen modulation of core-1 O- and N- glycans in human placenta.

Paraffin and cryo sections from first trimester placenta were used for lectin histochemistry using Peanut agglutinin (PNA) directed to core-1 O-glycans (disaccharide Galβ1-3GalNAc, named also Thomsen–Friedenreich [TF] antigen) and Concanavalin A (ConA) directed to N-glycans. HTR-8/SVneo cells were incubated under low (3%) or standard (20%) oxygen tension and levels of TF antigen and N-glycans were measured by FACS. Cultures were exposed to PNA and ConA and assayed for viability and proliferation.

ConA staining was widely distributed in all cellular elements of the villi, PNA staining was restricted in regions characterized by densely packed Ki-67 positive cytotrophoblastic cells. TF antigen expression level was significantly increased at 3% of oxygen, N-glycans expression was not influenced by oxygen tensions. Treatment of cell cultures with PNA resulted in significant increase of cell proliferation as compared to untreated or ConA- treated cultures.

In conclusion the expression of glycoconjugates bearing TF antigen is modulated by oxygen tension and the disaccharide has a functional role in the trophoblast cells proliferation.

Notes

Abstract 10

Generation of induced pluripotent stem cells from amnion epithelial cells

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Human Amniotic Epithelial (AE) cells express gene and surface markers typical of Embryonic Stem (ES) cells and have the ability to differentiate into cells from all three germ layers. Placenta is a readily available and non-controversial source of cells that can be used in regenerative medicine. AE cell and induced pluripotent stem (iPS) cell technologies would seem to be useful stem cell sources devoid of most of the ethical or religious concerns associated with the use of ES cells. We report the generation of iPS cell lines from primary human AE cells following exposure to lentiviral constructs carrying the reprogramming factors Oct4, Sox2, Klf4, c-Myc, Nanog, and Lin28 (AE-iPS). AE cells were analyzed for mRNA expression levels of stem cell markers (Oct4, Nanog, Sox2) by qRT-PCR immediately after isolation. After the reprogramming procedure we characterized the lines for markers of pluripotency, including alkaline phosphatase activity, nuclear Nanog and Oct4 staining. We performed a complete profiling of AE cells pre- and post-reprogramming procedure by Flow Cytometry for surface markers including: SSEA-3, SSEA-4, TRA1-60, TRA1-81, HLA-ABC, CD24, CD29, CD31, CD34, CD44, CD45, CD49f, CD73, CD105, CD90, CD117, CD133/2, CD146, CD166, EpCAM and ABCG2. FACS analysis revealed a morphological difference in forward scatter characterized by a compartment with a higher side scatter in AE, not present in AE-iPS thought to indicate a different level of cellular complexity and granularity. The percentage of SSEA-3 positive cells after reprogramming was three times higher than AE cells (30% vs 10%, respectively). Other embryonic markers (SSEA-4, TRA1-60 and TRA1-80) were also dramatically increased in AE-iPS cells (60-95%). We also observed that CD133/2 was highly expressed only after the reprogramming procedure.

Although the analysis is not complete on all lines, the colonies generated have the morphological features and the surface marker and gene expression profile of fully reprogrammed cells. In addition, teratoma formation has been confirmed on the 2 iPS lines created from normal human amnion, the others are pending. These studies confirm that iPS lines can be generated from primary human cells including amnion. In future studies, lines that can be confirmed to be successfully reprogrammed will be examined for their ability to differentiate in different lineage.

Selected for oral presentation

Notes

Abstract 11

The isolation and characterisation of term human fetal-derived and maternal-derived mesenchymal stromal cells

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The placenta is of interest to stem cell biologists since it is rich in mesenchymal stromal cells (MSC) and consists of both fetal (amnion and chorion membranes) and maternal (decidua) tissues. Fetal-derived MSC may have different biological properties from maternal MSC, given the differences in age at the time of their development, and such differences may have implications in the potential use of MSC as therapeutic agents. Therefore, we focused on isolating and characterising fetal and maternal MSC of the human placenta.

Male gender babies from elective term caesarean sections were collected, the amniotic and chorionic membranes were mechanically separated and the decidua tissue removed. The tissues were digested and the mononuclear cell fraction collected. The cells were either plated in culture or stained with primitive cell surface marker antibodies to determine if primitive cell populations could be isolated. Genotyping was also performed on cells that had been in culture to determine source of origin. Expanded cells were characterised according to cell surface phenotype and ability to differentiate into mesodermal lineages. Cells from the amnion, chorion and decidua had an adherent, fibroblast-like morphology and exhibited typical MSC characteristics such as cell phenotype and differentiation ability, although there was little differentiation into adipocytes. Very little cell surface expression of the primitive stem cell markers Tra1-60, Tra1-81 and SSEA-4 was found in freshly sorted cells. Genotyping showed the amnion cells were predominately fetal in origin and chorion and decidua cells were predominately maternal in origin. It remains to be determined if fetal MSC show greater therapeutic ability than maternal MSC.

Selected for oral presentation

Notes

Abstract 12

Amnionic mesenchymal stromal cells (AMSC) show a mesenchymal-epithelial phenotype and adopt endothelial characteristics under angiogenic conditions

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Even though AMSC play an important role in stem/progenitor cell research, their characterization is mostly limited to immunodetection by flow cytometry. Therefore, we performed immunohistochemical studies on cultured cells and term placental cryosections. Furthermore, we cultured the cells under angiogenic conditions to investigate their endothelial differentiation potential and examined the interaction with endothelial cells (EC).

AMSC express the common mesenchymal stem cell markers CD73, CD105 and CD90 and seem to adopt a mesenchymal-epithelial phenotype during culture: Shortly after isolation (1d), 92.1% (±5.6%) of the cells only express the mesenchymal marker vimentin, 1.3% (±1.4%) solely stain for the epithelial marker cytokeratin-7 and 5.7% (±3.4%) co-express these markers. After 5d, the double positive cells increase to 9.9% (±3.4%), while exclusive expression of cytokeratin or vimentin remains about the same (1.2% ±1.3% and 88.8% ±3.8%, respectively). After passage 1, all cells are vimentin-positive, while 50-80% co-express cytokeratin-7 and vimentin.

AMSC express vascular endothelial growth factor receptor-2 which can also be found on endothelial precursor cells. Angiogenic stimulation with endothelial growth medium containing VEGF enhances their proliferation potential and viability. They change their fibroblast-like morphology towards an endothelial, cobblestone-like phenotype. Even though they do not express the mature EC markers vWF and CD144, they take up DiI-AcLDL, a characteristic of EC. Induced AMSC form more stable and widespread networks than EC in a tube formation (matrigel) assay. In co-culture, they adhere to EC and stabilize their networks.

These data indicate angiogenic properties of AMSC which might be of therapeutic use in vascular biology.

Selected for oral presentation

Notes

Abstract 13

Immunoregulatory properties of human amniotic mesenchymal stromal cells: a comparison to human adipose derived stem cells

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It is widely recognized that mesenchymal stem cells (MSC) show immunomodulatory properties in vitro. MSC from amnion and adipose tissue are especially promising for tissue engineering due to easy procurement and abundance of material.

The aim of this study was to compare human amniotic mesenchymal stromal cells (hAMSC) and human adipose derived stem cells (ASC) regarding their immunoregulatory potential.

With regard to a possible cell-contact driven mechanism, cell surface expression profiles of MSC as well as their antiproliferative effect on PBMC were compared under normal and inflammatory culture conditions. Concerning a cell-contact independent mechanism, the effect of MSC on proliferation, activation markers and cytokine expression of indirectly co-cultured PBMC, T-cells as well as stimulated monocytes was observed.

In this study, we demonstrate that pre-treatment of hAMSC and ASC with IFN-gamma leads to expression or upregulation of MHC-II, PD-L1 and PD-L2. In direct co-cultures, pre-treated MSC even show enhanced antiproliferative properties. hAMSC and ASC exert similar effects on proliferation of indirectly co-cultured PBMC. Both MSC populations suppress expression of activation-induced soluble factors by PBMC. Additionally, the number of T-cells expressing CD69 is significantly upregulated in both co-cultures. Differentiation of monocytes into dendritic cells is strongly suppressed in the presence of hAMSC, whereas ASC exert only marginal inhibitory properties.

Overall, experimental data demonstrate that hAMSC and ASC are very similar in their ability to suppress proliferation and cytokine expression of PBMC, whereas their ability to suppress generation of dendritic cells is significantly different.

Notes

Abstract 14

Horse fetal adnexa: a source of mesenchymal (AMSCs) and epithelial stem cells (AESCs)

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For the first time, we separated homogeneous subpopulations of cells from intervascular and perivascular horse Warthon Jelly (WJ) using transwell of 8 µm pores and we described the characteristics of AESCs and AMSCs presumptive stem cells from horse amnion.

In the WJ, the large cells of the perivascular portion propagated slowly and passed 16.58 cell population doublings (PD) after 31 days, whereas in the same time range the small ones reached 19.49 PD. After the seventh passage, the proliferating traits of the two cell populations became similar. As a control, the unsieved perivascular portion passed 8.54 PD. On the contrary, in the intervascular portion the large cells propagated more rapidly in respect to the small ones (20.53 vs 13.66 PD) and the unsieved control (9.42 PD). The Colony Forming Unit Frequency (CFU-F) assay showed higher CFU-F for the small perivascular cells and the large intervascular cells (1:133 and 1:106 respectively).

AESCs displayed typical cuboidal morphology while AMSCs were fibroblast-like. The mean PD after 31 days were 13.08 and 26.5 respectively for AESCs and AMSCs. The mean frequency of CFU-F was respectively of 1:283 and 1:111 for AESCs and AMSCs.

All cellular lines expressed MSC mRNA markers (CD29, CD105, CD44) and were negative for CD34. Osteogenic, adipogenic and neurogenic differentiation were confirmed by specific stainings and by gene expression of differentiated cell lines.

In conclusion, size-sieving is a good tool to isolate more proliferative cells and equine WJ and amnion hold apparent expectations as sources of presumptive stem cells which may have widespread clinical applications in veterinary medicine.

Notes

Abstract 15

Human Wharton's jelly-derived mesenchymal stem cells express several immunomodulatory molecules both in their naïve state and hepatocyte-like differentiated progeny: prospects for their use in liver diseases.

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Wharton's jelly (WJ), the main constituent of umbilical cord, is a reliable source of mesenchymal stem cells (MSC). WJ-MSC show unique ability in crossing lineage borders. As other extraembryonic mesenchymal populations (placenta and amnion-derived cells), WJ-MSC express several immunomodulatory molecules, essential during the initial phases of human development. Indeed, our recent work pointed out the expression of non-classical HLA molecules as HLA-G in such cells, together with a favorable combination of B7 costimulators. Very few data in literature suggest that some of the immune features of the naïve cells are maintained after performing differentiation.

The aim of this work was extending the knowledge on the expression of immunomodulatory molecules by naïve and differentiated WJ-MSC. To this purpose, WJ-MSC underwent differentiation to osteoblasts, adipocytes and hepatocyte-like cells. Differentiated cells were characterized, by both RT-PCR, ICC and histological stains for the acquisistion of the desired phenotypical features. RT-PCR and ICC were used to investigate the differential expression of immune-related molecules in control and differentiated cells.

WJ-MSC resulted expressing diverse immunomodulatory molecules which spans from non-classical type I HLAs (i.e. HLA-E, -F, -G) , to further members of the B7 family, and of the CEA superfamily, for all of which in vivo immunomodulating functions are known. In addition, we demonstrated for the first time that the expression of these molecules is maintained after performing osteogenic, adipogenic or hepatogenic differentiation. Further experiments are undergoing to better evaluating the implications of these findings in the evolving field of liver regenerative medicine.

Selected for oral presentation

Notes

Abstract 16

Neural progenitors derived from the first-trimester human placenta cells mediate recovery of dopaminergic neurons and restoration of nigrostriatal circuit in the neonatal hypoxic-ischemic brain

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Placenta is a rich source of stem cells that can be used for the cell therapies. We isolated 17 proliferating mesenchymal stem cell-like cell lines from 19 first trimester human placenta (hPLC), which proliferated stably for 90~150 days in vitro. These cells, by enhanced cell-cell interaction, successfully differentiated into nestin expressing neural progenitors (hPLC-NP). In this report, we tested if the neural progenitors in vitro-differentiated from hPLC can mediate the recovery of dopaminergic neurons and motor coordination. Nigral dopaminergic neurons and the nigrostriatal circuit were destroyed by hypoxia-ischemia (HI) in the neonatal brain. Transplantation of hPLC-NP into bilateral striatum at 2 weeks after the insult recovered locomotor activity and in 8 weeks, the recipients exhibited near normal locomotor activity while, no improvement was observed in the control-grafted animals. Immunohistochemical analyses revealed that the implanted hPLC-NPs matured into ectodermal cells including the TH-expressing neurons in the recipient striatum. Further analyses showed that the substantia nigra of the recipient was indistinguishable from that of the normal animals in terms of TH-expressing cells. 5-bromodeoxyuridine was incorporated into several of the new TH-expressing neurons suggestive of the dopaminergic neurogenesis. Infusion of fluorogold (FG) into striatum confirmed that these newborn neurons have successfully restored the nigrostriatal circuit that was disrupted by the HI insult. Although, the implanted hPLC-NPs were matured in the recipient's brain, we propose that the recovery was mainly mediated by the resident progenitors because nestin was induced in the SNc of the recipient animals. Possibility of the migration of the transplanted cells into the host substantia nigra can be safely ruled out since TH-expressing cells expressed neither human nuclear antigen nor human mitochondrial antigen.

Relevance of these results to other dopaminergic degenerative disease needs to be confirmed, however, we propose that progenitors can induce regeneration of the diseased tissues by stimulating the resident tissue-specific progenitors as well as supplementing the lost tissues.

Selected for oral presentation

Notes

Abstract 17

Amniotic mesenchymal tissue cells inhibit tumor cell line proliferation

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We and others have demonstrated that amniotic membrane-derived cells strongly inhibit in vitro lymphocyte proliferation, abolish the production of inflammatory cytokines and suppress the generation and maturation of monocyte-derived dendritic cells. In this study, we aimed to investigate whether these cells may also exert suppressive effects on the proliferation of different tumor cell lines.

We have isolated cells from the mesenchymal layer of amniotic membrane (AMTC) and cultured them with KG1, KG1a, Jurkat, U937, Girardi heart, Hela and Saos cells. Tumor cell line proliferation was then assessed by measuring [3H]-Thymidine incorporation. We demonstrated that AMTC are able to inhibit the proliferation of different tumor cell lines of lymphoid and myeloid origin, as well as tumor cell lines of non-hematopoietic origin, both when cultured in contact and transwell settings, demonstrating the involvement of inhibitory soluble factor(s) in this suppression. This inhibition does not seem to be mediated by tumor cell line apoptosis, but likely to cell cycle arrest of the tumor cells in G0/G1 phase, as revealed by combined analysis with Annexin-V and IP staining, and BrdU and 7-AAD staining, respectively.

These findings reveal further interesting features of AMTC and suggest a possible application of these cells in controlling tumor cell proliferation.

Notes

Abstract 18

In vitro and in vivo differentiation of amniotic epithelial stem cells into hepatocyte-like cells

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The use of cell transplantation as an alternative to Orthotopic Liver Transplant for the treatment of liver diseases is still limited by the availability of useful cells. Stem cell-derived liver cells could be useful if they expressed the enzymes and functions needed for liver support. We investigated the differentiation of human Amniotic Epithelial cells (hAECs) into hepatocytes in vitro. We also tested the ability of rat-derived Amniotic Epithelial cells (rAECs) to integrate and differentiate into hepatic cells upon transplantation into a rat model of liver repopulation.

In vitro differentiation: The expression of mature liver genes was strongly increased in hAECs after culture on a Pig Liver-derived extracellular matrix sandwich system in the presence of EGF, HGF, bFGF, OSM, Dexamethasone and ITS. Differentiated cells were able to metabolize TE, HPC and ammonia, confirming the expression of functional liver enzymes.

In vivo transplant: After transplantation of rAECs into Retrorsine-treated animals, clusters of donor cells were present into the liver of recipient animals. Two, 6 and 12 months following transplants, donor cells had morphological appearance of mature hepatocytes with normal growth pattern. They expressed major liver proteins such as CYP2E1, 3A1 and Albumin.

Taken together, these data indicate that hAECs and rAECs have the potential to differentiate into mature hepatocytes both in vitro and in vivo, thus suggesting that these cells will be a useful source of cells for regeneration of liver tissue.

Notes

Abstract 19

The key role of decidualization in the immunomodulatory activities of human decidual stromal cells

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Decidual stromal cells (DSC) are the main cellular component of the decidua, the maternal tissue which is in close contact with the fetal trophoblast. We have isolated and maintained human DSC lines in culture and used them to characterize the antigen phenotype and properties of these cells. We previously reported that DSC are closely related to mesenchymal stem cells, have contractile activity and exert different immunological activities that may be involved in maternal–fetal cross-talk. Decidual stromal cells differentiate (decidualize) under the effect of the progesterone, changing their morphology to a rounder shape and secreting prolactin. Decidualization led DSC to a more immunoregulatory profile: it inhibited their phagocytic activity and secretion of IL-6, while increasing their secretion of IL-10 and expression of HLA-G. Decidualization also decreased the expression of CD21, CD54 and BAFF by DSC, and although DSC are resistant to many apoptosis-inducing factors, it induced apoptosis in these cells. The potential therapeutic use of DSC was supported by the finding that these cells survived in mice weeks after injection. However, in mice with trinitrobenzene sulfonic acid-induced colitis, decidualized DSC but not undifferentiated DSC showed a therapeutic effect. We also found that endometrial stromal cells, the cellular counterpart of DSC in the non-gestating uterus, exhibited an antigen phenotype equivalent to that of DSC, but ESC were less sensitive to the effects of progesterone.

Selected for oral presentation

Notes

Abstract 20

Transplantation of placenta-derived mesenchymal stem cells in immuno-competent mice submitted to myocardial infarction

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Death of cardiomyocytes due to myocardial infarction (MI) causes wall thinning and ventricular dilatation, which diminishes the ability of the heart to pump blood, leading to heart failure. This study aims to evaluate the role placenta-derived mesenchymal stem cells (pMSCs) in the treatment of cardiac failure in immunocompetent mice.

pMSCs were characterized as plastic-adherent and multipotent cells. They did not express hematopoietic or endothelial cells markers (CD45, CD34, CD14, HLA-DR, CD31, CD133), but express MSCs-associated molecules (CD90, CD105, CD73). pMSCs expressed pluripotency markers OCT-4, DMNT3b and KLF4, usually associated to embryonic stem cells, suggesting that their plasticity is superior to that of adult stem cells.

C57Bl/6 mice were submitted to MI through permanent occlusion of the anterior descending coronary artery. Two weeks after MI, mice were submitted to weekly intramyocardial injections containing pMSCs or vehicle during 3 weeks. Functional parameters were evaluated weekly by echocardiogram for 40 days after the beginning of treatment. However, after analysis of the ejection fraction, end systolic volume and end diastolic volume, we did not observe significant improvement in the pMSCs group, when compared to the vehicle group.

Although pMSCs did not improve cardiac function, it is important to determine for how long the cells remained at injection site, since long-term engraftment is probably necessary for functional improvement. As future perspectives, pMSCs, stably transduced with a viral construct expressing the luciferase gene under the control of ubiquitous promoter, will be used in a bioluminescence assay, to determine their biodistribution pattern and survival rate in vivo.

Notes

Abstract 21

Fetal human liver progenitor cells: a potential immune-privileged cellular source from non heart beating donors

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Therapeutically aborted fetuses are a precious source of stem cells, and generally, when younger than 20 gestational weeks old, are considered only special hospital waste. Meanwhile, the shortage of cadaveric donors for liver transplantation is leading physicians to seek therapeutic alternatives for reducing mortality in waiting lists.

The aims of this work are: 1) to establish, according to GMP guidelines, a reproducible and qualified method for isolating human liver progenitor cells; 2) to evaluate the cellular yield and viability; and 3) to characterize fetal liver stem cells.

From February 2007 to May 2010, 34 consecutive pregnant women donated, after signing informed consent, their therapeutically aborted fetuses to research. Liver digestion was performed with collagenase perfusion. A total number of 34 fetuses were obtained. They ranged between gestational weeks 17 and 22.5. Twenty seven fetal livers were finally processed. Seventeen had a cellular viability ≥ 70%,two <70%, and eight were necrotic. The cellular yield ranged between 3x108 and 3.3x109. The percentage of fetal liver cells expressing proliferation markers (Ki-67, PCNA) was 45-fold greater than that of adult hepatocytes. As gestational age increases, the percentage of immature bipotential progenitors significantly decreases. The mean HLA I expression (2.71%) of the fetal liver cells analyzed was significantly lower than that of control cells (HepG2= 95%; adult hepatocytes= 40%).

In conclusion, the vascular perfusion method can be considered a valid way of digesting the human fetal parenchyma and obtaining a high cellular yield. The low expression of HLA I antigens in fetal liver progenitor cells suggests a low immunogenicity, and thus potential clinical applications of these cells.

Notes

Abstract 22

Amniotic fluid stem cells as therapy for regenerating functional muscle in a muscular model of spinal muscular atrophy

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Recently, it has been shown that amniotic fluid contains a population of stem and progenitor cells able to differentiate into several different tissue-specific lines. In this study we used mice carrying homozygous deletion of Smn to skeletal muscle (HSA-Cre, SmnF7/F7 mice) mimicking the Spinal Muscular Atrophy, a recessive autosomal neuromuscular disorder with degeneration of motor neurons and muscle paralysis and atrophy.

GFP+ Amniotic Fluid Stem cells (AFS) have been systemically injected in HSA-Cre, SmnF7/F7 mice to test the in vivo myogenic potential and to investigate the possible therapeutic effect of AFS for the treatment of muscle-related diseases.

One month after transplantation, muscles from treated mice displayed physiological morphological appearance (low number of regenerating myofibers and dystrophin expression as in wild type mice). About 38% of fibers were GFP+ and by PCR, GFP expression was detected in different muscles, such as Tibialis, Gastrocnemius and Diaphragm. Instead, the untreated mice showed high number of centri-nucleated fibers and down regulation of dystrophin expression.

After physiological analyses treated mice recovered more than 75% of force when compared to the untreated (control). Tibialis anterior underwent cardiotoxin injection one month after AFS cells transplantation and 15 days later the cells generated new green fibers further proving functional integration. Finally, secondary transplants were performed injecting stripped satellite cells derived from primary transplanted mice directly into muscle. One month later about 30% fibers were found to be GFP+.

These results proved that AFS cells are able to renew the depleted muscle niche becoming new satellite cells themselves.

Selected for oral presentation

Notes

Abstract 23

Strategies to determine placental cell engraftment in different animal models

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Detection of cellular engraftment represents an important requirement in the field of in vivo transplantation. To this end, several approaches, such as molecular, histologic, cytofluorimetric and biochemical methods, have been undertaken. Cell visualization within tissues makes the histological approach an important tool for answering questions related to localization, vitality and phenotypical characterization of engrafted cells. The ability of Placenta-derived cells to engraft in xenogeneic animal models has been demonstrated, and strategies aimed at investigating this property at the morphological level could represent a useful tool to better understand this process. We set up and tested different methods for human placental cell visualization after xenogeneic transplantation into rat, mouse, and sheep tissues. Our proposed methods are based on performance of immunohistochemistry (using monoclonal anti-human nuclei and anti-human mithocondria antibodies) and in-situ hybridization (using anti-human ALU and Cen17 genomic sequences probes) on formalin-fixed paraffin-embedded tissues (FFPEs). The comparative efficacy of these methods was evaluated in terms of sensitivity and specificity, while cross-reactivity between markers in human placental cells and in host species tissues was also evaluated. Our studies allowed elucidation of the most appropriate strategies for cell detection, and we demonstrated that detection of human cells within sheep tissue was possible using Chromogenic in situ hybridization (CISH) with an anti-human ALU probe, while chimerism detection after transplantation into mouse or rat tissue could be achieved by application of CISH using an anti-Cen17 probe, or by Indirect immunofluorescence using a monoclonal anti-human mitochondria antibody.

Notes

Abstract 24

Application of human amniotic membrane on rat liver following left hepatectomy: evaluation of liver reaction

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Over past decades, the numerous unique characteristics of human amniotic membrane (hAM), such as its anti-inflammatory and antifibrotic properties, justified the frequent use of this material in human reparative surgery and experimental animal disease models. In order to investigate the potential of amniotic membrane to promote hepatic regeneration, we applied the membrane to the external surface of hepatectomized rat livers and assessed hepatocyte proliferation rate and tissue reaction at 1, 3, 7 and 14 days after surgery. Clinical and histopathological data were collected and compared with hepatectomized control rats which received no hAM transplantation and were sacrificed at the same intervals.

In both control and hAM-transplanted animals, a rapid rise in mitotic activity was first observed near the resection, followed by a higher peak in mitoses at day 3 in distant areas. The number of binucleated cells, which decreased after partial hepatectomy, increased during liver regeneration, and this was observed more so in hAM transplanted animals. In this group, at day 7, hAM was adherent to the liver capsule in association with interposed palisades of tightly packed fibroblast-like cells. Discrete areas of partial resorption of hAM were observed at day 14. The parenchyma did not show any morphologically appreciable signs of degeneration or inflammatory infiltration in hAM-transplanted animals.

Our data suggest that application of hAM membrane to the surface of resected livers does not impede normal recovery and actually induces a slight improvement in hepatocyte proliferation. Interestingly, the application of xenogeneic amniotic membrane in this study did not result in any evident signs of rejection.

Notes

Abstract 25

Isolation and characterization of mesenchymal stem cells from amniotic fluid and chorionic villi

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This study was design to assess amniotic- (AF-MSC) and chorionic villi-derived mesenchymal stem cells (CV-MSC) characteristics, in order to verify their possible applications for cellular therapy and regenerative medicine. Starting from 3 mL of amniotic fluid and approximately 5 mg of chorionic villi, the samples were analyzed for biological endpoints like: cell viability, proliferation rate, doubling time, immunophenotype, and differentiation potential. Genome stability, by karyotype analysis, genome-wide array-CGH and microsatellite analysis, were also explored. AF and CV samples showed the presence of cells with features of staminality and differentiation potential towards osteogenic, adipogenic and condrogenic phenotypes. Karyotype and microsatellite stabilities were assessed until the 15th and 27th culture passages, respectively. The frequency of the chromosomes aberrations at the different culture passages was not significantly different from the basal frequency found in primary cultures. Preliminary data obtained from array CGH analysis comparing DNA from early to late passages (3rd vs 27th) did not show any copy number variations of DNA segments, indicating that the in vitro culture did not induce any modification of the genome stability. Immunophenotyping of cultured MSC revealed two distinct expression patterns related to the two different prenatal sample sources. Our findings indicate that it is possible to isolate and extensively expand MSC from AF and CV and that the in vitro growth culture does not interfere with the DNA-repair systems since the DNA stability is maintained during in vitro expansion. Under these circumstances, AF- and CV-MSC could be suitable for therapeutic proposes. Moreover the use of cell bank technology, on native samples, might represent a life-long available autologous cell source for perinatal or adult regenerative medicine.

Notes

Abstract 26

Ability of polyurethane foams to support human placenta-derived mesenchymal cell adhesion and osteogenic differentiation

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The use of engineered constructs made up of cells cultured on porous scaffolds is an appealing alternative to current clinical therapies for bone tissue reconstruction. Mesenchymal cells hold great promise in this context, and human placenta represents an easily accessible source of pluripotent cells which is exempt from ethical debate.

The aim of this work was to evaluate the osteogenic differentiation capacity of human amniotic (hAMSCs) and chorionic (hCMSCs) mesenchymal stromal cells when cultured on polyurethane foams (PUFs) either with or without a coating of a-tricalcium phosphates (α-TCP PUF).

PUF matrix was synthesized by reacting a polyether-polyol mixture with MDI prepolymer, using Fe-acetyl-acetonate as the catalyst and water (2% w/wpolyol) as the expanding agent. The matrix was then coated by immersion in α-TCP suspension under magnetic stirring. hAMSCs and hCMSCs were isolated from two human term placentas and cultured on polyurethane foams or in glass chamber slides (control), in the presence of osteoinductive or control medium for up to 20 days. Cell morphology was investigated by SEM while scaffold colonization and cell differentiation were evaluated by routine and specialized histological stains.

Characterization of PUF allowed evaluation of foam density (0.127±0.003g/cm3), porosity (~90%) and average pore size (268µm). By SEM, good cell colonization and adhesion onto both PUF and α-TCP PUF was observed for all tested cell types. A clear adhesion of cells to the porous surface of PUF was confirmed by histological analysis, which also revealed the presence of calcium deposition around cells seeded on α-TCP PUF, even when cultured in the absence of osteoinductive medium.

Therefore, the tested polyurethane foams seem to be a valid scaffold to support adhesion and differentiation of placenta-derived mesenchymal cells.

Notes

Abstract 27

Mesenchymal transition of amnion epithelial cells

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Amnion epithelial cells (AEC) are a readily available cell source for potential use in regenerative medicine. Under certain conditions, subpopulations of AEC may express very early stem cell markers such as SSEA-3, SSEA-4, TRA1-60, TRA1-81, Oct-3/4, Nanog, SOX-2 and display stem cell behaviour, but usually AEC behave like mature epithelial cells. We therefore sought to induce epithelial-to-mesenchymal transition (EMT) in AEC to improve their capacity for cardiovascular regeneration.

Human AEC were derived from full-term placenta and expanded in customized media. To induce EMT, transforming growth factor-β (TGF- β) was added in different concentrations. EMT was then assessed by analysis of cell morphology, immunocytology staining, in vitro scratch wound assay, and Transwell cell migration assay.

Following primary expansion in customized media, AEC expressed stem cell markers and showed trilineage differentiation capacity. At higher passages under standard culture condition, however, AEC lost Oct-4 expression and behaved like mature epithelial cells. Nevertheless, 25ng/ml TGF- β added to the medium for 5-6 days was sufficient to induce striking changes in AEC morphology. Cells acquired an elongated, fibroblastoid shape, equivalent to that of mesenchymal stem cells. By immunocytology, TGF-β induced up-regulation of N-cadherin was confirmed. In the scratch wound assay, TGF- β stimulated AEC displayed an irregular migration pattern that lead to accelerated wound closure, and only stimulated AEC migrated to the lower Transwell chamber through an 8μm membrane.

In conclusion, under the influence of TGF-β, mature AEC undergo epithelial-to-mesenchymal transition and acquire a mesenchymal stem cell-like phenotype. EMT greatly enhances cell mobility and may thus help optimize AEC for use in cardiovascular cell therapy.

Notes

Abstract 28

Extracellular matrix: from the placental development at tissue engineering

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During the morphogenesis and development of tissues, a coordinated process of proliferation and differentiation of cells are requires. In this context, adequate relationships with the extracellular matrix (ECM) components are essential for normal development.

The ECM comprises a variety of versatile proteins arranged in a cell surface-associated network and its interaction with cells provide the basic principles of material sciences that could be applied to explain the interaction of the cells in artificial construct for tissue engineering.

Some examples are present that showed as the ECM is necessary for placenta in human and animal models during the pregnancy, as well artificial tissues construction develop for tissue engineering.

Placental tissues of human and rodent were obtained and process for morphological analysis. Primary cell cultures of stromal fibroblasts were generated from normal oral mucosa biopsies. A substitute of the stroma was constructed using fibrin. Extracellular matrix proteins were evaluated by immunohistochemistry.

During pregnancy the embryo and placental cells differentiate and promote several changes related with the normal development. During these processes, a coordinated distribution of collagens and glycoproteins were observed in different compartment of the placenta, as well in the artificial tissues construction.

The morphogenesis of the placental in mammals is associated with the presences of several ECM molecules, distributed with temporal-spaces differences. Similar changes were observed in the artificial construct. The knowledge of the ECM and their relationship with the cells may be useful to understandings the cells microenvironment in artificial tissue construction.

Notes

Abstract 29

Wharton's jelly derived mesenchymal stem cells: regenerative medicine beyond umbilical cord blood

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Umbilical cord blood (UCB) has been used as a source of hematopoietic stem cells (HSCs) for the transplantation of over 10,000 patients worldwide for the treatment of various disorders, including hematological malignancies. However, since UCB contains a finite number of mononuclear cells (MNCs), the likelihood of reaching a target cell dose is reduced drastically as the patient increases with age and weight. The result of low pre-transplant cell dose is delayed hematopoietic reconstitution, increased time-to-leukocyte recovery, increased likelihood of infections, and increased overall transplant-related morbidity and mortality. Novel approaches to overcome this limitation in UCB transplantations include multiple-unit transplantations and technologies to expand HSCs ex vivo. However, these approaches have not resulted in significant improvements in patient morbidity and mortality primarily due to complications, including increased graft vs. host disease (GvHD) outcomes and the lack of successful clinical demonstrations. One approach that is promising and has not been fully investigated is the co-transplantation of UCB-HSCs with mesenchymal stem cells (MSCs) derived from the Wharton's Jelly (WJ) of the umbilical cord (UC). We investigated this by co-transplanting 106 mononuclear UCB cells with 104 or 105 WJ-MSCs in sub-lethally irradiated NOD/SCID IL2R γ-null mice and observed a 3.5- and 6.0-, respective, fold increase in human CD45 expression in the bone marrow of transplanted mice, when compared to 106 mononuclear UCB cells transplanted alone. This data suggests a role for WJ-MSCs in UCB transplantation, allowing current UCB units that do not meet the minimum criteria for transplantation (based on total nucleated cell dose), effective for pediatric, and even possibly, adult patients.

Selected for oral presentation

Notes

Abstract 30

Role of human amniotic stromal cells in spinal cord injury repair

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Amniotic stromal cells were reported to possess stem cell features. In the present study, it was intended to test the potentials of this non-controversial source of stem cells in treating spinal cord injury. To evaluate, a xenotransplantation approach using human amniotic stromal cells transplantation in contusive spinal cord injury model of rats was used. Human amniotic stromal cells were isolated through collagenase treatment of amniotic membranes which were previously denuded of epithelial cell by trypsinization. Isolated cells were tested for their multipotentiality using standard osteogenic, chondrogenic and adipogenic induction methods. These cells were transplanted into the spinal cords of rats after contusion injury. Results were evaluated using behavioral, hisotological and histochemical methods. Either human mitochondrial antibody or PKH 27 labeling of transplanted cells was used to identify surviving cells.

Results indicate that human amniotic stromal cells did not cause improved functional recovery when compared with lesion group animals. Histochemical staining indicated the deposition of collagenous material at the transplantation site which might indicate the aberrant differentiation of transplanted cells towards osteo/chondrogenic lineage. A similar observation had been made earlier with rat amniotic stromal cells when used in spinal cord injury. Therefore, it is speculated that neural induction of these cells may be mandatory before using them for spinal cord injury repair. This could ensure proper differentiation of these cells paving a way for better functional recovery. The transplanted human amniotic stromal cells were found to survive in rat spinal cord for about 4 – 8 weeks without immune suppression and did not form any tumor. Thus further studies are required to verify the potential of these cells in treating spinal cord injury.

Notes

Abstract 31

Characterization and evaluation of fetal stem cells used for tissue engineering

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With regard to cardiovascular applications of the tissue engineering concept, several groups have demonstrated the principal feasibility to create functional living heart valves, blood vessels, and myocardial structures using autologous cell systems and rapidly degrading scaffold materials. Recent long-term in vivo studies demonstrated for the first time that the fabrication and implantation of living, autologous tissue engineered structures in a large animal model covering the full biological growth cycle is feasible. In today's cardiovascular clinical scenario, the highest medical need for a tissue engineering solution is in the field of pediatric applications treating congenital heart disease. In this context, the introduction of a living, growing replacement such as e.g. a tissue engineered heart valve made of the babies' own cells would substantially reduce today's severe therapeutic limitations, which are mainly due to the need for repeat reoperations to adapt the current artificial protheses to the somatic growth of the young patients. However, these reoperations are associated with substantial morbidity as well as mortality and beyond that, represent a significant trauma to the young patients and their families. Ideally, the cells to be used for babies with congenital heart disease - normally detected by prenatal ultrasound around week 20 - can be obtained already during pregnancy to provide the time for the tissue engineering process prior to birth. In recent in vitro studies we have demonstrated the feasibility to use various human fetal stem cells for tissue engineering of heart valves. In particular, amniotic fluid- and chorionic villi-derived cells have shown promising potential for the clinical realization of the congenital tissue engineering approach. Based on these results, the objective of our current research is to systematically investigate and to validate fetal stem cells with regard to their potential for tissue engineering applications.

Selected for oral presentation

Notes

Abstract 32

Immunomodulatory effects of placenta-derived multipotent cells towards lymphocytes of the innate and adaptive immune system

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Current sources of human stem cells include embryonic stem cells (ESCs) and adult stem cells (ASCs). However, concerns exist with either source: ESCs, with its significant ethical considerations, tumorigenicity concerns, and paucity of cell lines; and ASCs, which are possibly more limited in proliferative and differentiation potential. Thus, the search continues for an ethically conducive, easily accessible, and high-yielding source of stem cells. Our research group has isolated multipotent cells from the human term placenta, a temporary organ which is discarded after birth. Placenta-derived multipotent cells (PDMCs) exhibit similar markers as bone marrow mesenchymal stem cells, including CD105 and CD73, as well as ESC markers such as SSEA-4; and can differentiate into cell phenotypes representative of all three germ layers. Moreover, PDMCs have significant immunomodulatory effects towards allogeneic lymphocytes of both the innate and adaptive arm. PDMCs suppress allogeneic T lymphocyte proliferation, and express indoleamine 2,3-dioxygenase as well as intracellular HLA-G. Mechanistically, suppression of lymphocyte reactivity by PDMCs is not due to cell death, but decreased cell proliferation and increased numbers of regulatory T cells. Several effector functions of natural killer lymphocytes (NKs) are also suppressed by PDMCs, including interferon-γ (IFN-γ) secretion and cytotoxicity. Pretreatment of PDMCs with IFN-γ, a proinflammatory cytokine, surprisingly enhances such immunosuppressive effects. With its broad immunosuppressive properties and multilineage differentiation capacity, PDMCs may represent a potential cell source for therapeutic use.

Notes

Abstract 33

Effects of simvastatin on miRNA profile of multipotent mesenchymal stem cells from human amniotic membrane

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Multipotent mesenchymal stem cells (MSC) have beneficial effects against graft-versus-host disease (GVHD) when co-transplated with hematopoietic stem cells (HSC). Statin lipid-lowering drugs also are known to reduce acute GVHD that seem to be related to their effects on lymphocytes through TGF-β/SMAD pathway. SMADs in turn, can regulate the biogenesis of microRNAs (miRNAs), which are crucial post-transcriptional regulators of gene expression. We sought to verify if statins could affect miRNA expression and the molecular pathways associated to their targets, especially those that may be important for MSC immunomodulation. To do so, we treated MSC isolated from human placenta amniotic membranes with two concentrations of simvastatin and performed miRNA Microarray analysis (Agilent). We found 66 up and 59 down-regulated miRNAs in a dose-dependent way.

The top five up-regulated miRNAs were miR-126*, -135b, -496, -146b-5p and -516a-5p. The top five down-regulated miRNAs were miR-1267, -1201, -519b-3p, -942 and -96. Considering that predicted targets of these miRNAs are expected to be regulated by simvastatin, we used MirWalk miRNA database to predict these targets. Then we performed an Ingenuity Pathway Analysis with these targets, focusing on Cytokine signaling because soluble factors released by MSC are believed to be the key mechanism of their action. We found IL-8, Prolactin, CNTF, fMLP, FLT3, IL-17, CXCR4, IL-3, CCR3, IL-15 and B as the most represented cytokine signaling pathways. Some of these arekNF- already associated to MSC functions. We highlight Prolactin pathway, which is involved in many immunoregulatory processes, but its importance on MSC function is still unknown. The link between these pathways and their regulation by statins and miRNAs might represent another level of regulation of MSCs function.

Notes

Abstract 34

Amnion development and plasticity: insights from the mouse embryo

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The amnion is an avascular and bilayered extraembryonic membrane that surrounds the fetus of amniotes and contains the amniotic fluid. BMP signaling is essential for amnion development and homeostasis in the mouse. Embryos deficient in Smad5, an intracellular mediator of BMP signaling, develop an amnion thickening that becomes vascularized and haematopoietic. Some cells of the amniotic thickening express ectopically and de novo stem cell markers like Oct4, AP, SSEA-1, suggesting that amnion cells undergo an in situ change in fate. We demonstrate that Bmp/Smad5 signaling functions as an intracellular Nodal signaling antagonist. This is a novel mechanism essential for maintaining amnion fate and to prevent ectopic primitive streak formation.

To further explore the amnion signature and the mechanism that underlies amnion reprogramming in Smad5 mutant amnion we have recently undertaken a comparative whole transcriptome expression profiling study. Our first mRNASeq results will be presented.

The process of amnion development has been poorly described in primates and rodents, and conflicting descriptions exist. Understanding the morphogenesis and development of extra-embryonic tissues is crucial for correctly interpreting e.g. amnion plasticity, fate-mapping data and mutants with gastrulation defects. Moreover, the recent isolation from amnion of cells with stem cell features further argues for a better understanding of the process of amnion formation. Here, we revisit the highly dynamic process of amnion formation, present a comprehensive model and animation that provide a new framework for the interpretation of fate-map data, investigating complex defects in the amnion of mouse mutants and amniotic stem cell populations.

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