

TISSUE-SPECIFIC STEM CELLS

In Vitro Differentiation of Human Placenta-Derived Multipotent Cells into Hepatocyte-Like Cells

Chih-Cheng Chien^{a,b}, Betty Linju Yen^c, Fa-Kung Lee^e, Tsung-Hsuan Lai^{a,e}, Yao-Chang Chen^{c,d}, Shu-Hui Chan^a, Hsing-I Huang^{a,b}

^aCathay Medical Research Institute,

^cDepartment of Obstetrics and Gynecology, Cathay General Hospital, Taipei, Taiwan;

^bDepartment of Medicine, Fu-Jen Catholic University, Taipei, Taiwan;

^cStem Cell Research Center, National Health Research Institutes, Taipei, Taiwan;

^dDepartment of Forensic Medicine, National Taiwan University, Taipei, Taiwan

Key Words. Placenta • Differentiation • Hepatocyte • Regenerative medicine

Correspondence: Hsing-I Huang, Ph.D., Cathay Medical Research Institute, Cathay General Hospital, No. 32, Ln 160, Jian-Cheng Road, 22141, Hsi-Chih, Taipei, Taiwan, R.O.C. Telephone: 886-2-26907965, ext. 2312; Fax: 886-2-26907963; email: hihuang@cgh.org.tw

Received October 20, 2005; accepted for publication March 15, 2006.

First published online in *STEM CELLS EXPRESS* March 15, 2006.

Multipotent cells isolated from human term placenta (placenta-derived multipotent cells [PDMCs]) have been known to be able to differentiate into mesodermal lineage cells, including adipocytes and osteoclasts. The low infection rate and young age of placenta compared with other tissue origins of adult stem cells make these cells attractive target for cell-based therapy. However, the differentiation potential of PDMCs toward hepatic cells has not been evaluated yet. In this study, we cultivated PDMCs with hepatic differentiation medium to evaluate the ability of these cells in differentiating toward hepatic cells. After treatment, the morphologies of differentiated PDMCs changed to polygonal epithelial cell-like. The differentiated cells not only show the hepatocyte-like morphologies but also express hepatocyte-specific markers, including albumin and cytokeratin 18. The bioactivity assays revealed that these hepatocyte-like cells could uptake lipoprotein and store glycogen. Furthermore, the addition of rifampicin increased the gene expression of CYP3A4, which is similar with the activities of human liver cells. According to our previous results, PDMCs were capable of differentiating into mesodermal and ectodermal lineage cells. Our results indicate that PDMCs can differentiate into three germ layer cells, which is similar to embryonic stem cells. In conclusion, placenta might be an easily accessible source for progenitor cells that are capable of differentiating toward hepatocyte-like cells in vitro.

Placenta-Derived Multipotent Cells Exhibit Immunosuppressive Properties That Are Enhanced in the Presence of Interferon- γ

Chun-Jung Chang^a, Men-Luh Yen^b, Yao-Chang Chen^c, Chih-Cheng Chien^{d,e}, Hsing-I. Huang^{d,f}, Chyi-Huey Bai^g, B. Linju Yen^a

^aStem Cell Research Center, National Health Research Institutes, Zhunan, Taiwan;

^bDepartment of Primary Care Medicine and Department of Obstetrics/Gynecology, National Taiwan University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan;

^cDepartments of Laboratory Medicine and Forensic Medicine, National Taiwan University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan;

^dDepartment of Medicine, School of Medicine, Fu Jen Catholic University, Taipei, Taiwan;

^eCathay General Hospital Neihu;

^fCathay Medical Research Institute, Cathay General Hospital, Taipei, Taiwan;

^gCentral Laboratory, Shin Kong WHS Memorial Hospital, Taipei, Taiwan

Key Words. Mesenchymal stem cell • Placenta • Multilineage differentiation • Immunosuppression • Mixed lymphocyte culture • Interferon- γ • Human leukocyte antigen, class I, G • Indoleamine 2,3-dioxygenase

Correspondence: B. Linju Yen, M.D., Stem Cell Research Center, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 350, Taiwan. Telephone: +886-2-2653-4401, ext. 27502; Fax: +886-2-2792-9679; e-mail: blyen@nhri.org.tw

Received February 3, 2006; accepted for publication July 14, 2006.

Several types of nonhematopoietic stem cells, including bone marrow mesenchymal stem cells (BMMSCs) and embryonic stem cells, have been shown to have immunosuppressive properties. We show that human placenta-derived multipotent cells (PDMCs), which are isolated from a source without ethical concern and harbor multilineage differentiation potential, have strong immunosuppressive properties. PDMCs suppress both mitogen-induced and allogeneic lymphocyte proliferation in both CD4 and CD8 populations. The immunosuppression seen with PDMCs was significantly stronger than that with BMMSCs. Both PDMCs and BMMSCs express indoleamine 2,3-dioxygenase, but only PDMCs are positive for intracellular human leukocyte antigen-G (HLA). Mechanistically, suppression of lymphocyte reactivity by PDMCs is not due to cell death but to decreased cell proliferation and increased numbers of regulatory T cells. Addition of neutralizing antibodies to interleukin-10 and transforming growth factor (TGF)- β partially restored lymphocyte proliferation. Unlike BMMSCs, PDMCs treated with interferon- γ for 3 days only very minimally upregulated HLA-DR. On the contrary, PD-L1, a cell surface marker that plays an inhibitory role in T-cell activation, was upregulated and TGF- β expression was seen. The immunosuppressive properties of PDMCs, along with their multilineage differentiation potential, ease of accessibility, and abundant cell numbers, may render these cells as good potential sources for future therapeutic applications.

Isolation of Multipotent Cells from Human Term Placenta

B. Linju Yen^a, Hsing-I Huang^b, Chih-Cheng Chien^{b,c}, Hsiang-Yiang Jui^a, Bor-Sheng Ko^d, Ming Yao^d, Chia-Tung Shun^e, Men-luh Yen^{f,g}, Meng-Chou Lee^a, Yao-Chang Chen^a

^a Stem Cell Research Center, National Health Research Institutes, Taipei, Taiwan;

^b Cathay Medical Research Institute, and

^c Department of Anesthesiology, Cathay General Hospital, Taipei, Taiwan;

^d Department of Internal Medicine,

^e Departments of Forensic Medicine and Pathology,

^f Department of Primary Care Medicine, and

^g Department of Obstetrics and Gynecology, National Taiwan University Hospital and National Taiwan University, College of Medicine, Taipei, Taiwan

Key Words. Stem cell • Placenta • Multilineage differentiation Stage-specific embryonic antigen—4 (SSEA-4) • Tumor rejection antigens

Correspondence: Yao-Chang Chen, M.D., Stem Cell Research Center, National Health Research Institutes, No. 161, 4F, Min-Chuan E. Road, Taipei, 114, Taiwan. Telephone 886-2-2312-3456, ext. 5489; Fax: 886-2-2321-8438; e-mail: ycchenmd@nhri.org.tw

Current sources of stem cells include embryonic stem cells (ESCs) and adult stem cells (ASCs). However, concerns exist with either source: ESCs, with their significant ethical considerations, tumorigenicity, and paucity of cell lines; and ASCs, which are possibly more limited in potential. Thus, the search continues for an ethically conducive, easily accessible, and high-yielding source of stem cells. We have isolated a population of multipotent cells from the human term placenta, a temporary organ with fetal contributions that is discarded postpartum. These placenta-derived multipotent cells (PDMCs) exhibit many markers common to mesenchymal stem cells—including CD105/endoglin/SH-2, SH-3, and SH-4—and they lack hematopoietic-, endothelial-, and trophoblastic-specific cell markers. In addition, PDMCs exhibit ESC surface markers of SSEA-4, TRA-1-61, and TRA-1-80. Adipogenic, osteogenic, and neurogenic differentiation were achieved after culturing under the appropriate conditions. PDMCs could provide an ethically uncontroversial and easily accessible source of multipotent cells for future experimental and clinical applications.

Placenta has versatile 'stem cells'

Scientists looking for easier and less-controversial alternatives to stem cells from human embryos say they found a potential source in placentas saved during childbirth.

They describe primitive cells found in a part of the placenta called the amnion, which they coaxed into forming a variety of cell types and look like sought-after embryonic stem cells.

Placentas could provide a ready source of the cells, says the [University of Pittsburgh](#) team online in the journal [Stem Cells](#).

It is not yet certain that the cells they found are true stem cells, says Dr Stephen Strom, who worked on the study.

But they carry two important genes, Oct 4 and nanog, which so far have only been seen on embryonic stem cells.

"We were just blown away when we found those two genes expressed in those cells," Strom says.

"The presence of these two genes suggests these cells are pluripotent, which means they should be able to form any cell type in the body."

Stem cells are the body's master cells. So-called adult stem cells are found in the tissue and blood and are a source for renewing cells.

Embryonic stem cells are found in young embryos. While powerful, their use is controversial because some people believe destroying an embryo is immoral and unethical.

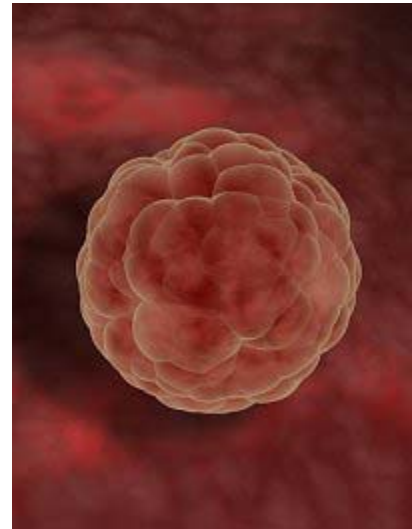
Supporters of embryonic stem-cell research say it may provide an important path to a new field of regenerative medicine, in which diseases ranging from juvenile diabetes to paralysis could be cured using transplants of carefully cultivated stem cells.

Where to look?

Strom and colleagues say they looked in a part of the placenta called the amnion, the outer membrane of the amniotic sac.

Other teams of researchers, notably Dr Anthony Atala of [Wake Forest University](#) in North Carolina, have found stem cells resembling embryonic cells in amniotic fluid. But research is still early and it is not known how useful those would be.

Strom says his cells are different from the ones the Wake Forest team found, and they may not be true stem cells because they did not form tumors in his experiments, as a true stem cell would.



In the future, we may be sourcing stem cells from placentas, rather than human blastocysts like this (Image: iStockphoto)

Strom says the cells he works with also do not appear to be immortal, unlike true stem cells.

Strom's team tested the cells in lab dishes, incubating them in various compounds, and coaxed them to form into what looked like heart cells, nerve cells, liver cells and pancreatic cells.

Strom's lab works specifically on liver transplants and he hopes to develop the cells to use them instead of donated liver. Pancreatic cells would be important because they could be used to treat diabetes.

The university has licensed the technology to biotechnology company Stemnion. The researchers are shareholders and will receive licence fees as part of the agreement.

Placenta Is A Rich Source Of Blood Stem Cells

ScienceDaily (Mar. 25, 2005) — Researchers at Children's Hospital Boston and the Dana-Farber Cancer Institute report a surprising finding about embryonic development: the blood system begins to form not only in the embryo itself, but also in the placenta, the organ that nurtures the baby in utero.

Meticulous experiments in mice revealed that the placenta harbors a large supply of hematopoietic (blood-forming) stem cells. These cells, which appear very early in development, are able to generate more blood stem cells and can give rise to a complete blood system when transplanted into an adult. Unlike other sites where blood stem cells are found during embryonic development, such as the liver, the stem cells in the placenta can increase in number without giving rise to mature, specialized cells.

"There must be something unique about the placenta that nurtures blood stem cells and discourages them from differentiating," says Dr. Stuart Orkin, a Howard Hughes Medical Institute investigator at Children's and DFCI, and a senior investigator of the study. "If we figure out what's special about the placental environment, we may learn how to grow blood stem cells in large numbers for clinical application."

Blood stem cells are used in treating blood cancers like leukemia and other blood diseases, and in patients receiving transplants, but growing them in quantity is difficult. The cells don't multiply readily in the laboratory, so they must be harvested from bone marrow by needle aspiration, a painful procedure, or coaxed into the blood and then collected. Both methods yield only a limited number of blood stem cells.

For more than a decade, scientists have believed that blood stem cells are made only in the embryo itself, within the region of the developing aorta. No role was suspected for the placenta, which has been seen as simply a place for nutrient exchange and waste

removal between mother and fetus. But rather than merely providing nutrients, Orkin says, the placenta may also provide an "infusion" of blood stem cells to the fetus.

"This research reveals a new organ for blood development," Orkin says. "It is surprising that this role for the placenta has been overlooked for so many years."

The study, published in the March issue of the journal *Developmental Cell*, found that blood stem cells appeared in the placenta early, with numbers peaking mid-gestation. Only the fetal liver, where blood stem cells are known to expand tremendously, had greater numbers of blood stem cells.

Children's Hospital Boston is home to the world's largest research enterprise based at a pediatric medical center, where its discoveries have benefited both children and adults for over 136 years. More than 500 scientists, including eight members of the National Academy of Sciences, nine members of the Institute of Medicine and 10 members of the Howard Hughes Medical Institute comprise Children's research community. Founded in 1869 as a 20-bed hospital for children, Children's Hospital Boston today is a 325-bed comprehensive center for pediatric and adolescent health care grounded in the values of excellence in patient care and sensitivity to the complex needs and diversity of children and families. Children's also is the primary pediatric teaching affiliate of Harvard Medical School. For more information about the hospital visit: <http://www.childrenshospital.org/research>.

First published online August 4, 2005

Submitted on December 15, 2004

Accepted on May 27, 2005

Stem Cell Characteristics of Amniotic Epithelial Cells

Toshio Miki ¹, Thomas Lehmann ¹, Hongbo Cai ¹, Donna B Stolz ², Stephen C Strom ^{3*}

¹ Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania

² Department of Cell Biology & Physiology and McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

³ Department of Pathology and McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

* To whom correspondence should be addressed. E-mail: strom@pitt.edu.

Abstract

Amniotic epithelial cells develop from the epiblast by 8 days after fertilization and prior to gastrulation opening the possibility that they might maintain the plasticity of pre-gastrulation embryo cells. Here we show that amniotic epithelial cells isolated from human term placenta express surface makers normally present on embryonic stem and germ cells. In addition, amniotic epithelial cells express the pluripotent stem cell specific transcription factors octamer-binding protein 4 (Oct-4), and nanog. Under certain culture conditions, amniotic epithelial cells form spheroid structures which retained stem cell characteristics. Amniotic epithelial cells do not require other cell derived feeder layers to maintain Oct-4 expression, do not express telomerase and are non-tumorigenic upon transplantation. Based on immunohistochemical and genetic analysis, amniotic epithelial cells have the potential to differentiate to all three germ layers-endoderm (liver, pancreas), mesoderm (cardiomyocyte), and ectoderm (neural cells) in vitro. Amnion derived from term placenta following live birth may be a useful and non-controversial source of stem cells for cell transplantation and regenerative medicine.

Key Words. Placenta, amniotic epithelial cell, stem cell, differentiation

First published online November 1, 2007

Stem Cells Vol. 26 No. 2 February 2008, pp. 300 -311
doi:10.1634/stemcells.2007-0594; www.StemCells.com
© 2008 [AlphaMed Press](http://www.AlphaMedPress.com)

TISSUE-SPECIFIC STEM CELLS

Concise Review: Isolation and Characterization of Cells from Human Term Placenta: Outcome of the First International Workshop on Placenta Derived Stem Cells

Ornella Parolini^a, Francesco Alviano^b, Gian Paolo Bagnara^b, Grozdana Bilic^c, Hans-Jörg Bühring^d, Marco Evangelista^a, Simone Hennerbichler^e, Bing Liu^f, Marta Magatti^a, Ning Mao^f, Toshio Miki^g, Fabio Marongiu^g, Hideaki Nakajima^h, Toshio Nikaidoⁱ, C. Bettina Portmann-Lanz^j, Venkatachalam Sankar^k, Maddalena Soncini^a, Guido Stadler^e, Daniel Surbek^j, Tsuneo A. Takahashi^h, Heinz Redl^e, Norio Sakuragawa^l, Susanne Wolbank^e, Steffen Zeisberger^c, Andreas Zisch^c, Stephen C. Strom^g

^aCentro di Ricerca E. Menni, Fondazione Poliambulanza, Istituto Ospedaliero, Brescia, Italy;

^bDepartment of Histology, Embryology and Applied Biology, University of Bologna, Bologna, Italy;

^cDepartment of Obstetrics, University Hospital Zurich, Zurich, Switzerland;
^dDepartment of Internal Medicine II, University Clinic of Tübingen, Tübingen, Germany;
^eRed Cross Blood Transfusion Service of Upper Austria/Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Linz-Vienna, Austria;
^fDepartment of Cell Biology, Institute of Basic Medical Sciences, Beijing, China;
^gDepartment of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA;
^hInstitute of Medical Science, University of Tokyo, Tokyo, Japan;
ⁱDepartment of Regenerative Medicine, University of Toyama Faculty of Medicine, Toyama, Japan;
^jDepartment of Obstetrics and Gynaecology, University of Berne, Berne, Switzerland;
^kDepartment of Anatomy, University of Madras, Chennai, India;
^lDepartment of Regenerative Medicine, Kitasato University, Kanagawa, Japan

Key Words. Human placenta • Fetal membranes • Amnion • Chorion • Mesenchymal stromal cells • Fetal tolerance

Correspondence: Ornella Parolini, Ph.D., Centro di Ricerca E. Menni, Fondazione Poliambulanza, Istituto Ospedaliero, Via Bissolati 57, 25124 Brescia, Italy. Telephone: 39-030-2455-754; Fax: 39-030-2455-704; e-mail: ornella.parolini@tin.it

Received July 24, 2007; accepted for publication October 18, 2007.
First published online in *STEM CELLS EXPRESS* November 1, 2007.

Placental tissue draws great interest as a source of cells for regenerative medicine because of the phenotypic plasticity of many of the cell types isolated from this tissue. Furthermore, placenta, which is involved in maintaining fetal tolerance, contains cells that display immunomodulatory properties. These two features could prove useful for future cell therapy-based clinical applications. Placental tissue is readily available and easily procured without invasive procedures, and its use does not elicit ethical debate. Numerous reports describing stem cells from different parts of the placenta, using nearly as numerous isolation and characterization procedures, have been published. Considering the complexity of the placenta, an urgent need exists to define, as clearly as possible, the region of origin and methods of isolation of cells derived from this tissue. On March 23–24, 2007, the first international Workshop on Placenta Derived Stem Cells was held in Brescia, Italy. Most of the research published in this area focuses on mesenchymal stromal cells isolated from various parts of the placenta or epithelial cells isolated from amniotic membrane. The aim of this review is to summarize and provide the state of the art of research in this field, addressing aspects such as cell isolation protocols and characteristics of these cells, as well as providing preliminary indications of the possibilities for use of these cells in future clinical applications.

Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta.

[In 't Anker PS](#), [Scherjon SA](#), [Kleijburg-van der Keur C](#), [de Groot-Swings GM](#), [Claas FH](#), [Fibbe WE](#), [Kanhai HH](#).

Department of Obstetrics, Lieden University Medical Center, Leiden, The Netherlands.
E.in_t_Anker@lumc.nl

Recently we reported that second-trimester amniotic fluid (AF) is an abundant source of fetal mesenchymal stem cells (MSCs). In this study, we analyze the origin of these MSCs and the presence of MSCs in human-term AF. In addition, different parts of the human placenta were studied for the presence of either fetal or maternal MSCs. We compared the phenotype and growth characteristics of MSCs derived from AF and placenta. Cells from human second-trimester (mean gestational age, 19(+2) [standard deviation, +/- 1(+3)] weeks, n = 10) and term third-trimester (mean gestational age, 38(+4) [standard deviation, +/- 1] weeks, n = 10) AF, amnion, decidua basalis, and decidua parietalis were cultured in M199 medium supplemented with 10% fetal calf serum and endothelial cell growth factor. Cultured cells were immunophenotypically characterized, the adipogenic and osteogenic differentiation capacity was tested, and the growth kinetics were analyzed. The origin of fetal and maternal cells was determined by molecular human leukocyte antigen typing. We successfully isolated MSCs from second-trimester AF, amnion, and decidua basalis as well as term amnion, decidua parietalis, and decidua basalis. In contrast, MSCs were cultured from only 2 out of 10 term AF samples. The phenotype of MSCs cultured from different fetal and maternal parts of the placenta was comparable. Maternal MSCs from second-trimester and term decidua basalis and parietalis showed a significantly higher expansion capacity than that of MSCs from adult bone marrow ($p < .05$). Our results indicate that both fetal and maternal MSCs can be isolated from the human placenta. Amnion is a novel source of fetal MSCs, likely contributing to the presence of MSCs in AF. Decidua basalis and decidua parietalis are sources for maternal MSCs. The expansion potency from both fetal and maternal placenta-derived MSCs was higher compared with adult bone marrow-derived MSCs.

PMID: 15579651 [PubMed - indexed for MEDLINE]

Amniotic fluid and placental stem cells.

[Delo DM](#), [De Coppi P](#), [Bartsch G Jr](#), [Atala A](#).

Wake Forest University School of Medicine, Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA.

Human amniotic fluid has been used in prenatal diagnosis for more than 70 years. It has proven to be a safe, reliable, and simple screening tool for a wide variety of developmental and genetic diseases. However, there is now evidence that amniotic fluid may have more use than only as a diagnostic tool and may be the source of a powerful therapy for a multitude of congenital and adult disorders. A subset of cells found in amniotic fluid and placenta has been isolated and found to be capable of maintaining prolonged undifferentiated proliferation as well as able to differentiate into multiple tissue types encompassing the three germ layers. It is possible that in the near future, we will see the development of therapies using progenitor cells isolated from amniotic fluid and placenta for the treatment of newborns with congenital malformations as well as of

adults, using cryopreserved amniotic fluid and placental stem cells. In this chapter, we describe a number of experiments that have isolated and characterized pluripotent progenitor cells from amniotic fluid and placenta. We also discuss various cell lines derived from amniotic fluid and placenta and future directions for this area of research.

PMID: 17141065 [PubMed - indexed for MEDLINE]

Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells.

[Can A, Karahuseyinoglu S.](#)

Department of Histology and Embryology, Ankara University School of Medicine, Sıhhiye, Ankara 06100, Turkey. alpcan@medicine.ankara.edu.tr

Human umbilical cord (UC) has been a tissue of increasing interest in recent years. Many groups have shown the stem cell potency of stromal cells isolated from the human UC mesenchymal tissue, namely, Wharton's jelly. Since UC is a postnatal organ discarded after birth, the collection of cells does not require an invasive procedure with ethical concerns. Stromal cells, as the dominant cells of this fetus-derived tissue, possess multipotent properties between embryonic stem cells and adult stem cells. They bear a relatively higher proliferation rate and self-renewal capacity. Although they share common surface markers with bone marrow-derived MSCs, they also express certain embryonic stem cell markers, albeit in low levels. Without any spontaneous differentiation, they can be successfully differentiated into mature adipocytes, osteoblasts, chondrocytes, skeletal myocytes, cardiomyocytes, neurons, and endothelial cells. While causing no immunorejection reaction, they effectively function in vivo as dopaminergic neurons, myocytes, and endothelial cells. Given these characteristics, particularly the plasticity and developmental flexibility, UC stromal cells are now considered an alternative source of stem cells and deserve to be examined in long-term clinical trials. This review first aims to document the published findings so far regarding the nature of human UC stroma with special emphasis on the spatial distribution and functional structure of stromal cells and matrix, which serves as a niche for residing cells, and, secondly, to assess the in vitro and in vivo experiments in which differential stem cell potencies were evaluated.

PMID: 17690177 [PubMed - indexed for MEDLINE]

Related Links

- [Biology of stem cells in human umbilical cord stroma: in situ and in vitro surveys.](#) [Stem Cells. 2007]
- [Human umbilical cord Wharton's Jelly-derived mesenchymal stem cells differentiation into nerve-like cells.](#) [Chin Med J (Engl). 2005]
- [Matrix cells from Wharton's jelly form neurons and glia.](#) [Stem Cells. 2003]

- [The potential of adipose-derived adult stem cells as a source of neuronal progenitor cells.](#) [Plast Reconstr Surg. 2005]
- [Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation.](#) [Biol Blood Marrow Transplant. 2007]

Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta.

[Igura K](#), [Zhang X](#), [Takahashi K](#), [Mitsuru A](#), [Yamaguchi S](#), [Takashi TA](#).

Division of Cell Processing, Institute of Medical Science, The University of Tokyo, Japan.

BACKGROUND: BM-derived mesenchymal stem cells (MSC) are attractive sources for autotransplantation with no risk of rejection, but the use of these cells has problems, including the necessity of harvesting BM from donors, the donors' age-dependency, limitation to autologous use and difficulty of use for patients with hereditary diseases. We report a method of isolating placenta-derived mesenchymal progenitor cells (PDMPC) that can be used as an alternative source of MSC. **METHODS:** We isolated PDMPC from human fetal chorionic villi using the explant culture method, from placentas collected after neonatal delivery (38-40 weeks of gestation). The PDMPC were characterized by morphologic and immunophenotypic analysis. The differentiation ability of mesenchymal and neural lineages was detected using specific culture conditions and determined by morphology, reverse transcription(RT)-PCR, histochemical staining and immunocytochemistry. **RESULTS:** The PDMPC all originated from fetal chorionic villi, as confirmed by fluorescence in situ hybridization analysis. The PDMPC population consisted of spindle-shaped cells and large flat cells. The PDMPC expressed CD13, CD44, CD73, CD90, CD105 and HLA class I as surface epitopes, but not CD31, CD34, CD45 and HLA-DR. These cells differentiated into osteocytes, chondrocytes and adipocytes under specific culture conditions, and were also induced to form neural-like cells. **DISCUSSION:** Our study shows that PDMPC can differentiate into mesenchymal lineages and be induced to form neural-like cells. Thus, PDMPC isolated from chorionic villi of placenta may provide a novel source for the research of stem and progenitor cells in placenta, cell therapy and regenerative medicine, particularly as a source of allogenic mesenchymal stem and progenitor cells with little ethical conflict and various advantages

PMID: 15770794 [PubMed - indexed for MEDLINE]



1: [Transplantation.](#) 2004 Nov 27;78(10):1439-48.

Comment in:

[Transplantation.](#) 2004 Nov 27;78(10):1411-2.

Engraftment potential of human amnion and chorion cells derived from term placenta.

[Bailo M](#), [Soncini M](#), [Vertua E](#), [Signoroni PB](#), [Sanzone S](#), [Lombardi G](#), [Arienti D](#), [Calamani F](#), [Zatti D](#), [Paul P](#), [Albertini A](#), [Zorzi F](#), [Cavagnini A](#), [Candotti F](#), [Wengler GS](#), [Parolini O](#).

Centro Ricerche Parco Scientifico E. Menni, Ospedale Poliambulanza, Via Romiglia, 4, I-25124 Brescia, Italy.

BACKGROUND: Fetal membranes are tissues of particular interest for several reasons, including their role in preventing rejection of the fetus and their early embryologic origin which may entail progenitor potential. The immunologic reactivity and the transplantation potential of amnion and chorion cells, however, remain to be elucidated. **METHODS:** Amnion and chorion cells were isolated from human term placenta and characterized by immunohistochemistry, flow cytometric analysis, and expression profile of relevant genes. The immunomodulatory characteristics of these cells were studied in allogeneic and xenogeneic mixed lymphocyte reactions and their engraftment potential analyzed by transplantation into neonatal swine and rats. Posttransplant chimerism was determined by polymerase chain reaction analysis with probes specific for human DNA. **RESULTS:** Phenotypic and gene expression studies indicated mesenchymal stem cell-like profiles in both amnion and chorion cells that were positive for neuronal, pulmonary, adhesion, and migration markers. In addition, cells isolated both from amnion and chorion did not induce allogeneic nor xenogeneic lymphocyte proliferation responses and were able to actively suppress lymphocyte responsiveness. Transplantation in neonatal swine and rats resulted in human microchimerism in various organs and tissues. **CONCLUSIONS:** Human amnion and chorion cells from term placenta can successfully engraft neonatal swine and rats. These results may be explained by the peculiar immunologic characteristics and mesenchymal stem cell-like phenotype of these cells. These findings suggest that amnion and chorion cells may represent an advantageous source of progenitor cells with potential applications in a variety of cell therapy and transplantation procedures.

PMID: 15599307 [PubMed - indexed for MEDLINE]

Related Links

- [Human amnion mesenchyme harbors cells with allogeneic T-cell suppression and stimulation capabilities.](#) [Stem Cells. 2008]
- [Expression of extracellular matrix metalloproteinase inducer in human placenta and fetal membranes at term labor.](#) [J Clin Endocrinol Metab. 2004]
- [Generation of human/rat xenograft animal model for the study of human donor stem cell behaviors in vivo.](#) [World J Gastroenterol. 2007]
- [The progesterone receptor in human term amniochorion and placenta is isoform C.](#) [Endocrinology. 2006]

- [Ultrastructural characteristics of human mesenchymal stromal \(stem\) cells derived from bone marrow and term placenta.](#) [Ultrastruct Pathol. 2007]

Mesengenic progenitor cells derived from human placenta.

[Wulf GG](#), [Viereck V](#), [Hemmerlein B](#), [Haase D](#), [Vehmeier K](#), [Pukrop T](#), [Glass B](#), [Emons G](#), [Trümper L](#).

Department of Hematology and Oncology, Georg August University Goettingen, 37075 Goettingen, Germany. gwulf@med.uni-goettingen.de

Progenitor cells with differentiation capacity along multiple mesengenic lineages are attractive tools for numerous purposes in regenerative medicine. Such mesengenic progenitor cells have been isolated from adult mammalian bone marrow, and we here report placental tissue as an alternative source for these cells. By means of dissection/proteinase digestion techniques, high numbers of viable mononuclear cells were harvested from human placenta at term, and a mesenchymal cell population with characteristic expression of CD9, CD29, and CD73 was obtained in culture. The in vitro growth behavior of such placenta-derived mesengenic cells was similar to that of human bone marrow mesengenic progenitor cells. After in vitro propagation for more than three passages the cells were exclusively of maternal origin. Differentiation experiments showed differentiation potential along osteogenic, chondrogenic, adipogenic, and myogenic lineages. In conclusion, we propose human term placenta as an easily accessible, ample source of multipotent mesengenic progenitor cells.

PMID: 15363170 [PubMed - indexed for MEDLINE]

Comparison of mesenchymal stem cells from human placenta and bone marrow.

[Zhang Y](#), [Li CD](#), [Jiang XX](#), [Li HL](#), [Tang PH](#), [Mao N](#).

Institute of Basic Medical Sciences, Academy of Military Medical Sciences, Beijing 100850, China. zhangyi@nic.bmi.ac.cn

BACKGROUND: Nowadays bone marrow represents the main source of mesenchymal stem cells (MSCs). We identified a new population of MSCs derived from human placenta and compared its biological characterization with bone marrow derived MSCs. **METHODS:** Mononucleated cells (MNC) were isolated from the human placenta tissue perfusate by density gradient fractionation. Individual colonies were selected and cultured in tissue dishes. At the same time, human bone marrow derived MSCs were

identified. Culture-expanded cells were characterized by immune phenotyping and cultured under conditions promoting differentiation to osteoblasts or adipocytes. The hematopoietic cytokines were assayed using reverse transcriptase polymerase chain reaction (RT-PCR). RESULTS: Human placental MSCs exhibited fibroblastoid morphology. Flow cytometric analyses showed that the placental MSC were CD29, CD44, CD73, CD105, CD166, HLA-ABC positive; but were negative for CD34, CD45, and HLA-DR. Functionally, they could be induced into adipocytes or osteocytes. Moreover, several hematopoietic cytokine mRNA was found in placenta-derived MSCs by RT-PCR analysis, including IL-6, M-CSF, Flt3-ligand and SCF. These results were consistent with the properties of bone marrow derived MSCs. CONCLUSION: These observations implicate the postpartum human placenta as an important and novel source of multipotent stem cells that could potentially be used for investigating mesenchymal differentiation and regulation of hematopoiesis.

PMID: 15198892 [PubMed - indexed for MEDLINE]

Placenta-Derived Multipotent Cells Differentiate into Neuronal and Glial Cells In Vitro.

[Yen BL](#), [Chien CC](#), [Chen YC](#), [Chen JT](#), [Huang JS](#), [Lee FK](#), [Huang HI](#).

Stem Cell Research Center, National Health Research Institutes, Taipei, Taiwan.

Stem cells have great potential for clinical application because of their self-renewal property and ability to differentiate into many types of cells, but because there are ethical and biological limitations with current sources of stem cells, the search continues for more suitable sources of multipotent cells. We have reported previously on a population of multipotent cells isolated from the human term placenta, an ethically unproblematic and easily available source of tissue. These placenta-derived multipotent cells (PDMCs) can differentiate into lineages of mesenchymal tissues, including osteoblasts and adipocytes, as well as non-mesenchymal tissue of neuron-like cells. We further examined the ability of PDMCs to differentiate into all 3 types of neural cells-neurons, astrocytes, and oligodendrocytes-under various induction conditions, including retinoic acid (RA), 1-methyl-3-isobutylxanthine (IBMX), and co-culture with neonatal rat brain cells. PDMCs exhibited outgrowth of processes and the expression of neuron-specific molecules such as neuron-specific enolase upon induction. Co-culture with neonatal rat brain cells also induced neural differentiation. Our results indicate that PDMCs can be differentiated into neural cell types of the human nervous system upon exposure to RA, IBMX, or primary rat brain cells.

PMID: 18092931 [PubMed - as supplied by publisher]

Placental villous stroma as a model system for myofibroblast differentiation.

[Kohnen G](#), [Kertschanska S](#), [Demir R](#), [Kaufmann P](#).

Department of Anatomy, RWTH Aachen, Germany.

Different subtypes of myofibroblasts have been described according to their cytoskeletal protein patterns. It is quite likely that these different subtypes represent distinct steps of differentiation. We propose the human placental stem villi as a particularly suitable model to study this differentiation process. During the course of pregnancy, different types of placental villi develop by differentiation of the mesenchymal stroma surrounding the fetal blood vessels. In order to characterise the differentiation of placental stromal cells in the human placenta, the expression patterns of the cytoskeletal proteins vimentin, desmin, alpha- and gamma-smooth muscle actin, pan-actin, smooth muscle myosin, and the monoclonal antibody GB 42, a marker of myofibroblasts, were investigated on placental tissue of different gestational age (7th-40th week of gestation). Proliferation patterns were assessed with the proliferation markers MIB 1 and PCNA. Additionally, dipeptidyl peptidase IV distribution was studied in term placenta and the ultrastructure of placental stromal cells was assessed by electron microscopy. Different subpopulations of extravascular stromal cells were distinguished according to typical co-expression patterns of cytoskeletal proteins. Around the fetal stem vessels in term placental villi they were arranged as concentric layers with increasing stage of differentiation. A variable layer of extravascular stromal cells lying beneath the trophoblast expressed vimentin (V) or vimentin and desmin (VD). They were mitotically active. The next layer co-expressed vimentin, desmin, and alpha-smooth muscle actin (VDA). More centrally towards the fetal vessels, extravascular stromal cells co-expressed vimentin, desmin, alpha- and gamma-smooth muscle actin, and GB 42 (VDAG). Cells close to the fetal vessels additionally co-expressed smooth muscle myosin (VDAGM). Ultrastructurally, V cells resembled typical mesenchymal cells. VD cells corresponded to fibroblasts, while VDA and VDAG cells developed features of myofibroblasts. Cells of the VDAGM-type revealed a smooth muscle cell-related ultrastructure. In earlier stages of pregnancy, stromal cell types with less complex expression patterns prevailed. The media smooth muscle cells of the fetal vessels showed a mixture of different co-expression patterns. These cells were separated from extravascular stromal cells by a layer of collagen fibres. The results obtained indicate a clearly defined spatial differentiation gradient with increasing cytoskeletal complexity in human placental stromal cells from the superficial trophoblast towards the blood vessels in the centre of the stem villi. The spatial distribution of the various stages of differentiation suggests that human placental villi could be a useful model for the study of the differentiation of myofibroblasts.

PMID: 8791101 [PubMed - indexed for MEDLINE]

Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration.

[Portmann-Lanz CB](#), [Schoeberlein A](#), [Huber A](#), [Sager R](#), [Malek A](#), [Holzgreve W](#), [Surbek DV](#).

Department of Clinical Research, University Women's Hospital, University of Berne, Berne, Switzerland.

OBJECTIVE: Mesenchymal stem cells (MSCs) have a broad differentiation potential. We aimed to determine if MSCs are present in fetal membranes and placental tissue and to assess their potential to differentiate into neurogenic and mesodermal lineages. **STUDY DESIGN:** MSCs isolated from first and third trimester chorion and amnion and first trimester chorionic villi and characterized morphologically and by fluorescence-activated cell sorting analysis. Their ability to mature under different culture conditions into various cells of mesodermal and neuroectodermal cell lines was assessed by immuno- and cytochemical staining. **RESULTS:** Independent of gestational age, cells isolated from fetal membranes and placenta showed typical MSC phenotype (positive for CD166, CD105, CD90, CD73, CD49e, CD44, CD29, CD13, MHC I; negative for CD14, CD34, CD45, MHC II) and were able to differentiate into mesodermal cells expressing cell markers/cytologic staining consistent with mature chondroblasts, osteoblasts, adipocytes, or myocytes and into neuronal cells presenting markers of various stages of maturation. The differentiation pattern was mainly dependent on cell type. **CONCLUSION:** Mesenchymal cells from chorion, amnion, and villous stroma can be differentiated into neurogenic, chondrogenic, osteogenic, adipogenic, and myogenic lineage. Placental tissue obtained during prenatal chorionic villous sampling or at delivery might be an ideal source for autologous stem cell graft for peripartum neuroregeneration and other clinical issues.

PMID: 16522395 [PubMed - indexed for MEDLINE]

Related Links

- [Term Amniotic membrane is a high throughput source for multipotent Mesenchymal Stem Cells with the ability to differentiate into endothelial cells in vitro.](#) [BMC Dev Biol. 2007]
- [Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta.](#) [Stem Cells. 2004]
- [\[Effect of human placenta derived mesenchymal stem cells on cord blood lymphocyte transformation\]](#) [Zhonghua Yi Xue Za Zhi. 2005]

- [Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta.](#) [Cytotherapy. 2004]
- [Characterization and neural differentiation of fetal lung mesenchymal stem cells.](#) [Cell Transplant. 2005]

First published online January 10, 2008

Stem Cells Vol. 26 No. 3 March 2008, pp. 831 -841

doi:10.1634/stemcells.2007-0758; www.StemCells.com

© 2008 [AlphaMed Press](#)

TRANSLATIONAL AND CLINICAL RESEARCH

Mesenchymal Stem Cells Effectively Deliver an Oncolytic Adenovirus to Intracranial Glioma

Adam M. Sonabend^a, Ilya V. Ulasov^a, Matthew A. Tyler^a, Angel A. Rivera^b, James M. Mathis^c, Maciej S. Lesniak^a

^aBrain Tumor Center, University of Chicago, Chicago, Illinois, USA;

^bDivision of Human Gene Therapy, University of Alabama at Birmingham, Birmingham, Alabama, USA;

^cDepartment of Cellular Biology and Anatomy, Louisiana Health Sciences Center, Shreveport, Louisiana, USA

Key Words. Glioma • Stem cells • Adenovirus • Oncolytic virus • Vector • Migration • Gene therapy

Correspondence: Maciej S. Lesniak, M.D., University of Chicago, Section of Neurosurgery, 5841 South Maryland Avenue, MC 3026, Chicago, IL 60637, USA. Telephone: 773-834-4757; Fax: 773-834-2608; e-mail: mlesniak@surgery.bsd.uchicago.edu

Received September 10, 2007; accepted for publication January 3, 2008.

First published online in *STEM CELLS EXPRESS* January 10, 2008.

Gene therapy represents a promising treatment alternative for patients with malignant gliomas. Nevertheless, in the setting of these highly infiltrative tumors, transgene delivery remains a challenge. Indeed, viral vehicles tested in clinical trials often target only those tumor cells that are adjacent to the injection site. In this study, we examined the feasibility of using human mesenchymal stem cells (hMSC) to deliver a replication-competent oncolytic adenovirus (CRAd) in a model of intracranial malignant glioma. To do so, CRAds with a chimeric 5/3 fiber or RGD backbone with or without CXCR4 promoter driving E1A were examined with respect to replication and toxicity in hMSC, human astrocytes, and the human glioma cell line U87MG by quantitative polymerase chain reaction and membrane integrity assay. CRAd delivery by virus-loaded hMSC was then evaluated in vitro and in an in vivo model of mice bearing intracranial U87MG xenografts. Our results show that hMSC are effectively infected by CRAds that use the CXCR4 promoter. CRAd-CXCR4-RGD had the highest replication, followed by CRAd-CXCR4-5/3, in hMSC, with comparable levels of

toxicity. In U87MG tumor cells, CRAd-CXCR4-5/3 showed the highest replication and toxicity. Virus-loaded hMSC effectively migrated in vitro and released CRAds that infected U87MG glioma cells. When injected away from the tumor site in vivo, hMSC migrated to the tumor and delivered 46-fold more viral copies than injection of CRAd-CXCR4-5/3 alone. Taken together, these results indicate that hMSC migrate and deliver CRAd to distant glioma cells. This delivery strategy should be explored further, as it could improve the outcome of oncolytic virotherapy for glioma.