Cytotherapy, 2011; 13: 1047–1056

informa
healthcare

Isolation and basic characterization of human term amnion and chorion mesenchymal stromal cells

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Abstract

Background aims. Emerging evidence suggests human placental membrane is a valuable source of mesenchymal stromal cells (MSC). Amnion and chorion are tissues of early embryologic origin that may entail progenitor potential. These tissues are abundantly available and ethically unobjectionable and, because they are discarded post-partum, they can be widely used for extensive research and eventually for therapeutic studies. *Methods.* We looked at the cells isolated from the six amnions and chorions of term placentas of gestational weeks 39 ± 1. Isolated cells were characterized by morphologic and immunophenotypic analysis. *Results.* With flow cytometry immunophenotype analysis, amnion- and chorion-derived cells were positive for MSC markers, and negative for hematopoietic markers. Immunocytochemical staining was positive for the embryonic cell markers Oct-3/4 and Rex-1. Oct-3/4 is a POU transcription factor that is expressed in embryonic stem (ES) cells and germ cells, and its expression is required to sustain cell self-renewal and pluripotency. Oct-3/4 is the most recognized marker for totipotent ES cells. Rex-1 is a zinc finger family transcription factor that is highly expressed in embryonic stem cells. It is one of several gene markers used to identify undifferentiated stem cells, and its expression is downregulated upon stem cell differentiation. Amnion- and chorion-derived cells were capable, under differentiation conditions, to differentiate into to mesoderm lineages. *Conclusions.* Phenotypic studies indicate MSC-like profiles in both amnion- and chorion-derived cells. Cells *in vitro* had fibroblastoid morphology. The *in vitro* growth behavior of such placenta-derived progenitor cells was similar to that of bone marrow MSC. Our results indicate that MSC can be easily isolated from the human term placenta. The human amniotic and chorion MSC maintained a marker profile similar to the mesenchymal progenitors and could be used for studies as an alternative source of MSC for further application in cellular therapy.

Key Words: *amnion, chorion, human term placenta, mesenchymal stromal cells*

Introduction

Multipotent mesenchymal stromal cells (MSC) are a promising cell resource for cell-based therapeutics because of their ability to self-renew and differentiate into specific functional cell types (1). There are many reports showing that bone marrow (BM)-derived MSC are able to differentiate into all mesodermic lineages: adipose and connective tissue, as well as bone and cartilage (2). Cell therapy with MSC has been used to treat a wide range of diseases (3). The procedures required to obtain the BM may be invasive and associated with appreciable morbidity, the cell numbers obtained can be low, and the differentiation potential may be dependent on the age of the donor (4). As an alternative to BM as a potential source of multipotent MSC are fetal membranes, the amnion and chorion of term human placenta. Cells that are phenotypically similar to BM MSC (2) have been isolated from unfractionated placenta (5–7), villous stroma of the para/umbilical area (8), the internal area of placental lobules (9), and from amnion and chorion membranes (10,11).

The human placenta is a fetomaternal entity that consists of a fetal component (the chorionic plate) and a maternal component (the deciduas). The amnion is the inner layer and is comprised primarily of two cell types: the epithelial cuboid cells and the columnar cells. The amniotic epithelial cells (AEC) on one side of the amnion create a continuous lining adjacent to the amniotic fluid, while on the other side of the amniotic epithelium is a thin layer of amniotic mesoderm (AM), throughout which a few fetal macrophages are

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(Received 26 November 2010; accepted 23 May 2011)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2011.592522

sporadically distributed (12). The chorion is composed of the inner chorionic mesoderm, similar to the mesenchymal region of the amnion, and an outer highly variable trophoblastic layer, with extravillous cytotrophoblast cells that represent the only residues of the former villi of the chorion frondosum (13).

MSC are primarily from the BM. There are five minimum criteria for defining human amniotic MSC (hAMSC) and human chorionic MSC (hCMSC) that originate from the extra-embryonic mesoderm of the amnion or chorion: (i) fetal origin (maternal contamination of 1% or less); (ii) formation of fibroblast colony-forming units; (iii) a specific surface antigen pattern, $CD90^+$ $CD73^+$ $CD105^+$ $CD45^-$ $CD34^-$ $CD14^-$ and $HLA\text{-}DR^-$; (iv) a differentiation potential to one or more lineages, osteogenic, adipogenic, chondrogenic or vascular/endothelial; and (v) adherence to plastic (14).

Transmission electron microscopy of hAMSC shows mesenchymal and epithelial characteristics. This hybrid phenotype is interpreted as a sign of multipotentiality and is not found in hCMSC, which are more primitive and metabolically quiescent. Specifically, if compared with hAMSC, transmission electron microscopy of hCMSC shows a simpler cytoplasmic organization. The most relevant features include the presence of stacks of rough endoplasmic reticulum cisternae, dispersed mitochondria and glycogen lakes. Features of higher specialization, such as the presence of assembled contractile filaments, prominence of endocytotic traffic and junctional communications, are lacking (14). Mixed populations of fetal membrane-derived cells express numerous cell-surface antigens and intracellular antigens similar to of BM-derived MSC (10). All express typical mesenchymal markers, but are negative for hematopoietic-, endothelial- and trophoblastic-specific cell markers, while they express stage-specific embryonic antigens (SSEA)-3 and −4 (15,16).

Both hAMSC and hCMSC express low HLA-ABC and no HLA-DR, indicating their immunoprivileged status (16,17). Several studies have revealed that MSC are not immunogenic and also exhibit immunomodulatory properties (18). The low immunogenicity of these cells is confirmed by clinical applications of amniotic membranes in surgical procedures, where they can be used as a biologic dressing (19) and for the treatment of corneal or conjunctiva destructive loss (20).

Knowledge about yields, phenotype and expansion of hAMSC and hCMSC is a prerequisite for their therapeutic application in regenerative medicine. We report on a protocol for the isolation and culture of hAMSC and hCMSC from term placenta. The aim of our study was to characterize and compare the differentiation potential of isolated amnion- and chorion-derived cells after culturing under appropriate conditions.

Methods

Isolation and primary culture of human amnion and chorion cells

Term (38–40-week gestation, $n = 6$) placentas from healthy donor mothers were obtained. Part of the amnion and chorion measuring 10×10 cm was then separated manually and washed extensively in phosphate-buffered saline (PBS), containing 100 IU penicillin/mL, 100 μg streptomycin/mL and 0.25 μg amphotericin B/mL (Invitrogen, Carlsbad, California, USA), before being cut into small pieces (*c.* 1.5×1.5 cm). Amnion fragments were incubated for 20 min at 37°C in Dulbecco's Minimal Essential Medium (MEM) (Biochrom AG, Berlin, Germany) containing 2.4 U/mL dispase (Invitrogen, GIBCO®, Carlsbad, California, USA) and 1% antibiotic–antimycotic solution. Cells were collected by centrifugation at 150 ***g*** for 15 min. Then they were digested with 1.0 mg/mL collagenase type II (Invitrogen, GIBCO) for approximately 2 h at 37°C in Dulbecco's MEM containing 100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg amphotericin (Invitrogen, GIBCO). Amnion fragments were later removed and the released cells were passed through a 40-μm cell strainer (BD Falcon™, Biosciences, Bedford, MA, USA). Cells were collected by centrifugation at 150 ***g*** for 15 min. We refer to these cells as hAMSC.

Chorion fragments, after mechanical removal of decidua, were incubated for 20 min at 37°C in Dulbecco's MEM supplemented with additives as described above. Dispersed chorion cells, called hCMSC, after filtration through a 40-μm cell strainer, were collected by centrifugation.

The cells were cultured with alpha-MEM medium (Biochrom AG) supplemented with 10% fetal bovine serum (FBS; Invitrogen, GIBCO) and 1% antibiotic–antimycotic solution in 75-cm^2 culture flasks (Sarstedt AG & Co., Nümbrecht, Germany). Cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2 atmosphere. Three to five days after initiating incubation, the small digested residues were removed and the culture was continued with fresh media added twice weekly. When cells were more than 80% confluent, they were recovered with 0.25% trypsin/ethylene diamine tetra acetic acid (EDTA) (Invitrogen, GIBCO). The cells were expanded for several passages (2–4).

Enrichment of $CD105^+$ cells

hAMSC and hCMSC were incubated with colloidal MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) coated with CD105 monoclonal antibodies for 15 min at 10°C. $CD105^+$ cells were then enriched using a MACS System (Miltenyi Biotec) according