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Human chorion-derived stem cells: changes in stem cell properties during serial passage

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Abstract

Background aims. Fetal membrane from human placenta tissue has been described as a potential source of stem cells. Despite abundant literature on amnion stem cells, there are limited studies on the stem cell properties of chorion-derived stem cells. *Methods.* The main aim was to determine the stemness properties of serial-passaged human chorion-derived stem cells (hCDSC). Quantitative polymerase chain reaction (PCR) was performed to reveal the following stemness gene expression in serial-passaged hCDSC: Oct-4, Sox-2, FGF-4, Rex-1, TERT, Nanog (3), Nestin, FZD-9, ABCG-2 and BST-1. Cell growth rate was evaluated from passage (P) 1 until P5. The colony-forming unit–fibroblast (CFU-F) frequency of P3 and P5 cells and multilineage differentiation potential of P5 cells were determined. The immunophenotype of hCDSC was compared using the surface markers CD9, CD31, CD34, CD44, CD45, CD73, CD90, CD117, HLA-ABC and HLA-DR, -DP and -DQ. Immunostaining for trophoblast markers was done on P0, P1, P3 and P5 cells to detect the contamination of trophoblasts in culture, while chromosomal abnormality was screened by cytogenetic analysis of P5 cells. *Results.* The surface markers for mesenchymal lineage in hCDSC were more highly expressed at P5 compared with P3 and P0, indicating the increased purity of these stem cells after serial passage. Indeed, all the stemness genes except TERT were expressed at P1, P3 and P5 hCDSC. Furthermore, human chorion contained high clonogenic precursors with a 1:30 CFU-F frequency. Successful adipogenic, chondrogenic and osteogenic differentiation demonstrated the multilineage potential of hCDSC. The karyotyping analysis showed hCDSC maintained chromosomal stability after serial passage. *Conclusions.* hCDSC retain multipotent potential even at later passages, hence are a promising source for cell therapy in the future.

Key Words: *cell differentiation, fetal stem cells, gene expression, human placenta, immunophenotype, quantitative polymerase chain reaction, serial passage*

Introduction

Adult stem cells have been considered to be developmentally committed and therefore restricted to produce specific cell lineages, namely those from the tissue in which the stem cell resides (1). Adult stem cells have been isolated from a variety of sources, such as bone marrow (BM) (2), lipoaspirate (3,4) and different parts of the placenta, the amnion (5), chorion, chorionic villi (6,7) and umbilical cord (8). Although many types of stem cells are available, the sources of stem cells selected for clinical use should possess the ability to renew, be easily isolated and be pluripotent, and have minimal ethical controversies. These properties are seen in human term placental-derived stem cells. These stem cells are available in abundance and do not involve any invasive procedure when harvesting. Because the placentas are mostly discarded anyway, isolating stem cells from these 'waste' tissues will not be of serious ethical concerns. Recent reports have shown that mesenchymal stromal cells (MSC) from the placenta do not elicit any immunologic reaction and could be used as autologous grafts for fetuses and newborns in peripartum tissue regeneration as well as *in utero* transplantation to treat genetic disorders (9,10).

The chorion is the membrane that is attached to the amnion by a spongy layer of loosely arranged collagen fibers. It consists of the mesodermal and trophoblastic region and is easily separated from the amnion (11). Some studies have characterized the stromal-associated surface markers, revealing the neurogenic, chondrogenic, osteogenic, adipogenic, myogenic (6) and angiogenic potency of chorion cells (12,13). However, the immunophenotypes of

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placenta-derived stem cells during serial passage have not been illustrated in any of the earlier studies. Immunophenotyping is essential to demonstrate the purity of the cell population as well as changes in the stem cell properties.

To the best of our knowledge, there is limited comprehensive data on mesenchymal progenitor stemness, in both *in vivo* and *ex vivo* cultivation. Although several molecular markers are available for committed progenitors and the end-stage phenotypes, there is at present no definite marker to identify the MSC.

Besides leaking the expression profile of stemness genes in stem cells isolated from the placenta, it is also important, for the purpose of clinical application, to generate large numbers of the desired cell types without compromising their properties. It is therefore essential to understand the expansion capability and potency of stem cells in serial passages. Hence we aimed to characterize the quantitative changes of the following stemness gene expressions: octamer-binding protein (Oct)-4, SRY-related HMG-box (Sox)-2, Fibroblast growth factor (FGF)-4, reduced expression (Rex)-1, telomerase reverse transcriptase (TERT), Nanog 3, Nestin, frizzled (FZD)-9, ATP-binding cassette, subfamily G (WHITE), member (ABCG)-2 and bone marrow stromal cell antigen (BST)-1, as well as the stem cell-surface markers of cultured human-derived chorion stem cells, by flow cytometry analysis. We also present findings regarding the growth rate, colony-forming unit–fibroblast (CFU-F) assay and multilineage differentiation (adipogenic, osteogenic and chondrogenic induction) ability of the serially passaged cells at passages (P) 3 and 5.

Methods

Human chorion-derived stem cell isolation and expansion

Human placenta (n = 6) was collected following delivery at the Universiti Kebangsaan Malaysia Medical Centre (Kuala Lumpur, Malaysia) after informed consent. All procedures in the study were reviewed and approved (approval number FF-215-2008) by the Research and Ethical Board of Universiti Kebangsaan Malaysia Medical Center. Upon receiving the placenta, the chorion was peeled away from the amnion, scrapped with gauze and rinsed with Dulbecco's phosphate-buffered saline (DPBS; Gibco-Invitrogen, Grand Island, NY, USA; http://www.invitrogen.com) to remove the decidual tissue. The chorion was cut into small pieces and digested with 0.3 % collagenase type I (Gibco-Invitrogen) in a shaker incubator at 37°C for 1 h. The digested tissue was centrifuged at 1200 r.p.m. for 10 min to yield the cell pellet. The cells were resuspended in equal volumes of Ham's F12 and Dulbecco's modified Eagle medium (DMEM/F-12), supplemented with 10% fetal bovine serum (FBS), 1 × glutamax, 50 μg/mL vitamin C and 1 × antibiotic–antimycotic (Gibco-Invitrogen), and cultured in T25 flasks (Falcon; BD Biosciences, San Jose, CA, USA; http://www.bdbiosciences.com). All cultures were maintained at 37°C in an incubator with 5% CO_2. The culture medium was changed every 3 days. When these primary cells (passage 0; P0) reached 80–90% confluence in the culture, the cells were trypsinized with 0.05% trypsin–ethylene diamine tetra acetic acid (EDTA) (Gibco-Invitrogen) and replated at an expansion degree of 1:4 under the same culture conditions (P1). Cells were passaged repeatedly after achieving a density of 80–90% until P).

Adipogenic differentiation

Human chorion-derived stem cells (hCDSC) at P5 (n = 6) were induced in adipogenic differentiation medium composed of DMEM/F-12 supplemented with 2% FBS, 1 × glutamax, 50 μg/mL vitamin C, 1 × antibiotic–antimycotic, 0.5 mM isobutylmethylxanthine (IBMX; Sigma-Aldrich, St Louis, MO, USA; http://www.sigmaaldrich.com), 1 μM dexamethasone (Sigma-Aldrich), 50 μg/mL indomethacin (Sigma-Aldrich) and 1 μM bovine insulin (Sigma-Aldrich). The cells were alternately cultured for 3 days each in adipocyte differentiation medium and normal medium (DMEM/F-12 plus 10% FBS) for 3 weeks. Cultures were rinsed with DPBS and fixed in 10% formalin solution. Differentiation into adipocytes was confirmed by the staining of neutral lipids with Oil Red O (Sigma-Aldrich).

Osteogenic differentiation

hCDSC at P5 (n = 6) were induced in osteogenic differentiation medium composed of DMEM/F-12 supplemented with 10% FBS, 1 × glutamax, 50 μg/mL vitamin C, 1 × antibiotic-antimycotic, 10 nM dexamethasone (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich) and 50 μg/mL sodium ascorbate 2-phospate (Sigma-Aldrich). The osteogenic differentiation medium was changed every 3 days for a period of 3 weeks. Cultures were rinsed with DPBS, fixed in 10% formalin solution, and osteogenic differentiation confirmed by the presence of calcium phosphate when stained with Alizarin Red S (Sigma-Aldrich).

Chondrogenic differentiation

hCDSC (n = 6) at P5 were grown in a palette culture system and induced in chondrogenic differentiation medium composed of DMEM/F-12 supplemented with 1% FBS, 1% glutamax, 50 μg/mL vitamin C, 1% antibiotic–antimycotic, 1%