POTENTIAL CLINICAL USE OF STEM CELLS FROM HUMAN PLACENTAL AND UMBILICAL CORD MATRIX FOR TISSUE REPAIR THROUGH ANGIOGENESIS

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INTRODUCTION

Mesenchymal stem cells (MSCs) are widely distributed in different parts of the placenta tissue. Several properties that make them of interest as a source of stem cells for therapeutic use are, they 1) can be isolated in large numbers, 2) are negative for CD34 and CD45, 3) grow robustly and can be frozen/thawed, 4) can be clonally expanded, and 5) can use for angiogenesis purpose.

OBJECTIVE

However the unique characteristics of stem cells from different parts of human placental tissue including the stemness status during serial-passage as well as in extensive expansion have not been defined in previous studies. Thus, in this study we compared the stemness profile of early passage as well as serial-passage of human chorion, chorionic villi- and umbilical cord matrix-derived stem cells using a few approaches including the real time PCR for the stemness and angiogenic-associated genes expression; CD markers detection using flow cytometry, CFU-F assay and multilineage differentiation testing towards adipogenesis and osteogenesis. We also determined the capability of hCDSC to express certain endothelial markers (CD31 and vWF) after culture in angiogenic induction media and the formation of pre-vascular like structure when cultured in 3D matrigel vascular model.

MATERIALS AND METHOD

Human placental stem cells were isolated and cultured in equal volume mix of Ham's F12 medium and Dulbecco's Modified Eagle Medium, supplemented with 10%FBS, 1% Glutamax, 1% Vitamin C and 1% Antibiotic antimycotic (FD+10% FBS). Quantitative real time RT-PCR was carried out to evaluate the differential mRNA expression of the following stemness-associated genes; Oct-4, Sox-2, FGF-4, Rex-1, TERT, Nanog-3, Nestin, FZD9, ABCG-2 and BST-1 for cells at passage 0, 1, 3 and 5. For angiogenic study, genes involved in angiogenesis; PGF, HGF, bFGF, angiopoietin-1, VEGF and endothelial differentiation; vWF, VEGFR2, ve-cadherin, PECAM-1, CD34 and eNOS were analysed.

Flow cytometry was performed on human chorion-derived stem cells at P0, P3 and P5. Accutase (Innovative Cell Technologies, San Diego, http://www.innovativecelltech.com) was used to detach the cells for 5 minutes. The harvested cells were centrifuged at 1200 rpm for 10 minutes and washed with 1X DPBS added with 0.5% Bovine Serum Albumin (Sigma-Aldrich). The cells

were filtered through 70 µm nylon membrane and viable cell density was determined by hemacytometer and trypan blue dye (Gibco-Invitrogen). A minimum of 2 X 10^5 cells were incubated with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated antibodies; CD9/PE, CD31/PE, CD34/FITC, CD44/FITC, CD45/FITC, CD73/PE, CD90/FITC, CD117/PE, HLA-ABC/FITC and HLA-DR DP DQ/FITC (BD Biosciences) for 30 minutes in the dark. Ten thousand events were acquired per antibody with Becton Dickinson FACSCalibur Flow Cytometer. The results were then analysed using CELLQuestPro acquisition software (BD Biosciences).

Human chorion-derived stem cells at P3 were plated at a density of 200 to 600 cells/cm^2 in 100 mm culture dishes (Falcon, BD Biosciences) with the medium being changed every 3 days for a period of 2 weeks and colonies which consisted of more than 50 toluidine blue-positive cells were counted.

Cultured cells at passage 5 were induced in standard adipogenic and osteogenic media followed by Alizarin Red S and Oil red O staining to test its multilineage capability. For angiogenic induction, hCDSC was cultured for 3 weeks in FD supplemented with 2% FBS + 10 ng/ml basic fibroblast growth factor and 50 ng/ml vascular endothelial growth factor (EDM). The cells were then transferred to Teflon coated slide and stained for the presence of vWF and CD31. Approximately 2 x 10^5 cells at P3, labelled with Qtracker 525 (Invitrogen) were also seeded in 200 ul of matrigel to form a 3D construct. The formation of pre-vascular like structure was observed using confocal imaging system (Nikon).

Numeric data were expressed as mean $\pm$ standard error of mean (SEM). Differences in quantitative PCR results and flow cytometry between two groups were tested for significance using Student's t-test. A p-value<0.05 was considered to be significant.

RESULTS AND DISCUSSION

The results confirmed expression of oct-4, sox-2, FGF4 and rex-1 in chorion, chorionic villi and umbilical cord matrix cells. Fetal cells from chorion showed the highest expression of all the stemness markers with significant higher expression of oct-4 and sox-2. Further characterisation of hCDSC revealed the maintenance of mRNA expression level after serial passage and differential expression of certain stemness genes. Besides, hCDSC showed high expression of bFGF and angiopoietin-1 while moderate to low expression of VEGF, VEGFR2, PGF, HGF and vWF. However, the endogenic genes (ve-cadherin, CD34, PECAM1, eNOS) were expressed at low level. Based on the optimised real time PCR protocol for angiogenic and endogenic mRNA expression, we managed to submit a patent application for AngioEnd Quantification Kit. Moreover, flow cytometric analyses clearly showed that hCDSC at various passages were positive for all mesenchymal surface markers tested. The expression of hematopoietic markers CD34 and CD117, leucocytes marker (CD45) and endothelial marker (CD31) were low at all three passages with a significant decrease in positivity in hCDSC of later passage. Human CDSC also demonstrated significant lower expression of MHC class II, HLA DR DP DQ at P3 and P5 when compared to P0. The CFU-F frequency of chorion cells at passage 3 (P3) scored 1:30 at seeding density of 200 cells/cm^2. Furthermore, the formation of lipid vacuoles and deposition of calcium after adipogenic and osteogenic induction provide evidence that hCDSC cultured in our

system has multilineage potential. In addition, hCDSC demonstrated positive staining for vWF after angiogenic induction in endothelial differentiation medium (EDM). Cultured hCDSC also showed the formation of pre-vascular like structure in 3D matrigel vascular model following confocal imaging after 3 days.

CONCLUSION

Stem cells isolated from human placental and umbilical cord matrix has potential clinical use in tissue repair through angiogenesis.