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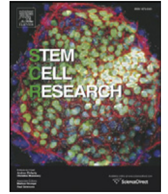

Isolation and comparative analysis of potential stem/progenitor cells from different regions of human umbilical cord

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ABSTRACT

Human umbilical cord (hUC) blood and tissue are non-invasive sources of potential stem/progenitor cells with similar cell surface properties as bone marrow stromal cells (BMSCs). While they are limited in cord blood, they may be more abundant in hUC. However, the hUC is an anatomically complex organ and the potential of cells in various sites of the hUC has not been fully explored. We dissected the hUC into its discrete sites and isolated hUC cells from the cord placenta junction (CPJ), cord tissue (CT), and Wharton's jelly (WJ). Isolated cells displayed fibroblastoid morphology, and expressed CD29, CD44, CD73, CD90, and CD105, and showed evidence of differentiation into multiple lineages in vitro. They also expressed low levels of pluripotency genes, OCT4, NANOG, SOX2 and KLF4. Passaging markedly affected cell proliferation with concomitant decreases in the expression of pluripotency and other markers, and an increase in chondrogenic markers. Microarray analysis further revealed the differences in the gene expression of CPJ-, CT- and WJ-hUC cells. Five coding and five lncRNA genes were differentially expressed in low vs. high passage hUC cells. Only MAEL was expressed at high levels in both low and high passage CPJ-hUC cells. They displayed a greater proliferation limit and a higher degree of multi-lineage differentiation in vitro and warrant further investigation to determine their full differentiation capacity, and therapeutic and regenerative medicine potential.

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1. Introduction

The successful isolation and culture of human embryonic stem cells (ESCs) was a landmark achievement, which generated significant interest in the field of stem cell biology. Pluripotent ESCs have the ability to self-renew indefinitely and to differentiate into ectoderm, mesoderm and endoderm (Carpenter et al., 2003; Hoffman and Carpenter, 2005; Itskovitz-Eldor, 2002). While they are the most primitive, both ethical and technical issues limit their therapeutic use (Fischbach and Fischbach, 2004). Furthermore, their potential to form teratomas is also a major concern (Goldring et al., 2011). These challenges prompted studies to search for alternative sources of stem cells that do not pose moral, ethical and safety dilemmas. Studies have shown the presence of adult stem cells (ASCs) in various organs and body tissues. Bone marrow (BM) has been a well-known source of ASCs, particularly hematopoietic stem cells (HSCs), and to a lesser extent skeletal stem cells [SSCs, also known as bone marrow-derived mesenchymal stem cells (Bianco and Robey, 2015)] and endothelial progenitors. BM-derived HSCs have been widely used for transplantation therapy to treat leukemia and blood disorders. These cells have the ability to self-renew, are multipotent and are able to differentiate into all blood lineages. Potential applications of bone marrow stromal cells (BMSCs), which contain the subset of SSCs (Robey et al., 2014), are limited because harvesting of BM involves an invasive and painful procedure with possible donor site morbidity. In addition, donor age, genetics, and exposure to environmental stress could cause a significant reduction of biological activity in BMSCs (D'Ippolito et al., 1999). Since a large number of cells are required for cell therapy to regenerate tissues, or to treat most human diseases and dysfunctions, it is imperative to search for new and robust sources of multipotent ASCs.

Recently, human umbilical cord (hUC) has been investigated as a source of cells with stem/progenitor cell properties. hUC is a desirable source of stem/progenitor cells as it is routinely discarded after delivery, collection is non-invasive and an abundant number of cells are present in cord tissue (Catacchio et al., 2013; Dominici et al., 2006; Weiss et al., 2006). The differentiation potential and non-immunogenic nature of hUC-derived cells could make them an ideal source for regenerative

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medicine (Ennis et al., 2008; Weiss et al., 2006). Stem/progenitor cells derived from peri-natal sources such as amniotic fluid, cord blood, cord tissue and placenta are less likely to be altered due to aging or environmental stresses. While the presence of fibroblastic cells with BMSC-like characteristics in cord blood is limited, umbilical cord has been found to be a promising source of potential stem/progenitor cells. However, the cord is an anatomically complex organ and isolation of potential stem/progenitor cells from its various sites has not been rigorously investigated.

In this study, we dissected the hUC into the cord-placenta junction (CPJ), cord tissue (CT), and Wharton's jelly (WJ). Cells isolated from these sites exhibited fibroblastoid morphology, expressed markers found on BMSCs and on other tissue-specific stem/progenitor cells. In vitro, they displayed signs of multipotency. In addition, they expressed pluripotency genes. CPJ-hUC cells showed higher proliferation capabilities as compared with CT- and WJ-hUC cells. These findings suggest that CPJ-hUC cells may have a potential use in tissue engineering and regenerative medicine. Nevertheless, further studies are warranted to fully explore the efficacy and differentiation potential of CPJ-hUC cells.

2. Materials and methods

2.1. Collection and processing of human umbilical cord samples

hUC samples ($n = 50$) were obtained from consented healthy donors through the Beaumont Hospital BioBank, Royal Oak, MI under an HIC (HIC #2012-101) approved protocol. The hUC samples were processed within 2–4 h of delivery. Briefly, the sample was rinsed in PBS several times until all the blood clots were removed. The hUC was then dissected to separate CPJ, (the region between the cord and placenta), CT (the outer layer of the cord), and WJ (the jelly-like tissue within the cord and surrounding the blood vessels). The CPJ, CT and WJ tissues were separately minced into approximately 1–2 mm pieces with the help of surgical and dissection tools, before culturing the explants.

2.2. Isolation of cells

The explants were cultured in 75 cm^2 culture flasks using culture medium (CM) including DMEM with 4500 mg/ml glucose and 2 mM L-glutamine (Invitrogen), supplemented with 10% FBS (Aleken Biologicals). The medium was changed every 3 days until the cells started to migrate from the explants (5–10 days after plating), after which medium was changed every 1–2 days. When explant cells populated approximately 2/3 of the flask, they were dissociated using TrypLE Select (Invitrogen) and were considered as P0 cells. They were then passaged (P1) in new culture flasks at a concentration of 1×10^4 cells/cm^2 for amplification. When these cells reached 70% confluency, they were harvested using TrypLE Select, cryopreserved, and sub-cultured for further studies.

2.3. Proliferation assay

Low passage (LP, P2–5) cells derived from CPJ, CT, and WJ were seeded in 24-well plates at a concentration of 5×10^3 cells/well. After predetermined time intervals (1–8 days), triplicate wells were treated with 5 mg/ml MTT solution (Sigma) for 2 h in the dark at 37 °C in 5% CO_2 incubator. The reaction was then stopped and the formazan complex was dissolved using 150 μl isopropanol with 10 μl of 0.1 N HCL on a shaker for 15 min in the dark. The optical density in each well was determined at 570 nm using an EPOCH plate reader (BioTek). The background absorbance of blank wells was subtracted from all the measured values.

2.4. Cell cycle analysis

LP cells were subjected to cell cycle analysis. Briefly, cells were grown to 70% confluency, tryspinized, and washed with PBS. Cells (10^6) were fixed with cold absolute ethanol and stored overnight at 4 °C, and then treated with 1 mg/ml RNase and PI staining solution (Sigma) for 40 min in the incubator at 37 °C in the dark. The DNA content was assessed by a Nexcelom Cellometer (Nexcelom Bioscience Lawrence) and results were analyzed using De Novo FCS Express 4 software.

2.5. Immunophenotyping

Flow cytometry was used to assess the cell surface marker profile of hUC cells. All experiments for FACS analysis were conducted with LP and high passage (HP, >P15) cells. Briefly, the cells were grown to 70% confluency, tryspinized, washed with PBS and pelleted. Cells (10^6) were then stained directly with FITC-conjugated antibodies against: CD34, CD44, CD45, CD90, or APC-conjugated antibodies against: CD29, CD73, CD105 (Becton Dickinson). Cells were stained single or dual labeled and then analyzed on a FACS Canto II (Becton Dickinson) using Diva Software (Beckton Dickinson).

2.6. Colony forming efficiency assay

Cells were seeded at a concentration of 1.6 cells/cm^2 in a petri dish in triplicate and cultured using CM. After 10–14 days, cells were washed with PBS, fixed in 4% paraformaldehyde for 30 min, stained with 0.1% crystal violet (Thermo Scientific) for 1 h, and then rinsed in tap water. Colonies consisting of a minimal cell number of 50 cells were counted. Data was recorded as total colony number per number of plated cells.

2.7. Lineage differentiation

LP cells were induced to differentiate along chondrogenic, osteogenic, and adipogenic lineages by culturing in specific differentiation media. Chondrogenic differentiation was induced in monolayer and pellet cultures (obtained by centrifugation of 2.5×10^5 cells at 3000 RPM for 10 min) using chondrogenic medium containing 20 ng TGFβ1, 10 ng insulin, 100 nM dexamethasone, and 100 μM ascorbic acid. After 3 weeks of culture, cells were stained with 1% toluidine blue and Periodic acid–Schiff (PAS) reagent (Thermo Scientific) to detect extracellular matrix produced by chondrogenic derivatives of hUC cells. Osteogenic differentiation was induced by using osteogenic medium containing 0.1 μM dexamethasone, 10 μM β-glycerophosphate, and 50 μM ascorbate-phosphate. After 3 weeks of culture, cells were stained with alizarin red stain (Sigma) and von Kossa stain (Thermo Scientific) to visualize calcium deposition. Adipogenic differentiation was induced using adipogenic medium containing 0.5 μM isobutyl-methylxanthine, 1 μM dexamethasone, 10 μM insulin, and 200 μM indomethacin. After 3 weeks of culture, cells were stained with Oil Red O (Sigma) to determine the presence of lipid droplets. Cell cultures using CM served as negative controls.

2.8. Sulfated glycosaminoglycans (sGAGs) assay

hUC cell pellets were incubated for 3 weeks in chondrogenic differentiation medium and digested at 55 °C for 16 h with 125 μg/ml papain (Sigma) in 100 mM sodium phosphate buffer containing 10 mM EDTA, pH 6.5. Cell lysates were vortexed multiple times and cleared by centrifugation. sGAGs were determined by incubating the lysate with dimethylmethylene blue (DMMB) dye in glycine/NaCl solution, pH 3.0 and the complex formed was quantified spectrophotometrically at absorbance 525 nm. Total sGAG content was determined by using the chondroitin sulfate as a standard.

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