Native Umbilical Cord Matrix Stem Cells Express Hepatic Markers and Differentiate Into Hepatocyte-like Cells

DAVID CAMPARD, PHILIPPE A. LYSY, MUSTAPHA NAJIMI, and ETIENNE MARC SOKAL

HPED Department, PEDI Unit, Laboratory of Pediatric Hepatology and Cell Therapy, Université Catholique de Louvain, Brussels, Belgium

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Background & Aims: **Umbilical cord matrix stem cells (UCMSCs) are able to differentiate into mesodermal and ectodermal lineages. The present study investigates the differentiation potential of human UCMSCs into hepatic lineage.** ***Methods:*** **We isolated human UCMSCs and characterized them in vitro by measuring their expansion potential, by assessing expression of mesenchymal stem cell (MSC) markers, and by evaluating their ability to differentiate into adipocytes and osteocytes. UCMSCs were thereafter subjected to a hepatogenic differentiation protocol. Expression of hepatic and MSC markers in differentiated cells was analyzed by reverse-transcription polymerase chain reaction, flow cytometry, and immunocytochemical assays and compared with undifferentiated UCMSCs and freshly isolated liver cells. UCMSCs were transplanted into livers of hepatectomized-SCID mice, and engraftment capacity was investigated by detection of human nucleus and mitochondria and human hepatic-specific proteins.** ***Results:*** **In vitro expanded UCMSCs constitutively expressed markers of hepatic lineage, including albumin, α-fetoprotein, cytokeratin-19, connexin-32, and dipeptidyl peptidase IV. In vitro-differentiated UCMSCs exhibited hepatocyte-like morphology, upregulated several hepatic markers, stored glycogen, produced urea, and exhibited an inducible CYP 3A4 activity. However, absence of some hepatic markers in differentiated UCMSCs, as HepPar1 or hepatocyte nuclear factor 4 (HNF-4), implied that their differentiation did not reach the level of mature hepatocytes. We also noticed that differentiated UCMSCs partially preserved MSC markers. Engraftment capacity of UCMSCs was observed, and expression of human albumin and α-fetoprotein was detected 2, 4, and 6 weeks after transplantation in mice livers, while cytokeratin 19 was completely down-regulated.** ***Conclusions:*** **We conclude that UCMSCs, with a newly demonstrated endodermic differentiation potential, might be an alternative source for liver-directed cell therapies.**

Liver cell transplantation is an emerging technique next to whole organ transplantation for the treatment of liver-based inborn errors of metabolism.[1–6] However, organ shortage remains a major limiting step of this procedure. More recent developments in stem cell technology have brought hope to identify new expandable sources of liver cells to be used for regenerative medicine. Embryonic stem cells can be considered the best model of multipotency, but their use remains subject to moral/ethical debates as well as safety concerns.[7] Adult stem cells are largely explored as a more acceptable source of cells. Recently we showed that stem-like cells isolated from the adult liver can differentiate into hepatocytes, although this source is also dependent on organ availability.[8,9] Several investigators have demonstrated the capacity of mesodermal cells such as multipotent adult progenitor cells,[10] mesenchymal stem cells (MSCs),[11,12] and fibroblasts[13] to differentiate into cells with features of hepatocytes, and the majority was isolated from bone marrow. However, the technique of bone marrow collection remains invasive and is not exempt of risks, even more so as bone marrow/MSCs are more easily obtained from young donors.[11] MSCs with hepatogenic capacities have also been isolated from umbilical cord blood,[14,15] but the yield of isolated cells is highly dependent on blood quality and remains limited compared with bone marrow.[16] Recently a bundle of data focused on a new promising source of MSCs derived from the matrix of the umbilical cord (Wharton's jelly).[17–23] Multipotency of umbilical cord matrix stem cells (UCMSCs) has widely been shown by conventional in vitro assays, inducing differentiation into adipocytes, osteocytes, and chondrocytes.[19,22] Furthermore, UCMSCs are also able to differentiate into neurons[19,20,22,24] and cardiomyocytes.[22,25,26] Moreover, in vivo experiments showed that transplanted

Abbreviations used in this paper: APC, Allophycocyanine; α1AT, α_1-antitrypsin; CK, cytokeratin; CYP, cytochrome P450; Cy7, Cyanin-7; DPPIV, dipeptidyl peptidase IV; FITC, fluorescein isothiocyanate; G6P, glucose-6-phosphatase; IMDM, Iscove's modified Dulbecco's medium; MSC, mesenchymal stem cell; PE, Phycoerythrin; PerCP, Peridinin-chlorophyll-protein complex; RT-PCR, reverse-transcription polymerase chain reaction; TAT, tyrosine aminotransferase; TDO, tryptophan 2,3-dioxygenase; UCMSC, umbilical cord matrix stem cell.

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UCMSCs were able to restore functional defects in rats with brain injury or retinal disease.[18,23,27] So far, differentiation of human UCMSCs into hepatic lineage has not been reported.

In the current study, we isolated MSCs from Wharton's jelly and characterized these cells for their morphology, growth potency, phenotypic profile, and capacity to differentiate into various lineages. We then evaluated the ability of UCMSCs to differentiate into hepatocytes, and differentiated cells were compared with native UCMSCs and adult human liver cells for expression of early and late hepatic markers and some hepatocyte-specific activities. The capacity of transplanted UCMSCs to engraft durably into the liver of hepatectomized SCID mice was also investigated.

Materials and Methods

Isolation and Expansion of UCMSCs

The present study was approved by the ethical committee of the Hospital and Faculty of Medicine of Université Catholique of Louvain. Umbilical cords were obtained from consenting patients delivering full-term infants (n = 15). Cords of 9.15–51.77 g were collected in 0.9% NaCl sterile solution and immediately transferred for cell isolation. Cords were cut in pieces of 3–4 cm, and sections were incised along their length to expose underlying Wharton's jelly. The umbilical vein and the 2 arteries were pulled away, and the remaining mesenchymal tissue was scraped off with a scalpel and centrifuged at 250*g* for 5 minutes at room temperature. The pellet was suspended in Hank's balanced salt solution with calcium and magnesium (Invitrogen, Merelbeke, Belgium) supplemented with 1 mg/mL collagenase type I (C-0130; Sigma, Bornem, Belgium), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen) and transferred to a 75-cm^2 flask with ventilated cap (Greiner Bio-One, Wemmel, Belgium). Tissues were digested at 37°C and 5% CO_2 atmosphere for 18–24 hours. The homogenate was diluted in Dulbecco's phosphate-buffered saline (Cambrex Bio Sciences, Verviers, Belgium) and centrifuged at 600*g* for 15 minutes. The cell pellet was resuspended in expansion medium composed of Dulbecco's modified Eagle medium low glucose (1 g/L D-glucose) with sodium pyruvate and L-glutamine (Invitrogen) supplemented with 10% fetal bovine serum (A15-101; PAA Laboratories GmbH, Pasching, Austria), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Viable cells were counted using trypan blue exclusion assay. The total number of viable isolated cells ranged between 1.8×10^5 and 3.6×10^6 (mean, $1.22 \times 10^6 \pm 1.09 \times 10^6$), and these were seeded in a single 25- or 75-cm^2 tissue culture flask (Greiner Bio-One) at a density of 4.8×10^3 to 1×10^4 cells/cm^2.

Plated cells were cultured in expansion medium at 37°C and 5% CO_2 in a fully humidified atmosphere. At 80%–90% of confluence, cells were detached from the flask using 0.05% trypsin/EDTA solution (Invitrogen). Cells were replated at a density of 8000 cells/cm^2 in expansion medium, and medium was changed twice a week.

Differentiation Procedures

Adipogenic and osteogenic differentiation. UCMSCs at passage 2 were plated at a density of 1×10^4 cells/cm^2 in expansion medium containing 1 μmol/L dexamethasone, 0.5 mmol/L isobutyl methylxanthine, 0.2 mmol/L indomethacin (all from Sigma) and 10 μg/mL insulin (Lilly Benelux SA, Brussels, Belgium) with medium change twice a week. After 4 weeks, lipid vesicles were revealed by Oil Red O staining.

UCMSCs at passage 2 were plated at a density of 3×10^4 cells/cm^2 in expansion medium containing 0.1 mol/L dexamethasone, 0.1 mmol/L ascorbate, and 10 mmol/L β-glycerophosphate (all from Sigma) with medium change twice a week. After 4 weeks, calcium deposition was evaluated by von Kossa and Alizarin Red stains. Tetracycline-labeled bone nodules were observed by inverted fluorescence microscopy (Axiovert S100; Carl Zeiss SA, Zaventem, Belgium) 1 day after refeeding cultures once with osteogenic medium containing 10 μg/mL tetracycline (7660; Sigma).

Hepatogenic differentiation. UCMSCs (from passage 2 to passage 6) were seeded at a density of 1.5×10^4 cells/cm^2 in 6- or 24-well plates coated with 5 μg/cm^2 of rat tail collagen type I (BD Biosciences, Bedford, MA) in Dulbecco's modified Eagle medium low glucose supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptomycin. Culture medium was switched 24 hours later to Iscove's modified Dulbecco's medium (IMDM; Invitrogen) containing 20 ng/mL epidermal growth factor (Peprotech EC Ltd, London, England) and 10 ng/mL basic fibroblast growth factor (Peprotech EC Ltd) for 2 days. Thereafter, induction of differentiation was triggered by culturing UCMSCs for 10 days with IMDM containing 20 ng/mL hepatocyte growth factor (Peprotech EC Ltd), 10 ng/mL basic fibroblast growth factor, 0.61 g/L nicotinamide (Sigma), and 1% insulin-transferrin-selenium premix (Invitrogen). The final maturation step consisted of treatment with IMDM containing 20 ng/mL oncostatin M (Peprotech EC Ltd), 1 μmol/L dexamethasone (Sigma), and 1% insulin-transferrin-selenium premix for 10 days. For each step, medium was changed every 3 days.

Flow Cytometry

Undifferentiated UCMSCs (at passage 4), differentiated UCMSCs, and liver cells were trypsinized and suspended at a concentration of 1×10^6 cells/mL in phosphate-buffered saline (PBS) 0.5% bovine serum albumin (Sigma) and then incubated at 4°C with antibodies against hematopoietic cell markers (CD45-phycoerythrin-