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In vitro differentiation of human umbilical cord blood mesenchymal stem cells into functioning hepatocytes

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Abstract Mesenchymal stem cells (MSCs) were isolated by gradient density centrifugation from umbilical cord blood. Spindle-shaped adherent cells were permitted to grow to 70% confluence in primary culture media which was reached by day 12. Induction of differentiation started by culturing cells with differentiation medium containing FGF-4 and HGF. Under hepatogenic conditions few cuboidal cells appeared in culture on day 7. From day 21 to day 28, most of cells became small and round. The control negative cells cultured in serum free media showed fibroblast-like morphology. Urea production and protein secretion by the differentiated hepatocyte-like cells were detected on day 21 and increased on day 28. Protein was significantly increased in comparison with control by day 28. The cells became positive for AFP at day 7 and positive cells could still be detected at days 21 and 28. The cells in the control group were stained negative for AFP. The cells expressed albumin gene at the 14th day that became markedly increased at the 28th day of culture with HGF and FGF-4. No albumin expression was observed in the 7th day sample and the control. This study demonstrated that UCB-derived MSCs had the ability to differentiate into functioning hepatocyte-like cells starting from the 7th day after culturing under hepatogenic conditions and became well functioning at days 21 and 28. These data indicated that UCB-derived MSCs can be a promising source of cell therapy for intractable liver diseases.

1. Introduction

Liver is dedicated to perform an extensive range of tissue specific functions including intermediary metabolism, detoxification of drugs or other xenobiotics as well as endogenous substrates, synthesis of plasma proteins and bile formation.[1] The principal

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cells of mature liver that carry out these functions are parenchymal hepatocytes which represent approximately 80% of total hepatic volume.[2]

Liver dysfunction is a major health problem in the world, and liver transplantation is the only conventional thriving treatment of end-stage liver disease.[3] Unfortunately, human donor livers are very limited and are unavailable to most people worldwide who necessitate whole organ transplantation. Hepatocytes transplantation and the use of hepatocytes with extracorporeal bioartificial liver supports are other alternatives.[4] However, the shortage of cell supplies and the long-term efficiency remains unclear which limits this strategy.[3]

There are requires for efficient *in vitro* models of hepatocytes developed from other sources with full metabolic function. Some cell types exhibit the potential to develop into viable hepatocytes such as embryonic stem cells (ESCs) and adult stem cells (ASCs).[5] Studies on ESCs were limited in clinical therapeutic applications as a result of ethical controversies. On the other hand, adult SCs (like bone marrow stem cells) can be propagated in large quantities and remain capable to differentiate into various tissue types[6] including hepatocyte lineage.[7]

Recently, human umbilical cord blood (HUCB) has been used as a rich, easily available and ethically acceptable source of stem cells (SCs). UCB can be collected from donors without invasive or painful procedure and is rarely infectious.[8] UCB-derived cells are also considered more primitive than BM-derived cells so they represent a more appropriate source for cellular therapies.[9]

There are 2 types of stem cells in HUCB: mesenchymal (MSCs) and hematopoietic. Lately it has been reported that UCB mesenchymal progenitor cells are capable of differentiating into hepatocytes.[10] Isolated MSCs from HUCB were induced to differentiate into hepatocyte-like cells under the effect of pro-hepatogenic conditions of different cytokines and growth factors including fibroblast growth factor (FGF) and hepatocyte growth factor (HGF).[10–13] Transformation of the fibroblast-like cells into round epithelioid cells expressing mRNAs of albumin (ALB), and alpha-fetoprotein took place under these hepatogenic conditions [10,11,13]. The functional capacity of the hepatocyte-like cells was assessed by albumin and urea secretion[10,12] and the ability to integrate DiI-acetylated low-density lipoprotein.[10,11]

The aim of this work was to study the functional activity of hepatocyte-like cells derived from HUCB-MSCs at different time points by reverse transcription–polymerase chain reaction (RT-PCR) for albumin gene expression level, immunocytochemical stain for detection of alpha-fetoprotein in the cell cytoplasm and biochemical assay of protein and urea levels secreted in the culture media.

2. Materials and methods

The study protocol was approved by the ethics committee of the Mansoura University.

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), recombinant human fibroblast growth factor (FGF)-4, penicillin/streptomycin/L-glutamine (100×), recombinant human hepatocyte growth factor (HGF), Ficoll-Paque (1077), all materials were purchased from Sigma, Egypt.

2.2. Collection of umbilical cord blood

UCB samples (No = 22) were obtained from Department of Obstetrics and gynecology, Mansoura University Hospital. Samples were collected after informed consent was given from full-term elective cesarean sections before placental separation. Family history of gene disorders and maternal fever was excluded. The umbilical cord was double clamped two inches apart from infant's abdomen, wiped with 70% alcohol followed by Betadine at needle insertion site to insure sterility. The needle insertion site was just above the clamp. The blood samples were obtained in special cord blood collection bags.[14]

2.3. Mesenchymal stem cell isolation and culture

The collected blood processed within 6 h of collection and the samples were transported in sterile tubes and diluted with phosphate buffer saline (PBS) in concentration 1:1. Mesenchymal stem cells were isolated using Ficoll gradient density centrifugation. Ficoll was added to the sample in concentration 3:1. Ficoll-Paque was normally placed at the bottom of a conical tube, and the blood was then slowly layered above it. Centrifugation of the tubes was done at 400 RCF for 30 min. The supernatant was discarded and the puffy coat layer which contained mononuclear cells was aspirated and transferred into other tube. PBS was added for washing in concentration 1:1 and centrifuged at 2000 RPM for 5 min. The supernatant was discarded.

The suspended precipitated cell pellet was cultured in primary complete culture media (containing 20 ml DMEM – 1% L-glutamine- 10% fetal bovine serum – 1 ml antibiotic) and plated in tissue culture flasks (25 cm^2). The samples were incubated in incubator (37 °C) with 5% CO2 level and were nourished every 3 days. Feeding step was performed by discarding the old media and adding 20 ml new complete media as mentioned before.

After culturing cells for 12 days in primary culture media, cells reached 70–80% confluence. The old media was discarded and 10 ml trypsin was added to each flask for 5 min. The flasks were shaken to ensure that the cells were completely detached from the flask wall, then the content of the flasks was put in tubes to be centrifuged for 5 min at 2000 RBM. The cell pellets appeared on the wall of the tube, the supernatant was discarded, finally the cells in the media were transferred into new flasks (25 cm^2) for subculture.[15]

2.4. Hepatogenic differentiation

To start induction of cell differentiation into hepatocytes, second- to third-passage cells, at approximately 70% confluence were treated with differentiation medium consisting of serum free DMEM supplied with 20 ng/mL HGF, 10 ng/ml FGF4 and 1% antibiotic penicillin and streptomycin 12. Feeding was carried out two times per week and hepatogenic differentiation was investigated at different time points (7th -14th -21st -28th day) after treatment with differentiation medium.

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