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Comparison of in vitro hepatogenic differentiation potential between various placenta-derived stem cells and other adult stem cells as an alternative source of functional hepatocytes ☆

Hyun-Jung Lee [a], Jieun Jung [b], Kyung Jin Cho [c], Chang Kyou Lee [c], Seong-Gyu Hwang [d], Gi Jin Kim [a,b,*]

[a] CHA Placenta Institute, CHA University, Seoul, Republic of Korea
[b] Department of Biomedical Science, CHA University, Seoul, Republic of Korea
[c] Department of Biomedical Science, College of Health Science, Korea University, Seoul, Republic of Korea
[d] Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, Republic of Korea

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ABSTRACT

Mesenchymal stem cells (MSCs) are powerful sources for cell therapy in regenerative medicine. The capability to obtain effective stem cell-derived hepatocytes would improve cell therapy for liver diseases. Recently, various placenta-derived stem cells (PDSCs) depending on the localization of placenta have been suggested as alternative sources of stem cells are similar to bone marrow-derived MSC (BM-MSCs) and adipose-derived MSC (AD-MSCs). However, comparative studies for the potentials of the hepatogenic differentiation among various MSCs largely lacking. Therefore, we investigated to compare the potentials for hepatogenic differentiation of PDSCs with BM-MSCs, AD-MSCs, and UCB-MSCs. Several MSCs were isolated from human term placenta, adipose tissue, and umbilical cord blood and characterized isolated MSCs and BM-MSCs was performed by quantitative reverse transcription-PCR (RT-PCR) and special stains after mesodermal differentiation. The hepatogenic potential of PDSCs was compared with AD-MSCs, UCB-MSCs, and BM-MSCs using RT-PCR, PAS stain, ICG up-take assays, albumin expression, urea production, and cytokine assays. MSCs isolated from different tissues all presented similar characteristics of MSCs. However, the proliferative potential of PDSCs and the expression of hepatogenic markers in differentiated PDSCs were higher than other MSCs. Interestingly, the expression of hepatocyte growth factor (HGF) increased in PDSCs after hepatogenic differentiation. Interestingly, stem cell factor (SCF) expression in chorionic plate-derived MSCs, one of the PDSCs, was significantly higher than in the other PDSCs. Taken together, the results of the present study suggest that MSCs isolated from various adult tissues can be induced to undergo hepatogenic differentiation in vitro, and that PDSCs may have the greatest potential for hepatogenic differentiation and proliferation. Therefore, PDSCs could be used as a stem cell source for cell therapy in liver diseases.
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1. Introduction

Hepatic failure is one of the major causes of morbidity and mortality worldwide. Although it is the best way to treat liver transplantation for acute and chronic hepatic failure patients, there are several obstacles (e.g., lack of donor organs, invasive procedure) Haydon and Neuberger (2000). Therapy using hepatocyte transplantation has emerged as an attractive treatment for patients with late-stage liver diseases Zaret and Grompe (2008). Mature hepatocytes isolated from the liver have important functions when they are transplanted; however, it is hard to expect any effective results from the transplantation of isolated hepatocytes due to the limited number of functional hepatocytes via their dedifferentiation during in vitro cultivation, limited ability to be cryopreserved and short-term survival (Elaut et al., 2006; Terry et al., 2007). Therefore, exploring other sources of cells to replace damaged hepatocytes in hepatogenic diseases is required.

Recently, stem cells have been spotlighted as alternative sources of hepatocytes because they have potential for hepatogenic differentiation (Cai et al., 2007; Cantz et al., 2008). Human embryonic stem cells (hESCs) have unlimited proliferation capacity in vitro as well as a potential for differentiation into all cell types under specialized differentiation conditions (Thomson et al., 1998). Many researchers have demonstrated that hESCs are capable of hepatogenic differentiation into hepatocytes or

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* Correspondence to: Department of Biomedical Science, CHA University, 606-1 Yeoksam 1-dong Kangnam-ku, Seoul 135-081, Republic of Korea. Tel.: +82 2 3468 3687; fax: +82 2 538 4102.
E-mail address: gjkim@cha.ac.kr (G.J. Kim).

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hepatocyte-like cells. However, the efficiency of their hepatogenic differentiation is still low and controversial according to the differentiation procedures and conditions (Cai et al., 2007). In particular, there are risks in using partially differentiated hESCs because they can induce teratoma formation due to the heterogenous population of partially differentiated hESCs Gallicano and Mishra (2010). In addition, Activin A is a necessary factor for hepatogenic specification of undifferentiated hESCs to induce the hepatogenic differentiation of hESCs in vitro because it can induce endodermal differentiation of ESCs. However, the application of Activin A for hepatogenic differentiation causes the loss of many cells via cell death due to the cytotoxicity of Activin A (Parashurama et al., 2008). In addition, collecting available hepatocytes or hepatocyte-like cells, and differentiating functional hepatocytes from hESCs are time-consuming processes (Cai et al., 2007; Hay et al., 2008). To enhance the potential for this approach, stem cell therapy requires a renewable cell source capable of functional hepatocytes(Hay et al., 2008). Therefore, hepatogenic differentiation using mesenchymal stem cells derived from various sources (e.g., bone marrow, adipose, cord blood, and placenta) has been developed to enhance the efficiency of hepatogenic differentiation using simple procedures, as well as to ensure the safety in stem cell application (Lee et al., 2004a; Snykers et al., 2006; Lee et al., 2010; Shin et al., 2010).

The plasticity means that MSCs derived from adult tissue can generate differentiated cell types of a different tissue. This ability is variously referred to as "plasticity" or "transdifferentiation" (Anderson et al., 2001; Krause et al., 2001; Barzilay et al., 2009). Although MSCs are capable of differentiating into mesodermal lineages such as osteoblasts, chondocyte and adipocytes, they are also able to give rise to multiple lineages including ectodermal (neurons) and endodermal (hepatocyte) cells (Jiang et al., 2002; Greco et al., 2007; Bianco et al., 2008). The potential for hepatogenic differentiation of various MSCs has been evaluated by measuring the expression of endodermal or hepatocyte markers including α-fetoprotein (AFP), hepatocyte nuclear factors (HNFs), albumin, and tyrosine aminotransferase (TAT), as well as urea production after inducing hepatogenic differentiation (Snykers et al., 2009). In addition, MSCs show therapeutic effects when transplanted into animal models with liver diseases, regardless of whether differentiated or undifferentiated MSCs are used (Banas et al., 2008; Shi et al., 2009; Lee et al., 2010). Furthermore, the hepatogenic differentiation potential of iPS cells, which could provide a source of autologus hepatocytes, has been introduced (Espejel et al., 2010; Sancho-Bru et al., 2011). But, hepatogenic differentiation potential of iPS cells should be studied on the efficiency and the safety because the generation of iPS is labor-intensive as well as based on virus gene delivery system. Due to the reason, there are no reports comparing the efficiency of hepatogenic differentiation among various MSCs derived from adult tissues including bone marrow, adipose tissue, cord blood and placenta.

Placenta-derived stem cells (PDSCs) have several advantages for use in cell-based therapy. They have a higher proliferative potential that is associated with short population doubling time, as well as ethical advantages. PDSCs contain several types of stem cells based on placental anatomy: chorionic villi (CV-MSCs), amnion (AE-MSCs), chorionic plate (CP-MSCs) and Wharton's jelly of the umbilical cord (WJ-MSCs) (Igura et al., 2004; Parolini et al., 2008; Troyer and Weiss, 2008). These placenta-derived MSCs have the ability to differentiate into various types of cells, including adipocytes, chondrocytes, osteocytes, neuronal cells and hepatocytes, given the appropriate induction conditions (Moon et al., 2008).

Therefore, we isolated several types of MSCs from human placenta, characterized the differences among several adult stem cells, and compared the hepatogenic differentiation of PDSCs including AE-MSCs, CP-MSCs, CV-MSCs, WJ-MSCs, AD-MSCs, BM-MSCs and UCB-MSCs.

2. Material and methods

2.1. Cell culture

The collection of placenta samples was approved by the Institutional Review Board of CHA General Hospital, Seoul, Korea. All participating women provided written, informed consent prior to the collection of samples. Placentas were collected from women who were free of medical, obstetrical, and surgical complications and who delivered at term ($\geq$ 37 gestational weeks). PDSCs including AE-MSC, CP-MSC, CV-MSC, and WJ-MSC were isolated from the placentas after term delivery. Various PDSCs were harvested, as described, with some modifications (Parolini et al., 2008; Kim et al., 2011). Harvested cells were cultured in Ham's F-12 medium/DMEM medium (Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS; Invitrogen), penicillin (100 U/ml), streptomycin (100 μg/ml) (Invitrogen), 25 ng/mL FGF-4 (Peprotech Inc) and 1 μg/mL heparin (Sigma-Aldrich) at 37 °C in a humidified atmosphere containing 5% CO_2. PDSCs were passaged every 48–72 h at a 1:3 ratio. AD-MSCs were kindly provided by Dr. Jong-Hyuk Seong (CHA University, Seoul Korea) and cultured with medium contained α-MEM (GIBCO-BRL, USA) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% fetal bovine serum (GIBCO-BRL, USA). Bone marrow-derived mesenchymal stem cells (BM-MSCs) were obtained from Cambrex Bioscience Walkersville (Cambrex BioScience Walkersville, Walkersville, MD) and cultured with medium contained α-MEM (GIBCO-BRL, USA) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 1 mM sodium pyruvate (GIBCO 11360070) and 10% fetal bovine serum (GIBCO-BRL, USA). UCB-MSCs were kindly provided by Dr. Young Soo Choi (CHA University, Seoul Korea) and were cultured with medium contained α-MEM (GIBCO-BRL, USA) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 20% fetal bovine serum (GIBCO-BRL, USA). Cells were split every 48–72 h at 1:3 ratio. To measure the growth of adult stem cells with respect to cell types, each cell type was seeded at a density of 2×10^4 cells into 60 mm dishes. At 0, 24, 48, 72, 96 and 120 h cells were digested with trypsin, stained with 0.2% trypan blue, and counted using a hemocytometer. The viability of all MSCs was determined by trypan blue exclusion. To analyze the growth kinetics of individual stem cells, cells were plated at 2×10^4 cells/cm^2 on culture dishes. Cell number was determined from each dish 24 h after plating and every 24 h until day 5.

2.2. Expression of stemness markers and lineage-specific markers of various MSCs by reverse transcription-PCR

Total RNA was extracted from the naïve stem cells and cells that had differentiated into osteogenic, adipogenic and hepatogenic lineages using RNeasy plus mini kits (QIAgen, Valencia, CA, USA), and 1 μg of RNA was reverse transcribed into cDNA using the Superscript Plus first-strand synthesis system (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cDNA samples were subjected to polymerase chain reaction (PCR) amplification. The PCR primers and the length of the amplified products are shown in Table 1. PCR conditions were as follows: initial denaturation (95 °C, 15 min), annealing (95 °C, 20 s; 55 °C, 58 °C, 59 °C or 60 °C, 40 s; 72 °C, 1 min) and final extension (72 °C, 5 min). All PCR reactions were performed using 40 cycles. The amplified PCR products were analyzed by electrophoresis on 1% agarose gels and stained with ethidium bromide for visualization.

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