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

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\textbf{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.

\section{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 hepatic diseases is required.

Recently, stem cells have been spotlighted as alternative sources of hepatocytes because they have potential for hepatic 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...
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 heterogeneous 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, chondrocyte 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 iPSCs, which could provide a source of autologous hepatocytes, has been introduced (Espejel et al., 2010; Sancho-Bru et al., 2011). But, hepatogenic differentiation potential of iPSCs should be studied on the efficiency and the safety because the generation of iPSCs 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 (≥ 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 μg/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% CO2. 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) 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 × 104 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 × 104 cells/cm² 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 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.
2.4. Hepatogenic differentiation of various MSCs

To direct the differentiation of AE-MSC, CP-MSC, CV-MSC, WJ-MSC, AD-MSC, BM-MSC, and UCB-MSC into hepatocytes in vitro, the cells were plated at a density of $2 \times 10^3$ cells/cm$^2$ on 0.1% collagen-coated dishes in culture medium (60% DMEM-LG, 40% MCDB201, 2% FBS, and 1% P/S) containing 20 ng/ml EGF and 10 ng/ml bFGF. Cells were grown to 60% confluence and then incubated for 7 days in basal medium supplemented with 2% FBS, 20 ng/ml HGF, 10 ng/ml bFGF, and 0.61 g/L nicotinamide. To induce maturation, the cells were treated with maturation medium (basal medium supplemented with 2% FBS, 1 μM dexamethasone, 50 mg/ml ITS$^+$ premix, and 20 ng/ml oncostatin M) for a further 2 weeks. The medium was replaced every 3 days. After hepatogenic differentiation, the hepatogenic induced cells were uptaken of CM-Dil for identification of hepatogenic differentiation. Subsequently, the induced cells were harvested for analysis of hepatocyte-specific gene expression using reverse transcription PCR.

2.5. CM-Dil uptake assay

After washing the cultured cells with PBS, a Vybrant CM-Dil cell labeling solution (Invitrogen cat.no V22888) was added to the plates at a final concentration of 5 μM CM-Dil, incubated at 37 °C for 20 min, rinsed three times with PBS, and then refilled with DMEM containing 10% FBS (GIBCO-BRL, USA).

2.6. ELISA assay

All adult stem cells were cultured to differentiate into hepatocytes using induction media. The cell culture supernatant was also collected and used as a negative control. Using the supernatant, an HGF ELISA assay (Peprotech cat.no 100-39-10),
urea assay (Bioassay systems DIUR-500) and SCF ELISA assay (Multiplex detection kit, Bio-rad, Hercules, CA, USA) were performed, following the manufacturer’s protocol.

2.7. Immunofluorescence staining analysis

Cells were fixed in 4% PFA and stained with an anti-albumin polyclonal antibody (1:50 dilution; Sigma-Aldrich, St Louis, MO) at 4 °C overnight. An Alexa 488-conjugated polyclonal antibody (Invitrogen) was used as a secondary antibody and DAPI staining was performed (Vector Laboratories, Burlingame, CA, USA).

2.8. Analysis of glycogen storage using periodic acid-schiff (PAS) staining

Culture dishes containing cells were fixed in 4% formaldehyde and oxidized in 1% periodic acid for 5 min, rinsed three times in dH2O, treated with Schiff’s reagent (SIGMA, St. Louis, MO, USA) for 15 min, and rinsed in dH2O for 5–10 min. Samples were counterstained with Mayer’s hematoxylin (DAKO, Glostrup, Denmark) for 1 min, rinsed in dH2O, and assessed using a light microscope.

2.9. Western blotting

Cells were washed with PBSs and lysed in 100 μl of cold Cell lysis buffer (Fermentas, Glen Burnie, Maryland, USA) with a protease inhibitor cocktail (Roche, Mannheim, Germany). Cell lysate was centrifuged at 13,000 × g for 10 min at 4 °C. The supernatant was harvested, and its protein concentration measured using a BCA protein assay kit (Pierce, Rockford, IL, USA). For electrophoresis, 30 μg of protein was dissolved in sample buffer (50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA, 0.02% Bromophenol blue), boiled for 5 min, and separated on an 8% SDS-PAGE reducing gel. Separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-rad, Hercules, CA, USA) using a trans-blot system (Bio-rad). Blots were blocked for 1 h in phosphate-buffered saline (PBS) containing 8% non-fat dry milk (BD, Sparks, MD, USA) and 0.05% Tween 20 at room temperature, washed three times with PBST, and incubated at 4 °C overnight with a primary antibody specific for human albumin (1:1000 dilution; SIGMA, St. Louis, MO, USA) in PBST containing 2% non-fat dry milk. The next day, blots were washed six times with PBST and incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies (1:10,000 dilution; Bio-rad, Hercules, CA, USA) in PBST containing 2% non-fat dry milk at room temperature. After being washed six times with PBST, the proteins were visualized with an ECL detection system (Amersham, Piscataway, NJ, USA).

2.10. Statistical analysis

Results are presented as the means ± SD. Statistical significance measured multiple comparisons were performed using the t-test with a significance level of P < 0.05.
3. Results

3.1. Growth kinetics of MSCs derived from placenta, adipose, bone marrow, and umbilical cord blood

MSCs derived from placenta (amnion, chorionic plate, chorionic villi, Wharton jelly), adipose, bone marrow, and umbilical cord blood were plated at \(2 \times 10^4\) cells/cm\(^2\) on culture dishes and cultured for 3, 4 days. In general, their morphologies were similar to the fibroblast phenotype without differences between the types of stem cells (Fig. 1a). The MSCs were assayed for cell proliferation activity. The growth curves of MSCs were derived as described in the Materials and Methods section from the first 5 day after plating. There were no differences among AE-MSC, CV-MSC, AD-MSC, BM-MSC, and UCB-MSCs. However, the CP-MSCs and WJ-MSCs exhibited a higher growth rate by 4th and 5th day after cell seeding (Fig. 1b). Growth rates of CP-MSCs and WJ-MSCs were over 2-fold greater than others MSCs at day 5. These findings indicate that CP-MSCs and WJ-MSCs derived from placenta have a strong proliferation activity.

3.2. Characterization of various MSCs derived from placenta, adipose tissue, bone marrow, and umbilical cord blood

The expressions of markers of stemness and the three germ lineage in seven types of MSCs were analyzed by RT-PCR. There were differences in the expression of self-renewal markers (e.g., Oct4, Nanog, Sox2) and germ lineage-specific markers (e.g., NFl, cardiac, AFP) among MSCs (Fig. 1c). Oct4 expression in WJ-MSCs, AD-MSCs, and BM-MSCs was strongly expressed. Furthermore, the three germ lineage-specific markers were all expressed in CP-MSCs, WJ-MSCs, and AD-MSCs. After inducing mesodermal line age differentiation, lineage-specific markers were analyzed by RT-PCR and chemical staining. As shown in Fig. 2, the expression of osteocalcin (OC), which is an osteocyte marker, increased in CP-MSCs, WJ-MSCs, BM-MSCs, and UCB-MSCs. In addition, the
expression of adiponectin, which is an adipose marker, was enhanced in CP-MSCs, WJ-MSCs, AD-MSC, and BM-MSCs (Fig. 2a). The differentiation potentials for osteogenic and adipogenic lineages were confirmed by von Kossa and Oil-Red O staining, respectively (Figs. 2b and c). These findings indicate that the characteristics of MSCs derived from various tissues are similar.

3.3. In vitro hepatogenic differentiation of various MSCs derived from placenta, adipose, bone marrow, and umbilical cord blood

Each type of adult stem cell was plated at $2 \times 10^4$ cells/cm$^2$ in culture dishes and cultured in culture medium with 2% FBS, after a pre-culture for 2 days in serum-free medium supplement with EGF, basic FGF, and BMP-4 to inhibit cell proliferation. Cell morphologies of all adult stem cells did not change during the initiation step, when cultures were treated with HGF, although the fibroblastic morphology was lost and the cells developed a broadened flattened shape. A polygonal shape developed during the maturation step when cells were exposed to medium containing oncostatin M and insulin, transferrin, and selenium (Fig. 3a). To determine whether differentiated cells expressed hepatogenic phenotype markers, total RNA was isolated from all MSCs after hepatogenic differentiation and the mRNA levels of several hepatogenic genes were examined by RT-PCR. Interestingly, the expression of AFP, which is expressed in the early stages of hepatogenesis, decreased in all MSCs with the exception of UCB-MSCs. The expression of albumin increased in hepatogenic differentiated cells. There was no difference in the expression of anti-trypsin and connexin 43 among MSCs. However, alterations of several markers related to hepatogenic function exhibited different patterns depending on the type of MSC (Fig. 2b). These findings suggest that the protocol for hepatogenic differentiation used in this study proved to be an effective method to induce the hepatogenic differentiation of adult stem cells, and that the potential for hepatogenic differentiation of MSCs derived from tissues could be different, although several markers correlated with functional hepatocytes were expressed.

![Fig. 3. Hepatogenic differentiation of several MSCs.](image-url)
3.4. Functional analysis of hepatocyte-like cells derived from placenta, adipose tissue, bone marrow, and umbilical cord blood after hepatogenic differentiation

To analyze hepatocyte-specific functions in the differentiated MSCs, we performed PAS staining, Western blots and ELISA assays. As shown on Fig. 4, the glycogen stores in the cytoplasm of hepatocyte differentiated MSCs were observed in almost all types of MSCs by PAS staining with the exception of UCB-MSCs (Fig. 4a). In addition, in vitro differentiation of MSCs into hepatocyte-like cells was identified by cellular uptake of indocyanine green (ICG) (Fig. 4b). The expression of albumin in all MSCs increased after hepatogenic differentiation and was especially higher in AE-MSCs, CP-MSCs, AD-MSCs, and UCB-MSCs (Fig. 4c). There were no differences in the expression pattern of albumin in WJ-MSCs and BM-MSCs. However, urea production was highly expressed in BM-MSCs after hepatogenic differentiation (Fig. 4d). These results indicate that the efficiency for hepatogenic differentiation of MSCs depends on the tissue origin could be different although they have a potential for hepatogenic differentiation.

3.5. Expression of HGF and SCF in MSCs derived from placenta, adipose tissue, bone marrow, and umbilical cord blood

Because several cytokines effectively induce hepatogenic differentiation through their alteration during differentiation, the expression of HGF and SCF was analyzed by ELISA. The expression of HGF was dramatically increased in all MSCs derived from placenta after inducing hepatogenic differentiation (Fig. 5a) and was higher than in other MSCs. Additionally, the expression of SCF increased in WJ-MSCs and BM-MSCs. However, urea production was highly expressed in BM-MSCs after hepatogenic differentiation (Fig. 4d). These results indicate that the efficiency for hepatogenic differentiation of MSCs depends on the tissue origin could be different although they have a potential for hepatogenic differentiation.

![Fig. 4. Functional assay for hepatogenic differentiation of several MSCs. (A) Glycogen storage using PAS staining of MSCs between undifferentiated (h) and differentiated (a–g) conditions. (B) ICG uptake by MSCs between undifferentiated (h) and differentiated (a–g) conditions (× 200). (C) Albumin expression of MSCs according to hepatogenic differentiation detected by Western blot. GAPDH was used as an internal control. (D) Urea production in MSCs according to hepatogenic differentiation using an ELISA assay. Scale bars: 5 μm, 25 μm. a: AE-MSC, b: CP-MSCs, c: CV-MSC, d: WJ-MSC, e: AD-MSC, f: BM-MSC, g: UCB-MSC, h: CP-MSC as a negative control.](image-url)
Recently, progenitor cells or stem cells could be isolated from human term placenta. These cells, referred to as PDSCs, can be expanded beyond 20 cell generations and can generate mesodermal cells, as well as neural and hepaticogenic cells. PDSCs express CD90, SH105, SH3, and SH4, as representative markers of MSCs, and are negative for CD34, CD45, and CD117 (c-kit), which are markers of hematopoietic stem cells isolated from bone marrow or UCB. The properties of PDSCs are similar to other MSCs isolated from some adult tissues. In particular, PDSCs have several advantages such as the earliest stage of adult stem cells, easiness to harvest cells without invasive procedures, their activities for self-renewal and multi-potent, and immunomodulatory properties (Li et al., 2007). In addition, PDSCs contains a variety of localization-specific MSCs (e.g., AE-MSCs, CV-MSCs and CP-MSCs) in placental tissues (Parolini et al., 2008). We reported previously that CP-MSCs isolated from human term placenta have the potential for self-renewal and hepaticogenic differentiation, and CP-MSCs have therapeutic effects in CCl₄-injured liver rat models through an anti-fibrotic effect due to the expression of MMP-9 (Lee et al., 2010). Since CP-MSCs and other MSCs share several stem cell properties, despite their different origins, these progenitor cells may possess an equivalent hepatogenic differentiation potential. However, their potential for hepaticogenic differentiation was controversial because hepatocytes originated from an endodermal lineage and hepaticogenic development progresses through a tightly regulated program of expression of transcriptional factors and cytokines in the stages of hepatocyte differentiation (Snykers et al., 2009). Therefore, it is important to use hepatocyte lineage-specific markers in representing stages in the potential sequence of molecular events of hepatocyte differentiation (Schwartz et al., 2002; Lee et al., 2004a).

We used a two-step differentiation protocol with sequential addition of bFGF, the cytokines OSM and HGF, and hormones (dexamethasone and insulin), which have been reported to be involved in the development and differentiation of hepatocytes (Talens-Visconti et al., 2006). As previously mentioned, HGF plays an essential role in the development and regeneration of the liver, bFGF is required to induce a hepatogenic fate in the foregut endoderm, and OSM increases hepatocyte size and hepatocyte maturation. The morphologic and phenotypic features and gene expression changes in all types of MSCs were compared (Fig. 3a).

Before hepaticogenic differentiation, MSCs have their distinct morphology, but the morphology of the cells induced to become hepatocytes is polygonal in shape. To identify the efficiency of hepaticogenic differentiation, the levels of gene expression of hepatogenic markers were determined by RT-PCR because their expression is primarily regulated at the transcriptional level. The expression of albumin, the abundant protein synthesized by mature hepatocytes, starts in early fetal hepatocytes and reaches the maximal level in adult hepatocytes. CYP2B6 (Cytochrome P450 2B6) is also considered specific to functional hepatocytes. TAT (tyrosine aminotransferase) is known to be a late marker of the hepatocyte lineage, but the level of TAT expression was only detected in PDSCs, not other stem cells (Fig. 3b). To confirm the functional activity of hepaticogenic differentiated MSCs, HGF secretion was measured at the final step of differentiation. HGF highly secretes in PDSCs including AE-MSC, CP-MSC, CV-MSC, and WJ-MSC after hepaticogenic differentiation, otherwise, AD-MSC, BM-MSC and UCB-MSC did not secrete HGF (Fig. 5a). These results indirectly suggest that PDSCs have a greater potential than other stem cells to differentiate into hepatocytes. Interestingly, the expression of SCF in CP-MSCs differentiated into hepatocytes dramatically increased (Fig. 5b). SCF is a hematopoietic factor, inducing leukocyte maturation and differentiation (Galli et al., 1994). In a previous report, SCF was shown to play a role in the liver’s recovery from a toxic injury, specifically an acetaminophen
(APAP)-induced hepatic injury (Simpson et al., 2003). Administration of exogenous SCF reduced mortality in APAP-treated mice, increased hepatocyte proliferation, and prevented hepatocyte apoptosis induced by APAP (Hu and Colletti (2008)). On the basis of these previous reports, we thought that SCF might play a role in hepatic differentiation as an important factor for hepatogenesis. In particular, increased SCF in CP-SCs after hepatic differentiation indicates that CP-SCs, out of several types of adult stem cells, have the best capacity for hepatic differentiation.

In summary, various MSCs derived from adult tissues are able to differentiate into functional hepatocyte-like cells in vitro regardless of their different origins. PDSCs have hepatogenic differentiation potential, and have a longer culture period and a higher proliferation capacity than other stem cells. Therefore, PDSCs could be used as a stem cell source for cell therapy in liver diseases.

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