Biochemical and Biophysical Research Communications 434 (2013) 235–240

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Transcriptional characterization of Wnt pathway during sequential hepatic differentiation of human embryonic stem cells and adipose tissue-derived stem cells

Jeonghoon Heo [a,f], Eun-Kyung Ahn [a,b], Hae-Gyeong Jeong [a], Young-Ho Kim [a,f], Sun-Hee Leem [b], Sang-Joon Lee [c,f], Eun-Kee Park [d], Mihi Yang [e,*]

[a] Department of Molecular Biology and Immunology, College of Medicine, Kosin University, Busan, Republic of Korea
[b] Department of Biology and Biomedical Science, Dong-A University, Busan, Republic of Korea
[c] Department of Ophthalmology, College of Medicine, Kosin University, Busan, Republic of Korea
[d] Department of Medical Humanities and Social Medicine, College of Medicine, Kosin University, Busan, Republic of Korea
[e] Research Center for Cell Fate Control, College of Pharmacy, Sookmyung Women's University, Seoul, Republic of Korea
[f] Institute for Medical Science, Kosin University, Busan, Republic of Korea

ARTICLE INFO

Article history:
Received 14 January 2013
Available online 28 March 2013

Keywords:
Human embryonic stem cell
Human adipose-derived stem cell
Hepatic differentiation
Wnt signaling

ABSTRACT

Human embryonic stem cells (hESs) and adipose-derived stem cells (hADSCs) are able to differentiate into hepatocytes. However, a role of Wnt signaling in hepatic differentiation of stem cells is unclear. This study characterized the transcriptional expression pattern of Wnt signaling genes during the sequential hepatocytes differentiation of hES and hADSC. The sequential hepatocytes differentiation of hES and hADSC was induced by three steps including induction, differentiation and maturation steps with the treatment of cytokines. Hepatocytes differentiation was more efficient in hES than hADSC in terms of the expression of hepatocyte-specific genes and the cellular uptake of ICG. The expression of WNT2B, WNT5A, and WISP1 increased at late hepatic differentiation of hES, but the expression of DKK1 and CCND1 decreased during early hepatic differentiation of hES. During hepatic differentiation of hADSC, the expression of WNT2B and WISP1 decreased, but the expression of WNT5B and DKK1 increased at late hepatic differentiation. These results showed that Wnt signaling appears to be activated in hepatic differentiation of hES, but repressed in hepatic differentiation of hADSC in a time-dependent manner, which suggests the differential regulation of Wnt signaling for hepatic differentiation of hES and hADSC.

1. Introduction

Stem cells are unspecialized cells that renew themselves for long periods and differentiated into the specific functional cells. The unique properties of stem cells have generated great interest for therapeutic use. Stem cells could be classified into embryonic stem cells (ESs) and adult stem cells (ASCs) based on their origin. Human embryonic stem cells (hESs) were isolated from the blastocysts of preimplantation embryos [1], and have a potential to develop into a living organism. hES retain the developmental potency and are able to differentiate into all of three germ layers *in vitro* and *in vivo* [1,2]. ASC are found among differentiated cells in a tissue and play a role in replacement for the lost or injured cells. Human adipose-derived stem cells (hADSCs) are ASC within the adipose stromal compartment similar to bone marrow-derived mesenchymal stem cells (MSCs) [3,4].

For clinical application of stem cells for hepatocyte transplantation, stem cells should be differentiated into homogeneous functional hepatocytes. Many studies have reported the differentiation of human hepatocyte-like cells from hES [5–8] or hADSC [9–12]. The *in vitro* approaches for hepatocyte differentiation from hES or hADSC involve the treatment of the cells with specific cytokines critical for liver development [5,11], the introduction of genes inducing hepatocyte differentiation [13], or the modification of culture environment by co-culturing with other cell types [14]. However, although various approaches to generate homogeneous functional hepatocytes have been developed, the efficiency of transplantable hepatic differentiation from hES or hADSC is still not sufficient. Moreover, the mechanisms underlying *in vitro* hepatic differentiation of hES and/or hADSC remain unclear.

Wnt signaling is involved in self-renewal and differentiation of stem cells, and stimulates intracellular signal transduction cascades [15,16]. Wnt signaling involved the canonical pathway for regulating cell fate determination and/or the non-canonical pathway for regulating planar polarity, cell adhesion and motility [17]. Canonical Wnt signals such as WNT2B, WNT3A and WNT7B induces the assembly of Frizzled receptors and LRP5-6 co-receptor

* Corresponding author. Address: Research Center for Cell Fate Control, College of Pharmacy, Sookmyung Women's University, 53-12 Chungpa-dong 2 Ka, Yongsan-ku, Seoul 140-742, Republic of Korea. Fax: +82 2 710 9871.
E-mail address: myang@sookmyung.ac.kr (M. Yang).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.02.109

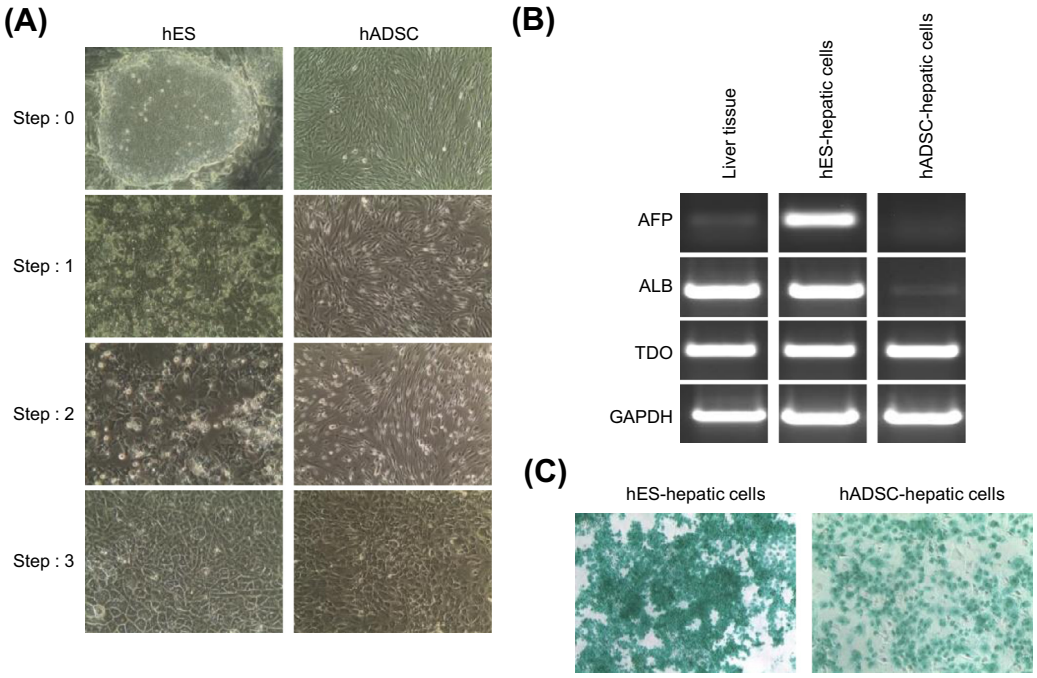

Fig. 1. Hepatic differentiation of hES and hADSC. (A) Morphological changes in three steps during hepatic differentiation (×100 magnification). (B) RT-PCR analysis of hepatocyte-specific gene expression in hES-hepatic cells and hADSC-hepatic cells. (C) Cellular uptake of ICG by hES-hepatic cells and hADSC-hepatic cells. Abbreviations: hES, human embryonic stem cells; hADSC, human adispose-derived stem cells; AFP, alpha-fetoprotein; ALB, albumin; TDO, tryptophan 2,3-dioxygenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

that inhibits the degradation of β-catenin by the glycogen synthase kinase-3β (GSK-3β). The accumulation of the stabilized β-catenin results in its nuclear translocation, where β-catenin binds with the T cell factor/lymphoid enhancer factor (TCF/LEF) family and activates the target genes such as MYC, CCND1, AXIN20, WISP1 and DKK1 [18]. Non-canonical Wnt signals such as WNT5A, WNT5B and WNT11are transduced through Frizzled receptors and ROR2/RYK co-receptors to the Rho family GTPases or the calcium ion-dependent cascades [19].

It has been known that Wnt signaling is involved in animal embryonic development, including the regulation of proliferation and cell fate determination of stem cells [20]. In addition, Wnt signaling regulates the neuronal differentiation of embryonic stem cells [21], the adipocytogenesis of bone marrow stem cells [22], and the osteoblastogenesis of mesenchymal stem cells [23]. Another evidences also showed that Wnt/β-catenin signaling plays a role in liver growth and development [24].

Wnt signaling appears to regulate the proliferation and fate determination of stem cells in a stage-specific and cellular context-dependent manner [25]. Therefore, the aim of this study was to characterize the transcriptional expression of Wnt signaling genes during the sequential hepatic differentiation process of hES and hADSC. The results showed that the transcriptional expression of Wnt signaling genes is associated with hepatocyte differentiation of stem cells in a time and stem cells type-dependent manner.

2. Materials and methods

2.1. Cell culture

The hES line (H9) was provided by the Wisconsin International Stem Cell Bank in the USA. H9 was maintained on mitomycin C (Sigma–Aldrich)-treated MEFs in Dulbecco's modified Eagle medium (DMEM)/F12 (Invitrogen) supplemented with 20% knock-out serum replacement (Invitrogen), 1 mM nonessential amino acid (Invitrogen), 0.1 mM 2-mercaptoethanol (Sigma), 4 ng/ml FGF2 (PeproTech) and 1% antibiotics. The media were changed every day.

hADSC were isolated from adipose tissue in breast fat pads of a female as approved by the Institution of Review Board. Briefly, the adipose tissue was rinsed with sterile phosphate-buffered saline (PBS) and minced for 5 min with fine scissors, and then incubated with 0.1% collagenase type I (Invitrogen) at 37 °C for 40 min. After adding the same volume of DMEM-10% FBS, the pellet was obtained by centrifugation at 260g for 7 min, resuspended in DMEM/F12 (Invitrogen) supplemented with 10% FBS, 10 ng/ml epidermal growth factor (EGF, Peprotech), 2 ng/ml FGF2 and 1% antibiotics. Non-adherent cells were removed by changing the medium after 24 h. At confluency, the cells were replated using 0.25% trypsin–EDTA (Invitrogen) at a ratio of 1:3. Cells at 3∼5 passages in culture were used for the experiments.

2.2. Induction of hepatocyte differentiation

Hepatic differentiation of hES was induced by three steps. In step 1, hES were cultured on matrigel-coated plates in the induction medium (IM) containing RPMI1640 (Hyclone) supplemented with 0.5% FBS, 100 ng/ml Activin A (PeproTech) and 1% antibiotics for 2 days. In step 2, the cells were cultured in the differentiation medium (DM) containing RPMI1640, 2% KOSR, 10 ng/ml bFGF, 10 ng/ml HGF (PeproTech) for 3 days at 37 °C and 5% CO_2. In step 3, the cells were cultured in the maturation medium (MM) containing William's Medium E (Invitrogen), 1% ITS premix (BD Biosciences), 0.05% bovine serum albumin (BSA, Fisher), 2 mM Ascorbic acid (Sigma), 1 μM hydrocortisone 21-hemisuccinate (StemCell Tech), 10 mM nicotinamide (Sigma), 1 μM dexamethasone (Invitrogen) for 24 days.

Hepatic differentiation of hADSC was induced by the three steps as described in the previous study [10]. In step 1 (induction step), hADSC were pre-cultured in DMEM supplemented with 20 ng/ml EGF and 10 ng/ml bFGF for 2 days. In step 2 (differentiation step), the cells were cultured in DMEM supplemented with 20 ng/ml