EXPERIMENTAL CELL RESEARCH 319 (2013) 1136–1145

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Research Article

Distinct transcriptional profiles of angioblasts derived from human embryonic stem cells

Sun-Hwa Song[a,b], *Woojin Jung*[c], *Koung Li Kim*[a], *Wonpyo Hong*[c], *Hyun Ok Kim*[d], *Kyung-Ah Lee*[b], *Ki-Young Lee*[c,*,1], *Wonhee Suh*[a,**,1]

[a]*College of Pharmacy, School of Medicine, Ajou University, San 5, Woncheon-Dong, Yeongtong-Gu, Suwon, Gyeonggi-do 443-749, South Korea*
[b]*Department of Biomedical Science, College of Life Science, CHA University, 198-1 Donggyo-dong, Pochon-si, Gyeonggi-do 487-010, South Korea*
[c]*Department of Biomedical Informatics, School of Medicine, Ajou University, San 5, Woncheon-Dong, Yeongtong-Gu, Suwon, Gyeonggi-do 443-749, South Korea*
[d]*Department of Laboratory Medicine, College of Medicine, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, South Korea*

ARTICLE INFORMATION

Article Chronology:
Received 13 November 2012
Received in revised form
20 February 2013
Accepted 21 February 2013
Available online 28 February 2013

Keywords:
Human embryonic stem cells
Angioblast
Microarray

ABSTRACT

Identification of differentially expressed genes in angioblasts derived from human embryonic stem cells (hESCs) is of great interest for elucidating the molecular mechanisms underlying human vasculogenesis. The aim of this study was to define hESC-derived angioblasts at the clonal level and to perform comparative transcriptional analysis to characterize their distinct gene expression profiles. In a clonal analysis performed in cell-specific differentiation media, hESC-derived $CD34^+CD31^+$ cells were identified as angioblasts in that they exhibited a significantly higher ability to form endothelial cell (EC) and smooth muscle cell (SMC) colonies than $CD34^+CD31^-$ and $CD34^-$ cell populations did. Microarray analysis showed that many genes involved in vascular development and signaling transduction were overexpressed in hESC-derived $CD34^+CD31^+$ cells, whereas those related to mitosis, the DNA damage response, and translation were substantially downregulated. In addition, comparative gene expression profiling of hESC-derived $CD34^+CD31^+$ cells and human somatic primary vascular cells demonstrated that hESC-derived $CD34^+CD31^+$ cells expressed key genes involved in the EC and SMC differentiation processes, which supports the result that hESC-derived $CD34^+CD31^+$ cells are bipotent angioblasts. Our results may provide insights into the identity and function of hESC-derived angioblasts and may also facilitate further investigation of the molecular mechanisms regulating human embryonic vasculogenesis.

© 2013 Elsevier Inc. All rights reserved.

Introduction

As human embryonic stem cells (hESCs) are derived from the inner cell mass of blastocysts and are capable of differentiating into all three germ layers under appropriate culture conditions, they have been considered to be a useful *in vitro* system for studies on human embryonic development [1]. Moreover, hESCs have been instrumental in investigating cell populations

*Corresponding author. Fax: +82 31 219 4477.
**Corresponding author. Fax: +82 31 219 3675.
E-mail addresses: kiylee@ajou.ac.kr (K.-Y. Lee), wonhee.suh@gmail.com (W. Suh).
[1] These authors equally contributed to this work.

0014-4827/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexcr.2013.02.019

that represent early stages of embryonic development and lineage commitment, i.e., stages that are difficult to access and study in human embryos. In the field of vascular biology, hESCs have been used to identify and characterize angioblasts, which are bipotent vascular precursors capable of differentiating into endothelial cells (ECs) and smooth muscle cells (SMCs) during the process of vascular development. Previous studies have shown that $CD34^+$ cells derived from hESCs have been considered to be candidate angioblasts, because the expression of CD34 coincides with that of several transcription factors involved in vascular development during hESC differentiation [2]. Moreover, hESC-derived $CD34^+$ cells give rise to both ECs and SMCs when they are cultured with specific growth factors and cytokines including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)–BB [3,4]. In *in vivo* experiments, transplanted hESC-derived $CD34^+$ cells have been found to differentiate into ECs and SMCs in injured tissues and enhance the neovessel formation and blood flow recovery [5,6].

Although CD34 has been frequently used for the identification and characterization of angioblasts derived from hESCs, it is not clear whether all $CD34^+$ cells derived from hESCs are angioblasts. Indeed, several studies have shown that hESC-derived $CD34^+$ cells may be heterogeneous and that only a subset of $CD34^+$ cells may function as angioblasts. When hESC-derived $CD34^+$ cells are cultured in endothelial growth medium containing a low level of VEGF or on collagen IV-coated dishes for endothelial differentiation, the number of ECs gradually decrease and other cell types emerge as the cell culture progresses [3,4]. Hence, it might be necessary to analyze the differentiation potential of hESC-derived $CD34^+$ cells at the clonal level in order to define the angioblast population.

Vascular differentiation of hESCs is a complicated and poorly defined process involving numerous molecular signal pathways triggered by various growth factors, an extracellular matrix, and a microenvironmental milieu. Understanding the mechanisms controlling human vascular development can be facilitated by elucidating the distinct gene expression profiles of hESC-derived angioblasts. In particular, comparative transcriptional analysis of hESC-derived angioblasts with other differentiating cells, and also with completely differentiated somatic vascular cells may help uncover or further define signaling pathways and the molecular mechanisms underlying vascular differentiation of hESCs. In this regard, the current study aimed to achieve more complete characterization of hESC-derived $CD34^+$ cells in order to define a subpopulation with angioblast characteristics. cDNA microarray experiments were performed to investigate the differential gene expression of hESC-derived angioblasts in order to obtain further insights on the process of vasculogenesis in human embryos.

Material and methods

Cell culture

HESCs (H9; Wicell Research Institute, Madison, WI) were grown on mitomycin C-treated mouse embryonic fibroblasts in DMEM/F12 (Gibco, Grand Island, NY) supplemented with 20% knockout serum replacement (Gibco), 1% penicillin-streptomycin (Gibco), 0.1 mM β-mercaptoethanol (Gibco), 1% non-essential amino acid (Gibco), and 5 ng/ml basic fibroblast growth factor (bFGF; ProSpec, Rehovot, Israel). Human vascular smooth muscle cells (HVSMCs; ScienCell Research Laboratories, Carlsbad, CA) were cultured in SMC growth medium (SMCM; ScienCell Research Laboratories). Human umbilical vein endothelial cells (HUVECs; ScienCell Research Laboratories) and human umbilical artery endothelial cells (HUAECs; ScienCell Research Laboratories) were cultured in endothelial growth medium (EGM)-2 (Lonza, Walkersville, MD).

Meosodermal differentiation of hESCs

After embryonic bodies (EBs) that formed from the hESC clumps were cultured on Matrigel (BD Biosciences, Bedford, MA) for two days, they were differentiated in medium I containing 10% knockout serum replacement, 10 ng/ml VEGF (R&D System, Minneapolis, MN), 10 ng/ml bone morphogenetic protein-4 (BMP-4; ProSpec), 5 ng/ml bFGF (ProSpec) and 3 ng/ml activin-A (ProSpec) in DMEM/F12 under hypoxic conditions (3% O2) for 8 days. Then, EBs were attached to a gelatin-coated dish and further differentiated for an additional 7 days in medium II containing 50 ng/ml VEGF, 20 ng/ml BMP-4, and 5 ng/ml bFGF in endothelial basal medium (Lonza) under normoxic conditions.

Real-time reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells using Trizol (Invitrogen, Carlsbad, CA) and cDNA was synthesized using the Superscript First-Strand Synthesis System (Invitrogen). cDNA was amplified using the SYBR Green PCR master mix (Applied Biosystems, Forster City, CA). Data were analyzed using the ΔΔ Ct method and normalized against values obtained for the housekeeping gene glyceraldehyd e-3-phosphate dehydrogenase (GAPDH). All reactions were performed in triplicate. Primer sequences are listed in Supplementary Table I.

Immunofluorescence staining

Cells were fixed in 4% paraformaldehyde (Sigma, Louis, MO), permeablized with 0.5% Triton X-100 (Sigma), and blocked in 10% normal goat or rabbit serum (Vector Laboratories Inc., Burlingame, CA). Cells were incubated with primary IgGs against CD34 (BD Bioscience), CD31 (Dako Inc., Carpinteria, CA), vascular endothelial-cadherin (VE-Cad, R&D Systems), von Willebrand factor (vWF, R&D System), smooth muscle22α (SM22α, Abcam, Cambridge, MA), α-smooth muscle actin (α-SMA, R&D Systems), or irrelevant non-specific IgGs. The cells were then incubated with Alexa Fluor 488- or 594-conjugated secondary IgGs (Invitrogen) and imaged using a fluorescence microscope (Nikon, Tokyo, Japan). Nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI).

Fluorescence-activated cell sorting (FACS) analysis

Cells pretreated with human-FcR blocking reagent (Miltenyi Biotec, Bergisch, Galdbach, Germany) were labeled with phycoerythrin (PE)-conjugated anti-human CD34 IgGs (Dako Inc.) and/or allophycocyanin (APC)-conjugated anti-human CD31 IgGs (BD bioscience) for 1 h at 4 °C. Negative controls were stained with the appropriate isotype-matched nonspecific IgGs. Cell surface expression of CD34

Download English Version:

https://daneshyari.com/en/article/10904336

Download Persian Version:

https://daneshyari.com/article/10904336

Daneshyari.com