Journal of Molecular and Cellular Cardiology 87 (2015) 17–26

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Novel zinc finger transcription factor ZFP580 promotes differentiation of bone marrow-derived endothelial progenitor cells into endothelial cells via eNOS/NO pathway

Shuping Wei [a,b,1], Jiawen Huang [c,1], Yuming Li [b], Juan Zhao [d], Yuyu Luo [d], Xiangyan Meng [d], Huiyan Sun [d], Xin Zhou [b], Mei Zhang [b,e,*], Wencheng Zhang [b,d,**]

[a] Department of Anatomy, Logistics University of Chinese People's Armed Police Force, Huizhihuan Road 1, Dongli District, Tianjin 300309, China
[b] Tianjin Key Laboratory of Cardiovascular Remodeling and Target Organ Injury, Tianjin 300162, China
[c] Tianjin Medical University, 22 Qixiangtai Road, Heping District, Tianjin 300070, China
[d] Department of Physiology and Pathophysiology, Logistics University of Chinese People's Armed Police Force, Huizhihuan Road 1, Dongli District, Tianjin 300309, China
[e] Department of Cardiology, The Affiliated Hospital Medical University of Chinese People's Armed Police Force, Tianjin 300162, China

ARTICLE INFO

Article history:
Received 12 January 2015
Received in revised form 30 June 2015
Accepted 5 August 2015
Available online 9 August 2015

Keywords:
ZFP580
Endothelial progenitor cells
Differentiation
Endothelial nitric oxide synthase

ABSTRACT

Background: The differentiation of endothelial progenitor cells (EPCs) plays a pivotal role in endothelial repair and re-endothelialization after vascular injury. However, the underlying mechanisms still remain largely elusive. Here, we investigated the role of the novel C2H2 zinc finger transcription factor ZFP580 in EPC differentiation and the molecular mechanisms behind EPC-mediated endothelial repair.
Methods: Bone marrow-derived EPCs were isolated, cultured, and identified. EPCs were infected with an adenovirus encoding ZFP580 or Ad-siRNA to silence ZFP580. Fluorescence-activated cell sorting (FACS) analysis was performed to analyze EPC surface makers. The expression of ZFP580, eNOS, VEGFR-2, CD31, CD34, CD45 and vWF was performed by Q-PCR, Western blot and immunostaining. NO donor SNAP or NOS inhibitor L-NAME was used to elucidate the possible molecular mechanism. Tube formation in vitro and angiogenesis assay in vivo were also used in this study.
Results: Both ZFP580 and eNOS were displayed dynamic expression during EPC differentiation. Overexpression of ZFP580 enhanced EPC differentiation, while knockdown suppressed it. ZFP580 also enhanced eNOS expression, and eNOS inhibition suppressed differentiation. Upregulation/knockdown of ZFP580 also enhanced/reduced endothelial tube formation from EPC in vitro, and angiogenesis in vivo in response to Matrigel plugs containing EPC.
Conclusions: ZFP580 promotes not only the differentiation of EPCs into ECs by increasing the expression of eNOS and the availability of nitric oxide, but also the vessel formation in vitro and in vivo. This might represent a novel mechanism of ZFP580 in EPC differentiation and its therapeutic value in the treatment of vascular disease.
© 2015 Published by Elsevier Ltd.

1. Introduction

Dysfunction of the vascular endothelium is a vital factor in the pathogenesis of vascular disease [1]. Endothelial cells (ECs) have the ability of cell division and migration not only in embryo but also in adult life. When a part of endothelium is injured and detached, neighboring endothelial cells proliferate, migrate, and cover the exposed surface. Furthermore, endothelial cells regenerate and new blood vessels are made in hypoxic lesions [2]. Endothelial progenitor cells (EPCs) are also demonstrated to play an important role for the vascular regeneration, the endothelial repair and the replacement of dysfunctional endothelium [3]. It is a new strategy used to cure the cardiovascular diseases by accelerating re-endothelialization and neoangiogenesis which EPCs/ECs participate in [4]. Since there is no clear definition for the term EPC, there are many different cell types that could fall under this definition. Key properties of these cells are colony formation in culture, attach to existing

Abbreviations: bFGF, recombinant rat basic fibroblast growth factor; C2H2, Cys2–His2; DiL-ac-LDL, Dil-conjugated acetylated low-density lipoprotein; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; EPCs, endothelial progenitor cells; FACS, fluorescence-activated cell sorting; HRP, horseradish peroxidase (HRP); KLF, Krüppel-like transcription factor; L-NAME, L-NG-nitroarginine methyl ester; MEP, megakaryocyte-erythroid progenitor; MNCs, mononuclear cells; Q-PCR, quantitative real-time polymerase chain reaction; SNAP, S-nitroso-N-acetylpenicillamine; TNF-α, tumor necrosis factor-α; UEA-I, *Ulex europaeus* lectin I; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2.

* Correspondence to: M. Zhang, Department of Cardiology, The Affiliated Hospital Medical University of Chinese People's Armed Police Force, Tianjin 300162, China.
** Correspondence to: W.C. Zhang, Department of Physiology and Pathophysiology, Logistics University of Chinese People's Armed Police Force, Huizhihuan Road 1, Dongli District, Tianjin 300309, China.
E-mail addresses: chyouyou@126.com (M. Zhang), wenchengzhang@yahoo.com (W. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.yjmcc.2015.08.004
0022-2828/© 2015 Published by Elsevier Ltd.

endothelial cells nearby hypoxic lesions, transmigrate into tissues, proliferate, differentiate, secrete angiogenic factors, and induce neovascularization [5,6]. Many transcription factors, growth factors, cytokines, and chemokines have been reported to be involved in the regulation of EPC/EC differentiation, migration and proliferation [7,8]. However, the mechanisms governing regulation of transcription during EPC differentiation into mature EC and participation in vascular repair remain largely unknown.

In order to better understand EPC/EC biology, we sought to identify core transcriptional factors regulating EPC differentiation. Among several transcriptional factors involved in the maintenance of EC function and cardiovascular stem/progenitor cell biology, the role of the Krüppel-like transcription factor (KLF) family in EPC biology emerged. KLFs play critical roles in differentiation, development, and maintenance of vascular integrity [9,10]. ZNF580, a novel zinc finger transcription factor, was initially cloned by our group from the human aorta tissue cDNA library [11]. The ZNF580 gene encodes 172-amino acid protein which contains three repeated tandem C2H2-type zinc finger domains at its carboxyl terminus. The protein structure of ZNF580 is similar to that of KLFs, which is also characterized by three tandem-repeated C2H2 zinc fingers in the C-terminus. Northern blot analysis showed that ZNF580 was ubiquitously expressed in human tissues [11]. Furthermore, the enhanced green fluorescent protein-ZNF580 was localized in the nuclei of MGC803, HEK293 and EA.hy926 endothelial cells [12,13]. In addition, ZNF580 played important roles in the migration and proliferation of endothelial cells [14]. ZFP580, the murine homologous gene of ZNF580, was subsequently cloned in our laboratory and investigated in rats [15]. Bioinformatics analysis revealed that ZFP580 is also a C2H2 (Cys2–His2) zinc-finger protein containing 172 amino acids, and 97% of the amino acid sequence is consistent with ZNF580. Furthermore, our previous study showed that ZFP580 had an anti-apoptotic effect following myocardial I/R injury. Both IHA hypoxia and HPC could up-regulate ZFP580 expression [16]. Therefore, we hypothesized that ZFP580 might be involved in vascular diseases, and can be used as a new molecular target for treating such diseases. However, a potential role for ZFP580 in EPC differentiation, and the mechanism by which it might act, has yet to be elucidated.

Endothelial nitric oxide synthase (eNOS) plays a major role in differentiation of EPCs [17]. At the cellular and molecular levels, neovascularization or re-endothelialization depends on the eNOS-derived nitric oxide (NO) bioavailability in ECs [18]. Multiple signaling pathways contribute to the process of EPC differentiation. Emerging evidence suggests that the eNOS/NO signaling pathway is involved in differentiation of EPCs into mature ECs [17,19,20]. In addition, our previous results of a luciferase reporter assay showed that ZNF580 acts as a positive modulator of eNOS gene promoter activity [21].

This study was designed to investigate the probable roles and underlying mechanisms of ZFP580 in EPC differentiation. Our results indicated that the transcription factor, ZFP580, plays a vital role in inducing differentiation of EPCs derived from bone marrow (BM-EPCs) into mature ECs, and thereby provides new insights into the expanded regulatory circuitry in EPC-mediated angiogenesis.

2. Materials and methods

2.1. Reagents

Vascular endothelial growth factor (VEGF) and recombinant rat basic fibroblast growth factor (bFGF) were purchased from PeproTech (London, UK). TRIzol, L-NG-Nitroarginine Methyl Ester (L-NAME), FITC-labeled *Ulex europaeus* lectin I (UEA-1), human fibronectin and S-nitroso-N-acetylpenicillamine (SNAP) were purchased from Sigma-Aldrich (St Louis, MO, USA). EGM-2 was purchased from Lonza (Walkersville, MD). Dil-conjugated acetylated low-density lipoprotein (DiL-ac-LDL) was purchased from Invitrogen (Carlsbad, CA). cGMP ELISA kits were purchased from Cell Biolab, Inc. (San Diego, CA, USA).

2.2. Bone marrow-derived EPC isolation, culture, and identification

Male Sprague–Dawley rats (1–2 month-old) provided by the Animal Center of the Academy of Military Medical Sciences (Beijing China) were used to perform this study. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication 8th Edition, 2011). The animal experimental protocol was approved by the Animal Care and Use Ethics Committee of the Logistics University of Chinese People's Armed Police Force. SD rats were sacrificed by intraperitoneal injection of ketamine (1 g/kg body weight) and xylazine (100 mg/kg). Bone marrow-derived endothelial progenitor cell (BM-EPC) isolation, culture, and identification were as previously described [22,23]. Briefly, total bone marrow-derived mononuclear cells (MNCs) were isolated from both femurs and tibias of male SD rats by Ficoll density gradient centrifugation. Isolated cells were subsequently plated on dishes precoated with fibronectin, and maintained in complete EGM-2 medium (supplemented with EGM-2 bullet kit, including 5% FBS, VEGF, recombinant rat bFGF). Cells were maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2). After 4 days, adherent cells were extensively washed to remove unattached cells and replaced with fresh media and culture medium was changed every 3 days. EPC identification and estimation of culture purity were determined by staining cells with FITC-labeled *Ulex europaeus* lectin I (UEA-1) and uptake of Dil-conjugated acetylated low-density lipoprotein (DiL-ac-LDL). EPCs were also characterized by immunofluorescence staining for the expression of CD31, CD45, and VEGFR-2. The fluorescent images were recorded under a laser scanning confocal microscope. They were additionally confirmed by demonstrating the expression of well-established cell surface markers like CD34 and VEGFR-2 by fluorescence-activated cell sorting analysis.

2.3. Cellular staining

For immunostaining, briefly, cells were washed with cold PBS, and then fixed with 4% (v/v) paraformaldehyde and then permeabilized with Triton X-100. Specific antibodies were used to detect CD31 (1:100 Abcam), CD45 (1:100 Abcam), endothelial nitric oxide synthase (eNOS) (1:100 Abcam) and VEGFR-2 (1:100 Abcam). Secondary, cells were exposed to FITC-and PE-conjugated anti-body (1:200 ImmunoReagents, Inc., China) at 37 °C for 2 h in the dark. Negative control cultures were incubated with isotype IgG instead of the first antibody. Nuclear staining was performed with DAPI (Sigma-Aldrich B2261, USA). Cell fluorescence was visualized using a fluorescence microscope (Radiance 2100, Bio-Rad, USA).

2.4. Flow cytometry

Fluorescence-activated cell sorting (FACS) analysis was used to detect cell surface markers. Cells were stained for 60 min at 48 °C, and then fixed with 2% paraformaldehyde. The surface markers investigated were FITC-conjugated CD34 (1:50 BD PharMingen), FITC-conjugated CD133 (1:50 Zhenjiang Hope Biotechnology Co.) and PE-conjugated CD31 (1:50 Zhenjiang Hope Biotechnology Co.). We used a biotinylated primary antibody directed against VEGFR-2 (1:50 Abcam), and streptavidin conjugated to allophycocyanin (APC, BD Pharmingen) as the secondary antibody. Isotype-identical antibodies served as negative controls.

2.5. Adenovirus infection of EPCs

Ad-ZFP580, a recombinant adenovirus encoding ZFP580, was generated by subcloning cDNA encoding the total length of ZFP580 into the adenoviral vector and the parental adenoviral vector was used as infection negative control (Ad-NC). In addition, an adenovirus encoding for siRNA-ZFP580 (Ad-siZFP580) was used to suppress

Download English Version:

https://daneshyari.com/en/article/8474049

Download Persian Version:

https://daneshyari.com/article/8474049

Daneshyari.com