Biomedicine & Pharmacotherapy 83 (2016) 100–106

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Gene expression profiling of common signal transduction pathways affected by rBMSCs/F92A-Cav1 in the lungs of rat with pulmonary arterial hypertension

Haiying Chen[a,b,1], Hongli Yang[a,1], Chong Xu[a], Hongmei Yue[b,c], Peng Xia[c], Pádraig Michael Strappe[d], Lei Wang[c], Li Pan[a], Wenqiang Tang[a], Shuangfeng Chen[a], Lexin Wang[c,d,*]

[a] Central laboratory, and key Laboratory of Oral and Maxillofacial-Head and Neck Medical Biology, Liaocheng People's Hospital, Liaocheng, Shandong, 252000, China
[b] Liaocheng People's Hospital and Clinical School of Taishan Medical University, Liaocheng, Shandong, China
[c] Department of Cardiology, Liaocheng People's Hospital and Affiliated Liaocheng People's Hospital of Shandong University, Liaocheng, Shandong, 252000, China
[d] School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW 2650, Australia

ARTICLE INFO

Article history:
Received 1 May 2016
Received in revised form 25 May 2016
Accepted 14 June 2016

Keywords:
Bone marrow derived mesenchymal stem cells
Endothelial nitric oxide synthase
Caveolin-1
Nitric oxide
Pulmonary arterial hypertension

ABSTRACT

Background: Pulmonary arterial hypertension (PAH) is associated with sustained vasoconstriction, inflammation and suppressed apoptosis of smooth muscle cells. Our previous studies have found that rat bone marrow-derived mesenchymal stem cells (rBMSCs) transduced with a mutant caveolin-1(F92A-Cav1) could enhance endothelial nitric oxide synthase (eNOS) activity and improve pulmonary vascular remodeling, but the potential mechanism is not yet fully explored. The present study was to investigate the gene expression profile upon rBMSCs/F92A-Cav1delivered to PAH rat to evaluate the role of F92A-Cav1 in its regulation.

Methods: PAH was induced with monocrotaline (MCT, 60 mg/kg) prior to delivery of lentiviral vector transduced rBMSCs expressing Cav1 or F92A-Cav1. Gene expression profiling was performed using Rat Signal Transduction PathwayFinder array. The expression changes of 84 key genes representing 10 signal transduction pathways in rat following rBMSCs/F92A-Cav1 treatment was examined.

Results: Screening with the Rat Signal Transduction PathwayFinder R^2 PCR Array system and subsequent western blot, immunohistochemistry or real time PCR analysis revealed that F92A-Cav1 modified rBMSCs can inhibit the inflammation factors (TNF-alpha, Icam1 and C/EBPdelta), pro-proliferation genes (c-Myc, Bcl2a1d, Notch1and Hey2), oxidative stress gene (Hmox1) and activate cell cycle arrested gene Cdkn1a, ameliorating inflammation and inhibiting cell proliferation in PAH rat.

Conclusion: rBMSCs/F92A-Cav1 inhibits inflammation and cell proliferation by regulating signaling pathways that related to inflammation, proliferation, cell cycle and oxidative stress.

1. Introduction

Pulmonary arterial hypertension (PAH) is a rare and rapidly progressive disease characterized by an inflammatory and vessel wall remodeling [1]. Hypertension and inflammation are the major risk factors that contribute to the development of endothelial dysfunction in PAH. The endothelial cells fail to maintain their homeostatic balance with consequent impaired vasodilators production, and over expression of vasoconstrictors and proliferators [2], resulting in increased pulmonary artery resistance, right ventricular failure and death [3]. Significant pulmonary vascular disease is a leading cause of death in patients with PAH.

Increasing evidence indicates that PAH is a vascular inflammatory disease with increased circulating levels of cytokines being reported in patients with PAH [4]. Endothelial cells injury and apoptosis is the first to occur under increased inflammation or other injury events, surviving endothelial cells promote a phenotypic change, leading to sustained vasoconstriction by disturbing different signaling pathways [5]. Therefore, early

* Corresponding author at: School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW 2650, Australia.
E-mail address: lwang@csu.edu.au (L. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biopha.2016.06.028
0753-3322/

intervention for endothelial dysfunction is important and may delay disease progression and improve survival and quality of life.

Nitric oxide (NO) is generated in endothelial cells by endothelial nitric oxide synthase (eNOS), genetic modification of adult stem cells to express eNOS may promote NO production and subsequent cellular responses [6]. NO exhibits diverse physiological actions, including vasodilation, anti-inflammation, anti-platelet, inhibiting proliferation and migration. Therefore, NO bioavailability reduction plays a central role in the development of endothelial dysfunction [2].

The eNOS function can be down-regulated by the scaffold domain of the caveolin-1 (Cav1), resulting in reduced NO production. Alternatively, modifying Cav1 scaffold domain, in particular Phenylalanine at position 92 (F92) is critical for the inhibitory actions of Cav1 toward eNOS, and mutation of F92 to Alanine has been shown to reduce eNOS inhibition and enhance NO production [7]. In our previous studies we found that NO production was increased in rat bone marrow derived mesenchymal stem cells (rBMSCs) transduced with a novel bicistronic lentiviral vector over-expressing mutant Cav1 (F92A) [8,9]. However, the exact key pathways involved in the improved pathological changes of PAH following rBMSCs treatment is yet to be elucidated. To explore the signaling pathways involved in rBMSCs/F92A-Cav1treatment for PAH rat, we systematically examined gene expression of common signal transduction pathways affected by rBMSCs/F92A-Cav1.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (certificate number SCXK Shandong 20090001) weighing 100–120 g were obtained from the animal experimental center of Shangdong University (Jinan, China). The experimental protocol was approved by the Institutional Animal Care and Use Committee (Liaocheng People's Hospital, Shandong, China). All studies with animals were conducted in accordance with the guidelines described in the National Institutes of Health "Guide for the Care and Use of Laboratory Animals"

2.2. Reagents

Monocrotaline (MCT) was purchased from sigma. RT^2 Profiler PCR Array, MasterMix (RT^2 SYBR Green QPCR), RNeasy Mini Kit and RNase-Free DNase Set were all obtained from Qiagen. pLVX-mCMV-zsgreen lentiviral vector backbone was purchased from Biowit Technologies (Shenzhen, China). The wt Cav1-GFP plasmid [10] were purchased from Addgene (Cambridge, MA) LV-Cav1, LV-F92A-Cav1constructed as we previous described [8].

2.3. Cell culture and treatment

rBMSCs were isolated, cultured and identified as we described previously [8]. rBMSCs and HEK 293 T cells (ATCC) were grown in DMEM-F12 and DMEM media respectively, supplemented with 10% FBS at 37 °C under a water-saturated 5% CO2 atmosphere. rBMSCs growing at an exponential phase were randomly divided into the following groups: control groups, Cav1 group (transduced with LV-Cav1 lentivirus), F92-Cav1 group (transduced with LV-F92-Cav1 lentivirus).

2.4. Lentiviral packaging and rBMSCs transduction

Lentiviral vector packaging and transduction was performed as we described previously with slight modification [8]. Briefly, lentiviral plasmids expressing the genes of interest, together with packaging plasmids psPax2, pRSV-Rev, VSV-G (all from addgene) were co-transfected into 70–80% confluent 293 T cells respectively with lipofectamine 2000 (Life Technologies), according to the manufacturer's instructions for the generation of LV-Cav1 and LV-F92A-Cav1 lentivirus. lipofectamine 2000/DNA complexes were added into 293 T cells with completed media and the addition of caffeine (final concentration of 4 mM) and sodium butyrate (final concentration of 1 mM) to achieve higher titer lentivirus [11]. The lentiviral particles were harvested at 48 h and 72 h post-transfection and the cell supernatants were centrifuged at 5000g for 30 min to remove cell debris, then filtered through a Steriflip-HV0.45 μm PVDF Filter Unit (Millipore, Billerica, MA, USA) and concentrated by PEG-it virus precipitation solution (SBI, USA) to obtain virus particles. The rBMSCs were transduced with the above mentioned lentiviral vectors at a multiplicity of infection (MOI) of 50 after the cells reached 60–70% confluence. Cells expressing fluorescence were observed by fluorescent microscopy (CKX71, Olympus) 5 days post transduction.

2.5. Animal model and cells transplantation

Rat models of PAH were established by a subcutaneous injection of monocrotaline (MCT, 60 mg/kg). The rats were randomly divided into three groups: PAH groups (only MCT), Cav1 groups (rBMSCs transduced with Cav1 lentivirus) and F92A-Cav1 groups (rBMSCs transduced with F92A-Cav1 lentivirus). Gene modified rBMSCs were transfused to LV-Cav1and F92A-Cav1 rat groups by tail intravenous injection at the 2 weeks after MCT injection. For the control groups, rats received saline instead of MCT.

2.6. Pulmonary hemodynamic measurements

Under general anesthesia with chloral hydrate (400 mg/kg) administered subcutaneously, pulmonary hemodynamic measurements were performed by right heart catheterization 3 weeks after cell delivery to the PAH rat. The external jugular vein was isolated and a 2Fr microtip catheter (Millar Instruments, Houston, TX) was advanced into the right ventricle (RV) to obtain pressure measurements. For pulmonary arterial pressure (PAP), RV systolic pressure (RVSP) was measured with a MP150 Systems (BIOPAC Systems, Inc, Goleta, CA) and was used to estimate the pulmonary arterial pressure. Rats were euthanized with an overdose of chloral hydrate (4 g/kg) after hemodynamic measurements was finished. Left lungs were dissected, and lung tissue samples were immediately immersed in RNAlater RNA stabilization reagent (Qiagen) for PCR array analysis.

2.7. Sample preparation

Lung samples (20 mg) were disrupted and homogenized using a gentleMACS™ Dissociator (Miltenyi Biotec, Auburn CA) in RNeasy lysis buffer RLT (Qiagen) with β-Mercaptoethanol (β-ME). Total RNA from each groups (control group, PAH group, Cav1 group and F92A-Cav1 group) was extracted from lung tissues using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol, and 1 μg of total RNA was then reverse transcribed to first-strand complementary DNA (cDNA), according to the protocol provided by the RT^2 First Strand Kit (Qiagen).

2.8. Real-time PCR-based array analysis

The Rat Signal Transduction PathwayFinder™ RT2Profiler™ PCR Array (Qiagen, PARN-014ZA), which includes the primers of 84 key genes representative of ten different signal transduction pathways, was used to examine the major signal transduction