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Research paper

Chicoric acid prevents PDGF-BB-induced VSMC dedifferentiation, proliferation and migration by suppressing ROS/NFκB/mTOR/P70S6K signaling cascade

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ABSTRACT

Phenotypic switch of vascular smooth muscle cells (VSMCs) is characterized by increased expressions of VSMC synthetic markers and decreased levels of VSMC contractile markers, which is an important step for VSMC proliferation and migration during the development and progression of cardiovascular diseases including atherosclerosis. Chicoric acid (CA) is identified to exert powerful cardiovascular protective effects. However, little is known about the effects of CA on VSMC biology. Herein, in cultured VSMCs, we showed that pretreatment with CA dose-dependently suppressed platelet-derived growth factor type BB (PDGF-BB)-induced VSMC phenotypic alteration, proliferation and migration. Mechanistically, PDGF-BB-treated VSMCs exhibited higher mammalian target of rapamycin (mTOR) and P70S6K phosphorylation, which was attenuated by CA pretreatment, diphenyleneiodonium chloride (DPI), reactive oxygen species (ROS) scavenger N-acetyl-L-cysteine (NAC) and nuclear factor-κB (NFκB) inhibitor Bay117082. PDGF-BB-triggered ROS production and p65-NFκB activation were inhibited by CA. In addition, both NAC and DPI abolished PDGF-BB-evoked p65-NFκB nuclear translocation, phosphorylation and degradation of Inhibitor κBα (IκBα). Of note, blockade of ROS/NFκB/mTOR/P70S6K signaling cascade prevented PDGF-BB-evoked VSMC phenotypic transformation, proliferation and migration. CA treatment prevented intimal hyperplasia and vascular remodeling in rat models of carotid artery ligation in vivo. These results suggest that CA impedes PDGF-BB-induced VSMC phenotypic switching, proliferation, migration and neointima formation via inhibition of ROS/NFκB/mTOR/P70S6K signaling cascade.

1. Introduction

In mature and normal blood vessels, vascular smooth muscle cells (VSMCs) are characterized to be a highly quiescent and contractile phenotype associated with elevated levels of contractile markers proteins such as α-smooth muscle actin (α-SMA), SM22α and smooth muscle myosin heavy chain (SMMHC) [1,2]. In atherosclerosis and arterial restenosis, VSMCs can switch to be a dedifferentiated, proliferative, and migratory phenotype by downregulating gene expressions of VSMC contractile markers and upregulating synthetic protein expressions of osteopontin (OPN) [3,4]. Accumulating evidence indicates that VSMC phenotypic switching is widely observed in atherosclerosis, intimal hyperplasia, hypertension and postangioplasty restenosis [5,6]. Unraveling the potential mechanisms of VSMC phenotypic switching may provide novel therapeutic target for the prevention and treatment of these diseases.

The aberrant VSMC proliferation and migration are core events in the pathophysiology of many cardiovascular diseases including atherosclerosis and restenosis after angioplasty [5]. The VSMC phenotypic switching is closely linked with excessive proliferation and migration of VSMCs, which is believed to a common vascular pathological condition in atherosclerosis, restenosis and vein bypass graft failure [3]. Therapeutic strategies against VSMC phenotypic switching, proliferation and migration may be beneficial for VSMC-related pathological conditions. In response to vascular injury, the activated inflammatory cells, platelets and VSMCs release the growth factors, especially platelet-

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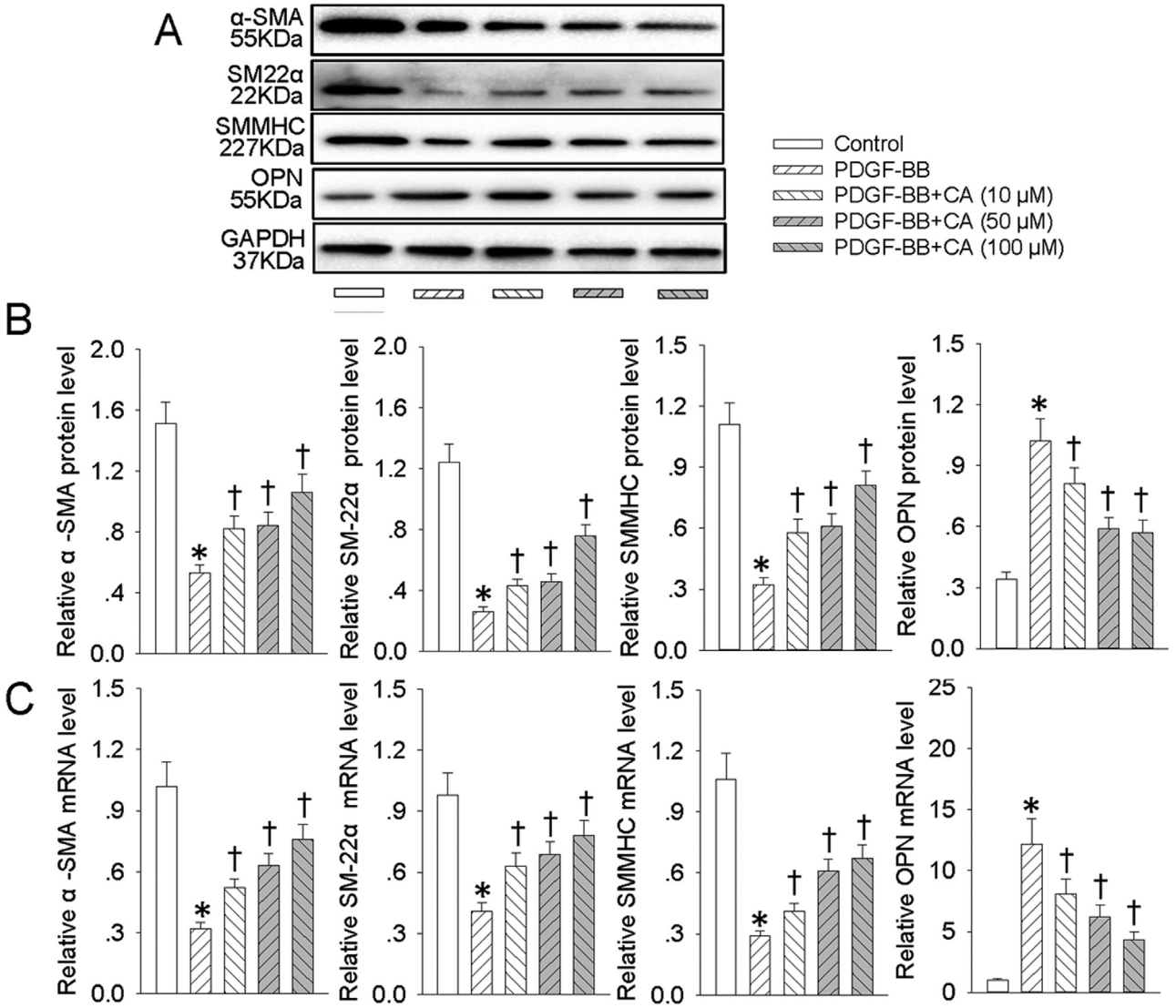

Fig. 1. CA abrogated PDGF-BB-induced VSMC dedifferentiation. VSMCs were pretreated with various concentrations (10, 50 and 100 μM) of CA for 6 h followed by stimulation with PDGF-BB (20 ng/mL) for 24 h. (**A**) Western blot was employed to quantitate the expression levels of contractile protein α-SMA, SMMHC, SM22α and synthetic proteins OPN. (**B**) Bar graph showing the relative protein level of α-SMA, SMMHC, SM22α and OPN. (**C**) Bar graph showing the relative mRNA level ofα-SMA, SMMHC, SM22α and OPN. Values are mean ± SE. * $P < 0.05$ vs. Control, † $P < 0.05$ vs. PDGF-BB. $n = 6$ for each group.

derived growth factor (PDGF), thereby leading to a switch of VSMCs from a contractile phenotype to a synthetic phenotype [7,8]. PDGF family is composed of five proteins including PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD [9], among which, PDGF-BB is described to be one of the most potent stimulants for VSMC proliferation and migration [10]. Therefore, PDGF-BB was utilized to induce VSMC dedifferentiation in this study.

Chicoric acid (CA) is isolated and purified from plant and vegetables, which is reported to possess antioxidant and anti-inflammatory activities [11,12]. CA is established to be a new potential antidiabetic agent by stimulating insulin secretion [13]. CA functions as a regulator of cellular apoptosis, growth, differentiation, and immune response [14–16]. A recent study demonstrates that CA is a potent anti-atherosclerotic ingredient via attenuating oxidized low-density lipoprotein (oxLDL)-facilitated endothelial dysfunction [11]. However, no investigations were conducted concerning the effects of CA on the phenotypic switch, proliferation and migration of VSMCs. Therefore, this study was designed to explore the roles and molecular mechanisms of CA in the regulation of VSMC physiology.

2. Material and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA, and fetal bovine serum (FBS) were obtained from Gibco BRL (Carlsbad, CA, USA). Recombinant human PDGF-BB was purchased from R&D Systems (Minneapolis, MN, USA). Chicoric acid (CA), rapamycin, diphenyleneiodonium chloride (DPI) and dihydroethidium (DHE) were bought from Sigma (St. Louis, MO, USA). Cell counting kit-8 (CCK-8) kits, NFκB inhibitor BAY 11-7082 and N-acetyl-lcysteine (NAC) were obtained from Beyotime Institute of Biotechnology (Shanghai, China). 5-Ethynyl-2′-deoxyuridine (EdU) Apollo kits were purchased from RiboBio (Guangzhou, China). Mitochondria-targeted antioxidant mitoquinone was purchased from Suzhou Vosun Chemical (Jiangsu, China) [17]. The transwell system was obtained from Corning (Corning, Inc., Cypress, CA). Antibodies against α-SMA, SM22α, PCNA, cyclin D1, P27 and horseradish peroxidase conjugated secondary antibodies were purchased from Proteintech Group, Inc (Wuhan, China). Antibodies against p65-NFκB, IκBα, and phosphor-IκBα were obtained from Cell Signaling Technology (Beverly, MA, USA). Antibodies against total or