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Cartilage oligomeric matrix protein prevents vascular aging and vascular smooth muscle cells senescence

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ABSTRACT

Aging-related vascular dysfunction contributes to cardiovascular morbidity and mortality. Cartilage oligomeric matrix protein (COMP), a vascular extracellular matrix protein, has been described as a negative regulatory factor for the vascular aging-related processes including atherosclerosis and vascular calcification. However, whether COMP is implicated in the process of vascular aging remains unclear. Here, we identified a novel function of COMP in preventing vascular aging and vascular smooth muscle cells (VSMCs) senescence. Firstly, vascular COMP expression was decreased in three different senescence-accelerated mouse models and was also declining with age. *COMP*$^{-/-}$ mice displayed elevated senescence-associated markers expression, including p53, p21 and p16, in the aortas compared with their wild type (WT) littermates. In accordance, COMP deficiency induced aging-related vascular dysfunction as evidenced by the significantly reduced phenylephrine-induced contraction and increased vascular stiffness as evaluated by pulse wave velocity. The aortic wall of *COMP*$^{-/-}$ mice was susceptible to senescence by displaying senescence-associated β-galactosidase (SA β-gal) activity induced by peri-adventitial application of $CaCl_2$ to the abdominal aorta. *In vitro*, COMP knockdown by small interfering (si) RNA led to the elevation of p53, p21 and p16 as well as SA β-gal activity in VSMCs after H_2O_2 stimulation. VSMCs isolated from COMP$^{-/-}$ mice showed elevated senescence-associated markers expression and supplement of COMP adenovirus to COMP-deficient VSMCs greatly rescued cellular senescence. Taken together, these findings revealed the essential role of COMP in retarding the development of vascular aging and VSMC senescence.

1. Introduction

Vascular aging is a major risk factor for a variety of cardiovascular diseases including atherosclerosis, vascular calcification and hypertension, etc [1]. Aging vasculature exhibits the reduced arterial compliance and increased stiffness, as well as the impaired function of contraction as a result of senescence-related arterial alterations in cells, matrix, and biomolecules [1]. The occurrence of cellular senescence, particularly in vascular smooth muscle cells (VSMCs) upon stress, has been demonstrated in human atherosclerotic, calcifying and hypertensive arteries [2], as evidenced by the positive staining of senescence-associated β-galactosidase (SA β-gal) and the expression of senescence-related signaling hallmarks, such as high levels of p53, p21, p16 and phosphorylated p38. Consequently, VSMC senescence promotes pathogenesis of atherosclerosis, vascular calcification and other aging-related vascular diseases [3]. Multiple triggers have been identified nowadays to induce VSMC senescence, such as DNA damage, telomere shortening, oxidative stress, oncogene activation, the loss of tumor suppressors, epigenetic stress, angiotensin II and mitochondrial dysfunction [2,4]. However, the mechanism by which VSMCs counteract the multiple triggers of senescence and maintain cellular homeostasis following environmental stimuli remains less understood. An investigation of the endogenous modulators of vascular aging is favorable for the prevention and

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treatment of vascular aging and aging-related vascular diseases.

The extracellular matrix (ECM) constitutes an active and dynamic microenvironment for VSMCs and plays a fundamental role in the regulation of vascular function in normal and pathophysiological conditions. The collagen content increases, whereas elastin decreases with age, giving rise to a corresponding elastin fragmentation and progressive medial degeneration, aortic stiffening and aging-related reduction of vascular complications [5]. Nevertheless, a limited number of reports have recognized the effects of proteoglycans and glycoproteins, which constitute more than half of the human aortic ECM as demonstrated by recent proteomics analysis, on vascular aging [6–8]. Cartilage oligomeric matrix protein (COMP), a 524 kDa pentameric noncollagenous glycoprotein, is a matricellular protein found in both the musculoskeletal and cardiovascular systems. Our recent studies have demonstrated that COMP plays critical roles in the maintenance of vascular homeostasis. COMP maintains the contractile phenotype of VSMCs via integrin α7β1 and prevents osteochondrogenic transdifferentiation of VSMCs by directly binding to BMP-2, which inhibits post injury neointima formation and vascular calcification [9,10]. We recently also demonstrated that COMP negatively regulates atherosclerosis and lesional calcification formation via direct interaction with integrin β3 [11]. As COMP is involved in aging-related vascular diseases including atherosclerosis and vascular calcification, we asked whether COMP is implicated in vascular aging.

2. Materials and methods

2.1. Reagents

Antibodies against COMP, p53, p21, p16 and β-actin were purchased from Abcam (Cambridge, UK). IRDye-conjugated secondary antibodies for western blotting were purchased from Rockland, Inc. (Gilbertsville, PA, USA). Other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless specified.

2.2. Animals

COMP$^{-/-}$ mice with a C57BL/6J background were kindly provided by Professor Äke Oldberg (Department of Cell and Molecular Biology, University of Lund, Sweden) [12]. Aortas of *Zmpste24*$^{-/-}$ mice and their WT littermates were provided by Professor Zhongjun Zhou (Department of Biochemistry, University of Hong Kong, Hong Kong) [13]. *ApoE*$^{-/-}$, SAM-R1 and SAM-P8 mice were purchased from the Department of Laboratory Animal Science, Peking University Health Science Center. All animal studies followed the guidelines of the Animal Care and Use Committee of Peking University.

2.3. Western blotting

Western blot was performed as previously described [14]. Cells and mouse tissue extracts that contained equal amounts of total protein were resolved by 10% or 15% SDS-PAGE and subsequently transferred onto nitrocellulose membranes. The membranes were blocked with 5% milk in TBST, followed by incubation with primary antibody at 4 °C overnight. Following 1 h of incubation with IRDye-conjugated secondary antibodies, the membranes were subjected to an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA) to detect the immunofluorescence signal.

2.4. Aortic ring myograph

A myograph was performed as previously described [15]. Briefly, the aortic rings were isolated and removed from mice following euthanasia. The dissected vessels were immediately placed in Krebs-Henseleit buffer, and then cleaned of additional connective tissues. The aorta was sectioned into 1.5–1.8 mm rings and subsequently subjected to vascular tension experiments. The cumulative dose responses to phenylephrine were obtained to characterize vasocontraction. Data are expressed as percentages to KCl-induced contraction.

2.5. Measurement of pulse wave velocity

The *in vivo* pulse wave velocity (PWV) was measured using an ECG-triggered 10-MHz Doppler probe [16]. The animals were anesthetized with isoflurane and maintained during the measurement by mask ventilation of 1.5% isoflurane with a coupled charcoal scavenging system. The animals were positioned supine with their limbs taped to electrocardiogram electrodes, which were incorporated into a temperature-controlled printed circuit board. The aortic arch blood flow and the abdominal aorta blood flow were captured, and the separation distance between them was measured. The PWV was calculated as a quotient of the separation distance and time difference between pulse arrivals, as measured from the ECG R-peaks.

2.6. $CaCl_2$-induced mouse abdominal aortic calcification

Mice were anesthetized with isoflurane and the infrarenal abdominal aortas were treated with periadventitial application of 0.2 mol/L $CaCl_2$ for 15 min as described previously [10]. Control mice were treated with 0.2 mol/L NaCl. After 7 days, the abdominal aorta was harvested and sectioned for SA β-gal staining. Analysis of calcium content and von Kossa staining were used to assess the efficiency of the model as described previously [10].

2.7. Senescence-associated β-galactosidase (SA β-gal) staining

SA β-gal staining was performed using a Senescence Detection Kit (Medical & Biological Laboratories CO., LTD). For the abdominal aortas, mice were perfused via the left ventricle with phosphate-buffered saline (PBS). Aortas were harvested and incubated in fixative solution for 1 h and in SA β-gal staining solution (prepared according to the manufacture's protocol) at 37 °C for 16 h and then cryosectioned at 10 μm. SA β-gal activity was identified based on positively stained blue cells.

Cells were washed with PBS and fixed with fixative solution for 15 min at room temperature. Then cells were incubated with staining solution at 37 °C for 16 h. Senescent cells were blue-stained as observed under light microcopy. Cells were counterstained with Hoechst 33342 to count the total number.

2.8. Cell culture

The rat smooth muscle embryonic thoracic aorta cell line A7r5 was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and maintained in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2. H_2O_2 treatment was performed by adding 10 μM H_2O_2 for 2 days. Primary mouse thoracic VSMCs were isolated by collagenase digestion as described previously [17]. Briefly, following mouse euthanasia, thoracic aortas were harvested and cut into small pieces. To remove the endothelial cells, these pieces were digested with 1 mg/ml trypsin (Hyclone) at 37 °C for 10 min. After centrifugation, the precipitate was resuspended with 10 mg/ml collagenase type I (Gibco) for 6–8 h. Gelatin was pre-laid onto the culture dishes before the cells were seeded. The cell cultures contained >95%

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