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Sulfatase 1 mediates the attenuation of Ang II-induced hypertensive effects by CCL5 in vascular smooth muscle cells from spontaneously hypertensive rats

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ABSTRACT

Extracellular sulfatases, sulfatase 1 (Sulf1) and sulfatase 2 (Sulf2), play a pivotal role in cell signaling and carcinogenesis. Chemokine CCL5 inhibits Ang II-induced hypertensive mediators via angiotensin II (Ang II) type 2 receptor (AT_2 R) pathway in vascular smooth muscle cells (VSMCs) from spontaneously hypertensive rats (SHR). In this study, we investigated the effect of Sulfs on anti-hypertensive effects of CCL5 in SHR VSMCs. CCL5 attenuated Ang II-induced inhibition of sulfatase activity in SHR VSMCs. Inhibition of Ang II-induced 12-lipoxygenase (12-LO) and endothelin-1 (ET-1) expression by CCL5 was reduced in Sulf1 small interfering RNA (siRNA)-transfected SHR VSMCs. In addition, attenuation of Ang II-induced dimethylarginine dimethylaminohydrolase-1 (DDAH-1) inhibition by CCL5 was reduced in Sulf1 siRNA-transfected SHR VSMCs. Downregulation of Sulf2 did not affect inhibitory effects of CCL5 on Ang II-induced 12-LO and ET-1 expression and Ang II-induced inhibition of DDAH-1 expression in SHR VSMCs. Downregulation of Sulf1 abrogated the expression of CCL5-induced AT_2 R messenger RNA (mRNA) and synergistic effect of CCL5 on Ang II-induced AT_2 R expression in SHR VSMCs. These findings suggest that Sulf1 is a potential up-regulatory factor in anti-hypertensive actions of CCL5 via AT_2 R pathway on Ang II-induced hypertensive effects in SHR VSMCs.

1. Introduction

Extracellular sulfatases (Sulfs) are well known as hydrolytic enzymes that control cell metabolism and signaling [1]. Sulfs regulate cell signaling by remodeling heparan sulfate proteoglycans (HSPGs) on the surface of cells. Sulf-mediated removal of 6-*O*-sulfate groups from HSPGs results in the release of bound growth factors, which initiate signaling pathways [1,2]. Therefore, the activity of Sulfs plays a pivotal role in both cell survival and proliferation. Aside from cell signaling, Sulfs modulate cellular processes such as cell development, muscle regeneration, neuromodulation, and tumor growth [1,3–5]. Sulfs are named as heparan sulfate 6-*O*-endosulfatases [2,6], and there are two types of heparan sulfate 6-*O*-endosulfatases: sulfatase 1 (Sulf1) or sulfatase FP1 and sulfatase 2 (Sulf2) or sulfatase FP2 [6]. Sulf1 and Sulf2 double knockout mice show significant developmental defects as well as reduced body weight [7,8]. Although Sulf1 and Sulf2 are structurally similar, they have reverse effects in tumor cells. Sulf1 inhibits angiogenesis and proliferation in cancer cells [9,10], whereas Sulf2 promotes angiogenesis and tumorigenic effects [11,12]. Thus, Sulfs have been suggested as therapeutic targets for cancer therapy.

Although the reduction in HSPG levels in the glomerular basement membrane has been related to hypertension [13], the role of Sulfs in hypertension development or maintenance is questionable. It has been reported that the maintenance of normal 6-*O*-sulfation levels by Sulf1 is important for the function of vascular smooth muscle cells (VSMCs) [14]. HSPGs are found in vascular walls, and overexpression or knockdown of the Sulf1 gene in normal VSMCs inhibits adhesion and increases of proliferation and apoptosis. Thus, Sulfs may play a functional role in hypertensive VSMCs.

The chemokines CC-chemokine ligand (CCL)-2 and CXC-chemokine ligand (CXCL)-8 play a major role in the development of hypertension [15–18]. Our previous studies show that CCL5 downregulates the

Abbreviations: CCL, CC-chemokine ligand; CXCL, CXC-chemokine ligand; Sulf1, sulfatase 1; Sulf2, sulfatase 2; VSMC, vascular smooth muscle cell; SHR, spontaneously hypertensive rat; Ang II, angiotensin II; AT_1 R, Ang II type 1 receptor; AT_2 R, Ang II type 2 receptor; 12-LO, 12-lipoxygenase; ET-1, endothelin-1; DDAH-1, dimethylarginine dimethylaminohydrolase-1; Sulfs, sulfatases; HSPGs, heparan sulfate proteoglycans; AMPK, AMP-activated protein kinase; ADMA, asymmetric (N^G,N^G) dimethylarginine; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; cDNA, complementary DNA; siRNA, small interfering RNA; SEM, standard error of the means; eNOS, endothelial nitric oxide synthase

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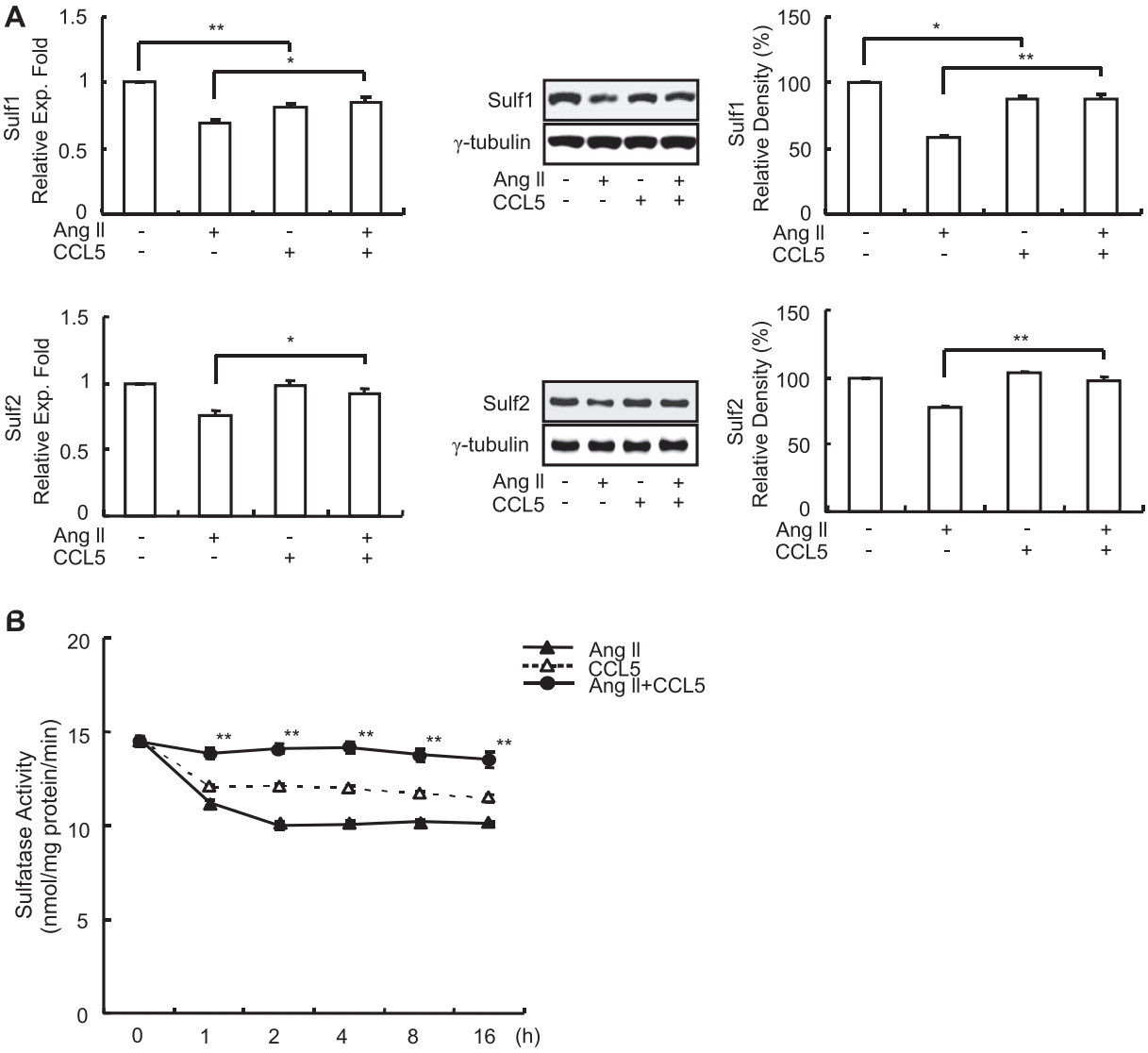

Fig. 1. CCL5 attenuates the inhibition of Ang II-induced sulfatase expressions and activities in VSMCs from SHR. (A) VSMCs were untreated or treated with Ang II (0.1 μmol/L) and/or CCL5 (100 ng/mL) for 2 h. Total RNAs and cell lysates were isolated and Sulf mRNAs and protein expressions determined by real-time PCR and immunoblotting, respectively. Data shown are representatives of three independent experiments. Bars represent the means ± SEM of three independent experiments. Statistical significance was tested by ANOVA or Kruskal-Wallis test, followed by the Bonferroni test. $^{*}p < 0.05$, $^{**}p < 0.01$. (B) VSMCs were untreated or treated with Ang II (0.1 μmol/L) and/or CCL5 (100 ng/mL) for the indicated time. Cell lysates were isolated and sulfatase activity assay was performed. Bars represent the means ± SEM of three independent experiments. $^{**}p < 0.01$ versus VSMCs treated with Ang II.

expression of angiotensin II (Ang II)-induced hypertensive mediators, 12-lipoxygenase (LO) and endothelin-1 (ET-1), in VSMCs obtained from spontaneously hypertensive rats (SHR) [19,20]. In addition, CCL5 up-regulates AMP-activated protein kinase (AMPK) activity and interleukin-10 (IL-10) and dimethylarginine dimethylaminohydrolase-1 (DDAH-1) expression [20–22]. Furthermore, CCL5 injection inhibits the elevation in blood pressure of the developing hypertension-state SHR [23]. Therefore, CCL5 is most likely to play a down-regulatory role in Ang II-induced vascular hypertension, which is contrary to the up-regulatory roles of chemokines CCL2 and CXCL8 in pathophysiologic features of hypertension [15–18].

Based on our previous studies, which demonstrated CCL5-mediated downregulation of Ang II-induced 12-LO and ET-1 production and up-regulation of DDAH-1 expression, we examined the relationship between CCL5-induced anti-hypertensive effects and Sulfs in VSMCs from SHR.

2. Materials and methods

2.1. Reagents

Total RNA extraction kit was purchased from iNtRON (Biotechnology, Seoul, Korea). Ang II was supplied by Calbiochem (San Diego, CA, USA). PD123319 was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) and CCL5, from R&D systems (Minneapolis, MN). LightCycler FastStart DNA SYBR Green I Mix was purchased from Roche (Mannheim, Germany) and Lipofectamine 2000, from Invitrogen (Carlsbad, CA, USA). Primer sequences for Ang II type 1 receptor (AT_1 R), Ang II subtype 2 receptor (AT_2 R), Sulf1, Sulf2, 12-LO, ET-1, DDAH-1, and β-actin were synthesized at Bionics (Daejeon, Korea). Sulf1, Sulf2, 12-LO, ET-1, and DDAH-1 antibodies were supplied by Santa Cruz Biotechnology (California, USA). AT_2 R antibody was purchased from Abcam (Cambridge, UK) and monoclonal anti-γ-tubulin antibody, from Sigma-Aldrich (St. Louis, MO, USA). Rat Sulf1 small interfering RNA (siRNA) Sulf2 siRNA sequences were purchased from Bioneer technology (Daejeon, Korea) and Santa Cruz Biotechnology (California,