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ACE2/Ang-(1–7) signaling and vascular remodeling

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The renin-angiotensin system (RAS) regulates vascular tone and plays a critical role in vascular remodeling, which is the result of a complex interplay of alterations in vascular tone and structure. Inhibition of the RAS has led to important pharmacological tools to prevent and treat vascular diseases such as hypertension, diabetic vasculopathy and atherosclerosis. Angiotensin converting enzyme 2 (ACE2) was recently identified as a multifunctional monocarboxypeptidase responsible for the conversion of angiotensin (Ang) II to Ang-(1–7). The ACE2/Ang-(1–7) signaling has been shown to prevent cellular proliferation, pathological hypertrophy, oxidative stress and vascular fibrosis. Thus, the ACE2/Ang-(1–7) signaling is deemed to be beneficial to the cardiovascular system as a negative regulator of the RAS. The addition of the ACE2/Ang-(1–7) signaling to the complexities of the RAS may lead to the development of novel therapeutics for the treatment of hypertension and other vascular diseases. The present review considers recent findings regarding the ACE2/Ang-(1–7) signaling and focuses on its regulatory roles in processes related to proliferation, inflammation, vascular fibrosis and remodeling, providing proof of principle for the potential use of ACE2 as a novel therapy for vascular disorders related to vascular remodeling.

angiotensin converting enzyme 2, inflammation, vascular remodeling, angiotensin II, oxidative stress

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The renin-angiotensin system (RAS) regulates vascular tone and plays a critical role in adaptive and maladaptive vascular remodeling [1–3]. Vascular remodeling is the result of a complex interplay of alterations in vascular tone and structure, including changes in both the cellular and non-cellular components that depend on the pathological condition, inflammation, endothelial dysfunction and extracellular matrix (ECM) synthesis or degradation [1,4,5]. The RAS consists of a series of enzymatic reactions culminating in the generation of angiotensin (Ang) II in plasma as well as in various tissues including the heart and vasculature. The detrimental effects of Ang II almost mediated via the Ang II type 1 (AT1) receptor [6–8]. Inhibition of the Ang II/AT1 signaling has led to important pharmacological tools to prevent and treat vascular diseases related to vascular remodeling.

Angiotensin converting enzyme 2 (ACE2), a multifunctional monocarboxypeptidase, was recently identified as a negative regulator of the RAS [2,9,10]. The classical pathway of the RAS involving the ACE-Ang II-AT1 receptor axis is now antagonized by the second arm constituted by the ACE2-Ang-(1–7)-Mas receptor axis. The balance between ACE and ACE2 is the key factor in regulating angiotensin levels [11–13]. ACE2 cleaves several important bio-

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logical peptides such as Ang I, Ang II, Apelin-13, Apelin-17, Apelin-36, and [des-Arg9]-bradykinin [1,12,14–16]. ACE2 can cleave Ang I to generate the inactive Ang-(1–9) peptide, which can then be converted to the vasodilator peptide Ang-(1–7) by ACE or neutral endopeptidase (NEP) [17–19]. ACE2 also directly metabolizes Ang II to generate the beneficial heptapeptide Ang-(1–7). Ang-(1–7) is a biologically active metabolite of the RAS whose actions are often opposite to those attributed to the Ang II/AT1 signaling (Figure 1) [13,20,21]. There is increasing interest regarding the protective role of the ACE2/Ang-(1–7) signaling in vascular disease. In this review, we focus on regulatory roles of the ACE2/Ang-(1–7) signaling in proliferation, inflammation, vascular fibrosis and remodeling.

1 ACE2/Ang-(1–7) signaling and vascular proliferation and hypertrophy

The Ang II/AT1 signaling has been shown to be aberrantly activated in vascular hypertrophy and remodeling by promoting vascular smooth muscle cells (VSMC) growth, transdifferentiation and proliferation (Figure 1), eliciting a variety of biological actions of the RAS in the vascular homeostasis [1,12,22,23]. As a specific Ang II-degredating enzyme, ACE2 suppresses VSMC proliferation and vascular hypertrophy. Loss of ACE2 led to vascular proliferation and elevated migration of SMC while ACE2 overexpression inhibited vascular proliferation and hypertrophy by preventing aortic wall thickening [1,4,10,24–27]. The Janus kinase 2 (JAK2)/signal transducer and activators of transcription 3 (STAT3) signaling cascades play a key role in VSMC growth and vascular remodeling (Figure 1) [10,28,29]. In our previous studies [4,6,10,12], we revealed that administration of human recombinant ACE2 (hrACE2) significantly abolished the Ang II-mediated cardiovascular proliferation and remodeling in association with the prevention of the JAK-STAT-SOCS signaling (Figure 1, Table 1). Inhibition of ACE2 by DX600 obviously facilitated Ang II-mediated VSMC proliferation [6,30]. Moreover, we demonstrated previously that ACE2 deficiency led to greater increases in Ang II-mediated profilin-1 expression in aortas of ACE2-mutant mice associated with enhanced phosphorylation levels of Akt and extracellular signal-regulated kinase 1/2 (ERK1/2)/mitogen activated protein kinases (MAPK) [1]. Conversely, ACE2 overexpression resulted in reduction of profilin-1 expression and downregulation of Akt/ERK phosphorylated signaling [1,10,31] (Table 1). The actin-binding protein profilin-1 has recently been linked to VSMC proliferation, vascular pathology and vascular diseases via the modulation of actin polymerization and cytoskeleton remodeling [1,6,32–34]. Compared with nontransgenic controls, profilin-1 overexpression results in vascular hypertrophy and remodeling characterized with higher medial thickness and VSMC proliferation in aorta of profilin-1 transgenic mice with activation of the ERK/MAPK phosphorylation signaling [34,35]. Intriguingly, downregulation of profilin-1 with profilin-1 siRNA and rhACE2 largely abolished Ang II-mediated VSMC proliferation and oxidative stress [6]. These findings confirm that the ACE2/Ang-(1–7) signaling exerts its beneficial effects on vascular proliferation and hypertrophy via the modulation of JAK2-STAT3-SOCS3 and profilin-1/ERK signaling pathways.

In our previous work, Ang-(1–7) treatment strikingly improved the pressure overload-induced cardiovascular hypertrophy and remodeling in the ACE2-mutant mice via the suppression of activation of ERK1/2 and STAT3 phosphorylation signaling (Table 1) [19]. Ang-(1–7) has been shown to inhibit VSMC proliferation and oppose the mitogenic effects of Ang II [25]. Strawn and his co-workers reported that Ang-(1–7) largely inhibited VSMC proliferation of carotid arteries in adult male Sprague-Dawley rats. Ang-(1–7) supplement partially blunted the Ang II-, or platelet-derived growth factor (PDGF)-stimulated VSMC proliferation [25,26,36]. In addition, Ang-(1–7) promoted the release of prostacyclin from VSMC isolated from the aortas of hypertensive rats (Table 1) [37]. Michiya and colleagues [38] have investigated that reduced vascular medial thickness and attenuated vascular hypertrophy were observed in aortas of spontaneously hypertensive rats (SHR) combined with increased levels of ACE2 and Ang-(1–7) during blockade of Ang II receptors. Treatment with azilsartan, an AT1 receptor blocker, or Ang-(1–7) attenuated neointimal area and VSMC proliferation as well as augmented mRNA expression of ACE2 in mice with vascular injury induced by polyethylene-cuff placement around the mouse femoral artery [39]. The above observations imply protective effects of the ACE2/Ang-(1–7) signaling on vascular proliferation and hypertrophy.

2 ACE2/Ang-(1–7) signaling and vascular inflammation and oxidative stress

The effects of vascular inflammation and oxidative stress in the initiation and progression of cardiovascular diseases have been well recognized [1,39]. Generally, activation of NADPH oxidase is a central mediator of the pathological effects of Ang II, contributing to enhanced production of reactive oxygen species (ROS) and activation of pro-inflammatory transcription factors and vascular injury (Figure 1) [40–42]. An important evidence for the relevance of the ACE2/Ang-(1–7) signaling as a potent target to suppress inflammation comes from the observation that administration of XNT (1-[(2-dimethylamino)ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl)sulfonyloxy]-9H-xanthene-9-one), a small molecule ACE2 agonist, improved the

endothelial function of vessels of both hypertensive and diabetic rats accompanied by attenuation of oxidative stress (Table 1) [43]. In a previous report (Table 1), we demonstrated that loss of ACE2 led to marked increases in the Ang II-induced aortic expression of inflammatory cytokines and chemokines, including monocyte chemoattractant protein 1 (MCP-1), interleukin-1β (IL-1β), and IL-6 [1]. We also found that loss of ACE2 resulted in greater activation of NADPH oxidase and ROS production in mice aortas with enhanced expression of profilin-1 [1]. Profilin-1 overexpression has been revealed to aggravate vascular inflammation and vascular remodeling [44]. In the hypertensive rat model, rhACE2 delivered over a 14-day period partly corrected the hypertension, the NADPH oxidase activation and the increased superoxide generation in the aortas with a drastic reduction in plasma Ang II/Ang-(1–7) peptide ratio [45]. We have previously reported [4,6,12,15,19] that administration of rhACE2 or Ang-(1–7) prevented Ang II-mediated activation of NADPH oxidase and profilin-1 expression, contributing to reduction of ROS generation in VSMC or pressure-overloaded ACE2-null mice (Table 1).

ACE2 overexpression prevented the Ang II-induced increases in proinflammatory reaction and activation of NADPH oxidase in cultured VSMC (Table 1), and these protective effects of ACE2 could be blocked by the co-treatment with Ang-(1–7)/Mas antagonist A-779 [6,46,47]. Elevated production of ROS in response to increased RAS activity in the vasculature resulted in heightened transcription of nuclear factor-κB (NF-κB) (Figure 1), and these latter further promoted activation of NADPH oxidase and endothelial lesion via increasing levels of vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), MCP-1 and IL-6 (Figure 1) [48,49]. Sahara et al. [50] have revealed that treating ACE2-mutant mice with TNF-α triggered up-regulated expression of inflammatory factors, including MCP-1, macrophage inflammatory protein (MIP)-1α, MIP-2α (Table 1). In the cerebral artery of rats (Table 1), Ang-(1–7) infusion led to reduced oxidative stress with reduction of NF-κB activity [51]. The ACE2/Ang-(1–7) signaling has been exhibited to be the counter-regulator of Ang II in the context of leukocytes recruitment [52,53]. Expression and release of inflammatory factors were obviously enhanced in macrophages from ACE2-deficiency mice with accelerated monocytes adhesion to vascular endothelial cells (ECs) and promotion of the EC inflammation (Table 1) [54]. In aortic adventitial fibroblasts (AFs), Ang II stimulated monocytes recruitment through pathway involving fibroblasts-derived MCP-1 and IL-6, and these monocytes further augmented production of proinflammatory cytokines [55]. Treatment with azilsartan or Ang-(1–7) downregulated the mRNA levels of MCP-1, TNF-α, and IL-1β, and superoxide anion production in the injured artery [39]. ACE2 overexpression inhibited macrophages function and Ang II-mediated proinflammatory factors [56], further supporting the hypothesis that the ACE2/Ang-(1–7) signaling might be a promising avenue for developing cardiovascular disease therapeutic agents.

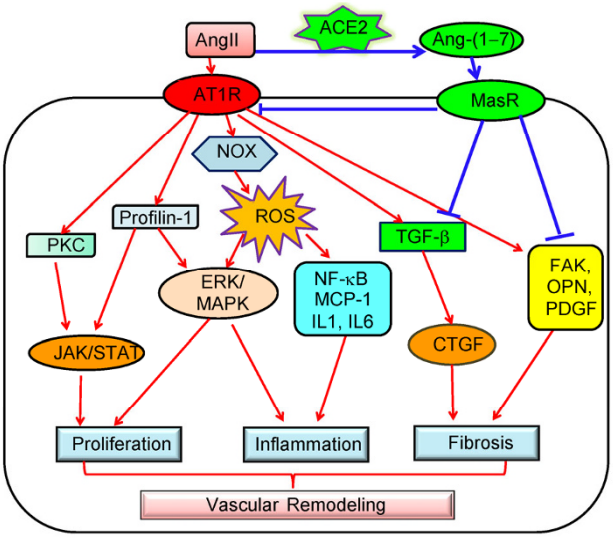

Figure 1 (color online) The roles and mechanisms of the ACE2/Ang-(1–7) signaling in the vascular proliferation, inflammation, fibrosis and remodeling. ACE2, angiotensin converting enzyme 2; Ang II, angiotensin II; Ang-(1–7), angiotensin-(1-7); ERK, extracellular signal-regulated kinase; MAPK, mitogen activated protein kinases; ROS, reactive oxygen species; IL-1, interleukin-1; IL-6, interleukin-6; JAK, janus kinase; STAT, signal transducer and activators of transcription; PKC, protein kinase C; NF-κB, nuclear factor-κB; OPN, osteopontin; MCP-1, monocyte chemoattractant protein 1; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β.

3 ACE2/Ang-(1–7) signaling and vascular fibrosis

The ACE2/Ang-(1–7) signaling has been exhibited to be a negative modulator of vascular fibrosis by modulation of fibroblast density, fibrogenic pathways and the production of ECM proteins such as collagen and matrix metalloproteinases (MMPs) (Table 1). Loss of ACE2 augmented Ang II-mediated expression of fibrosis-associated genes such as transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), procollagen type I and procollagen type III [12,13]. ECM deposition and cell migration both are adverse effects of the TGF-β-CTGF signaling associated activation of multifunctional matrix cellular factor [57–60]. There is good evidence that Ang II acting on AT1 receptor contributes dramatically to fibroblasts proliferation and expression of ECM proteins by activation of the TGF-β-CTGF signaling. Ang II-induced activation of FAK, which was highly expressed in cultured VSMCs, led to cell adhension to the ECM and activation of cytoskeletal proteins, finally influencing vascular cell shape and movement [61]. Moreover, Ang II promoted expression of osteopontin (OPN)

Table 1 The regulatory roles of ACE2/Ang-(1–7) signaling in the vascular system[a)]

Experiment models	Strategy used	Effects	References
HUASMC *in vitro*	rhACE2 treatment	↓ VSMC proliferation ↓ ERK1/2, ↓ JAK/STAT	[6]
Mice *in vivo*	rhACE2 treatment	↓ ERK1/2, PKC	[12]
Rat aorta *in vivo*	ACE2 overexpression	↓ neointimal formation	[65]
Mice VSMC *in vitro*	ACE2 inhibitor	↑ ERK1/2	[30]
Rat VSMC *in vitro*	Ang-(1–7) treatment	↓ VSMC proliferation	[25]
Mice vascular *in vivo*	Ang-(1–7) infusion	↓ ERK1/2, STAT3 ↓ NADPH oxidase	[19]
Rat aorta *in vitro*	Ang-(1–7) treatment	↑ prostacyclin	[37]
ApoEKO mice *in vivo*	Ang-(1–7) infusion	↓ ROS, eNOS ↑ endothelial function	[64]
Mice aorta *in vivo*	ACE2 deletion	↑ inflammation ↑ MMP-2, -9	[54]
Mice aorta *in vivo*	ACE2 deletion	↑ NADPH, ROS ↑ profilin-1; Akt/ERK	[15] [1]
Mice VSMC *in vitro*	ACE2 overexpression	↓ NADPH oxidase	[46]
Rat pulmonary artery *in vivo*	XNT administration	↓ IL-1β, IL-6, TNF-α ↓ MCP-1, TGF-β	[24]
Rat vascular *in vivo*	Ang-(1–7) infusion	↓ NF-κB; ROS	[51]
ACE2KO/mice aorta *in vivo*	TNF-α treatment	↑ MCP-1, MIP-1α, ↑ MIP-2α	[50]
Akita mice *in vivo*	ACE2 deletion	↑ MMP-2,-9,-12,-13	[4]

a) VSMCs, vascular smooth muscle cells; KO, knockout; Akt, protein kinase B; ERK, extracellular signal-regulated kinase; ROS, reactive oxygen species; MMPs, matrix metalloproteinases; MIP, macrophage inflammatory protein; eNOS, endothelial nitric oxide synthetase; NADPH, nicotinamide adenine dinucleotide phosphate; JAK, janus kinase; STAT, signal transducer and activators of transcription; XNT, ACE2 agonist; PKC, protein kinase C; MCP-1, monocyte chemoattractant protein 1; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6.

which acts as ECM protein influencing VSMC adhesion and migration (Figure 1) [62]. The anti-fibrotic effects of ACE2 in VSMCs were primarily executed through the Mas receptor, as the Mas-deficient mice exhibited tendency to pro-fibrosis in cardiovascular system [63]. Long-term infusion of Ang-(1–7) exerted vasoprotective and atheroprotective effects in the ApoE knockout mice model with increased eNOS expression and improvement of endothelial function (Table 1) [64]. In addition, Ang-(1–7) treatment has been shown to attenuate neointimal formation by structural recovery of endothelium and exert the atheroprotective effects through acting on both the AT2 and the Mas receptor (Table 1) [65]. The interesting interaction between the ACE2/Ang-(1–7) signaling and AT2 receptor has been well documented and this crosstalk greatly broadens our understanding of the RAS.

Studies have suggested that the ACE2/Ang-(1–7) signaling blocks the key pro-fibrogenic signaling initiated by Ang II [4,53]. In addition to the decrease in the plasma Ang II level and the increase in Ang-(1–7) level, the protective aspects of ACE2 were partly due to its down-modulation of MMPs [16]. Genetic ACE2 deficiency in ApoE knockout mice resulted in increased vascular atherosclerosis via raising expression of VCAM-1, MCP-1 and MMP-2, and MMP-9 (Table 1) [54]. The increased activities of MMPs, especially MMP-2 and MMP-9, contribute dramatically to the synthesis and deposition of ECM proteins in the cardiovascular system [12,58,66]. In our recent study [4,9,12,13,66], we demonstrated that ACE2 served as a protective agent against diabetes-induced cardiovascular complications. Loss of ACE2 led to greater activation of pro-MMP2, MMP2, MMP-9, MMP-12, MMP-13 and MMP-14 in the Akita/ACE2 double mutant mice, resulting in degradation of ECM (Table 1). While enhancement of ACE2 by AT1 receptor blockade rescued the cardiovascular remodeling and dysfunction and normalized the altered fibrosis-associated signaling pathways in cardiovascular system [4]. The anti-fibrosis effect of ACE2 appears promising for management of patients with hypertension, atherosclerosis, and aneurysm and so on. It will be of great significance to illuminate the crosstalk between the ACE2 and vascular fibrosis.

4 Conclusion and perspectives

The development of vascular remodeling is associated with multiple interactions of cell signaling. Activation of the tissue and systemic RAS and the generation of Ang II play a key role in vascular diseases. Consistent with increased Ang II action via AT1 receptor, Ang II-induced high levels of oxidative stress, inflammation and fibrosis come into being, which are the initial steps of vascular injury and then contribute to VSMC proliferation, vascular remodeling and dysfunction [3,15,53]. ACE2 is the first known homolog of

human ACE and functions as a pleiotropic monocarboxypeptidase responsible for the conversion of Ang II to Ang-(1–7). The ACE2/Ang-(1–7) signaling contends against the formation of Ang II and counterbalances the Ang II/AT1-mediated vascular proliferation, hypertrophy and remodeling, thereby functioning as a negative regulator of the RAS in cardiovascular system. Remarkable effects of ACE2 on attenuation of Ang II-induced VSMC proliferation, oxidative stress and inflammation in vasculature lead to the development of ACE2 as a potential novel medicine for treatment of cardiovascular disease. The beneficial effects of ACE2/Ang-(1–7) signaling were demonstrated in the clinically relevant model of pressure-overload- and Ang II-induced vascular remodeling and injury. The addition of the ACE2/Ang-(1–7) signaling to the complexities of the RAS may lead to the development of a novel therapeutic approach for patients with hypertension and other vascular diseases related to vascular remodeling. In future studies, a greater understanding of the processes involved in vascular proliferation, fibrosis and pathological remodeling, together with the mechanisms through which signaling pathways interact, will facilitate the exploitation of new therapeutic medicine to more efficiently control vascular remodeling.

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