Loss of Angiotensin-Converting Enzyme 2 Exacerbates Myocardial Injury via Activation of the CTGF-Fractalkine Signaling Pathway

Bei Song, BSc; Zhen-Zhou Zhang, BSc; Jiu-Chang Zhong, MD*; Xi-Yong Yu, MD, PhD; Gavin Y. Oudit, MD, PhD; Hai-Yan Jin, MD; Lin Lu, MD; Ying-Le Xu, BSc; Zamaneh Kassiri, PhD; Wei-Feng Shen, MD; Ping-Jin Gao, MD, PhD; Ding-Liang Zhu, MD, PhD

**Background:** Angiotensin-converting enzyme 2 (ACE2) has been implicated in human heart failure, but the mechanism remains elusive. We hypothesized that ACE2 deficiency would exacerbate angiotensin (Ang) II-mediated myocardial injury.

**Methods and Results:** 10-week-old ACE2 knockout (ACE2KO) and wild-type mice received by mini-osmotic pump either AngII (1.5 mg · kg⁻¹ · day⁻¹) or saline for 2 weeks. ACE2 deficiency triggered greater increases in the expression of connective tissue growth factor (CTGF), fractalkine (FKN) and phosphorylated ERK1/2 in AngII-treated ACE2KO hearts. These changes were associated with greater activation of matrix metalloproteinase (MMP) 2, MMP9 and MT1-MMP and exacerbation of myocardial injury and dysfunction. In cultured cardiofibroblasts, exposure to AngII (100 nmol/L) for 30 min resulted in marked increases in superoxide production and expression of CTGF, FKN and phosphorylated ERK1/2, which were strikingly prevented by recombinant human ACE2 (rhACE2; 1 mg/ml) and the CTGF-neutralizing antibody (5 μg/ml), but were aggravated by ACE2 inhibitor DX600 (0.5 μmol/L). These protective effects of rhACE2 were eradicated by the Ang-(1–7) antagonist A779 (1 μmol/L). More intriguingly, rhACE2 treatment significantly abolished AngII-mediated increases in MMP2, MMP9 and MT1-MMP in cardiofibroblasts.

**Conclusions:** Loss of ACE2 exacerbates AngII-mediated inflammation, myocardial injury and dysfunction in ACE2-deficient hearts via activation of the CTGF-FKN-ERK and MMP signaling. ACE2 gene may represent a potential candidate to prevent and treat myocardial injury and heart diseases. (*Circ J* 2013; 77: 2997–3006)

**Key Words:** Angiotensin-converting enzyme 2; Connective tissue growth factor; Fractalkine; Matrix metalloproteinase; Myocardial injury
of matrix metalloproteinase (MMP), an effect that may also drive pathological remodeling and cardiac injury.\(^5\) In mice with cardiac remodeling and injury, MMP-2, MMP-9 and membrane type 1 (MT1)-MMP, a cell surface activator of MMP-2, were overexpressed in the heart associated with activation of CTGF signaling.\(^5,9,11\) However, the roles of the CTGF-FKN and MMP signaling pathways involved in myocardial injury remain to be fully elucidated.

A new counterbalancing arm of the RAS is now known to exist, in which angiotensin-converting enzyme 2 (ACE2) functioning essentially as a negative regulator of the RAS.\(^2\) Grades AngII to the beneficial heptapeptide Ang-(1–7), thereby providing potential therapeutic opportunities to block harmful effects of AngII.\(^3,7,8,19\) A new counterbalancing arm of the RAS is now known to exist, in which angiotensin-converting enzyme 2 (ACE2) degrades AngII to the beneficial heptapeptide Ang-(1–7), thereby functioning essentially as a negative regulator of the RAS.\(^3,15,19\) In the heart, ACE2 is expressed in cardiomyocytes, fibroblasts, and endothelial cells.\(^3,8,11,18\) Our previous studies have demonstrated that ACE2 overexpression prevents AngII-mediated cardiovascular inflammation and injury associated with reduced MMP2 level.\(^3,8,11\) Suggesting a critical role of ACE2 in the regulation of cardiovascular injury and dysfunction. However, the exact roles and mechanisms of ACE2 in the cardiovascular system remain largely unknown. In this work, we assessed the hypothesis that loss of ACE2 would accelerate myocardial injury. We randomized ACE2 knockout mice (ACE2KO, Ace2\(^{−/−}\)) and wild-type littermates (WT, Ace2\(^{+/+}\)) to either AngII or saline infusion, as in a previous study.\(^3,20\)

### Methods

**Experimental Animals and Protocols**

10-week-old male WT and ACE2KO mice were randomized to either AngII (1.5 mg·kg\(^{-1}\)·day\(^{-1}\)) or saline (control) infusion with an osmotic minipump (model 1002, Alzet Corp, Palo Alto, CA, USA) for 2 weeks, as in previous studies.\(^3,20\) Systolic blood pressure (SBP) was measured noninvasively by the tail-cuff method with an IITC Blood Pressure Monitoring System (Life Science Inc, St. Petersburg, Fl, USA). Plasma AngII and Ang-(1–7) levels were measured by radioimmunoassay as described previously.\(^3,11\) All experiments were approved and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996), the Animal Research Ethics Committee at Shanghai Jiao Tong University School of Medicine and the Canadian Council on Animal Care.

**Echocardiography and Myocardial Ultrastructure Observation**

Transtheracic echocardiography was performed and analyzed in a blinded manner, as described previously, with a Vevo 770 high-resolution imaging system equipped with a 30-MHz transducer (RMV-707B; Visualsonics, Toronto, ON, Canada).\(^3,8\) For transmission electron microscopic analysis, samples of mouse heart tissues (left ventricles) were immediately cut into small pieces and immersed in 2.5% glutaraldehyde as described previously.\(^19\) The myocardial ultrastructure of the WT and ACE2KO mice was observed on a HITACHI-600 electron microscope (Hitachi, Japan).

**Culture of Cardiofibroblasts**

Adult murine LV cardiofibroblasts were isolated and cultured as described previously.\(^3\) The cardiofibroblasts were put into serum-free DMEM for 18 h prior to treatment. Recombinant human ACE2 (rhACE2; 1 mg/ml), the Ang-(1–7) antagonist, A779 (1 μmol/L; Sigma-Aldrich, St. Louis, MO, USA), ACE2 inhibitor DX600 (0.5 μmol/L), ERK1/2 inhibitor PD98059 (10 μmol/L), Ang-(1–7) (100 nmol/L), CTGF-neutralizing antibody (monoclonal anti-CTGF, 5 μg/ml; Sigma-Aldrich), and IgG (5 μg/ml) were added to the cardiofibroblasts for 30 min prior to 30-min exposure to AngII (100 nmol/L; Sigma-Aldrich). The treated cells further underwent dihydroethidium (DHE) staining or were collected for TaqMan real-time polymerase chain reaction (PCR) and Western blotting analyses.

**Gelatin Zymography and TaqMan Real-Time PCR Analysis**

Pro- and cleaved forms of MMP2 and MMP9 were detected by gelatin zymography analysis as described previously.\(^11\) The mRNA expression levels were evaluated by TaqMan real-time reverse transcription PCR as described before.\(^5,9,11\) Total RNA was extracted from heart tissues and cardiofibroblasts using TRIzol reagent. The primers and probes for α-smooth muscle actin (α-SMA), MCP-1, tumor necrosis factor-α (TNFα), MMP2, MMP9 and MT1-MMP are listed in Table S1.

**Western Blot Analysis**

The proteins from heart tissues or cardiofibroblasts were mea-
Loss of ACE2 Exacerbates Myocardial Injury

2999

Loss of ACE2 Exacerbates Myocardial Injury

20

Described previously. Data were calculated as the changes in the rate of luminescence per minute.

Statistical Analysis

Values are shown as mean ± SEM. All statistical analyses were performed with SPSS software (Version 11.5) either by Student’s t test for comparison between groups or by ANOVA followed by the Student-Newman-Keuls test for multiple-comparison testing as appropriate. A value of P<0.05 was considered statistically significant.

Results

Loss of ACE2 Facilitated AngII-Mediated Hypertension and Myocardial Inflammation With Greater Activation of the CTGF-FKN and ERK Signaling

We firstly evaluated the effects of ACE2 deficiency on the SBP and cardiac CTGF-FKN signaling. As shown in Table, compared with WT mice, SBP was obviously elevated in

Measurement of Superoxide Production and NADPH Oxidase Activity

Oxidative stress is generally identified by indirect markers of the oxidant injury, such as superoxide. To evaluate superoxide production in cardiofibroblasts, the oxidative fluorescent dye, DHE, was used as described previously. The NADPH oxidase activity in cardiofibroblasts was quantified by lucigenin-enhanced chemiluminescence assay using a Berthold FB12 luminometer (Berthold Technologies, Germany) at 37°C, as described previously. Data were calculated as the changes in the rate of luminescence per minute.

Figure 1. Cardiac expression of CTGF, FKN, phosphorylated ERK1/2 and inflammatory cytokines in mice. (A–C) Representative Western blot images of the cardiac expression of CTGF (A), FKN (B) and phosphorylated ERK1/2 (C) in wild-type (WT) and angiotensin-converting enzyme 2 (ACE2) knockout (KO) mice in response to angiotensin (AngII) (n=5 for each group). (D–F) TagMan real-time PCR analysis shows mRNA expression of inflammatory cytokines MCP-1 (D), TNFα (E) and α-SMA (F) in the heart of mice (n=6 for the Ace2+/y control group and Ace2−/y control group; n=8 for the Ace2+/y AngII group and Ace2−/y AngII group). α-tubulin or 18S rRNA was used as an endogenous control. *P<0.01 compared with corresponding control group; #P<0.05 compared with Ace2+/y AngII group; ØP<0.05 compared with Ace2+/y control group. R.E., relative expression; CTGF, connective tissue growth factor; FKN, fractalkine; ERK1/2, extracellular signal-regulated kinase 1/2; MCP-1, monocyte chemoattractant protein-1; TNF, tumor necrosis factor; SMA, smooth muscle actin.
ACE2 mutant mice associated with a marked increase in the plasma AngII level and a reduction in the plasma Ang-(1–7) level (n=6; P<0.05). Chronic AngII infusion resulted in a predicted pressor response in both WT and ACE2-deficient mice (n=6–8; P<0.01, respectively). Moreover, loss of ACE2 resulted in greater increases in SBP at 2 weeks after AngII infusion in ACE2KO mice than in WT mice (n=6–8; P<0.05, respectively), together with a greater elevation in the AngII level and lower Ang-(1–7) level (Table).

Activation of the RAS has also been linked to inflammation, another key determinant of adverse myocardial injury.11 We next investigated AngII-induced cardiac inflammation and the associated signaling cascades in ACE2-deficiency status. Compared with WT mice, there were marked increases in the expression of CTGF (Figure 1A), FKN (Figure 1B), phosphorylated ERK1/2 (Figure 1C) and MCP-1 (Figure 1D) in ACE2-deficient hearts (n=5–6; P<0.05, respectively). Western blotting and TaqMan real-time PCR analyses revealed that lack of ACE2 resulted in greater increases in AngII-induced expression of CTGF (Figure 1A), FKN (Figure 1B), phos-
Loss of ACE2 Exacerbates Myocardial Injury

<table>
<thead>
<tr>
<th>Ace2+/y Con (x7400)</th>
<th>Ace2+/y Ang II (x7400)</th>
<th>Ace2−/y Con (x7400)</th>
<th>Ace2−/y Ang II (x7400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image](Image66x535 to 520x723)</td>
<td>![Image](Image66x535 to 520x723)</td>
<td>![Image](Image66x535 to 520x723)</td>
<td>![Image](Image66x535 to 520x723)</td>
</tr>
</tbody>
</table>

**Figure 3.** Myocardial ultrastructural changes in wild-type (WT; Ace2+/+) and ACE2 knockout (KO; Ace2−/−) mice by transmission electron microscopy (A–D, ×7,400; E–H, ×17,500). Compared with the WT mice, myocardial ultrastructural injury was aggravated in ACE2KO mice in response to angiotensin (Ang) II, characterized by disruption or dissolution of myocardial myofilaments, myofilaments arranged irregularly and loosely (pink arrow), and vacuolar degeneration and swollen mitochondria (yellow star).

**Loss of ACE2 Exacerbated AngII-Mediated Cardiac Hypertrophy and Dysfunction**

The echocardiographic data revealed normal systolic function with no change in LV fractional shortening (FS) or ejection fraction (EF) between WT and ACE2KO mice (Table). There were obvious increases in LV posterior wall thickness (LVPWT) and the ratio of LV weight (LVW) and body weight (BW) in ACE2KO mice (Table; n=6; P<0.05, respectively). Notably, chronic stimulation by AngII resulted in greater concentric remodeling in the ACE2KO mice with greater increases in LVPWT, LVW and the LVW/BW ratio (Table), as well as mRNA expression of α-SMA (Figure 1E; n=6–8; P<0.05, respectively). Consistent with the exacerbation of AngII pathological hypertrophy in ACE2KO mice, AngII-induced cardiac dysfunction was greater in ACE2KO mice, based on the decreased LVFS and LVEF (Table; n=8; P<0.05 or P<0.01, respectively), indicating that loss of ACE2 facilitated AngII-mediated pathological hypertrophy and cardiac dysfunction in ACE2-null mice.

**Loss of ACE2 Exacerbated AngII-Induced Myocardial Ultrastructure Injury With Greater Activation of MMP Signaling**

TaqMan real-time PCR analysis revealed that loss of ACE2 resulted in greater increases in AngII-induced mRNA expression of MMP2 (Figure 2A), MMP9 (Figure 2B) and MT1-MMP (Figure 2C) in ACE2-deficient hearts (n=6–8; P<0.05, respectively). Gelatin zymography demonstrated markedly increased levels of MMP2 (cleaved- and pro-MMP2) and MMP9 (Figure 2D) in ACE2-deficient hearts in response to AngII (n=5–6; P<0.05, respectively). Increased levels of MMP2 and MMP9 were confirmed independently by Western blotting analysis (Figure 2E), which showed substantially greater increases in the ACE2-deficient hearts (n=5; P<0.05, respectively). Although we have not provided direct evidence for collagen degradation, these increases in MMP expression and activities provide some evidence for increased degradation and dissolution of myocardial myofilaments in the ACE2-deficient hearts as assessed by ultrastructure observation with transmission electron microscopic analysis (Figures 3D,H). Compared with WT mice (Figures 3A,E), more severe myocardial ultrastructural injury was observed in ACE2KO hearts, including disarranged myofilaments and mild swollen mitochondria (Figures 3C,G). Notably, in response to chronic stimulation by AngII, loss of ACE2 resulted in aggravated myocardial ultrastructural injury in ACE2-null mice (Figures 3D,H), characterized by disruption or dissolution of myocardial myofilaments and mitochondrial vacuolar degeneration with membrane lysis.

**Inhibition of ACE2 Facilitated AngII-Mediated Oxidative Stress and Inflammation in Cardiofibroblasts With Greater Activation of the CTGF-FKN-ERK Signaling**

Enhanced oxidative stress has been linked to activation of MMPs and degradation of key components of the ECM, thereby contributing to myocardial injury.21 In cultured cardiofibroblasts, exposure to AngII (100 nmol/L) resulted in significant increases in the expression of CTGF (Figures 4A,D), FKN (Figures 4B,E), phosphorylated ERK 1/2 (Figures 4C,F) and MCP-1 (Figure 6A) as well as superoxide production (Figure 5) and NADPH oxidase activity (Figure 5), which were largely aggravated by pharmacological inhibition of ACE2 with DX600 (0.5 μmol/L) (n=5–6; P<0.01 or P<0.05, respectively). More intriguingly, pretreatment with Ang-(1–7) (100 nmol/L), ERK1/2 inhibitor PD98059 (10 μmol/L) and CTGF-neutraliz-
Treatment With rhACE2 Suppressed AngII-Mediated Oxidative Stress, Inflammation and Activation of the CTGF-FKN-ERK and MMP Signaling in Cardiomyocytes

As shown in Figure 6, TaqMan real-time PCR revealed that stimulation with AngII (100 nmol/L) resulted in significant increases in the expression of MCP-1, TNFα, α-SMA, MMP2, MMP9, and MT1-MMP in cultured cardiomyocytes (n=5–6; P<0.01, respectively), which were strikingly prevented by treatment with rhACE2 (1 mg/ml) but were largely aggravated by ACE2 inhibitor DX600 (0.5 μmol/L) (n=5–6; P<0.05, respectively). Decreased levels of MMP2 and MMP9 after treatment with rhACE2 (1 mg/ml) were confirmed by Western blot analysis, which showed reduction in the AngII-mediated increased protein expressions of MMP2 and MMP9 in cardiomyocytes in response to rhACE2 (Figure 6G) (n=5–6; P<0.05, respectively). Intriguingly, pretreatment with rhACE2 (1 mg/ml) dramatically prevented AngII-induced increases in superoxide production, NADPH oxidase activity (Figure 5) and expression of CTGF (Figure 4D), FKN (Figure 4E) and phosphorylated ERK1/2 (Figure 4F) (n=5–6; P<0.05, respectively), indicating the detrimental effect of ACE2 inhibition on AngII-mediated oxidative stress via the CTGF-FKN-ERK signaling.
Loss of ACE2 Exacerbates Myocardial Injury

22, 23 CTGF has been shown to enhance the level of phosphorylated ERK1/2 and promote the expression of the inflammatory chemokine, FKN (also called CX3CL1), which is a newly identified membrane-bound chemokine that plays a vital role in the transition from compensated ventricular remodeling hypertrophy to heart failure.

Moreover, FKN augments myocardial injury and accelerates the progress of heart failure with activation of MAPK/ERK signaling through the G protein-coupled chemokine receptor CX3CR1. 24 The activation of MAPK pathways plays a key role in the progression to cardiac hypertrophy and heart failure. 3 Intriguingly, implications implied regulatory roles of ACE2 in oxidative stress, and the CTGF-FKN-ERK and MMP signaling pathways.

Discussion

The major findings in our study are the regulatory roles of ACE2 in the CTGF-FKN-ERK signaling pathway in mice with myocardial hypertrophy and cardiac injury. It is well established that CTGF is a matricellular protein that regulates diverse cellular processes such as ECM deposition, cell migration, survival and proliferation, leading to cardiac remodeling and structural injury. 22,23 CTGF has been shown to enhance the level of phosphorylated ERK1/2 and promote the expression of the inflammatory chemokine, FKN (also called CX3CL1), which is a newly identified membrane-bound chemokine that plays a vital role in the transition from compensated ventricular remodeling hypertrophy to heart failure. 10 Moreover, FKN augments myocardial injury and accelerates the progress of heart failure with activation of MAPK/ERK signaling through the G protein-coupled chemokine receptor CX3CR1. 24 The activation of MAPK pathways plays a key role in the progression to cardiac hypertrophy and heart failure. 3 Intriguingly,
Figure 6. Effects of rhACE2 and DX600 on inflammation and matrix metalloproteinase (MMP) activation in cardiofibroblasts. Taq-Man real-time PCR (A–F) and representative Western blot (G) show the expression of MCP-1 (A), TNFα (B), α-SMA (C), membrane type 1 (MT1)-MMP (D), MMP2 (E,G) and MMP9 (F,G) in cardiofibroblasts treated with angiotensin II (AngII: 100nmol/L) in the absence and presence of rhACE2 (1mg/ml) and the ACE2 inhibitor DX600 (0.5μmol/L) (n=5 for control group; n=6 for all other groups). **P<0.01 compared with control group; #P<0.05 compared with AngII group. R.E., relative expression; rhACE2, recombinant human angiotensin-converting enzyme 2; SMA, smooth muscle actin; TNF, tumor necrosis factor.

activation of endogenous ACE2 by treatment with the ACE2 agonist, XNT, or rhACE2 has been shown to improve cardiac hypertrophy and dysfunction with downregulation of MAPK/ERK signaling and upregulation of the ACE2/ACE ratio.3,18 In this work, we demonstrated that in AngII-induced hypertensive mice with myocardi hypertrophy and ultrastructural deterioration, the levels of CTGF and FKN were upregulated in the heart, with marked increases in phosphorylated ERK1/2 and expression of MCP-1. Our data confirm the activation of the myocardial CTGF-FKN-ERK signaling pathway in Ace2–/– mutants. This is in agreement with previous reports demonstrating that activation of the CTGF signaling plays an important role in the development of cardiac hypertrophy and myocardial injury with enhanced expression of FKN and phosphorylated ERK1/2.10,23 More importantly, absence of ACE2 triggers greater increases in cardiac CTGF-FKN and ERK phosphorylation signaling in ACE2-null mice in response to chronic AngII infusion. These changes were associated with exacerbation of AngII-induced myocardial hypertrophy and ultrastructural injury, characterized by disruption or dissolution of myocardial myofibrils and mitochondrial vacuolar degeneration. Consistent with exacerbation of AngII pathological hypertrophy and ultrastructural injury, AngII-induced cardiac dysfunction was greater in ACE2KO mice than
in WT mice, on the basis of decreased LVFS and LVEF by echocardiographic analysis, likely driven by the greater elevation in AngII level and lower Ang-(1–7) level in ACE2KO mice. These findings indicated that loss of ACE2 exacerbates AngII-mediated inflammation, myocardial hypertrophy and ultrastructural injury associated with greater activation of the CTGF-FKN-ERK signaling pathway.

The reduction in plasma Ang-(1–7) level coupled with the increase in plasma AngII level in response to ACE2 deficiency is likely a key mechanism of pathological hypertrophy and cardiac dysfunction. As a critical enzyme in the metabolism of AngII, ACE2 serves to directly balance the levels of AngII and Ang-(1–7), turning the balance within the RAS cascade from pro-inflammatory, pro-oxidative and pro-hypertrophic to anti-inflammatory, anti-oxidative, and anti-hypertrophic actions.\textsuperscript{7,16,25–28} AngII/AT1 receptor signaling is critically involved in activation of CTGF signaling, inflammatory chemokines and cytokines, which may in turn trigger oxidative stress, cardiovascular remodeling, restructuring and myocardial injury.\textsuperscript{12,26,27} The importance of Ang-(1–7) has been highlighted in recent studies showing that Ang-(1–7) can suppress AngII-mediated myocardial hypertrophy and remodeling independent of blood pressure.\textsuperscript{3,16,29–31} Genetic deletion of the Ang-(1–7) receptor, Mas, leads to a marked impairment of cardiac function with higher levels of phosphorylated ERK1/2 in the hearts of adult Mas\textsuperscript{−/−} mice.\textsuperscript{31} In the present study, we showed that ACE2 deficiency is linked with higher levels of CTGF, FKN and phosphorylated ERK1/2, as well as augmentation of inflammation and cardiac hypertrophy, characterized by marked increases in the LVW/BW ratio and LVPWT. The pivotal role of ACE2 as a negative regulator of AngII-mediated signaling in inflammation and myocardial injury further confirms that ACE2 inhibition could facilitate the adverse effects of AngII.\textsuperscript{1,3,6,18,31,32} In our present study, the in vitro cultured cardiofibroblast data clearly showed a direct role of ACE2/Ang-(1–7) signaling in modulating the pathological effects of AngII. To evaluate a more direct effect of ACE2, we examined the effect of rhACE2 and ACE2 inhibitor, DX600, on AngII effects in cardiofibroblasts. Exposure of these cells to AngII resulted in significant increases in superoxide production, NADPH oxidase activity and expression of CTGF, FKN and phosphorylated ERK1/2, which were strikingly prevented by administration of rhACE2 but were largely aggravated by DX600. In addition, pretreatment with the CTGF-neutralizing antibody, ERK inhibitor PD98059, and Ang-(1–7) significantly abrogated AngII-induced upregulation of superoxide production, NADPH oxidase activity and the expression of CTGF, FKN and phosphorylated ERK1/2 in cardiofibroblasts, indicating the protective effect of ACE2 against AngII-mediated myocardial inflammation and oxidant injury through modulation of the CTGF-FKN-ERK signaling pathways. More importantly, the cellular protective effects of rhACE2 were completely suppressed in cardiofibroblasts by the Ang-(1–7) antagonist, A779, suggesting the beneficial effect of rhACE2 on the pathological actions of AngII via Ang-(1–7) signaling.

Evidence is emerging that members of the MMP family can serve as potential markers of pathological remodeling and myocardial injury.\textsuperscript{1,5,31,33} Based on their metalloproteinase function and substrate specificity, MMPs have been shown to be involved in normal developmental processes such as cardiac development, and when dysregulated, their activities can lead to a variety of cardiovascular processes, including myocardial hypertrophy and structural injury.\textsuperscript{5,11,34} Overexpression of MMPs in the cardiovascular system results in increased levels of MMP activity and subsequent increases in the synthesis and deposition of ECM proteins. Particularly, MMP-2 and MMP-9 contribute to the degradation and reorganization of ECM components and are involved in the pathophysiology of cardiovascular remodeling and injury.\textsuperscript{1,5,31,33} In our previous work,\textsuperscript{11} we showed that in ACE2 deficient-myocardial infarction mice, loss of ACE2 leads to increased MMP2 and MMP9 levels with MMP2 activation, as well as increased neutrophilic infiltration in the infarct and peri-infarct regions, ultimately resulting in a disrupted ECM and ventricular dysfunction. In this study, we demonstrated that loss of ACE2 augmented AngII-induced CTGF expression and myocardial ultrastructural injury in ACE2-deficient hearts, with greater activation of MMP2, MMP9 and MT1-MMP. This is in agreement with other reports demonstrating that enhanced CTGF is associated with increased MMP activation in mice with cardiac hypertrophy and myocardial injury.\textsuperscript{1,5} AngII-mediated oxidative stress is known to activate MMPs, leading to degradation of ECM proteins and cardiac injury.\textsuperscript{5,21,33} In our in vitro study, the downregulation of AngII-induced oxidative stress by rhACE2 was responsible for inhibition of MMP activation in cardiofibroblasts, including MMP2, MMP9 and MT1-MMP. Conversely, pharmacological inhibition of ACE2 by DX600 largely aggravated AngII-induced enhancement of superoxide production and NADPH oxidase activity in cardiofibroblasts associated with MMP activation. Taken together, our data demonstrate that loss of ACE2 leads to greater increases in AngII-mediated inflammation and activation of the CTGF-FKN-ERK and MMP signaling, ultimately contributing to adverse myocardial injury.

In conclusion, we provide the first evidence that loss of ACE2 exacerbates AngII-mediated inflammation, myocardial injury and cardiac dysfunction associated with greater activation of the CTGF-FKN-ERK and MMP signaling. ACE2 exerts protective effects on AngII-induced cellular oxidative stress and inflammation with the suppression of the CTGF-FKN-ERK and MMP signaling pathways in cardiofibroblasts. These results indicate a critical role of ACE2 in the prevention of myocardial inflammation and ultrastructural deterioration and support the notion that ACE2/Ang-(1–7) signaling is a negative regulator of the RAS. Targeting ACE2 has potential therapeutic importance for modulating myocardial injury and heart failure. Future investigations are required to more precisely clarify the role of ACE2 as a biomarker of myocardial injury and cardiovascular disease and assess whether measurement of ACE2 will improve heart disease risk prediction.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No.s 81170246, 81370362, 3097522 & 81070103), the Shanghai Pujiang Talents Program of Shanghai Science and Technology Committee (11PJ1408300), the Canadian Institute for Health Research (86602 & 84279) and Scientific Research Project of Health Bureau of Shanghai (2011347). Dr Zhong is one of the SMC Morningstar Distinguished Young Scholars of Shanghai Jiao Tong University in China and a Scholar of Alberta Innovates-Health Solution in Canada. We gratefully acknowledge technical assistance from the University of Alberta.

Disclosures

The authors have no disclosures.

References


15. Iwai M, Nakaoka H, Senba I, Kanno H, Moritani T, Horiuchi M. Possible involvement of angiotensin-converting enzyme 2 and Mas activation in inhibitory effects of angiotensin II Type 1 receptor blockade on vascular remodeling. Hypertension 2012; 60: 137 – 144.


**Supplementary Files**

**Methods**

**Table S1.** Primer and probe sequences for TaqMan real-time PCR analysis*

Please find supplementary file(s); http://dx.doi.org/10.1253/circj.CJ-13-08050