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Research Article

ACE2 deficiency induced perivascular fibrosis and cardiac hypertrophy during postnatal development in mice

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Abstract

In order to investigate the role of angiotensin-converting enzyme 2 (ACE2) in cardiac development, we examined the effects of ACE2 deficiency on postnatal development of the heart using ACE2-knockout (ACE2KO) mice. Heart samples of wild type (WT; C57BL/6J) mice and ACE2KO mice were taken at 1, 4, and 10 weeks of age. In WT mice, expression of ACE2 mRNA increased from 1 week to 10 weeks. A similar increase was observed in immunostaining of ACE2 in the heart, in which ACE2 was strongly expressed in coronary arteries. Compared with WT mice, heart weight was greater in ACE2KO mice at 4 weeks, and coronary artery thickening and perivascular fibrosis were also already enhanced from 4 weeks. Consistent with the increase of fibrosis, cardiac expression of collagen and TIMP was higher, and expression of MMP was lower in ACE2KO mice at 4 weeks. In addition, TGF-β mRNA was also higher, and lower expression of PPARγ mRNA was observed at 4 weeks in ACE2KO mice. These results suggest that ACE2 plays an important role in postnatal development of the heart, and that lack of ACE2 enhances coronary artery remodeling with an increase in perivascular fibrosis and cardiac hypertrophy already around the weaning period. J Am Soc Hypertens 2013;7(4):259–266.

Keywords: ACE2; development; fibrosis; cardiac hypertrophy.

Introduction

The renin-angiotensin system (RAS) plays a critical role in the pathogenesis of cardiovascular diseases. Recent studies have established a new regulatory axis in RAS, in which angiotensin (Ang)-(1-7) is finally produced from Ang I or Ang II by the catalytic activity of angiotensin-converting enzyme 2 (ACE2). The discovery that Ang-(1-7) opposes the pressor, proliferative, profibrotic, and prothrombotic actions mediated by Ang II via the Ang type 1 (AT_1) receptor has contributed to the realization that the RAS is composed of two opposing arms.[1,2] ACE2 activity regulates the ACE2/Ang-(1-7)/Mas axis and appears to play an important role in cardioprotection.[3]

In previous studies, it was shown that targeted disruption of ACE2 in mice results in a severe cardiac contractility defect, increased Ang II level, and upregulation of hypoxia-induced genes in the heart, suggesting that ACE2 is an essential regulator of cardiac function in vivo.[4] Oudit et al reported that the age-dependent cardiomyopathy in ACE2-null mice is related to increased Ang II-mediated oxidative stress and neutrophilic infiltration via the AT_1 receptors.[5] Treatment of cardiomyocytes with Ang-(1-7) prevented Ang II-induced hypertrophy by modulating calcineurin/nuclear factor of the activated T-cell signaling cascade.[6] In addition, Mas-deficient mice showed impaired left ventricle (LV) function and increased

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LV end-diastolic dimension, with significantly higher coronary vessel resistance.[7]

These results suggest that ACE2 may play an important role in cardiac remodeling in adult mice. The effect of ACE2 deficiency on postnatal development of the heart during the weaning period has not yet been fully clarified. In the present study, we examined the possibility that ACE2 might be involved in the regulation of cardiac postnatal development. To assess this hypothesis, we used ACE2-null mice at 1, 4, and 10 weeks of age and examined cardiac hypertrophy, coronary artery thickness, and perivascular fibrosis, focusing on a variety of possible factors involved in cardiovascular postnatal development.

Methods

Animals

ACE2-deficient (ACE2KO) mice (C57BL/6J background, provided by Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan)[8] at 1, 4, and 10 weeks of age were used. Wild-type (WT; C57BL/6J) mice were used as control. The mice were housed in a room where lighting was controlled (12 hours on, 12 hours off) and room temperature was kept at 24°C. They were given free access to standard laboratory chow (CE2 rodent diet, CLEA Japan, Inc., Tokyo, Japan) and water. An angiotensin II type 1 receptor blocker (ARB), olmesartan (provided by Daiichisankyo Co, Ltd, Tokyo, Japan), was orally administered at a dose of 1.0 mg/kg per day to the mice of 4 weeks of age for 6 weeks.[9] Blood pressure was measured at 10 weeks of age by the indirect tail-cuff method with a blood pressure monitor (BP-98A-L; Softron, Tokyo, Japan), with mice kept in a temperature-controlled holder at 37°C, as previously described.[10] In this study, blood pressure at 1 and 4 weeks of age could not be determined since the size of animals was too small to determine blood pressure by tail-cuff method. The Animal Studies Committee of Ehime University approved the experimental protocol.

Quantitative Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

Heart samples were obtained after sacrifice of mice under deep anesthesia. Total RNA was extracted with Sepasol-RNA I Super G (Nacalai Tesque, Kyoto, Japan) according to the manufacturer's protocol. Total RNA (2 μg) was converted into cDNA with a SuperScript VILO cDNA Synthesis Kit (Life Technologies Japan, Tokyo, Japan). Real-time RT-PCR was performed using a SYBR Premix Ex Taq Kit (Takara Bio Inc., Shiga, Japan) and Thermal Cycler Dice Real Time System TP800 (Takara Bio Inc., Shiga, Japan). Amplification was carried out with an initial denaturation step of 30 seconds at 95°C, followed by 40 cycles consisting of 5 seconds at 95°C for denaturation and 30 seconds at 60°C for annealing. The level of target gene expression was calculated using the second derivative method and normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression in each sample. The sequence of the RT-PCR primers used in this study is listed in the Table.

Morphometric Analysis

Heart samples were fixed in formalin and embedded in paraffin. The middle segment of the heart was cut into cross sections. The sections were stained with hematoxylin-eosin for measurement of left ventricular wall thickness, and van

Table
PCR primers for quantitative RT-PCR assay

Target	Forward (5′ to 3′)	Reverse (5′ to 3′)
ACE2	TGTGTCTGATGTCATTCCTAGAAGTG	AGGCTGGTAAGGTGGCTCAAG
Mas	GTCATCATCTTCATAGCCATCCTCAG	GTTCTTCCGTATCTTCACCACCAA
Collagen I	ATCTCCTGGTGCTGAGGAC	ACCTTGTTTGCCAGGTTCAC
Collagen III	AGGCAACAGTGGTTCTCCTG	GACCTCGTGCTCCAGTTAGC
MMP9	TGAACAAGGTGGACCATGAGGTGA	TAGAGACTTGCACTGCACGGTTGA
MMP13	TGGCTTAGAGGTGACTGGCAAACT	TATTCACCCACATCAGGCACTCCA
TIMP1	TCCCTTGCAAACTGGAGAGTGACA	AAGCAAAGTGACGGTTCTGGTAGT
TIMP2	TTTCTAGCCACACCAGGCAGATGA	GGTTTGCTGGGAAGGCATTTGAGT
TGF-β	CCTGGATACCAACTATTGCTTCAGC	TCCAAATATAGGGGCAGGGTCC
EGF	GGGAGTCTGCCTGCGGATG	CTTTGCCCTGTGCCCATTCC
HGF	GTCAGCACCATCAAGGCAAGG	ACCAGGAACAATGACACCAAGAAC
VEGF	CAGATCATGCGGATCAAACCTCA	TTCTGTCTTTCTTTGGTCTGCATTC
IGF-1	TGGACCAGAGACCCTTTGCG	AGGTGCCCTCCGAATGCTG
PPARγ	TGGAGACCGCCCAGGCTTG	GTCTGTCATCTTCTGGAGCACCTT
GAPDH	TGCGACTTCAACAGCAACTC	ATGTAGGCCATGAGGTCCAC

ACE, angiotensin-converting enzyme; EGF, epidermal growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; MMP, matrix metalloproteinase; PPAR, peroxisome proliferator-activated receptor; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; VEGF, vascular endothelial growth factor.