Angiotensin-Converting Enzyme 2 Priming Enhances the Function of Endothelial Progenitor Cells and Their Therapeutic Efficacy

Ji Chen, Xiang Xiao, Shuzhen Chen, Cheng Zhang, Jianying Chen, Dan Yi, Vinayak Shenoy, Mohan K. Raizada, Bin Zhao, Yanfang Chen

Abstract—Angiotensin-converting enzyme 2 (ACE2) is a newly discovered enzyme catalyzing Angiotensin II into Angiotensin 1-7. Angiotensin II has been reported to impair endothelial progenitor cell (EPC) function and is detrimental to stroke. Here, we studied the role of ACE2 in regulating EPC function in vitro and in vivo. EPCs were cultured from human renin and angiotensinogen transgenic (R+A+) mice and their controls (R−A−). In vitro experiments, EPCs were transduced with lentivirus-ACE2 or lentivirus-green fluorescence protein. The effects of ACE2 overexpression on EPC function and endothelial NO synthase (eNOS)/nicotinamide adenine dinucleotide phosphate oxidase (Nox) expression were determined. ACE2, eNOS, and Nox inhibitors were used for pathway validation. In in vivo studies, the therapeutic efficacy of EPCs overexpressing ACE2 was determined at day 7 after ischemic stroke induced by middle cerebral artery occlusion. We found that (1) lentivirus-ACE2 transduction resulted in a 4-fold increase of ACE2 expression in EPCs. This was accompanied with an increase in eNOS expression and NO production, and a decrease in Nox2 and -4 expression and reactive oxygen species production. (2) ACE2 overexpression improved the abilities of EPC migration and tube formation, which were impaired in R+A+ mice. These effects were inhibited by ACE2 or eNOS inhibitor and further enhanced by Nox inhibitor. (3) Transfusion of lentivirus-ACE2–primed EPCs reduced cerebral infarct volume and neurological deficits, and increased cerebral microvascular density and angiogenesis. Our data demonstrate that ACE2 improves EPC function, via regulating eNOS and Nox pathways, and enhances the efficacy of EPC-based therapy for ischemic stroke. (Hypertension. 2013;61:681-689.)

Key Words: angiogenesis ■ angiotensin-converting enzyme 2 ■ cell therapy ■ endothelial progenitor cells ■ ischemic stroke

Angiotensin-converting enzyme 2 (ACE2) is a newly discovered enzyme of the renin–angiotensin system, which catalyzes the conversion of angiotensin I (Ang I) into Ang 1-9 and Ang II into Ang 1-7. Accumulating evidence demonstrates that ACE2 provides vascular protective effects by counteracting the deleterious effects of Ang II and possess great potential for developing new avenues to treat vascular diseases and arterial hypertension. Endothelial progenitor cells (EPCs), which are defined as bone marrow (BM)-derived immature cells with the ability to differentiate into mature ECs, are suggested to play important roles in vascular homeostasis and angiogenesis. Reduced number and impaired function of circulating EPCs are associated with poor cardiovascular outcomes in patients with coronary artery disease. Ang II induces EPC senescence via activation of nicotinamide adenine dinucleotide phosphate oxidase. Our previous study shows that the BM-derived EPCs are reduced and dysfunctional in human renin and angiotensinogen transgenic (R+A+) mice, and that blockade of Ang II/type 1 angiotensin receptor signaling with losartan is able to improve these defects. Endothelial NO synthase (eNOS) downregulation and decreased NO generation can also induce impairment of EPC function and senescence. Although the role of ACE2 in counteracting the effects of Ang II has been well detailed, the role of ACE2 in EPC function and the specific mechanism have not been explored.
In this study, we determined the role of ACE2 in regulating EPC function. We analyzed the eNOS/NO and Nox/ROS pathways to explore the underlying mechanisms. To further investigate the possible implication of ACE2 overexpression in EPCs, we evaluated the therapeutic efficiency of EPCs overexpressing ACE2 on experimental ischemic stroke.

**Methods and Materials**

**Culture and Characterization of Early Outgrowth EPCs**

BM-derived EPCs were generated from R+/A+ mice (R+/A+ EPCs) and their controls (R−A--; R−A− EPCs) and characterized as we reported previously.14 After 7 days of culture, cells double-positive for Di-acLDL (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine [Dil]-labeled acetylated low-density lipoprotein), and Bs-Lectin staining were considered as early outgrowth EPCs.14 In addition, EPCs were stained with phycoerythrin (PE)-conjugated cluster of differentiation (CD)31 (10 μL; BD Bioscience, San Jose, CA), PE-conjugated CD34 (10 μL; AbD Serotec), fluorescein isothiocyanate (FITC)-conjugated vascular endothelial-Cadherin (1 μL; ebioscience), PE-Cy7-conjugated vascular endothelial growth factor receptor-2 (5 μL; BD Bioscience), PE-conjugated von Willebrand Factor (10 μL; BD Bioscience), FITC-conjugated CD133 (5 μL; BD Bioscience), FITC-conjugated CD45 (1 μL; ebioscience) antibodies. The phenotype of EPCs was analyzed by flow cytometry (Accuri C6 flow cytometer) as we described before.13

**EPCs Transduction With Lentivirus-ACE2 and Pathway Blocking Studies**

The lentivirus containing murine ACE2 cDNA (Lenti-ACE2) and lentivirus containing green fluorescence protein (Lenti-GFP) were produced as previously described.15 EPCs were transduced with Lenti-ACE2 (Lenti-ACE2–EPCs) or Lenti-GFP (Lenti-GFP–EPCs) as previously reported.14 In brief, after cultivation for 7 days, EPCs were cultured in 6-well plates (1×10⁴ cells/well) and incubated with serum-free EPC culture medium containing the lentivirus (at 5×10⁶ cells/mL) in dark for 24 hours. Transduction efficiency (the percentage of GFP-expressing cells) was quantitated by direct counting using an optical grid.

For blocking experiments, after transduction with Lenti-GFP or Lenti-ACE2, EPCs were incubated with ACE2 inhibitor (DX600, 1 μmol/L), eNOS inhibitor (l-NAME, 1 mmol/L), or Nox inhibitor (apocynin, 10 μmol/L) for 24 hours. Then, the EPCs were harvested for the analyses of function and gene expression.

**EPC Proliferate Assay**

The EPC proliferation was performed according to the manufacturer’s protocol (Cell proliferation enzyme-linked immunosorbent assay, bro-moedoxyuridine [BrdU; colorimetric], Roche) as previously reported.17 Briefly, EPCs were seeded on 96-well plates (1×10⁴ cells/well). The cells were grown in complete culture medium, which was changed after 12 hours with complete media supplemented with 10 μg/mL BrdU. BrdU incorporation was measured after 24 hours using a microtiter-plate reader (Packard) at 450 nm with a reference wavelength of 690 nm. BrdU uptake was calculated as the percentage of the untransduced EPCs.

**Real-Time Reverse Transcriptase Polymerase Chain Reaction**

The levels of ACE2 in EPCs transduced with Lenti-ACE2 were determined using real-time reverse transcriptase polymerase chain reaction method.18 EPC total mRNAs were isolated using RNeasy Mini kit and reverse-transcribed with the high capacity cDNA archive kit (Qiagen). The real-time reverse transcriptase polymerase chain reaction was run using SYBR Green reagents (Qiagen). The primer sequences for ACE2 were 5′-AAAGCTGACATAAACTGCGCTCCCTGTGGCTCCTTC-3′ and 5′-AAGTCGACCATAAAAGGAAGTCTGAGCATCATACTG-3′. β-actin was chosen as the housekeeping gene for normalizing the data of gene expression. The mRNA level of ACE2 in EPCs from R−A− mice transduced with Lenti-GFP was defined as 100%.

**EPC Function Assays**

The migration and tube formation abilities of EPCs were evaluated by using Boyden chamber (Chemicon) and tube formation assay kit (Chemicon) methods as we previously described.15

**Measurement of ROS Generation**

Intracellular ROS generation in EPCs was determined by dihydroethidium staining as previously described.20 Cells were incubated with dihydroethidium (2 μmol/L) in dark for 30 minutes. After washing with phosphate buffered saline, cells were then lysed with 50 μL lysis buffer on ice. The lysates were transferred into black 96-well plates for fluorescence measurement using a spectrofluorometer.

**Determination of NO Production**

The membrane-permeable indicator dianisofluorescein diacetate (Invitrogen, Grand Island, NY) was used to assess NO production released by EPCs.20 Briefly, the EPCs were loaded with 2 μmol/L dianisofluorescein diacetate in serum-free endothelial cell basal medium-2 (37°C for 30 minutes), washed twice with phosphate buffered saline, and incubated with dianisofluorescein diacetate-free in endothelial cell basal medium-2 (20 minutes) for deestification of the indicator. Dianisofluorescein fluorescence was measured using a spectrofluorometer.

**Animals and Procedures**

**Animals**

Male adult (8–10 weeks of age; weight ranges from 25–32 g) R+/A+ mice and their age-matched controls (R−A--) with C57BL/6J genetic background were used for all experiments. The strains were maintained in our laboratory (founders were from Dr Curt D. Sigmund’s laboratory at the University of Iowa).21,22 Mice were maintained in a 22°C room with a 12-hour light/dark cycle and fed with standard chow and drinking water ad libitum. All experimental procedures were approved by the Wright State University Laboratory Animal Care and Use Committee and were in accordance with the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health.

**Middle Cerebral Artery Occlusion Surgery and EPC Transfusion**

Focal ischemic stroke was induced in animals by middle cerebral artery occlusion (MCAO) surgery under anesthesia by inhaling 2.5% isofluran as we reported previously.11 Two hours after MCAO, mice were injected via the tail vein with Lenti-GFP–EPCs or Lenti-ACE2–EPCs (2×10⁶ cells/100 μL in phosphate buffered saline) or the same volume of phosphate buffered saline as previously described.15,23 EPCs were donated from R−A− mice. Pain and discomfort were minimized by an initial injection of Buprenorphine (0.1 mg/kg, SC) followed with another 2 injections every 12 hours.

**BrdU Labeling**

To label the new generated cells, mice were injected with BrdU (IP, 65 μg/g per day) immediately after EPC infusion for 7 continuous days.24

**Functional Evaluation of Neurological Deficits**

On day 7, the neurological deficit score of each mouse was evaluated by using the 5-point scale method as previously described.22

**Measurements of Cerebral Infarct Volume and Microvascular Density**

On day 7 after EPC transfusion, the brains were immediately collected and fixed in 4% paraformaldehyde overnight and in 4% paraformaldehyde plus 30% sucrose for 3 days. The brains were then cut into coronal sections (20 μm). As we previously described,21,22 cerebral
ischemic damage and cerebral microvascular density (cMVD) in the peri-infarct area were revealed by Fluoro-Jade (0.001%; Histo-chem) and CD31 (1:50; Invitrogen) staining, respectively.

**Immunofluorescence Analysis**

Brain coronal sections were incubated with BrdU (1:50; Abcam), CD31 (1:50; BD Biosciences), or GFP (1:50; Santa Cruz) antibody overnight at 4°C. Next, brain sections were reacted with Cy5 (blue, for BrdU), Cy3 (red, CD31), or FITC (green, for GFP) conjugated secondary antibodies (1:250; Invitrogen) for 30 minutes at room temperature in the dark. The positive cells in the peri-infarct area of each section were visualized using confocal microscopy (Leica TCS SP2). Angiogenesis was determined as BrdU+CD31+ cells according to previous reports.24,25 In vivo tracking of transfused EPCs were recognized as GFP+ cells. Cell counting was performed from photographs in 6 random microscopic fields (200×) by an investigator who was unaware of grouping. The average of 5 sections from rostral to caudal represented the data for sample.

**Western Blot Analysis**

Proteins from EPCs were isolated with lysis buffer (Roche Diagnostic). The antibodies used were anti-ACE2 (1:1000; Cell Signaling Technology), eNOS (1:1000; Abcam), Nox2 (1:1000; Abcam), or Nox4 (1:250; Abcam) at 4°C overnight. β-actin (1:4000; Sigma) was used to normalize protein loading.

**Statistical Analysis**

All data, excepting neurological deficit scores, are presented as mean±SE. The neurological deficit scores were expressed as median (range). The neurological deficit scores among different groups were compared by the Kruskal–Wallis test. When the Kruskal–Wallis test showed a significant difference, the Mann–Whitney test was applied. For the rest of the measurements, comparisons for 2 groups were performed by the Student t test. Multiple comparisons were analyzed by 1- or 2-way ANOVA. For all tests, a P<0.05 was considered significant.

**Results**

**Characterization of EPCs**

As we reported previously, BM-derived EPCs were defined as the cells uptaking Di-LDL and binding with Bs-Lectin,13 as well as expressing specific surface markers. The EPCs showed positive to CD34, vascular endothelial growth factor receptor-2, and CD133 and negative to CD31, vascular endothelial Cadherin, von Willebrand Factor, CD45, and CD146 (Figure 1). There were no difference regarding cell markers expressed on the EPCs from the R−A− and R+A+ mice.

**The Effects of Lentivirus Transduction on EPC Proliferation**

The transduction efficiency was ≈94±2% (Figure 2A). Lentiviral transduction decreased EPC proliferation rate by 15%. The proliferation rate of Lenti-ACE2–EPCs was increased when compared with Lenti-GFP–EPCs (by 35%) or untransduced EPCs (by 20%; Figure 2B).

**Lenti-ACE2 Transduction Increases ACE2 Expression in EPCs**

The basal levels of ACE2 mRNA and ACE2 protein expression were not different in the EPCs derived from R−A− and R+A+ mice. Lenti-ACE2 transduction induced a 4-fold upregulation of ACE2 in EPCs at both mRNA (P<0.01; Figure 2D) and protein levels (P<0.01; Figure 2C and 2E).

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![Figure 1](http://hyper.ahajournals.org/) The phenotype of cultured endothelial progenitor cell (EPCs). Representative flow plot showing the expression of CD34, vascular endothelial growth factor receptor-2, CD133, CD31, vascular endothelial (VE)-Cadherin, von Willebrand Factor (vWF), CD45, and CD146 on the EPCs cultured from R−A− (A) and R+A+ mice (B).
Transduction of EPCs With Lenti-ACE2 Increases eNOS Expression and Decreases Nox2, 4 Expressions

At basal, the level of eNOS expression was lower (0.24±0.02 versus 0.18±0.01; P<0.05; Figure 3A), whereas levels of Nox2 (0.36±0.03 versus 0.48±0.03; P<0.05; Figure 3B) and Nox4 (0.34±0.02 versus 0.46±0.03; P<0.01; Figure 3C) were higher in the EPCs generated from R+A+ mice. Transduction of Lenti-ACE2 increased eNOS, and decreased Nox2 and Nox4 expressions in EPCs from both R+A+ (by 61%, 27%, and 28%, respectively) and R−A− (by 62%, 44%, and 50%, respectively) mice (P<0.01). In addition, DX600 (ACE2 inhibitor) was able to partially block those effects of Lenti-ACE2 on EPCs (P<0.05 or 0.01; Figure 3).

Transduction of EPCs With Lenti-ACE2 Increases NO and Decreases ROS Production

The NO production was higher (versus P<0.05; Figure 4A), whereas the ROS production was lower (P<0.05; Figure 4B) in the EPCs generated from R+A+ mice. Lenti-ACE2 transduction increased 25% NO production, and decreased 48% ROS production in EPCs derived from R−A− mice, and induced 32% increase of NO and 28% decrease of ROS production from R+A+ mice (P<0.01). Again, DX600 partially blocked those effects of Lenti-ACE2 on EPCs (P<0.05). In addition, apocynin (Nox inhibitor) decreased the ROS production, whereas l-NAME (eNOS inhibitor) blocked the NO production in Lenti-ACE2–primed EPCs (P<0.05; Figure 4).
Infusion of Lenti-ACE2–EPCs Enhances the Efficacy in Decreasing Ischemic Injury and Neurological Deficit Score

The infarct volume of MCAO–induced stroke was exaggerated in R+A+ mice (P<0.05; Figure 6A and 6B). Lenti-GFP–EPC transfection decreased infarct volume in both R−A− (by 33%; P<0.01) and R+A+ mice (by 20%; P<0.05; Figure 6A and 6B) and in neurological deficit score (P<0.05; Figure 6C). Furthermore, transfection of Lenti-ACE2–EPCs was able to further decrease the infarct volume in R−A− (by 30%; P<0.05) and R+A+ mice (by 40%; P<0.05; Figure 6A and 6B) and further improve neurological motor function (P<0.05 or 0.01; Figure 6C).

Infusion of Lenti-ACE2–EPCs Enhances the Efficacy in Increasing cMVD in the Peri-Infarct Area

At basal, the cMVD in the peri-infarct area was decreased by 41% in R+A+ mice (P<0.05; Figure 7A and 7B). Infusion of Lenti-GFP–EPCs was able to increase the cMVD in peri-infarct area by 22% in R−A− mice and 30% in R+A+ mice (P<0.01; Figure 7B). Transfection of Lenti-ACE2–EPCs could further induce a 17% increase in cMVD in the peri-infarct area in R−A− mice (P<0.01; Figure 7B), and 55% in R+A+ mice (P<0.05).

Figure 4. The effects of angiotensin-converting enzyme 2 (ACE2) overexpression on reactive oxygen species (ROS) and NO production of endothelial progenitor cell (EPCs). The ROS (A) and NO (B) production in EPCs transduced with Lenti-green fluorescence protein (GFP) or Lenti-ACE2. Cells were incubated with DX600 (1 μmol/L), apocynin (APO) (10 μmol/L), or L-NAME (1 mmol/L), P<0.05, **P<0.01 vs Lenti-GFP; ††P<0.05, †P<0.01 vs R−A− EPCs; #P<0.05 vs Lenti-ACE2, n=6 per group.

Infusion of Lenti-ACE2–EPCs Enhances the Efficacy in Promoting Angiogenesis in the Peri-Infarct Area

Figure 8A shows representative images of in vivo tracking of transfused EPCs (GFP+ cells) in the peri-infarct area in different groups on day 7 after MCAO surgery. The number of GFP+ cells was more in Lenti-ACE2–EPCs transfection group than in Lenti-GFP–EPC transfection group (P<0.01; Figure 8C).

Figure 8B shows representative micrographs of angiogenesis (BrdU+CD31+ cells) in the peri-infarct area. Lenti-GFP–EPC transfection promoted angiogenesis on day 7 in R−A− mice (by 30%; P<0.01). Transfusion of Lenti-ACE2–EPCs further promoted angiogenesis in both R−A− (by 81%; P<0.01) and R+A+ mice (by 300%; P<0.01; Figure 8D).

Discussion

EPCs have emerged as a pivotal type of cells for maintaining endothelial homeostasis and replenishing injured ECs, and have been shown to substantially contribute to endothelial regeneration and functional restoration.26 It has been recently documented that Ang II reduces EPC number and induces EPC dysfunction in vitro and in vivo.3 In addition, studies indicated that ACE inhibitors and type 1 angiotensin receptor blockers have protective effects on EPCs.27,28 A recent study showed that overexpressing ACE2 in blood vessels improves
EC function. However, whether ACE2 counteracts the effects of Ang II on EPCs has not been explored.

In this study, we demonstrated that the abilities of migration and tube formation of BM-derived EPCs from R+A+ mice are impaired. This result is consistent with previous studies showing that Ang II via type 1 angiotensin receptor decreases the differentiation and accelerates the senescence of BM-derived EPCs. Here, we also found that the eNOS expression and NO production are lower, whereas Nox2 and -4 expression and ROS production are higher in the EPCs generated from R+A+ mice. These findings are supported by previous evidence demonstrating that the renin–angiotensin system plays a key role in modulating EC function through regulating NO and ROS production. Recently, it has been suggested that Ang II inhibits Akt–induced eNOS activation and NO release in ECs and that Ang II infusion decreases NO production in aorta by causing eNOS uncoupling.

One of the major findings in the present study is that ACE2 overexpression in EPCs generated from both R−A− and R+A+ mice enhances their abilities of tube formation and migration. Interestingly, those effects are more evident in the EPCs from R+A+ mice and can be partially abolished by ACE2 inhibitor (DX600), suggesting that ACE2 might counteract the effect of Ang II on EPCs. We assume that the changes with ACE2 overexpression might mainly reflect the elevation of Ang 1-7 in addition to the reduction of Ang II. This is supported by a recent report showing that ACE2 overexpression in myocytes and fibroblasts induces Ang II reduction and Ang 1-7 elevation in cell lysates. DX600 is a large peptide which would not traverse the cell membrane. Because of the presence of intracellular renin–angiotensin system peptidases, the incomplete reversal with DX600 would reflect that intracellular expression of ACE2 regulates the intracellular ratio of Ang II to Ang 1-7.

At the same time, our data show that ACE2 overexpression downregulates the expression of Nox2/Nox4 and alleviates the ROS production, whereas it upregulates the expression of eNOS and elevates the NO production. These findings are in agreement with other studies. ACE2/Ang 1-7 counter-regulates Ang II–induced Nox activation in ECs and prevents ROS overproduction and senescence in EPCs. The eNOS also has been reported to regulate the mobilization and function of EPCs. We speculate that the dual effects of ACE2 overexpression on stimulating NO and reducing ROS...
production may benefit from the reduced formation of peroxynitrite. There is a study reported that ACE2 deficiency enhances Ang II–mediated peroxynitrite production.39 In addition, both the decreased ROS formation and enhanced ROS scavenging enzymes might account for the reduction of ROS production.40,41 To further confirm whether reduction of oxidative stress and upregulation of eNOS associated with the ACE2 mediated improvement of EPC function, we conducted the pathway block studies. As we expected, the beneficial effects of ACE2 overexpression on EPCs were partially blocked by NOS inhibition and further enhanced by a Nox inhibitor. These data add new information to previous reports showing the protective role of ACE2 in cells. For example, ACE2 overexpression in the human monocyte cell line attenuates Ang II–induced release of inflammatory factors.1 Transduction of cardiac fibroblasts with lenti-ACE2 results in a significant attenuation of both basal and hypoxia/reoxygenation–induced collagen production and inflammatory cytokine production.16

To explore the function of EPCs overexpressing ACE2 in vivo, we investigated the therapeutic efficacy of Lenti-ACE2–primed EPCs on experimental ischemic stroke mice. Transfused EPCs were tracked in vivo, suggesting recruitment of transfused EPCs to the peri-infarct area. Our data demonstrate for the first time that transfection of ACE2–primed EPCs is able to enhance the efficacy of nonprimed EPCs in attenuating cerebral damage (decreasing the infarct volume, improving neurological deficits) and promoting cerebral repair (increasing cMVD and angiogenesis). Angiogenesis is a vital component of wound repair. EPCs are believed to play an important role in maintaining endothelial integrity and vascular homeostasis, and to participate in angiogenesis, which represents an important endogenous tissue repair mechanism. Introduction or mobilization of EPCs can restore tissue vascularization after ischemic stroke and reestablish endothelial integrity.13,42 In this study, we double-stained the brain slides with CD31 and BrdU to measure the newly generated ECs for the index of angiogenesis, which is
commonly used in the similar studies.\textsuperscript{24,25} Our results showed that infusion of Lenti-GFP–EPCs increased angiogenesis in R–A– mice, but not in R+A+ mice. Although the detailed mechanism has not been explored in this study, we tentatively attribute this to the high level of Ang II in the R+A+ mice,\textsuperscript{22} which impairs the function of EPC.\textsuperscript{9,11} Of note, we further discovered that infusion of EPCs overexpressing ACE2 promotes angiogenesis in both R–A– and R+A+ mice, which might benefit from the ACE2 catalyzing Ang II into Ang 1-7. This is supported by other reports showing that Ang 1-7 improves endothelial function\textsuperscript{5,9} and ameliorates Ang II–Induced EC apoptosis.\textsuperscript{43} Although it is unclear whether the improvement of angiogenesis is exclusively mediated by EPC integration or paracrine effects, our in vivo results imply that overexpression of ACE2 in EPCs could enhance the beneficial effect of EPC-based therapy for ischemic stroke. Future studies are warranted to determine the fate of transplanted EPC populations by analyzing the dynamics of transplanted cells in living animals.

In summary, both the in vitro functional and in vivo therapeutic findings of present study demonstrate that overexpression of ACE2 in EPCs enhances EPC function via eNOS/NO and Nox/ROS signaling pathways. Transfusion of Lenti-Ace2–primed EPCs might provide a novel approach for treating ischemic stroke, especially in those with Ang II activation.

Perspectives

Our findings show that ACE2 enhances EPC function via regulating eNOS/NO and Nox/ROS pathways, and improves EPC-based therapeutic effects on ischemic stroke. This study depicts the new aspect of ACE2 gene function and the underlying mechanisms. Our data provide a strong rationale for using ACE2 target to develop vaso-protective drugs and to enhance the efficacy of EPC-based therapy. In addition, further study on the effect of ACE2 on EPCs should provide important insights into the pathogenesis of vascular disease and development of novel therapeutic interventions.

Sources of Funding

This work was supported by the National Heart, Lung, and Blood Institute (HL-098637, to Y.C.; HL-093567, to M.M.; and HL-56921, to M.K.R.), and the National Natural Science Foundation of China (#81271214, #81270195, #81070878).

Disclosures

None.

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### Novelty and Significance

**What Is New?**

- This study demonstrates the protective role of angiotensin-converting enzyme 2 (ACE2) in endothelial progenitor cell (EPC) function in vitro and the beneficial effects of ACE2–primed EPCs on treating ischemic stroke in vivo.

**What Is Relevant?**

- The present study supports the hypothesis that overexpressing ACE2 could improve EPC function and enhance the efficacy of EPC-based therapy for ischemic stroke via counteracting the detrimental effects of angiotensin II.

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**Summary**

ACE2 overexpression enhances EPC function via modulating nicotinamide adenine dinucleotide phosphate oxidase/reactive oxygen species and endothelial NO synthase/NO signaling pathways, and improves the therapeutic efficacy of EPC transfusion for ischemic stroke by reducing ischemic injury and promoting recovery. Our findings provide novel insight into ACE2 as a potential target for new drug development.
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_Hypertension_. 2013;61:681-689; originally published online December 24, 2012; doi: 10.1161/HYPERTENSIONAHA.111.00202

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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