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: angioinversion  ■ 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 possesses great potential for developing new avenues to treat vascular diseases.1,2 Endothelial cell (EC) dysfunction/injury is well known as the common pathological basis for vascular diseases. It has been reported that ACE2 alleviates the development of early atherosclerotic lesions by improving EC function.3 The antiatherosclerotic effect of ACE2 involves in downregulation of the Ang II–activated reactive oxygen species (ROS). Nicotinamide adenine dinucleotide phosphate oxidase (Nox)-derived ROS plays a key role in Ang II–induced endothelial damage.4 Oxidative stress–induced EC dysfunction is accompanied by the reduction of NO bioavailability and plays an important role in cerebrovascular disease.5 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.7 Reduced number and impaired function of circulating EPCs are associated with poor cardiovascular outcomes in patients with coronary artery disease.8 It is recently reported that Ang II through type 1 angiotensin receptor activation and oxidative stress impairs EPC function in vitro and in vivo.9 Ang II induces EPC senescence via activation of nicotinamide adenine dinucleotide phosphate oxidase.10 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.11 Endothelial NO synthase (eNOS) downregulation and decreased NO generation can also induce impairment of EPC function and senescence.12 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 [DiI]-labeled acetylated low-density lipoprotein), and B-s-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 lentiviruses containing green fluorescent protein (Lenti-GFP) were produced as previously described.15 EPCs were transduced with Lenti-ACE2 (Lenti-ACE2–EPCs) or Lenti-GFP (Lenti-GFP–EPCs) according to the manufacturer’s instructions.16 In brief, after cultivation for 7 days, EPCs were cultured in 6-well plates (1×10^4 cells/well) and incubated with serum-free EPC culture medium containing the lentivirus (at 5×10^6 particles/mL) for 24 hours. Transduction efficiency (the percentage of GFP-expressing cells) was quantified 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, bromodeoxyuridine [BrdU], colorimetric, Roche) as previously reported.17 Briefly, EPCs were seeded on 96-well plates (1×10^4 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. 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 RNaseasy 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′-AAGCTGGATAGCCAGGTTCCTGCTGCCGTCCTTCC-3′ and 5′-AAGTCGACCTAAAAGGAAGTCTGAGCATCATC-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.13

Measurement of ROS Generation
Intracellular ROS generation in EPCs was determined by dihydroethidium staining as previously described.19 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 diaminofluorescein diacetate (Invitrogen, Grand Island, NY) was used to assess NO production released by EPCs.20 Briefly, the EPCs were loaded with 2 μmol/L diaminofluorescein diacetate in serum-free endothelial cell basal medium-2 (37°C for 30 minutes), washed twice with phosphate buffered saline, and incubated with diaminofluorescein diacetate-free endothelial cell basal medium-2 (20 minutes) for deesterification of the indicator. Diaminofluorescein 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% isoflurane as we reported previously.13 Two hours after MCAO, mice were injected via the tail vein with Lenti-GFP–EPCs or Lenti-ACE2–EPCs (2×10^6 cells/100 μL in phosphate buffered saline) or the same volume of phosphate buffered saline as previously described.13,23 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.21

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,15,22 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. 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 U tests were 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, 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).
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 control; P<0.05 or 0.01) in Lenti-ACE2–primed EPCs from R−A− mice (by 32% increase of NO and 28% decrease of ROS production) whereas the ROS production was lower (P<0.05; Figure 4A) whereas levels of Nox2 (0.36±0.03 versus 0.48±0.03; P<0.05; Figure 4B) and Nox4 (0.34±0.02 versus 0.46±0.03; P<0.03; Figure 4C) were higher in the EPCs generated from R+A+ mice. Lenti-ACE2 transduction, whereas 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).

Lenti-ACE2 Transduction Enhances EPC Function via Both eNOS and Nox Pathways

As shown in Figure 5, the abilities of EPC migration and tube formation were attenuated in EPCs from R+A+ mice by 31% and 26%, respectively (P<0.01 or 0.05). Lenti-ACE2 transduction enhanced those functions of EPCs from both R+A+ (by 47% and 52%, respectively) and R−A− (by 34% and 50%, respectively) mice (P<0.01). Moreover, DX600 abolished and l-NAME partially blocked Lenti-ACE2–induced enhancement of those functions in EPCs (P<0.01). Apocynin further enhanced those functions in Lenti-ACE2–primed EPCs from R−A− mice, but not in Lenti-ACE2–primed EPCs from R−A− mice.
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, transfusion 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). Transfusion 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).

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. It has been recently documented that Ang II reduces EPC number and induces EPC dysfunction in vitro and in vivo. In addition, studies indicated that ACE inhibitors and type 1 angiotensin receptor blockers have protective effects on EPCs. 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.\textsuperscript{39} In addition, both the decreased ROS formation and enhanced ROS scavenging enzymes might account for the reduction of ROS production.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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. 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, which impairs the function of EPC.

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 infusion of Lenti-GFP–EPCs increased angiogenesis in both R−A− and 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, which might benefit from the ACE2 catalyzing Ang II into Ang 1-7.

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 Lentivirus-induced 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 vasoprotective 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.

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**Disclosures**

None.

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**Novelty and Significance**

**What Is New?**

- This study demonstrates the protective role of angiotensin-converting en-
  zyme 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.

**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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