CXCR7 Upregulation Is Required for Early Endothelial Progenitor Cell–Mediated Endothelial Repair in Patients With Hypertension

Xiao-Yu Zhang,* Chen Su,* Zheng Cao,* Shi-Yue Xu, Wen-Hao Xia, Wen-Li Xie, Long Chen, Bing-Bo Yu, Bin Zhang, Yan Wang, Jun Tao

Abstract—Dysfunction of early endothelial progenitor cells (EPCs) is responsible for impaired endothelial repair capacity after arterial injury in patients with hypertension. Here, we hypothesized that diminished signaling of CXC chemokine receptor 7 (CXCR7) contributes to the reduced EPC functions, and enhanced CXCR7 expression restores the capacities of EPCs from hypertensive patients. CXCR7 expression of EPCs from hypertensive patients was significantly reduced when compared with that from healthy subjects. Meanwhile, the phosphorylation of p38 mitogen-activated protein kinase, a downstream signaling of CXCR7, was elevated, which increased cleaved caspase-3 level of EPCs. CXCR7 gene transfer augmented CXCR7 expression and decreased the phosphorylation of p38 mitogen-activated protein kinase, which was paralleled to EPC functional upregulation of in vitro adhesion, antiapoptosis activities, and in vivo re-endothelialization capacity in a nude mouse model of carotid artery injury. The enhanced in vitro and in vivo functions of EPCs were markedly inhibited by neutralizing monoclonal antibody against CXCR7, which was blocked by p38 mitogen-activated protein kinase inhibitor SB203580. Downregulation of cleaved caspase-3 level induced by CXCR7 gene transfer or SB203580 pretreatment improved EPC functions. Furthermore, we found that lercanidipine, a dihydropyridine calcium channel antagonist, enhanced CXCR7 expression and facilitated in vitro and in vivo functions of EPCs. Our study demonstrated for the first time that diminished CXCR7 signal at least partially contributes to the reduced in vitro functions and in vivo re-endothelialization capacity of EPCs from hypertensive patients. Upregulation of CXCR7 expression induced by gene transfer or lercanidipine treatment may be a novel therapeutic target for increased endothelial repair capacity in hypertension. (Hypertension. 2014;63:383-389.) • Online Data Supplement

Key Words: CXCR7 protein, human • endothelial progenitor cells • endothelial repair • gene transfer techniques • hypertension • lercanidipine • p38 mitogen-activated protein kinases

Hypertension is one of the major risk factors for cardiovascular diseases. Accumulating evidence indicates that patients with hypertension are associated with abnormalities in endothelial structure and function. It is generally accepted that loss of endothelial integrity leads to the initiation and development of cardiovascular diseases. Therefore, maintenance of endothelial integrity may be an important strategy for the optimal treatment of hypertension aimed at reducing the high incidence of hypertension-related cardiovascular diseases.

Endothelial progenitor cells (EPCs) derived from bone marrow play a critical role in maintaining the integrity of vascular endothelium after arterial injury. Based on the phenotypic difference, EPCs are divided into early and late EPCs. Previous studies have demonstrated that decline in number and function of early EPCs is responsible for the loss of normal endothelial homeostasis in hypertension. However, the molecular mechanism underlying the diminished EPC-based endothelial reparative capacity after arterial injury is not completely clear. Data reported herein have proved that chemokine receptors are highly related to the process of EPC participating in endothelial repair. Among them, chemokine receptor 4 (CXCR4) has long been considered to exclusively act via stromal-derived factor 1 (SDF-1), which is shown to regulate many biological processes. Indeed, recent studies demonstrated that hypertensive patients display impaired endothelial repair capacity of EPCs after vascular injury and diminished CXCR4 signaling contributes to detrimental EPC-based maintenance of endothelial integrity. However, as the newly discovered second receptor of SDF-1, CXCR7 also plays an essential role in the vascular system including vascular formation and protection, endothelial cell growth, and survival. Previous studies reported that CXCR7
binds to SDF-1 with higher affinity than CXCR4.\(^{17,18}\) We hypothesized that CXCR7 signaling may also influence hypertension-related EPC functions other than CXCR4 signaling. However, until now there were no data available on CXCR7 signaling alteration and its potential molecular mechanism related to vascular endothelial repair capacity of EPCs from hypertensive patients. We hypothesized that reduced CXCR7 signaling expression leads to the fall in endothelial repair capacity of EPCs, and CXCR7 signaling upregulation contributes to the restoration of EPC-mediated endothelial reparative capacity in patients with essential hypertension. To test these assumptions, CXCR7 signaling and its relation to endothelial repair capacity of early EPCs were examined in hypertensive patients and normal subjects. EPCs from hypertensive patients were treated with CXCR7 gene transfer. Human EPCs were tested in vitro for the ability to affect CXCR7 signaling, as well as migration and adhesion functions. We also assessed the effect of transplantation of in vitro CXCR7 gene–transferred EPCs from hypertensive patients on in vivo re-endothelialization after wire-mediated injury of the carotid artery in nude mice. Furthermore, we investigated the effects of EPCs treated with lercanidipine, a dihydropyridine calcium channel antagonist, on the change in CXCR7 signaling and re-endothelialization capacity of EPCs from hypertensive patients. The aim of the present study is to provide novel information on the loss of EPC-related endothelial repair capacity in hypertension and to evaluate whether upregulation of CXCR7 expression can be used as a potential new therapeutic target to improve the endothelium-reparative capacity of human EPCs from patients with hypertension.

**Methods**

Detailed methods for the present study are provided in the online-only Data Supplement.

**Results**

**Baseline Characteristics**

As listed in Table S1 in the online-only Data Supplement, baseline characteristics were not significantly different between healthy subjects and hypertensive patients with the exception of systolic blood pressure and diastolic blood pressure.

**Downregulation of CXCR7 Results in Reduced In Vitro Functions and In Vivo Endothelial Repair Capacity of EPCs From Hypertensive Patients**

We evaluated in vitro functions of early EPCs to investigate whether CXCR7 was related to the dysfunction of EPCs from hypertensive patients. Both the basal level of migration and adhesion onto tumor necrosis factor-α–prestimulated monolayer human umbilical vein endothelial cells (HUVECs) were significantly lower in EPCs from hypertensive patients than those from healthy subjects (Figure 1A and 1B). Transplantation with EPCs from healthy subjects markedly increased re-endothelialization of denuded carotid arteries in nude mice (Figure 1C and 1D) when compared with PBS. Moreover, in vivo re-endothelialization capacity of EPCs from hypertensive patients was significantly reduced in comparison with EPCs from healthy subjects (40.3±3.5% versus 66.7±1.7%; \(P<0.001\); Figure 1C and 1D). Our data showed that the expression of CXCR7 in EPCs from hypertensive patients was significantly lower than that from healthy subjects, which was consistent with in vivo re-endothelialization capacity (Figure 1E). Confocal laser scanning microscopy revealed that transplanted CM-Dil–labeled early EPCs were attached to the sites in endothelial repair zone of the injured carotid artery but not in the uninjured carotid artery (Figure S1).

**Ad5/CXCR7 Transfection Upregulates CXCR7 Expression of EPCs**

The most direct way to upregulate CXCR7 in EPCs is gene transfer. To confirm the effect of CXCR7 on the regulation of in vitro functions and in vivo endothelial repair capacity of EPCs, we transduced EPCs from hypertensive patients with Ad5 vector encoding CXCR7 cDNA. At 48 hours after transduction, the CXCR7 expression was obviously increased in Ad5/CXCR7-transduced EPCs from patients with cardiovascular disease compared with Ad5/enhanced green fluorescent protein (EGFP)-transduced EPCs or nontransduced EPCs from hypertensive patients (Figure S2), which were confirmed by real-time polymerase chain reaction and Western blot.

**CXCR7 Gene Transfer Increases the In Vitro Adhesion Function and In Vivo Re-Endothelialization Capacity of EPCs From Hypertensive Patients**

In the migration assay, there are no significant differences on the basal migration capacity among nontransduced EPCs, Ad5/
CXCR7-transduced EPCs, and Ad5/EGFP-transduced EPCs with or without SDF-1 induced (Figure 2A and 2B). In the quiescent HUVEC monolayer, adhesion of CM-DiI–labeled EPCs had no obvious difference among nontransduced EPCs, Ad5/EGFP-transduced EPCs, and Ad5/CXCR7-transduced EPCs. However, in tumor necrosis factor-α–activated HUVECs, adhesion of Ad5/CXCR7-transduced EPCs from hypertensive patients exceeded Ad5/EGFP-transduced EPCs or nontransduced EPCs (Figure 2C and 2D).

In addition, Ad5/CXCR7-transduced EPCs increased the re-endothelialization areas of denuded carotid arteries in contrast with nontransduced EPCs and Ad5/EGFP-transduced EPCs (76.9±3.8% versus 43.4±3.6% or 46.6±1.7%; P<0.01; Figure 2E and 2F). Therefore, these data suggested that the CXCR7 enhanced impaired adhesion function and re-endothelialization capacity of EPCs from hypertensive patients but not migration.

CXCR7-Mediated p38 Mitogen-Activated Protein Kinase Leads to the Improvement of In Vitro Functions and In Vivo Endothelial Repair Capacity of EPCs From Hypertensive Patients

Considering p38 mitogen-activated protein kinase (p38 MAPK) is a key molecular signaling in the cellular survival, migration, and metastasis,19,20 we hypothesized that p38 MAPK signaling pathway is involved in CXCR7 downstream regulating EPC-mediated re-endothelialization. To further define the mechanistic link between CXCR7 and p38 MAPK, we tested the level of phosphorylated p38 (p-p38). Our data showed that the levels of p-p38 were significantly higher in EPCs from hypertensive patients and Ad5/EGFP-transduced EPCs compared with EPCs from healthy subjects or Ad5/CXCR7-transduced EPCs from hypertensive patients (Figure 3A). Moreover, paralleling with Ad5/CXCR7-transduced, p38 MAPK inhibitor SB203580 can markedly attenuate the effect of upregulation of p38 MAPK phosphorylation level (Figure 3B).

To evaluate the effect of upregulation of p38 MAPK phosphorylation level on migration and adhesion, EPCs were also treated with CXCR7-mAb and SB203580. Compared with Ad/CXCR7 EPCs, CXCR7-mAb was significantly able to inhibit the migration (Figure 3C), adhesion function (Figure 3D), and in vivo re-endothelialization capacity (76.8±2.3% versus 51.6±3.4%; P<0.05; Figure 3E and 3F). Furthermore, SB203580 can restore the CXCR7 mAb-induced decrease of migration (Figure 3C), adhesion function (Figure 3D), and re-endothelialization activity (68.6±3.2% versus 51.6±3.4%; P<0.05; Figure 3E and 3F). Indeed, our data demonstrated that abnormality in CXCR7/p38 MAPK signaling pathway is, at least in part, responsible for the fall in EPC-mediated endothelial repair capacity in hypertension.

CXCR7/p38 MAPK Signaling Influences Apoptosis and Cleaved Caspase-3 Expression of EPCs

To investigate the effect of CXCR7/p38 MAPK signaling on EPC survival, we test apoptosis and cleaved caspase-3 level of EPCs. Our data indicated that increased CXCR7 expression in EPCs from hypertensive patients by Ad5/CXCR7 gene transfer can reduce apoptosis rate (Figure S3A and S3B). Meanwhile, CXCR7-mAb markedly attenuated the antiapoptosis capacity of Ad5/CXCR7-transduced EPCs and this effect can be inhibited by SB203580 (Figure S3D). The expression of cleaved caspase-3 was significantly increased in EPCs from hypertensive patients compared with EPCs from healthy subjects and Ad5/CXCR7-transduced EPCs from hypertensive patients (Figure S3C), which was consistent with the level of p38 MAPK. In addition, SB203580 can reduce its increased expression in EPCs treated with CXCR7-mAb (Figure S3E).Therefore, the results suggested CXCR7/p38 MAPK signaling mediates EPC survival.
Lercanidipine Facilitates Proliferation and Antiapoptosis Capacity of EPCs From Hypertensive Patients

We then investigated whether antihypertensive lercanidipine treatment can increase EPC functions from hypertensive patients. After 24- and 48-hour incubation in the absence or presence of lercanidipine (1, 10, 25, 50, and 100 μmol/L), cells were harvested and viability was examined by the CCK8 (cell counting kit-8) test. Lercanidipine began to increase in cell number in a concentration at 1, 10, 25, and 50 μmol/L and inhibited proliferation at 100 μmol/L (Figure 4A). Especially, EPC growth was significantly increased after treatment with 25 μmol/L lercanidipine compared with control group. Interestingly, lercanidipine enhanced antiapoptosis capacity of EPCs in a concentration at 1, 10, 25, and 50 μmol/L and increased apoptosis rate at 100 μmol/L, which is consistent with its effect on proliferation (Figure 4B). Collectively, these results suggested that lercanidipine increases EPCs proliferation and survival and does not exert a cytotoxic effect on EPCs under lower concentration.

Lercanidipine-Mediated CXCR7 Signaling Is Involved in Enhancement of In Vitro Functions and In Vivo Endothelial Repair Capacity of EPCs From Hypertensive Patients

We then investigated the effect of lercanidipine in regulating CXCR7 expression in relation to the capabilities of EPCs from hypertensive patients. After 24- and 48-hour incubation in the absence or presence of lercanidipine (1, 10, 25, 50, and 100 μmol/L), cells were harvested and viability was examined by the CCK8 test. Lercanidipine began to increase in cell number in a concentration at 1, 10, 25, and 50 μmol/L and inhibited proliferation at 100 μmol/L (Figure 4A). Especially, EPC growth was significantly increased after treatment with 25 μmol/L lercanidipine compared with control group. Interestingly, lercanidipine enhanced antiapoptosis capacity of EPCs in a concentration at 1, 10, 25, and 50 μmol/L and increased apoptosis rate at 100 μmol/L, which is consistent with its effect on proliferation (Figure 4B). Collectively, these results suggested that lercanidipine increases EPCs proliferation and survival and does not exert a cytotoxic effect on EPCs under lower concentration.

Discussion

Our present study demonstrates that decline in CXCR7 signaling expression contributes to impaired endothelial repair capacity of EPCs from hypertensive patients. Downregulation of CXCR7 leads to the activation of its downstream signaling p38 MAPK, which increases caspase-3 level of EPCs. Ad5/CXCR7 gene transfer treatment enhances CXCR7 expression and decreases p38 MAPK phosphorylation and...
caspase-3 expression, which is paralleled to the enhanced in vitro adhesion and antiapoptosis functions, as well as in vivo re-endothelialization capacity of EPCs from hypertensive patients. The augmentation of in vitro and in vivo capacities of EPCs is inhibited by CXCR7-mAb, which can be attenuated by p38 MAPK inhibitor SB203580. Lercanidipine, a dihydropyridine calcium channel antagonist, also facilitates CXCR7 expression of EPCs from hypertensive patients, and this alteration is correlated with accelerated in vivo re-endothelialization in a nude mouse model of carotid artery.

Our present study demonstrates for the first time that diminished CXCR7 expression with upregulation of p38 MAPK/caspase-3 signaling pathway is, at least in part, involved in the reduced function of EPCs from hypertensive patients. CXCR7 gene transfer or lercanidipine treatment contributes to EPC-mediated improvement of endothelial repair capacity in patients with hypertension via regulating CXCR7/p38 MAPK/caspase-3 signaling.

Previous study showed that dysfunction of EPCs from hypertensive patients leads to impaired re-endothelialization capacity after arterial injury. Our recent data demonstrated that deterioration of CXCR4/JAK-2 (janus kinase-2) signaling contributes to the fall in EPC-mediated endothelium-reparative function of hypertensive patients. However, the study also indicated that CXCR7 has greater binding affinity for SDF-1 than CXCR4. Furthermore, emerging data suggested that CXCR7 is essential for the regulation of cellular biological functions. For example, it has been reported that CXCR7 enhanced survival, as well as adhesive and invasive activities of prostate cancer cells. CXCR7 mediated resistance to apoptosis in human glioma cells. CXCR7 protected human renal progenitor cells from apoptotic challenges and regulated renal progenitor cells survival. More importantly, recent investigations demonstrated that CXCR7 is an important signaling molecule and plays a pivotal role in the regulation of adhesion and survival functions of in vitro EPCs from both rats and normal human beings. However, the molecular pathway underlying CXCR7-mediated biological effect of EPCs related to endothelial repair capacity is still unclear in hypertension.

It is generally accepted that patients with hypertension display impaired EPC-based re-endothelialization capacity after arterial injury. However, the underlying molecular mechanism remained to be further elucidated. Given the close association between CXCR7 and EPC function, therefore, the deep insight into the role of CXCR7 signaling in the modulation of re-endothelialization capacity of EPCs may be of important clinical implication for our further understanding of the pathogenesis of EPC dysfunction in hypertension and developing a novel potential therapeutic target. Here, we found that CXCR7 expression of EPC is significantly reduced in hypertensive patients compared with normal subjects. Furthermore, the phosphorylation of p38 MAPK in EPCs, involved in the CXCR7 downstream signaling, was also enhanced, which increased the cleaved caspase-3 level of EPCs from hypertensive patients. In parallel, both in vitro adhesion, antiapoptosis activities, and in vivo re-endothelialization capacity were markedly decreased in EPCs from hypertensive patients. Our data reported here for the first time suggest that abnormality in CXCR7/p38 MAPK/caspase-3 signaling is related to the decline in EPC-based endothelial repair capacity in patient with hypertension.

To further demonstrate the role of CXCR7/p38 MAPK/caspase-3 signaling pathway in maintenance of EPC-mediated endothelial repair capacity in hypertensive patients, we upregulated the expression of CXCR7 by Ad5/CXCR7 gene transfer. Indeed, our data showed that Ad5/CXCR7 gene transfer...
transfer can increase CXCR7 signaling expression of EPCs from hypertensive patients. In parallel, this CXCR7 expression augmentation is consistent with increased in vitro EPC adhesion function, antiapoptosis capacity, and accelerated in vivo re-endothelialization. Furthermore, upregulation of in vitro function and in vivo re-endothelialization capacity induced by Ad5/CXCR7 gene transfer is blocked by CXCR7-mAb, which can be reversed by p38 MAPK inhibitor SB203580. These effects are paralleled to the downregulation of caspase-3 level in EPCs. Our current study demonstrated that reduced CXCR7 expression with subsequently activated downstream p38 MAPK signaling after accelerated cellular apoptosis induced by cleaved caspase-3 elevation is, at least in part, responsible for the fall in EPC-mediated endothelial repair capacity in hypertension, providing the novel insight into the molecular mechanism for our understanding of EPC dysfunction in hypertension. Therefore, novel pharmacological intervention targeted to enhance CXCR7 expression has important clinical relevance for the maintenance of endothelial integrity and function in patients with hypertension.

Lercanidipine has long been used to treat patients with hypertension. Previous studies have shown that lercanidipine possesses several vascular protections.26–28 However, the impact of lercanidipine on CXCR7 signaling expression of EPCs and its relation to endothelial protection in hypertensive patients is unclear. We hypothesized that lercanidipine might facilitate the expression of CXCR7 signaling in EPCs, which contributes to EPC-mediated re-endothelialization after arterial injury. Indeed, our data showed that lercanidipine can upregulate CXCR7 signaling expression of EPCs from patients with hypertension. In parallel, the augmentation is consistent with increased in vitro EPC adhesion function, antiapoptosis capacity, and accelerated in vivo re-endothelialization. Similar with CXCR7 gene transfer, upregulation of EPC functions mediated by lercanidipine treatment can be also attenuated by CXCR7-mAb. Nevertheless, we found that Ad/CXCR7-induced upregulation of CXCR7 in EPCs failed to mediate EPC migration consistent with previous reports that CXCR7 has no effect on EPC migration,23,25 whereas lercanidipine can increase EPC migration as well as proliferation capacities. This disparity of EPC functional activities between CXCR7 gene transfer and lercanidipine treatment is unclear and beyond the present study. Future study will be under investigation in our laboratory to answer this question. Collectively, our present study, taken with previous investigations, demonstrates for the first time that the effect of lercanidipine on CXCR7/p38 MAPK signaling of EPCs can account for its beneficial vascular protection in addition to its antihypertensive effect.

Our present study still had some limitations. First, our previous study has confirmed that diminished CXCR4/JAK-2 signaling plays an important role in impaired function of EPCs from hypertensive patients; here, we found that CXCR7/p38 MAPK/caspase-3 signaling may also contribute to the maintenance EPC function. Which signaling pathway is more important in diminished EPC-mediated endothelial repair capacity in hypertension needs to be further studied. Second, some recent studies have suggested that CXCR7 and CXCR4 can form CXCR7/CXCR4 homo- and heterodimers to modulate SDF-1.17,29 Research into the interaction between CXCR7 and CXCR4 is warranted in the next study. Third, our data showed that abnormality of CXCR7 signaling of EPCs is involved in the impaired re-endothelialization capacity of EPCs. However, the exact mechanism responsible for diminished CXCR7 expression remains to be further investigated.

**Perspectives**

Our study demonstrated that abnormalities in CXCR7/p38 MAPK/caspase-3 signaling pathway at least partially lead to the reduction of re-endothelialization capacity of EPCs from hypertensive patients. Upregulation of CXCR7 induced by gene transfer or lercanidipine treatment may be an effective intervention target to improve endothelial repair capacity via modulating CXCR7/p38 MAPK/caspase-3 signaling pathway.

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**Disclosures**

None.

**References**

Novelty and Significance

What Is New?
- This study advances our knowledge of the molecular mechanism underlying the diminished endothelium-reparative capacity of EPCs from hypertensive patients after arterial injury and demonstrates CXCR7 as a potential therapeutic target for improved EPC-based endothelial repair capacity in hypertension.

Summary
The present study demonstrates for the first time that reduced CXCR7 expression contributes to the loss of EPC-related endothelial repair capacity in patients with hypertension and provides a novel therapeutic target for increased endothelial repair capacity in hypertension.

What Is Relevant?
- This study advances our knowledge of the molecular mechanism underlying the diminished endothelium-reparative capacity of EPCs from hypertensive patients after arterial injury and demonstrates CXCR7 as a potential therapeutic target for improved EPC-based endothelial repair capacity in hypertension.
CXCR7 Upregulation Is Required for Early Endothelial Progenitor Cell–Mediated Endothelial Repair in Patients With Hypertension

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CXCR7 Upregulation is Required for Early Endothelial Progenitor Cells Mediated Endothelial Repair in Patients with Hypertension

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Short title: CXCR7 and EPC functions in hypertension

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Supplemental Materials and Methods

Subjects’ Characteristics

15 consecutive outpatients with newly diagnosed hypertension (BPs ≥ 140/90 mmHg) and 15 age-matched healthy subjects were enrolled into the study after informed consent was obtained. Exclusion criteria included malignancy, cardiovascular events, or active inflammatory disease and those with other cardiovascular risk factors or taking other medications. Blood pressure measurements were performed according to JNC-7. All of the subjects underwent 3 blood pressure measurements in 2 different visits, after 30 min of rest, and the measurements were spaced by 5- to 10-min intervals, on both the left and right arm, in the sitting and lying positions. The baseline characteristics are shown in Table S1. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China).

EPC culture, materials and cell labelling

Early EPCs were isolated and cultured as previously described in detail.1-3 After 7 days of culture, EPCs were defined as cells dually positive for 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindo-carbocyanine (DiI)-acetylated low density lipoprotein (acLDL) uptake (0.02mg/ml; Invitrogen, Carlsbad, CA, USA) and BS-1 lectin binding (0.01mg/ml; Sigma-Aldrich, St. Louis, MO, USA). Endothelial markers of cultured EPCs were also examined by flow cytometry analysis using CD31 (BD Pharmingen), von Willebrand factor (vWF) and kinase-insert domain receptor (KDR) (R&D Systems Inc) as previously described.2,3 Based on the isolation and cultivation protocol, the adherent mononuclear cells were identified as early EPCs.2,4 Adherent cells were maintained for 7 days and then were used for the following experiments. Neutralizing monoclonal antibody against CXCR7 (CXCR7-mAb), IgG2a isotype control (R&D System, Minneapolis, MN, USA), and p38MAPK inhibitor SB203580 (Sigma-Aldrich, St Louis, MO, USA) were used as blocking agents in this study.

EPC gene transfer

After 7 days in culture, cells were transduced with the adenovirus serotype 5 (Ad5) encoding the human CXCR7 gene (Ad5/CXCR7) or enhanced green fluorescent protein gene (Ad5/EGFP) (GeneChem company, Ltd, Shanghai). Human EPCs were transduced with Ad5/CXCR7 or Ad5/EGFP for 90 min in culture medium without serum. After transduction, cells were washed with PBS and incubated with EPC medium for 48h before subsequent experiments.

Lercanidipine preparation

Lercanidipine was kindly provided by Lee's Pharmaceutical Holdings Limited. It was dissolved in DMSO. The final concentration of the solvent was <1% (vol/vol.%).

EPC proliferation

EPCs were planted in 96-well plates (5×10^3 cells per well) and cultured for 24h in EBM-2 medium (Lonza, Basel, Switzerland) supplemented with 2% FBS for 24h and 48h with or without lercanidipine. The medium was replaced with 100µL of fresh medium containing 10µL of CCK8 (Dojindo Molecular Technologies, Japan) solution, and the cells were subsequently incubated for 2h. Then the absorbance at 450 nm in each well was recorded on an Infinite F200 Multimode plate reader (Tecan, Männedorf, Switzerland).
EPC migration assay in vitro
In brief, a total of $2 \times 10^4$ EPCs were isolated, resuspended in 250µL EBM-2 and pipetted at the 7th day in the upper chamber of a modified Boyden chamber (Costar Transwell ® assay, 8 µm pore size, Corning, NY). The chamber was placed in a 24-well culture dish containing 500µL EBM-2 supplemented with either PBS or 100 ng/mL SDF-1. After 24h incubation at 37°C, transmigrated cells were counted by independent investigators blinded to treatment randomly.

EPC adhesion assay in vitro
A monolayer of human umbilical vein endothelial cells (HUVECs) was prepared 48h before the assay by plating $2 \times 10^5$ cells in each well of a four well plate. HUVECs were pretreated with or without 1 ng/mL tumor necrosis factor-α (TNF-α, Peprotech) for 12h. Then $1 \times 10^5$ CM-DiI (CellTracker® CM-DiI, Invitrogen)-labelled EPCs were added to each well and incubated for 3h at 37°C. Non-attached cells were gently removed with PBS, and adherent EPCs were fixed with 4% paraformaldehyde and counted by independent investigators blinded to treatment randomly.

Assay for EPC apoptosis
EPC apoptosis induced by serum starvation was detected by AnnexinV-FLUOS staining kit (KeyGen Biotech, Nanjing, China) to determine whether CXCR7 exerted a survival effect on EPCs. Briefly, EPCs were cultured with serum free EBM-2 medium for 24h. Annexin V-FITC and propidium iodide (PI) were added to the washed cells ($1 \times 10^6$ cells/mL in FACS buffer) for 15 min at room temperature in the dark. FACS buffer was added, and cells were analyzed immediately by flow cytometry analysis.

Real-time PCR assay for mRNA level of CXCR7 gene
Total RNA was extracted with the High pure RNA isolation kit(Roche, Indianapolis, USA). The first strand cDNA were synthesized using PrimeScript® RT reagent Kit (Takara Biotechnology, Japan). The mRNA expression of CXCR7 was quantified using the $2^{-\Delta\Delta CT}$ analytical method in triplicate using a StepOne Plus real-time PCR System (ABI, USA). The mRNA level of GAPDH gene was measured in each sample as an internal normalization standard. The primer CXCR7 A (sense) is 5’-TCTGCATCTCTTCCGACTACTCA-3’, and the primer CXCR7 B (anti-sense) is 5’-GTAGAGCAGGACGCTTTTGTT-3’. Real-time PCR was performed in a 20µL reaction mixture containing primers, FastStart Universal SYBR Green master (ROX) reagent (Roche Applied Science, Mannheim, Germany) and 2µL cDNA sample.

Western blot analysis
Total EPC protein were extracted and quantified by cytoBuster TM protein extraction reagent (Merk, Darmstadt, Germany) and bicinechonic acid protein assay kit (Invitrogen, Carlsbad, CA, USA) separately. Protein extracts were subjected to SDS–PAGE, transferred to polyvinylidene fluoride membranes (Roche, Indianapolis, IN, USA). The following antibodies were used: rabbit anti-CXCR7 antibody (1:250; abCAM, Cambridge, MA, USA), rabbit anti-phospho-p38MAPK and anti- p38MAPK antibody (1:1000; Cell Signaling Technology), and rabbit anti-GADPH antibody (1:3000; Cell Signaling Technology). Proteins were visualized with HRP-conjugated anti-rabbit IgG (1:2000; Cell Signaling Technology), followed by use of the ECL chemiluminescence system (Cell Signaling Technology).
Animals model and in vivo reendothelialization assay

Male NRMInu/nu athymic nude mice (SLAC Laboratory Animal Center, Shanghai, China), aged 8–10 weeks, were used to allow injection of human EPCs. Animals were anaesthetized with ketamine (100 mg/kg IP) and xylazine (5 mg/kg IP). Surgery was carried out using a dissecting microscope. The left carotid artery was exposed via a midline incision on the ventral side of the neck. The bifurcation of the carotid artery was located, and two ligatures were placed around the external carotid artery, which was then tied off with the distal ligature. An incision hole was made between the two ligatures to introduce the denudation device. The curved flexible wire (0.35 mm diameter) was introduced into the common carotid artery and passed three times in order to denude endothelium. The wire was then removed, and the external carotid artery was tied off proximal to the incision hole with the proximal ligature.

After 7 days culture, EPCs (5×10⁵ cells) were resuspended in 100μL of pre-warmed PBS (37°C) and transplanted 3h after carotid artery injury via tail vein injection with a 27 G needle. The same volume of PBS as a control was injected into placebo mice. Three days after carotid artery injury, endothelial regeneration was evaluated by staining denuded areas with 50µL of solution containing 5% Evans Blue dye via tail vein injection.

After 7 days culture, PBS and EPCs were labeled with CM-DiI (CellTracker™ CM-DiI, Invitrogen) and injected into the tail vein of nude mice with carotid injury to examine the homing to the uninjured and injured carotid artery. After 24 hours the animals were sacrificed, blood was immediately removed and the injured sections of the carotid arteries from two groups were dissected. The carotid arteries were opened and laid on microscope slide, then incubated with 4% paraformaldehyde for 20 minutes and 1% TritonX-100 for 15 minutes, sequentially. The carotid arteries were stained with BS-1 lectin (0.01 mg/ml) for 1h and DAPI (100 ng/ml) for 15 minutes. Confocal laser scanning microscopy (ZEISS,LSM710) analysis was used to obtain selective images with increasing depth and detect homing of CM-DiI-labeled EPCs to the site of vascular injury.

All experimental protocols complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and the Animal Care and Use Committees of Sun Yat-sen University. Our study conformed to the ethical principles outlined in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China).

Statistical Analysis

All results are expressed as mean value ± SD. Statistical significance was evaluated by means of a Student $t$ test or ANOVA. $P<0.05$ was considered statistically significant. All statistical analyses used SPSS statistical software (SPSS version 13.0).

Supplemental References

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### Supplemental Table

#### Supplemental Table S1. Subjects’ Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Hypertension patients (n=15)</th>
<th>Control subjects (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>47.9±7.1</td>
<td>47.1±10.7</td>
<td>0.113</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>10</td>
<td>0.682</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>100</td>
<td>0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2±2.4</td>
<td>23.5±2.3</td>
<td>0.914</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>68.9±13.4</td>
<td>72.7±11.5</td>
<td>0.875</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>164.8±12.6</td>
<td>119.7±7.3</td>
<td>0.041*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>96.3±10.3</td>
<td>78.1±6.3</td>
<td>0.040*</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.0±0.9</td>
<td>4.5±1.0</td>
<td>0.439</td>
</tr>
<tr>
<td>Blood urea nitrogen (mmol/L)</td>
<td>4.9±1.9</td>
<td>4.4±0.7</td>
<td>0.100</td>
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<tr>
<td>Creatinine (µmol/L)</td>
<td>70.8±23.6</td>
<td>68.9±18.8</td>
<td>0.943</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>324.5±78.9</td>
<td>294.5±71.4</td>
<td>0.527</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.5±0.8</td>
<td>4.9±0.9</td>
<td>0.517</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.3±0.5</td>
<td>1.3±0.4</td>
<td>0.072</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.8±0.8</td>
<td>3.3±0.7</td>
<td>0.650</td>
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<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.1±0.3</td>
<td>1.2±0.3</td>
<td>0.830</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. *P < 0.05 vs. control group baseline. CVD = cardiovascular disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
Supplemental Figures and Figure Legends

Supplementary Figure S1

**Figure S1.** Assessments of the transplanted early EPCs homing to the injured and uninjured carotid arteries. **A,** Confocal laser scanning microscopy analysis (magnification: 100) of the uninjured (I) and injured carotid arteries (II). The endothelial layer was visualized by β-lectin (green) of the uninjured (I) and injured (II) carotid artery, and DAPI was used for nuclei staining (blue). **B-C:** Confocal laser scanning microscopy analysis was used to obtain serial images with increasing depth of the carotid arteries (magnification: 400). CM-Dil-labeled early EPCs were injected into the tail vein of nude mice with carotid injury. CM-Dil-labeled early EPCs (red signal) attached to beneath the endothelial layer, as indicated by selective images with increasing depth of the two different reendothelialized zones of the carotid artery (**C:a-b-c** and **D:d-e-f**). In the subendothelial layer of the endothelial repair zone of the carotid artery, fluorescence-labeled EPCs (red signal) were clearly detectable (**C:e** and **D:f**).
Supplementary Figure S2

Figure S2. Ad5/CXCR7 gene transfer upregulates CXCR7 expression of EPCs in hypertension. A: Representative photographs and quantitative analyses of CXCR7 mRNA level in EPCs at 48 hours after transduction measured by Real-time PCR. (*P<0.001 vs. Non-transduced hypertensive EPCs; n=6 per group). B: Representative photographs and quantitative analyses of CXCR7 protein expression at 48 hours after transduction measured by western blot. (*P<0.01 vs. Non-transduced hypertensive EPCs; n=6 per group).

Figure S3. CXCR7/p38 MAPK signaling is related to apoptosis and cleaved caspase 3 expression of EPCs. A-B: Representative photographs (A) and quantification analyses (B) apoptosis cells rate measured by flow cytometry. (*P<0.01 vs. Healthy EPCs; n=3 per group). C: Representative photographs and quantitative analyses of cleaved caspase 3 protein.
expression EPCs. (*$P<0.01$ vs. C Healthy EPCs; n=6 per group). **D:** Quantification analyses of apoptosis cells rate measured by flow cytometry. (*$P<0.01$ vs. Ad/CXCR7- hypertensive EPCs; n=3 per group). **E:** Representative photographs and quantitative analyses of cleaved caspase 3 protein expression in CAD EPCs with/without CXCR7 mAb and/or SB203580. (*$P<0.001$ vs. Hypertensive EPCs; $#P<0.001$ vs. Ad/CXCR7- hypertensive EPCs+CXCR7 mAb; n=6 per group).