Loss of Endothelial CXCR7 Impairs Vascular Homeostasis and Cardiac Remodeling After Myocardial Infarction

Implications for Cardiovascular Drug Discovery

BACKGROUND: Genome-wide association studies identified the association of the *CXCL12* genetic locus (which encodes the chemokine CXCL12, also known as stromal cell–derived factor 1) with coronary artery disease and myocardial infarction (MI). Unlike CXCR4, the classic receptor for CXCL12, the function of CXCR7 (the most recently identified receptor) in vascular responses to injury and in MI remains unclear.

METHODS: Tissue expression of CXCR7 was examined in arteries from mice and humans. Mice that harbored floxed *CXCR7* and *Cdh5*-promoter driven *CreERT2* were treated with tamoxifen to induce endothelium-restricted deletion of CXCR7. The resulting conditional knockout mice and littermate controls were studied for arterial response to angioplasty wire injury and cardiac response to coronary artery ligation. The role of CXCR7 in endothelial cell proliferation and angiogenesis was determined in vitro with cells from mice and humans. The effects of adenoviral delivery of *CXCR7* gene and pharmacological activation of CXCR7 were evaluated in mice subjected to MI.

RESULTS: Injured arteries from both humans and mice exhibited endothelial CXCR7 expression. Conditional endothelial CXCR7 deletion promoted neointimal formation without altering plasma lipid levels after endothelial injury and exacerbated heart functional impairment after MI, with increased both mortality and infarct sizes. Mechanistically, the exacerbated responses in vascular and cardiac remodeling are attributable to the key role of CXCR7 in promoting endothelial proliferation and angiogenesis. Impressively, the impaired post-MI cardiac remodeling occurred with elevated levels of CXCL12, which was previously thought to mediate cardiac protection by exclusively engaging its cognate receptor, CXCR4. In addition, both *CXCR7* gene delivery via left ventricular injection and treatment with a CXCR7 agonist offered cardiac protection after MI.

CONCLUSIONS: CXCR7 represents a novel regulator of vascular homeostasis that functions in the endothelial compartment with sufficient capacity to affect cardiac function and remodeling after MI. Activation of CXCR7 may have therapeutic potential for clinical restenosis after percutaneous coronary intervention and for heart remodeling after MI.

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Clinical Perspective

What Is New?

- This study shows that CXCR7 is expressed in injured arteries and that endothelium-specific deletion of CXCR7 exacerbates neointimal formation after arterial wire injury, a procedure mimicking percutaneous coronary intervention, and impairs post–myocardial infarction survival, heart function, and remodeling.
- CXCR7 plays a pivotal role in promoting endothelial proliferation and angiogenesis.
- *CXCR7* gene delivery or pharmacological CXCR7 activation offers cardiac protection after myocardial infarction.

What Are the Clinical Implications?

- CXCR7 represents a novel regulator of vascular remodeling and myocardial infarction that functions in the endothelial compartment.
- Activation of CXCR7 bears therapeutic potential to prevent/treat heart failure after myocardial infarction and clinical restenosis after percutaneous coronary intervention.

The genetic locus of *CXCL12*, which encodes the C-X-C motif chemokine 12, also known as stromal cell–derived factor 1, has been associated with coronary artery disease and myocardial infarction (MI) in genome-wide association studies.[1] Two receptors have been found to ligate CXCL12, CXCR4 (the classic G protein–coupled receptor) and CXCR7 (a recently deorphanized receptor). Although CXCR4 is known to participate in vascular remodeling,[2–5] atherosclerosis,[6] and MI,[7–9] the function of CXCR7 in cardiovascular disease is largely unknown.

CXCR7, which is phylogenetically closely related to chemokine receptors, binds CXCL12 with a higher affinity than CXCR4, but it fails to couple to G proteins to induce typical chemokine receptor–mediated cellular responses.[10] CXCR7 has been proposed to serve as a scavenger receptor for CXCL12 by mediating effective ligand internalization and degradation.[11–13] However, combined evidence suggests that CXCR7 may have signaling activity beyond ligand scavenging, including in tumor cell growth and organ regeneration.[14–17]

In humans, CXCR7 is expressed in the brain, heart, kidney, endothelium, and tumor cells.[10,18] It is extensively expressed in the endothelium of tumor blood vessels[14] and is inducible by hypoxia.[19] The CXCR7 protein is not expressed on human or mouse blood leukocytes.[20] Mice lacking CXCR7 die perinatally of heart valve malformation, a phenotype that is recapitulated in mice that have endothelium-specific deficiency of CXCR7.[21] Using hyperlipidemic *Apoe*$^{-/-}$ mice, Li and colleagues[22] elegantly demonstrated that global knockout of CXCR7 exacerbated atherosclerotic lesions as a result of a defect in the cholesterol uptake by adipose tissue. However, the role of endothelial CXCR7 in the vascular response to injury and cardiac remodeling after MI remains unexplored.

In this study, we reported that the endothelium-specific deletion of CXCR7 exacerbated neointimal formation after endothelial denudation injury and impaired post-MI survival, heart function, and remodeling, which is consistent with the key role of CXCR7 in endothelial proliferation and angiogenesis.

METHODS

Animal Study

CXCR7 floxed mice harboring inducible *Cre* cassette under *Cdh5* promoter (*CXCR7*$^{f/f}$ *Cdh5-CreERT2*$^{+}$, labeled cKO) and littermate controls (*CXCR7*$^{f/f}$ *Cdh5-CreERT2*–) were treated with tamoxifen to induce endothelium-restricted deletion of CXCR7. To study arterial response to endothelial denudation injury, the mice were subjected to injury at femoral artery by angioplasty wire, as we described previously.[23] Hind-limb ischemia model was used to evaluate angiogenesis in vivo. An improved procedure for coronary artery ligation without ventilation was used to study the role of CXCR7 in MI, as detailed in the online-only Data Supplement. Overexpression of CXCR7 in the infarcted heart was conducted by injecting recombinant adenovirus into the left ventricular cavity ≈1 minute before coronary artery ligation. TC14012, a CXCR7 agonist, was administered by intraperitoneal injection after the ligation.

Cell Study

Mouse and human endothelial cells were used, as appropriate, for analysis of cell proliferation, wound healing, and tubule formation. The mouse endothelial cells were isolated from mouse lung or aorta. Human aorta endothelial cells were isolated in a manner similar to that for mouse aortic endothelial cells. Human umbilical vein endothelial cells were obtained from AllCells. The CXCL12 concentration was determined with a human CXCL12 ELISA kit (R&D Systems). Calcium release was examined in mouse aortic endothelial cells. The calcium was fluorescently stained with Cal-520 dye (AAT Bioquest) and monitored with a FlexStation3 Microplate Reader (Molecular Devices).

Statistical Analysis

Statistical analysis was performed with SPSS Statistics 22.0 (IBM) or GraphPad Prism 5 (GraphPad Software Inc). When only 2 means were compared, the Student *t* test was used for normally distributed variables and the Mann-Whitney test used for nonnormally distributed variables. Comparisons of multiple groups were made by ANOVA, as detailed in the online-only Data Supplement. The log-rank (Mantel-Cox) test was used to compare survival curve. Data are expressed as mean±SEM. Differences were considered statistically significant at $P<0.05$.

See the online-only Data Supplement for a detailed description of the methods. Ethics approval was obtained from the Institutional Review Board, Fuwai Hospital, National Center for Cardiovascular Diseases, China.

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RESULTS

Endothelial Expression of CXCR7 in Injured Arteries of Mice and Humans

First, we examined vascular expression of CXCR7 in mice. Low levels of CXCR7 were expressed in discrete endothelial cells in healthy mice (Figure 1A). However, in injured arteries, CXCR7 expression was upregulated and observed mainly in the endothelial cells of neointima, colocalizing with von Willebrand factor, an endothelial marker (Figure 1B). Human aorta from the patients who underwent aortic dissection showed CXCR7 expression in lesional endothelial cells, particularly in lesion shoulder regions (Figure 1C) and in some microvessels (Figure 1D).

Loss of Endothelial CXCR7 Exacerbates Neointimal Formation After Endothelial Denudation Injury in Mice

Basal arterial expression of CXCR7 was similar between cKO mice and littermate controls before tamoxifen induction (Figure I in the online-only Data Supplement). After tamoxifen treatment, the mice were subjected to endothelial denudation injury at femoral artery by angioplasty wire. This injury induces vascular hyperplasia response that mimics clinical restenosis after percutaneous coronary intervention. All mice were without the genetic manipulation of lipid metabolism and were fed a normal chow diet. Endothelial CXCR7 mRNA expression was essentially abolished in the endothelial cells that were isolated from the conditional knockout mice (Figure 2A). This was further confirmed via immunostaining of the injured arteries (Figure 2B). Deletion of CXCR7 did not alter the expression of CXCR4 and CXCL12 (Figure II in the online-only Data Supplement). The loss of endothelial CXCR7 significantly increased neointimal area and the ratio of neointima to media without altering media thickness (Figure 2C–2F). Endothelial CXCR7 deletion did not change body weight or plasma lipids in these normolipidemic mice (Table).

CXCR7 Increases Proliferation of Interleukin-1β–Treated Endothelial Cells and Promotes Endothelial Regeneration After Endothelial Denudation Injury

Early vascular changes were examined on day 7 after the endothelial injury. Endothelial CXCR7-deficient mice exhibited impaired re-endothelialization (Figure 3A and 3B). A reduction in infiltrated monocytes was also observed (Figure 3C and 3D). It is interesting to note that platelet-derived growth factor-BB expression was enhanced in the intima layer (Figure 3E and 3F) and in plasma (Figure III in the online-only Data Supplement).

In cultured endothelial cells that were isolated from mice, stimulation with interleukin (IL)-1β upregulated the

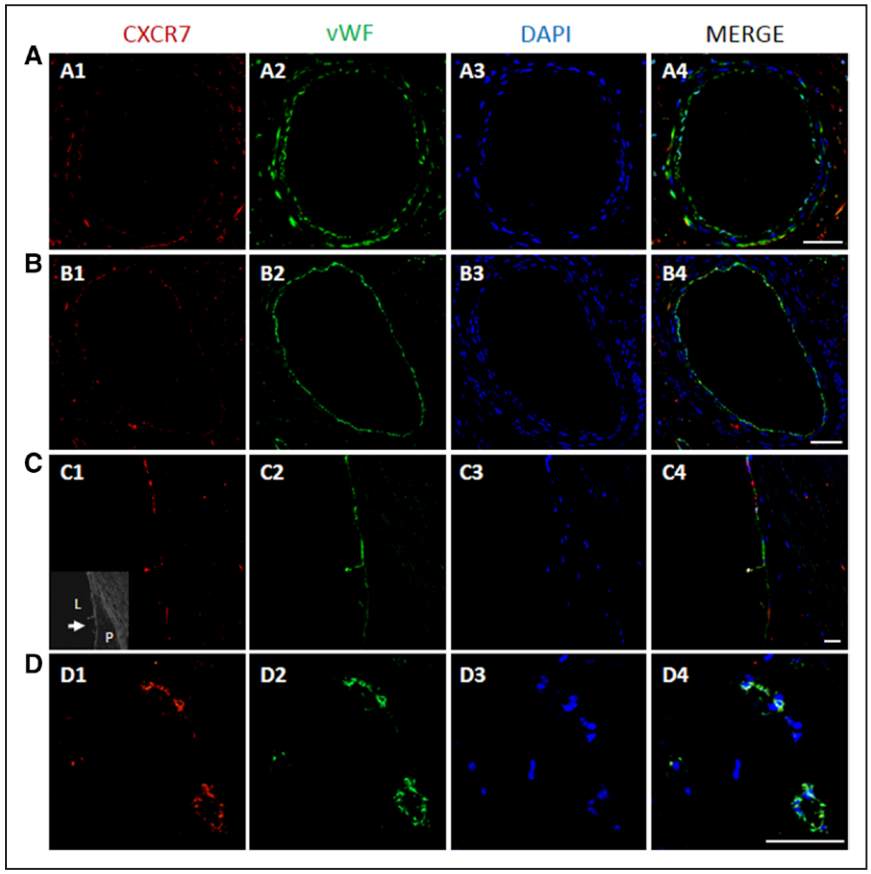

Figure 1. Human and mouse injured arteries expressed CXCR7 in endothelial cells.
CXCR7 (red) and von Willebrand factor (vWF; endothelial marker; green) were immunofluorescently stained in mouse healthy femoral arteries (**A**) and hyperplasia femoral arteries 28 days after wire injury (**B**) and in aorta sections from patients who underwent aortic dissection (**C** and **D**). DAPI stains nuclei in blue. The 3 colors were merged in the far **right**. The **inset** in **C1** shows the staining location in **C** at low magnification. The **arrow** points to an advanced lesion. Representative sections from 3 independent stainings are shown. Bar=50 μm. L indicates lumen; and P, plaque.

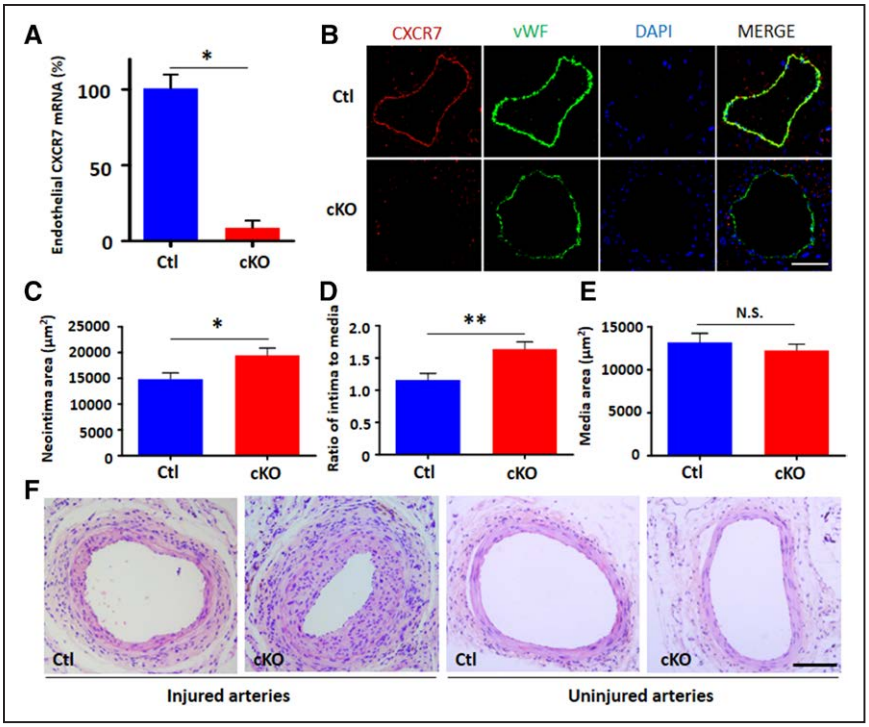

Figure 2. Inducible deletion of endothelial CXCR7 increased neointimal formation after endothelial denudation injury. CXCR7 mRNA expression in the mouse lung endothelial cells isolated from endothelial CXCR7 conditional knockout mice (cKO) and littermate controls (Ctl) was detected via reverse transcription–polymerase chain reaction (**A**). Immunofluorescent staining of CXCR7 (red) and von Willebrand factor (vWF; green) was performed in arteries of cKO and Ctl after wire-denudation injury (**B**). DAPI stains nuclei in blue. In cKO, neointimal formation (**C**) and ratio of intima to media (**D**) were increased, and media thickness was unaltered (**E**). Representative hematoxylin and eosin staining of the injured and uninjured arteries from cKO and Ctl (**F**). n=12 cKO and 15 Ctl. Bar=100 μm. **P*<0.05. ***P*<0.01.

expression of CXCR7 (Figure 4A and 4B), CXCR4, and CXCL12 (Figure IV in the online-only Data Supplement). IL-1β promoted cell proliferation in CXCR7-competent endothelial cells but not in CXCR7-deficient cells (Figure 4C and 4D). Furthermore, we treated endothelial cells with a CXCR7-specific antagonist, CCX771 (which has an IC_{50} of ≈5.3 nmol/L, with no effect on CXCL12 binding to CXCR4),[24] and a control compound, CCX704. CCX771 inhibited proliferation of both lung- (Figure 4C) and aorta- (Figure 4D) derived endothelial cells. It is notable that no significant difference in proliferation was found when endothelial cells were treated with CXCR4 siRNA (Figure V in the online-only Data Supplement) or AMD3100, a CXCR4 antagonist (which has an IC_{50} of ≈44 nmol/L,[24] does not affect CXCL12 binding to CXCR7,[10] or shows weak binding to CXCR7 [Ki ≈34.5 μmol/L[25]]; Figure VI in the online-only Data Supplement). Therefore, the endothelial cell proliferation is CXCR7 dependent. The underlying signaling pathway was then explored. Inhibition of Raf1, extracellular signal-regulated kinase, phosphatidylinositol-3-kinase, or signal transducer and activator of transcription 3, but not adenylate cyclase, prohibited the inhibitory effect of CCX771 on the endothelial cell proliferation (Figure 4E). CXCR7 knockdown by siRNA (Figure V in the online-only Data Supplement) also inhibited the proliferation and exhibited similar downstream signaling (Figure 4F). Consistently, CCX771 treatment or CXCR7 knockdown reduced phosphorylation of Raf1, extracellular signal-regulated kinase, AKT, and signal transducer and activator of transcription 3 (Figure 4G–4I). Phosphorylated Jun N-terminal kinase and P38 were not changed with CXCR7 blockade (Figure VII in the online-

Table. Plasma Chemicals and Body Weight in Mice

	Control (n=6)	cKO (n=7)	*P* Value
Plasma glucose, mmol/L	11.3±1.22	13.0±1.46	0.376
FFA, mmol/L	1.09±0.12	1.13±0.11	0.797
Triglycerides, mmol/L	1.14±0.17	1.06±0.11	0.675
Total cholesterol, mmol/L	1.73±0.18	1.90±0.14	0.456
HDL-C, mmol/L	1.31±0.16	1.50±0.14	0.367
LDL-C, mmol/L	0.25±0.03	0.22±0.01	0.225
ALT, IU/L	27.9±2.82	29.6±3.45	0.705
Body weight, g	21.3±1.20	23.4±1.32	0.273

Data are expressed as mean±SEM. ALT indicates alanine aminotransferase; cKO, conditional knockout; FFA, free fatty acids; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

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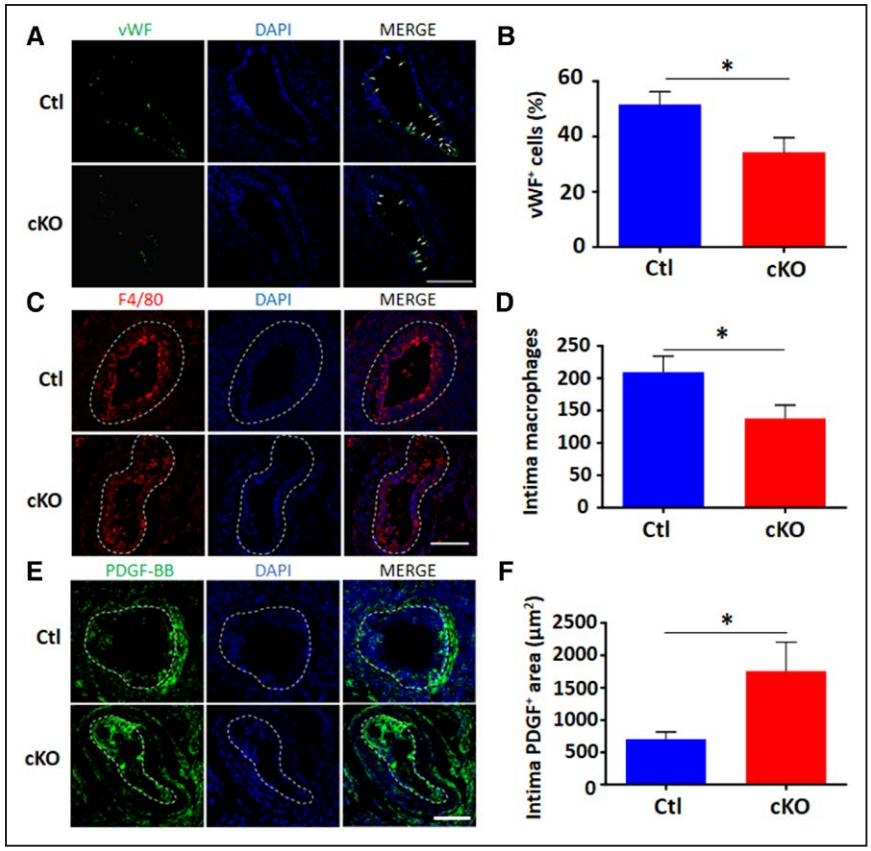

Figure 3. Endothelial deletion of CXCR7 impaired re-endothelialization after denudation via wire injury.
Immunofluorescent staining (**A**, **C**, and **E**) and corresponding quantification (**B**, **D**, and **F**) of endothelial cells (**A;** von Willebrand factor [vWF]; green), intimal macrophage (**C;** F4/80; red), and platelet-derived growth factor (PDGF)-BB expression (**E**; green) were performed with arteries on day 7 after wire injury. **Arrows** denote endothelialization. Bar=100 μm. n=10 conditional knockout (cKO) and 9 control (Ctl). **P*<0.05.

only Data Supplement). The progrowth effect was less clear without the IL-1β treatment (Figure VIII in the online-only Data Supplement). In addition, in human umbilical vein endothelial cells, CCX771 or siRNA knockdown of CXCR7 suppressed cell proliferation in the presence of IL-1β (Figure 4J and 4K). Similar results were also obtained with tumor necrosis factor-α (Figure IX in the online-only Data Supplement).

Hence, CXCR7 is inducible by inflammatory stimulation and promotes inflammation-associated endothelial proliferation in vitro. This may contribute to the endothelial regeneration after denudation injury, which limits the vascular stenotic response to injury. This observation led us to ask whether CXCR7 plays a role in angiogenesis, an endothelial regenerative process that forms new blood vessels in response to tissue ischemia.[26]

Endothelial CXCR7 Is Pivotal to Angiogenesis

Tubule formation was used to examine CXCR7 in endothelial angiogenic response in vitro. CXCR7 blockade by siRNA significantly inhibited angiogenesis in human umbilical and aorta endothelial cells and in mouse endothelial cells (Figure 5A–5D and Figure VI in the online-only Data Supplement). In the mouse model of hind-limb ischemia, endothelial CXCR7 deletion significantly attenuated blood flow recovery after femoral artery ligation, as examined by laser Doppler imaging (Figure 5E–5G). Further histology staining of endothelium shows a reduced vascular number in the ischemic gastrocnemius (Figure 5H and 5I). Therefore, endothelial CXCR7 plays a critical role in promoting ischemia-induced angiogenesis, which was previously thought to be mediated by the exclusive interaction of CXCL12 with CXCR4.[27]

Loss of Endothelial CXCR7 Impairs Heart Function After MI and Increases Mortality and Infarct Size With Reduced Vascular Density

Next, we investigated the function of endothelial CXCR7 in MI (Figure 6). It is striking that compared with control mice, deletion of endothelial CXCR7 (cKO) significantly shortened survival time and reduced cumulative survival rate (Figure 6A). The cKO mice exhibited significantly impaired heart function and remodeling after MI, with no alterations in baseline heart characteristics (Figure 6B–6F and Tables I and II in the online-only Data Supplement). The endothelial CXCR7 deficiency in the hearts of cKO mice was verified by immunofluorescent staining (Figure 6G). The cKO mice showed increased infarct size (Figure 6H and 6I), which coincided with reduced vascular density in the infarcted region (Figure 6J and 6K). This suggests that impaired angiogenesis may cause the functional defect and enhanced cardiac fibrosis. It is notable that despite the known effect of CXCL12 in cardioprotection after MI,[10,11,28] endothelial CXCR7 dele-

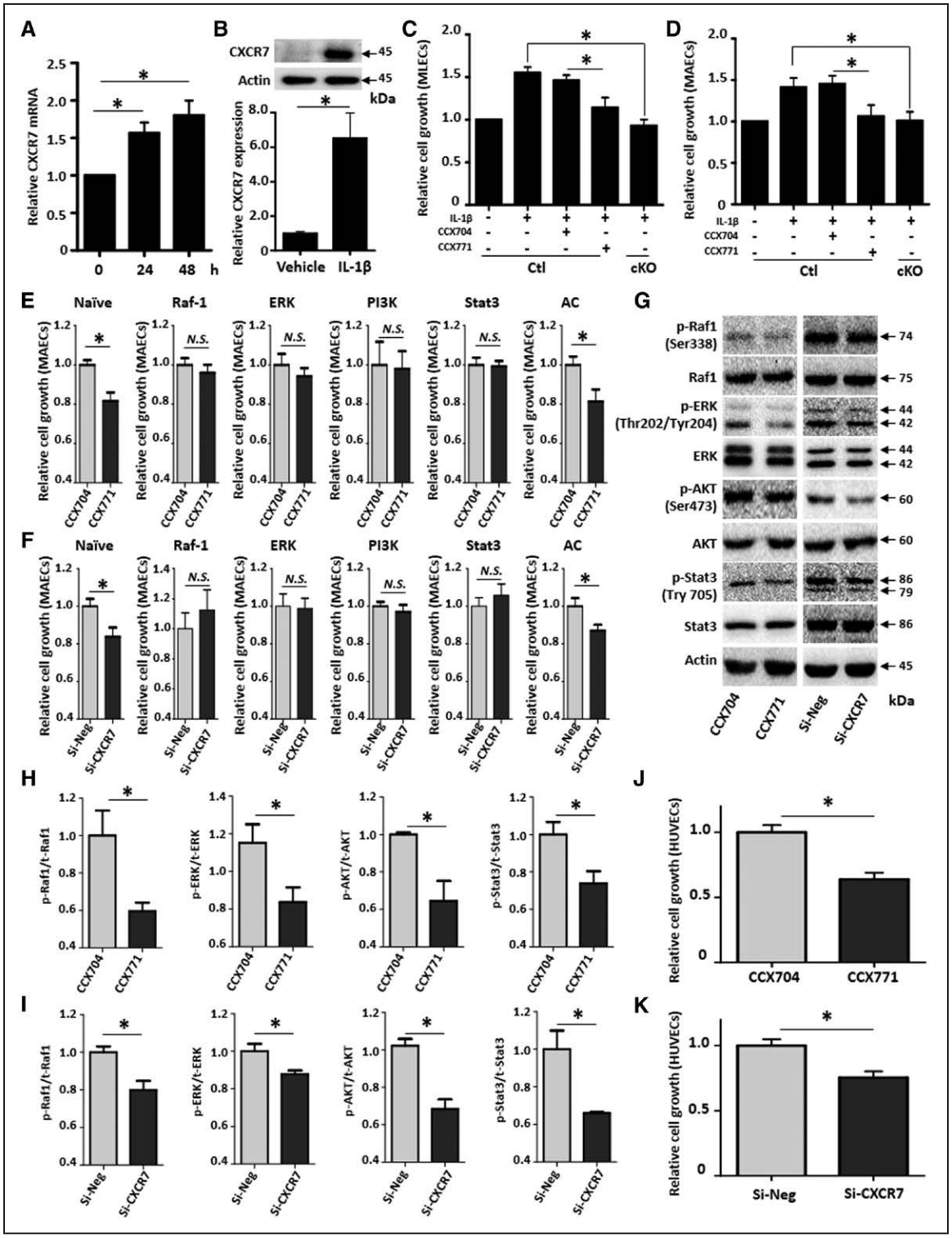

Figure 4. CXCR7 blockade impaired endothelial proliferation in vitro.
Interleukin (IL)-1β (10 ng/mL) treatment increased CXCR7 mRNA (**A**) and protein (**B**) level in mouse lung endothelial cells (MLECs). IL-1β (10 ng/mL) promoted cell growth. Pharmacological inhibition (CCX771) or genetic deletion of CXCR7 suppressed the cell proliferation in both MLECs (**C**) and mouse aortic endothelial cells (MAECs; **D**). In MAECs, inhibition of Raf1, extracellular signal-regulated kinase (ERK), phosphatidylinositol-3-kinase (PI3K), and signal transducer and activator of transcription 3 (Stat3), but not adenylate cyclase (AC), abolished the inhibitory effect of CCX771 (**E**) or CXCR7 knockdown (**F**) on cell proliferation. Western blot analysis (**G**) showed that phosphorylation of Raf1, ERK, AKT, and Stat3 was reduced by CCX771 (**H**) or CXCR7 knockdown (**I**). In human umbilical vein endothelial cells, with the stimulation of IL-1β, CCX771 (**J**) or CXCR7 knockdown (**K**) decreased cell proliferation. Each experiment was performed no less than 3 times. **P*<0.05. ***P*<0.01.

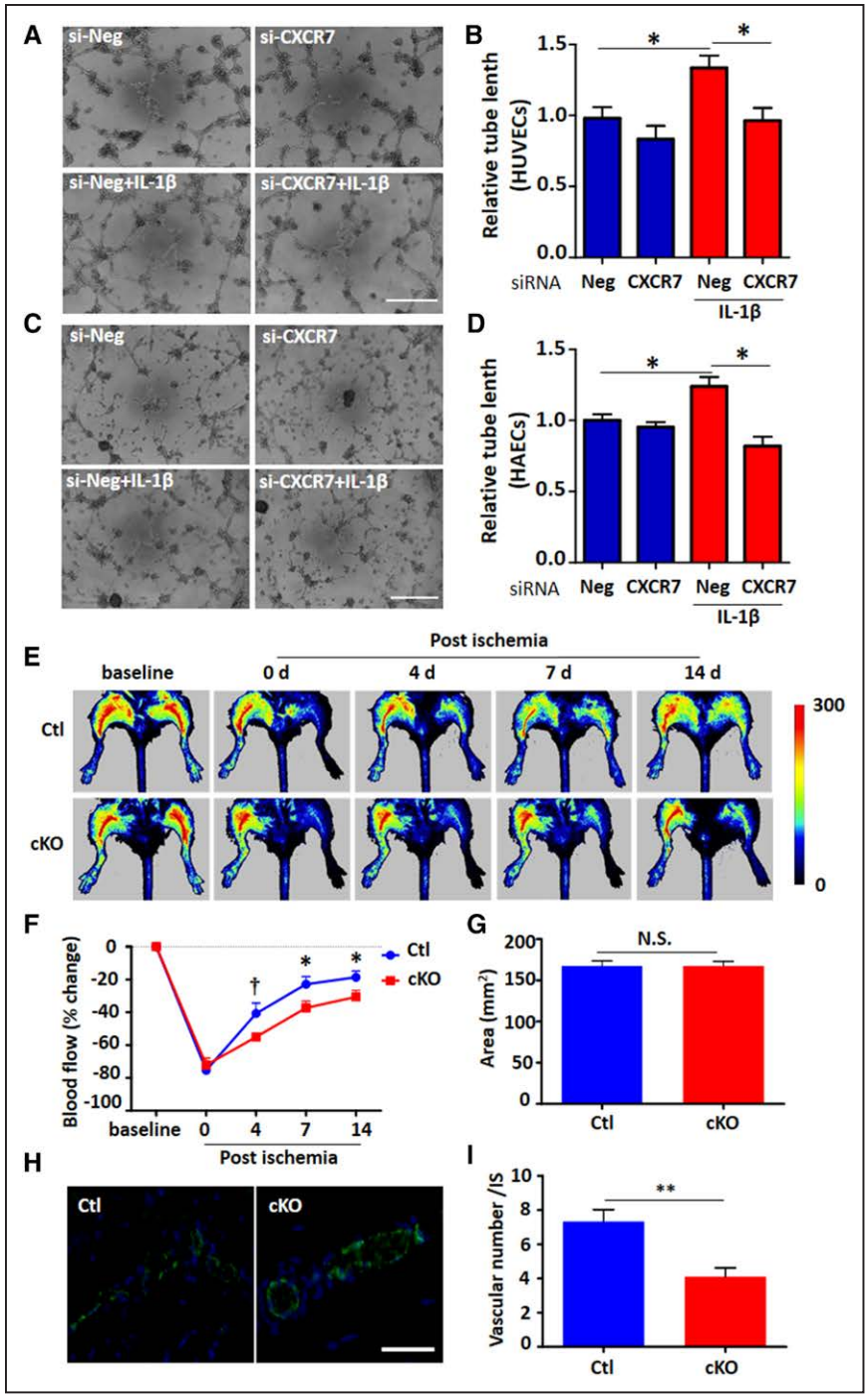

Figure 5. Endothelial deletion of CXCR7 attenuated angiogenesis and functional recovery of blood flow in mice that were subjected to hind-limb ischemia.

Tube formation was analyzed in human umbilical vein endothelial cells (HUVECs; **A** and **B**) or human aorta endothelial cells (HAECs; **C** and **D**) that were pretreated with or without interleukin (IL)-1β for 6 hours and transfected with si-CXCR7 or negative si-RNA (si-Neg). Bar=500 μm. The blood flow of the mice subjected to hind-limb ischemia was monitored at indicated time points in a blinded manner (**E**). Endothelial deletion of CXCR7 attenuated hind-limb blood flow recovery (**F**; n=8). The monitored hind-limb area was the same between the 2 groups (**G**). On day 21 after ischemia, the vascular density in spatium intermusculare (IS) of gastrocnemius was detected via immunostaining of von Willebrand factor (**H;** green). DAPI (blue) stained the nucleus. The average vascular number in spatium intermusculare was quantified (**I**; n=3). Bar=50 μm in **H**. **P*<0.05. ***P*<0.01. †*P*=0.053.

tion impaired post-MI cardiac function with paradoxically elevated CXCL12 levels (Figure 7).

Gain of Function of CXCR7 Affords Cardiac Protection After MI

To explore the role of gain of function of CXCR7 in MI, we constructed a recombinant adenovirus that expresses CXCR7 and injected the viral particles into the left ventricular cavity after MI (Figure 8A–8D). Compared with the control vector, delivery of the adenovirus that expresses CXCR7 improved heart function and reduced infarct size after MI (Figure 8C and 8D and Table III in the online-only Data Supplement).

TC14012, a CXCR7 agonist with CXCR4-antagonizing activity,[29] dose dependently promoted endothelial cell proliferation in vitro (Figure 8E). TC14012 modulated the proliferation signaling pathways (Figure X in the online-only Data Supplement) similarly to those affected by CXCR7 blockade (Figure 4E and 4F). It is impressive that TC14012 treatment significantly reduced the infarct size after MI (Figure 8F and 8G).

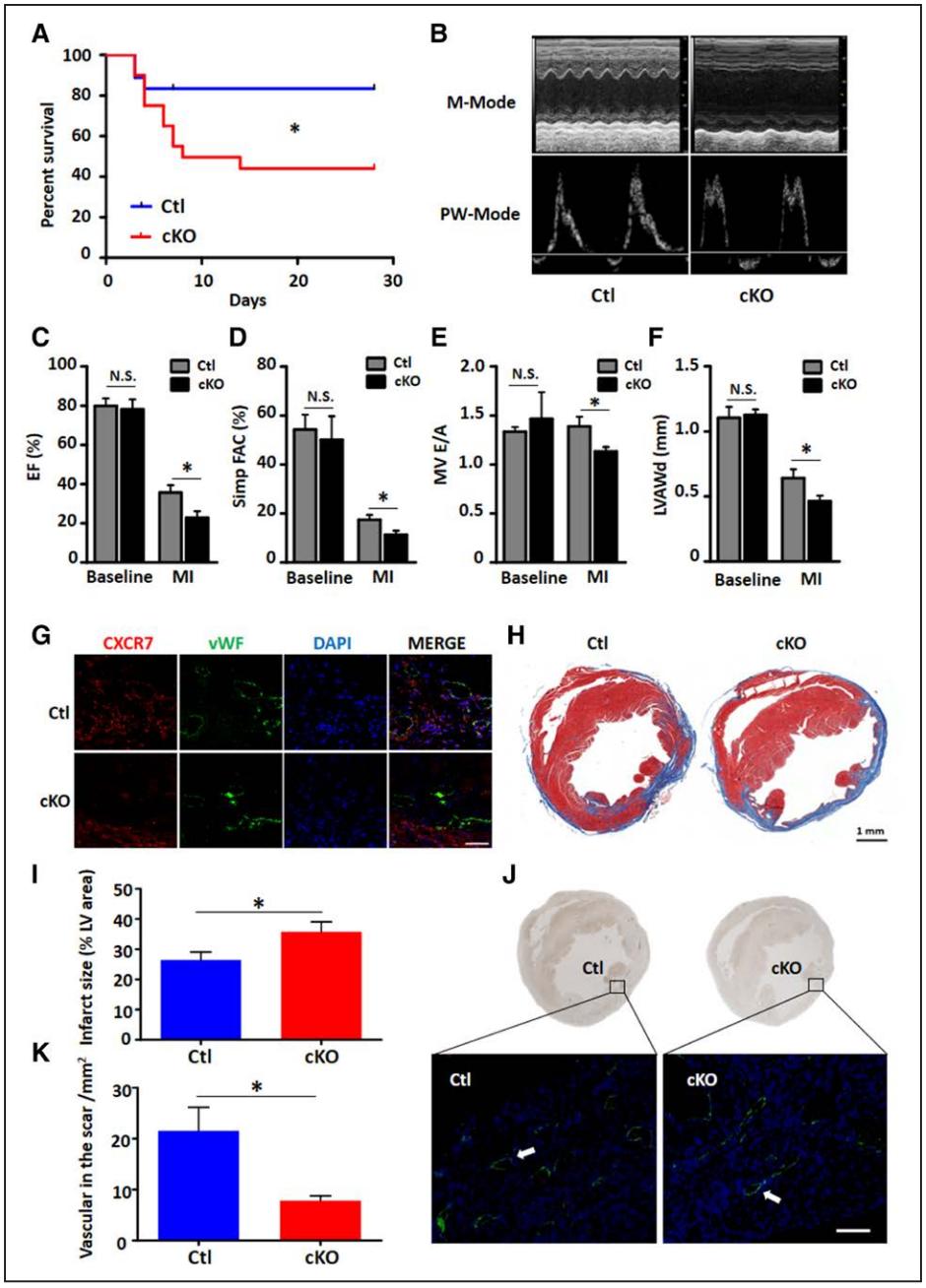

Figure 6. Endothelial CXCR7 deletion in mice impaired cardiac function, reduced survival rate, and increased infarction size after myocardial infarction (MI).

Survival curve (**A**; Kaplan-Meier method) indicates that conditional knockout (cKO) mice had reduced survival time and a greater cumulative death rate within 30 days after MI (n=18 cKO, 20 control [Ctl]). Mouse cardiac function was evaluated in a blinded study 7 days after MI surgery by an ultrasound professional. **B**, Representative echocardiograph from Ctl and cKO mice. Ejection fraction (EF; **C**), left ventricular fractional area change (FAC; **D**), mitral ratio of peak early to late diastolic filling velocity (E/A; **E**), and left ventricular end-diastolic anterior wall thickness (LVAWd; **F**) were all decreased in cKO mice (**C**–**F**: *t* test; n=12 each group). Immunofluorescent staining of the infarcted heart showed endothelial expression of CXCR7 in the Ctl mice but not in the cKO mice (**G**). Masson staining of the hearts isolated 28 days after MI surgery shows enhanced infarct size in the cKO group (**H** and **I**; n=8 cKO, 9 Ctl). Immunostaining of endothelial cells (von Willebrand factor positive [vWF+], green; **arrows** show the representative vasculature) in the ischemic area revealed significantly reduced vascular density in cKO (**J** and **K**; n=3). Bar=50 μm in **G** and **J**. **P*<0.05.

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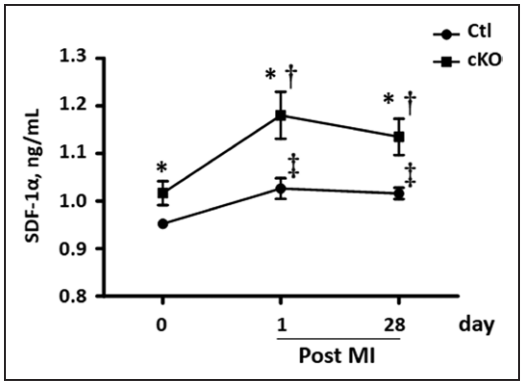

Figure 7. Elevated plasma CXCL12 in mice after myocardial infarction (MI).
MI increased plasma levels of CXCL12 in both control (Ctl) and conditional knockout (cKO) mice. Compared with Ctl, cKO exhibited higher plasma CXCL12 levels before (denoted as 0) and after MI. SDL-1α indicates stromal cell–derived factor-1α. **P*<0.05 vs Ctl. †*P*<0.05 vs 0 in cKO. ‡*P*<0.05 vs 0 in Ctl.

DISCUSSION

Endothelial CXCR7 Regulates Vascular Homeostasis and Cardiac Remodeling Independently of Lipid Traits

This study demonstrates that CXCR7 plays a key role in maintaining endothelial integrity, through which it regulates vascular response to endothelial denudation injury and cardiac remodeling after MI. Loss of endothelial CXCR7 promoted vascular stenosis (Figure 2), an effect attributable to attenuated endothelial repair (Figure 3). This in vivo observation is compatible with a direct signaling effect of CXCR7 in promoting endothelial proliferation (Figure 4). Angiogenesis, an endothelial cell–dependent process by which new blood vessels are formed, is essential in revascularization after MI[26] for promoting tissue regeneration after ischemic insult. As shown here, endothelial CXCR7 plays a critical role in ischemia-induced angiogenesis (Figure 5). It is important to note that loss of this signal resulted in functional and structural impairment in the heart after MI (Figure 6). Furthermore, loss of endothelial CXCR7 modulates vascular homeostasis without altering blood lipids (Table), which is different from global deletion of CXCR7 in a hyperlipidemic mouse model.[22] It is interesting that genome-wide association studies of blood lipid levels fail to detect a signal from CXCL12 pathway[30] despite its consistent association with coronary artery disease and MI.[1] Therefore, endothelial CXCR7 constitutes a novel regulator of vascular homeostasis and possesses sufficient capacity to affect cardiac function after MI independently of lipid traits.

The function of CXCR7 in promoting endothelial cell proliferation and angiogenesis is particularly associated with inflammatory condition. CXCR7 is inducible by both IL-1β and tumor necrosis factor-α and promotes endothelial proliferation and angiogenesis in the presence of these inflammatory cytokines (Figures 4 and 5 and Figures VI and IX in the online-only Data Supplement). This is also consistent with our in vivo observation of hind-limb ischemia (Figure 5) and MI (Figure 6) in which inflammation is an intrinsic pathological component.

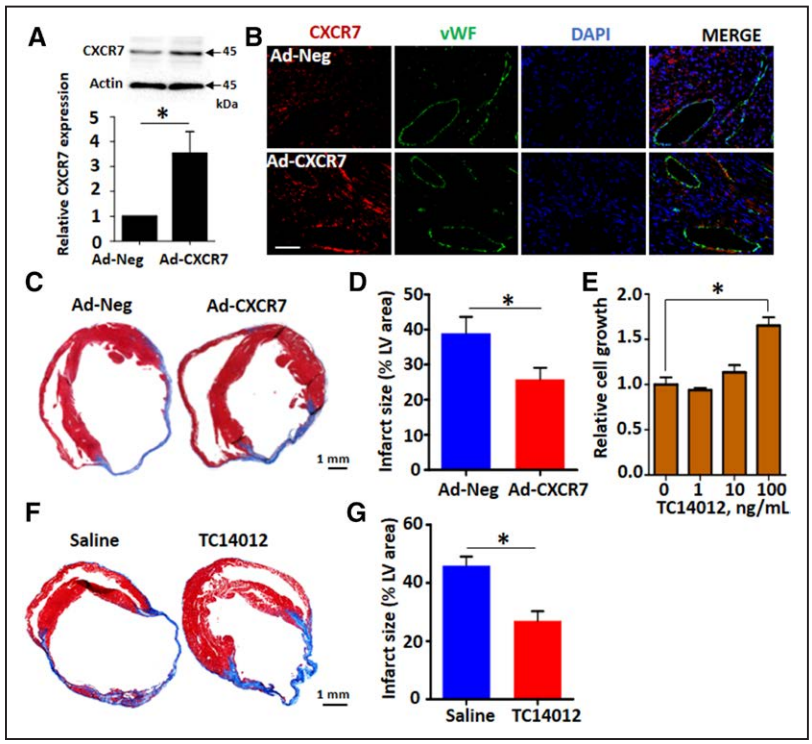

Figure 8. Adenoviral delivery of *CXCR7* gene or pharmacological CXCR7 activation protected adverse cardiac remodeling after myocardial infarction (MI).
Recombinant adenovirus expressing CXCR7 (Ad-CXCR7) was constructed, and the expression of CXCR7 protein was verified in 293T cell lines (**A**). Mice were injected via the left ventricle (LV) with adenovirus negative for CXCR7 (Ad-Neg) or Ad-CXCR7, and tissue expression of CXCR7 was examined (**B**; bar=50 μm). Infarct size was decreased in mice injected with Ad-CXCR7 (**C** and **D**; n=10 Ad-Neg, 11 Ad-CXCR7). TC14012, a CXCR7 agonist, promoted the proliferation of mouse aortic endothelial cells that were treated with interleukin-1β (**E**). Post-MI treatment of TC14012 reduced the infarct size in mice (**F** and **G**; n=6 saline, 7 TC14012). **P*<0.05.

CXCR7 in Post-MI Remodeling: Beyond the CXCL12-CXCR4 Axis

It is known that CXCL12-CXCR4 axis promotes myocyte survival and angiogenesis, and clinical delivery of CXCL12 represents a promising strategy for treating ischemic heart disease.[7,8,27,28] The function of CXCL12 has been thought to be mediated via its exclusive partner, CXCR4. In this study, endothelium-restricted deletion of CXCR7, the second receptor for CXCL12 that was most recently deorphanized, impaired post-MI heart function and increased cardiovascular mortality and infarct size, coinciding with suppressed vascular density in the ischemic region. More impressive is that these changes occurred with elevated CXCL12 levels (Figure 7), which, presumably, allows enhanced availability to CXCR4. Hence, the proangiogenic and cardiac protective effect of endogenous CXCL12-CXCR4 axis in MI appears to be superimposed by the loss of endothelial CXCR7. Actually, previous studies show a complex picture of the role of CXCR4 in the cardiac function after MI. Hypomorphic mutation of CXCR4 ($CXCR4^{+/-}$) reduced infarct size and angiogenesis after MI without changing postinfarction systolic dysfunction, which was associated with higher levels of cardiomyocyte-protective phosphatidylserine in the infarcted heart.[9] Tissue-specific disruption of CXCR4 in cardiomyocytes did not affect cardiac remodeling or vascular density after MI.[31] Our data indicate that CXCR7 is essential during the functional recovery of heart after MI (Figure 6), likely through its role in promoting inflammation/injury–stimulated endothelial proliferation and ischemia-induced angiogenic response (Figures 4 and 5).

Another intriguing explanation might be that CXCR7 deletion abrogates a function of CXCR4/CXCR7 heterodimer on the endothelium, as inferred from an in vitro overexpression system.[32] Nevertheless, CXCR7 knockdown inhibited endothelial cell proliferation and angiogenesis without affecting CXCR4-dependent calcium release or migration (Figure VI in the online-only Data Supplement). These data clearly demonstrate a distinctive role of CXCR7 in the examined endothelial cell function, although the current evidence cannot rule out possibilities of a direct functional interaction between CXCR7 and CXCR4 under other conditions. Further studies indicate that β-arrestin2 is required in transducing CXCR7 signal inside endothelial cells, which may activate proliferation pathways (Figure 4 and Figures VI and XI in the online-only Data Supplement).

Conclusions

Endothelial CXCR7 regulates vascular homeostasis, which limits pathological vascular response to endothelial injury and affords cardiac protection after MI. This is mechanistically attributable to a direct signaling effect of CXCR7 in promoting endothelial proliferation and angiogenesis. This discovery provides a new dimension toward understanding of the causality issues among CXCL12 genetic locus, plasma levels, and cardiovascular risk.[33–35] The function of endothelial CXCR7 is schematically summarized in Figure XII in the online-only Data Supplement. CXCR7 activation/overexpression may be a new therapeutic strategy for clinical restenosis after percutaneous coronary intervention and for heart failure after MI.

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DISCLOSURES

None.

AFFILIATIONS

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FOOTNOTES

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