Am J Physiol Heart Circ Physiol 310: H705–H715, 2016.
First published January 22, 2016; doi:10.1152/ajpheart.00587.2015.

Targeted delivery of human iPS-ECs overexpressing IL-8 receptors inhibits neointimal and inflammatory responses to vascular injury in the rat

Samantha Giordano,[1] Xiangmin Zhao,[2] Daisy Xing,[1] Fadi Hage,[1,3] Suzanne Oparil,[1] John P. Cooke,[4] Jieun Lee,[5] Karina H. Nakayama,[6,7] Ngan F. Huang,[6,7,8] and Yiu-Fai Chen[1]

[1]*Vascular Biology and Hypertension Program, Division of Cardiovascular Disease, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama;* [2]*Department of Pulmonary, Critical Care, Sleep and Allergy, College of Medicine, University of Illinois at Chicago, Chicago, Illinois;* [3]*Division of Cardiology, Birmingham Veterans Affairs Medical Center, Birmingham, Alabama;* [4]*Houston Methodist Research Institute, Houston, Texas;* [5]*Division of Cardiovascular Medicine, Stanford University, Stanford, California;* [6]*Cardiovascular Institute, Stanford University, Stanford, California;* [7]*Veterans Affairs Palo Alto Health Care System, Palo Alto, California; and* [8]*Department of Cardiothoracic Surgery, Stanford University, Stanford, California*

Submitted 24 July 2015; accepted in final form 14 January 2016

Giordano S, Zhao X, Xing D, Hage F, Oparil S, Cooke JP, Lee J, Nakayama KH, Huang NF, Chen YF. Targeted delivery of human iPS-ECs overexpressing IL-8 receptors inhibits neointimal and inflammatory responses to vascular injury in the rat. *Am J Physiol Heart Circ Physiol* 310: H705–H715, 2016. First published January 22, 2016; doi:10.1152/ajpheart.00587.2015.—Interleukin-8 (IL8) is highly expressed by injured arteries in a variety of diseases and is a chemoattractant for neutrophils which express IL8 receptors IL8RA and RB (IL8RA/B) on their membranes. Neutrophils interact with the damaged endothelium and initiate an inflammatory cascade at the site of injury. We have generated a novel translational targeted cell therapy for acute vascular injury using adenoviral vectors to overexpress IL8RA/B and green fluorescent protein (GFP) on the surface of endothelial cells (ECs) derived from human induced pluripotent stem cells (HiPS-IL8RA/B-ECs). We hypothesize that HiPS-IL8RA/B-ECs transfused intravenously into rats with balloon injury of the carotid artery will target to the injured site and compete with neutrophils, thus inhibiting inflammation and neointima formation. Young adult male Sprague-Dawley rats underwent balloon injury of the right carotid artery and received intravenous transfusion of saline vehicle, 1.5×10^6 HiPS-ECs, 1.5×10^6 HiPS-Null-ECs, or 1.5×10^6 HiPS-IL8RA/B-ECs immediately after endoluminal injury. Tissue distribution of HiPS-IL8RA/B-ECs was analyzed by a novel GFP DNA qPCR method. Cytokine and chemokine expression and leukocyte infiltration were measured in injured and uninjured arteries at 24 h postinjury by ELISA and immunohistochemistry, respectively. Neointimal, medial areas, and reendothelialization were measured 14 days postinjury. HiPS-IL8RA/B-ECs homed to injured arteries, inhibited inflammatory mediator expression and inflammatory cell infiltration, accelerated reendothelialization, and attenuated neointima formation after endoluminal injury while control HiPS-ECs and HiPS-Null-ECs did not. HiPS-IL8RA/B-ECs transfused into rats with endoluminal carotid artery injury target to the injured artery and provide a novel strategy to treat vascular injury.

targeted cell therapy; human induced pluripotent stem cells; endothelial cells; vascular injury; inflammation vascular injury; restenosis

NEW & NOTEWORTHY

This novel study uses endothelial cells derived from human induced pluripotent stem cells (HiPS-ECs) as proof of concept for autologous cell therapy for vascular damage by targeting cells to the area of inflammation via the overexpression of interleukin 8 (IL-8) receptors on the cell surface.

INFLAMMATION plays an integral role in many forms of vascular disease, including the response to acute endoluminal vascular injury (20, 22). Inflammatory mediators, including cytokines, chemokines, and adhesion molecules, are the main effectors of the early inflammatory response. Our previous studies have demonstrated increased expression of a variety of chemokines, e.g., cytokine-induced neutrophil chemoattractant-2β [CINC-2β, equivalent to interleukin 8 (IL8) in humans] and monocyte chemoattractant protein-1 (MCP-1), in the balloon injured rat carotid artery within 2 h of the insult (13). The increased chemokine expression was followed by the infiltration of a variety of cell types, including neutrophils and monocytes/macrophages, into the adventitial and periadventitial domains of the injured artery within 24 h postinjury (11, 21). Further, we observed migration of adventitial fibroblasts through the medial layer into the neointima at 7–14 days postinjury (11, 14).

IL8 is highly expressed and released by the injured vasculature and functions as a chemoattractant for neutrophils, which express IL8 receptors A and B (IL8RA/B) (also called CXCR1 and CXCR2, respectively) on their membranes. Neutrophils interact with the damaged endothelium at the site of injury and initiate a proinflammatory cascade, causing monocyte/macrophage and T cell recruitment to the injured vessel (12, 21). Previous works have shown that after endoluminal injury there is a significant increase in IL8 (12, 21). Our laboratory has overexpressed IL8RA/B on the surface of rat aortic endothelial cells (IL8RA/B-RAECs) to target the RAECs to sites of injury, thus competing with neutrophils and attenuating the inflammatory response and the extent of vascular damage (5, 20, 24). To test the targeting effects of overexpression of IL8RA/B on RAECs in vivo, we utilized the rat carotid balloon injury model which has previously been shown to stimulate IL8 expression in the injured artery (20). Transfusion with IL8RA/B-RAECs caused decreases in inflammatory cytokine expression, neutrophil and monocytes/macrophage infiltration, and neointima formation in balloon-injured arteries of rats compared with injured arteries of rats treated with empty adenovirus (Null) transduced RAECs (Null-RAECs) or RAECs overexpressing either IL8RA or RB alone (20).

Address for reprint requests and other correspondence: Y.-F. Chen, 1008 Zeigler Research Bldg., 703 19th St. South, Dept. of Medicine, Univ. of Alabama at Birmingham, Birmingham, AL 35294 (e-mail: yfchen@uab.edu).

In the current study, we extended the targeted cell delivery strategy from adult IL8RA/B-RAECs to a stem cell type, ECs derived from human induced pluripotent stem cells (HiPS-EC), which is more applicable for future translational and/or clinical studies. We tested the effectiveness of HiPS-ECs transduced with adenoviruses to overexpress IL8RA/B (HiPS-IL8RA/B-ECs) in attenuating the inflammatory response, accelerating reendothelialization, and inhibiting neointima formation in the rat carotid injury model. Using a novel PCR detection method, we demonstrated that intravenously transfused HiPS-IL8RA/B-ECs are highly localized to the injured carotid artery. Further, we demonstrated, for the first time, that xenotransplantation of HiPS-IL8RA/B-ECs inhibited inflammation and decreased neointima formation in the rat carotid injury model.

MATERIALS AND METHODS

Animal and procedures. Twelve-week-old male Sprague-Dawley rats (Charles River Laboratories) were maintained at constant humidity (60 ± 5%), temperature (24 ± 1°C), and light cycle (6 AM to 6 PM) and fed a standard rat pellet diet ad libitum (5, 20, 24). Protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Alabama at Birmingham (UAB) and were consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Rats were weighed before surgery and before being euthanized at either 24 h or 14 days postinjury.

One day before injury, rats were anesthetized with ketamine and xylazine (80 and 5 mg/kg, respectively) and a PE-10 cannula was placed in the right femoral vein (15). On the following day, rats were anesthetized with ketamine and xylazine and underwent balloon injury of the right carotid artery (RCA); the left carotid artery (LCA) was used as an uninjured control (3). Human induced pluripotent stem cell-endothelial cells (HiPS-ECs), HiPS-ECs transduced with empty (Null) adenovirus (HiPS-Null-EC), or HiPS-ECs overexpressing IL8RA/B (HiPS-IL8RA/B-ECs) (total 1.5×10^6 cells/rat) were transfused via the femoral vein cannula 1, 3 and 5 h (0.5×10^6 cells in 500 μl saline for each injection) postinjury.

Generation of HiPS-ECs. HiPS-ECs were generated and characterized according to our previous publications (7, 8, 17, 18, 23). HiPS cells were derived from human foreskin fibroblasts using the retroviral constructs encoding the Yamanaka factors (19). To differentiate the HiPS cells into HiPS-ECs, cells were cultured in ultra-low attachment dishes containing differentiation media for 4 days to form embryoid bodies (EBs). EBs were seeded on 0.2% gelatin-coated dishes and grown in differentiation media for an additional 10 days. Single cell suspensions were incubated with PE-conjugated anti-human CD31 antibody, and flow cytometry was used to purify the ECs. EC derivation was confirmed by antibody staining for EC markers, including von Willebrand factor (vWF), vascular endothelial-cadherin (VE-cadherin), endothelial nitric oxide synthase (eNOS), and CD31. The purity of the HiPS-EC was >90% by phenotype and vWF and CD31 immunostaining (see Figs. 1 and 3).

Plasmid, adenoviral vector generation, and HiPS-EC transduction. HiPS-ECs overexpressing IL8RA/B were generated using adenoviral vectors containing IL8RA or RB and green fluorescent protein (GFP) genes using the AdEasy Adenoviral Vector System (Stratagene) as previously described (6, 20).

HiPS-ECs were grown in EC growth medium (cat. no. MCDB-131C, Vec Technologies) to 80% confluence and treated with a mixture of adenoviruses containing IL8RA-GFP and IL8RB-GFP or adenovirus containing only GFP (Null-GFP) as a control. Eighteen hours after adenoviral transduction, fresh media was added to the cells, and 48 h after transduction fluorescence imaging of the transduced ECs exhibited green fluorescence, confirming GFP expression.

In vitro characterization of HiPS-ECs transduced with IL8RA-GFP, IL8RB-GFP, or Null-GFP adenoviruses. To confirm the expression of IL8RA/B in transduced HiPS-ECs, cells were fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100 in PBS, and stained with selective primary antibodies against IL8RA (cat. no. ab60254, Abcam) or IL8RB (cat. no. ab24963, Abcam), and a Texas Red-labeled goat anti-mouse IgG secondary antibody (cat. no. 115-

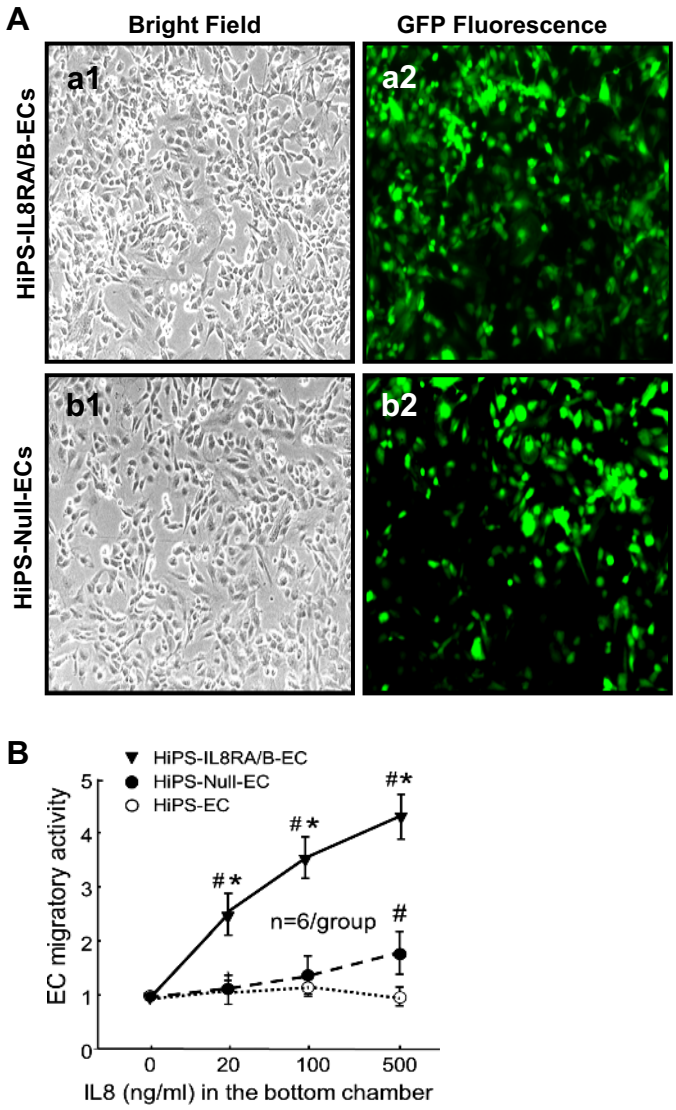

Fig. 1. *A*: adenoviral transduction of human induced pluripotent stem cell-endothelial cells (HiPS-ECs) and overexpression of interleukin 8 receptor A (IL8RA) and receptor B (IL8RB) in transduced HiPS-ECs. Bright field (*Aa1*) and fluorescence photomicrographs (*Aa2*) of ECs derived from human induced pluripotent stem cells (HiPS-ECs) 48 h after transduction with adenoviruses carrying IL8RA or RB genes (HiPS-IL8RA/B-ECs) and green fluorescent protein (GFP) gene. *Ab1* and *Ab2*: HiPS-Null-ECs are HiPS-ECs transduced with empty (Null) adenovirus with GFP gene. Over 90% of the cells were GFP positive after transduction (transduction efficiency: > 90%). *B*: HiPS-IL8RA/B-ECs migration toward IL8 in vitro. In vitro migratory activities of HiPS-IL8RA/B-ECs, HiPS-Null-ECs, and HiPS-ECs (without adenoviral transduction) toward IL8 were assessed by Boyden double-chamber technique. The bottom chambers contained graded concentrations of IL8 and the monolayer of ECs in the top chambers were incubated for 12 h. Data are plotted with the baseline migration of HiPS-ECs as 1 toward various concentrations of IL8 (*x*-axis in log scale). Results are means ± SE. #$P < 0.05$ compared with respective untransduced HiPS-EC groups (open circles). *$P < 0.05$ compared with respective HiPS-Null-EC groups (closed circles).

075-003, Jackson ImmunoResearch Lab) and counterstained with DAPI (4′,6-diamidino-2-phenylindole, Sigma) as a nuclear marker (20). Stained ECs were mounted for fluorescent microscopic analysis with a computerized Zeiss-Axioskop system.

A Boyden chamber assay was used to assess the effects of overexpression of IL8RA/B on migratory activity of HiPS-ECs as described previously (20). Briefly, HiPS-IL8RA/B-ECs, HiPS-Null-EC, or nontransduced HiPS-ECs were plated in the top well of the Boyden chamber and then exposed to various concentrations of IL8 in the bottom well. Cell migration was determined by the number of cells that migrated from the top to the bottom well in 12 h.

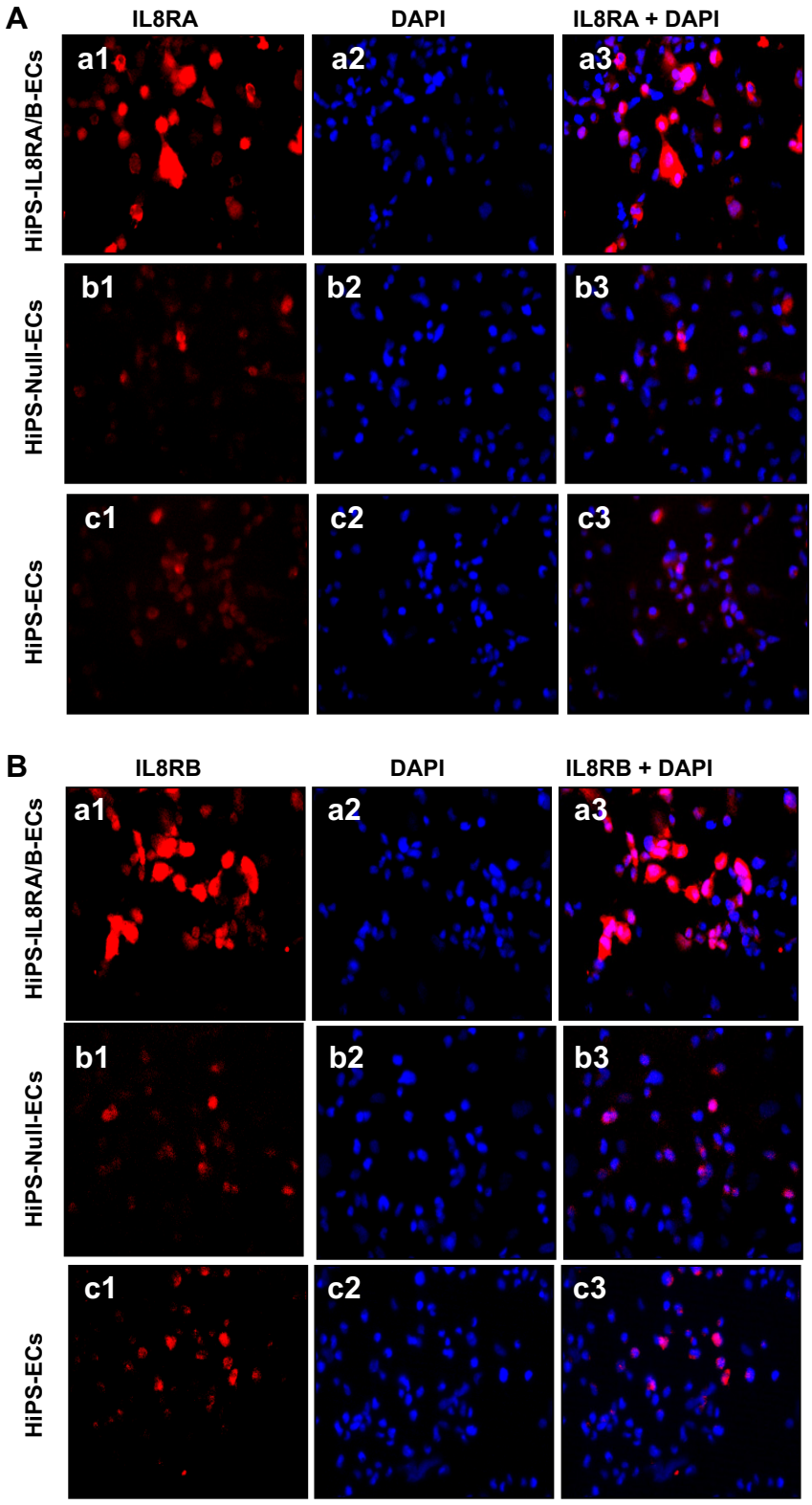

Fig. 2. IL8RA and RB expression in HiPS-ECs with or without adenoviral IL8RA/B transduction. *A*: fluorescence micrographs showing expression of IL8RA (red color, immunostained with IL8RA primary antibody) in HiPS-IL8RA/B-ECs (*a1*), HiPS-Null-ECs (*b1*), and HiPS-ECs (*c1*) (without transduction) 48 h after adenoviral transduction. *a2*, *b2*, and *c2*: DAPI staining of the nuclei of cells in *a1*, *b1*, and *c1*, respectively. *a3*, *b3*, and *c3*: merged images of IL8RA and DAPI, respectively. *B*: fluorescence micrographs showing expression of IL8RB (red color, immunostained with IL8RB primary antibody) in HiPS-IL8RA/B-ECs (*a1*), HiPS-Null-ECs (*b1*), and HiPS-ECs (*c1*) 48 h after adenoviral transduction. *a2*, *b2*, and *c2*: DAPI staining of the nuclei of cells in *a1*, *b1*, and *c1*, respectively. *a3*, *b3*, and *c3*: merged images of IL8RB and DAPI, respectively.

In vivo cell transfusion regimen. HiPS-IL8RA/B-ECs or HiPS-Null-ECs were grown in 100-mm culture dishes until >90% of cells expressed GFP. Cells were washed with 0.9% saline, collected with a cell scraper, dispersed by gentle pipetting and concentrated by centrifugation at 370 *g*. Cells from a single culture dish were resuspended in 1.5 ml saline. Following carotid artery injury, rats were randomly divided into 4 groups which received *1*) saline (Vehicle-control), *2*) HiPS-ECs (nontransduced EC control), *3*) HiPS-Null-ECs (ECs transduced with empty adenovirus), or *4*) HiPS-IL8RA/B-ECs (ECs transduced with adenoviruses carrying IL8RA or RB constructs). Each rat

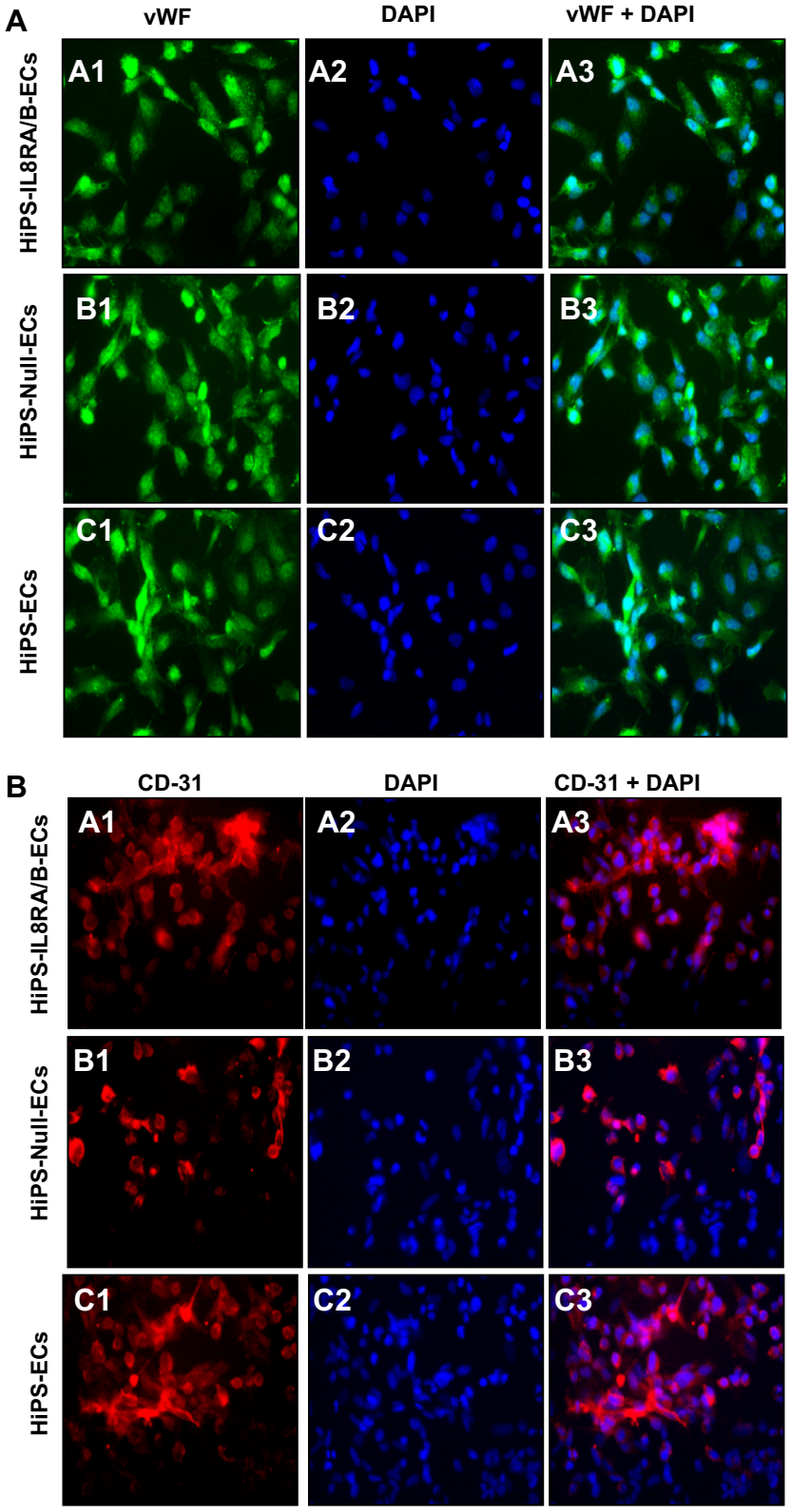

Fig. 3. vWF and CD31 expression in HiPS-ECs with or without adenoviral IL8RA/B transduction. *A*: fluorescence micrographs showing expression of vWF (green color, immunostained with vWF primary antibody) in HiPS-IL8RA/B-ECs (*A1*), HiPS-Null-ECs (*B1*), and HiPS-ECs (C*1*) (without transduction) 48 h after adenoviral transduction. *A2*, *B2*, and *C2*: DAPI staining of the nuclei of cells in *A1*, *B1*, and *C1*, respectively. *A3*, *B3*, and *C3*: merged images of vWF and DAPI, respectively. *B*: fluorescence micrographs showing expression of CD31 (red color, immunostained with CD31 primary antibodies) in HiPS-IL8RA/B-ECs (*A1*), HiPS-Null-ECs (*B1*), and HiPS-ECs (*C1*) 48 h after adenoviral transduction. *A2*, *B2*, and *C2*: DAPI staining of the nuclei of cells in *A1*, *B1*, and *C1*, respectively. *A3*, *B3*, and *C3*: Merged images of CD31 and DAPI, respectively.

(n = 6–10/group) was transfused with a total of 1.5×10^6 cells in 1.5 ml of saline through the femoral venous cannula at 1, 3, and 5 h after carotid artery injury. Vehicle-control rats received 1.5 ml of saline.

In vivo HiPS-EC tracking. One group of rats was euthanized 24 h after carotid injury and transfused with HiPS-IL8RA/B-ECs, HiPS-Null-ECs, or HiPS-ECs for cell tracking. The injured right carotid artery (RCA) and uninjured left carotid artery (LCA) were harvested, DNA was extracted, and real-time qPCR was used for quantitation of the GFP DNA. The levels of GFP DNA were used as an index of the distribution of HiPS-ECs transduced with IL8RA/B or Null adenoviruses in the RCA and LCA. GAPDH DNA was used to normalize the data. Data were normalized to the injured RCA, and background signals of the nontransduced HiPS-EC and vehicle controls were subtracted. GFP DNA in whole RCA and LCA was measured with real-time qPCR. The GFP/GAPDH DNA ratios were normalized using the average value in RCA of HiPS-IL8RA/B group as 1. Values in the RCA and LCA of the vehicle control group (transfused with saline) represent the background noise of PCR. The PCR primers for GFP were forward 5′-ATC CTG TTA CCA GTG GCT GC-3′ and reverse 5′-CGC TTA CCG GAT ACC TGT CC-3′; and for GAPDH were forward 5′-TGC CAC TCA GAA GAC TGT GG-3′ and reverse 5′-ACG GAT ACA TTG GGG GTA GG-3′.

Immunohistochemical imaging of GFP and CD31 was conducted in paraffin-embedded cross sections of injured RCA using primary antibodies against CD31 (cat. no. ab28364, Abcam) and GFP (cat. no. MS128-P1ABX, Thermo Scientific) and anti-mouse IgG (cat. no. BA-2000, Vector Laboratories), anti-rabbit IgG (cat. no. BA-2000, Vector Laboratories) secondary antibodies and the nova-RED detection kit (cat. no. SK-4800, Vector Laboratories).

To determine cell attachment to the endoluminal surface of the injured RCA in vivo, a rat received intravenous transfusion of 1.5×10^6 HiPS-IL8RA/B-ECs immediately after injury. The RCA was harvested 30 min after transfusion, cut longitudinally and imaged via confocal fluorescence microscopy. GFP fluorescence showed HiPS-IL8RA/RB-EC bound to the endoluminal surface of the artery.

Immunohistochemical assessment of infiltration of neutrophils and monocytes/macrophages into injured carotid arteries. Injured RCA and uninjured LCA from a second group of rats were formalin perfusion-fixed at 24 h postinjury and embedded in paraffin for immunohistochemical analysis as described previously (20). Neutrophils and monocytes/macrophages were immunostained with specific antibodies against myeloperoxidase (MPO) (cat. no. sc-59600 Santa Cruz Biotechnology) and ED1 (cat. no. MCA341R Ab Serotec), respectively.

Real-time qRT-PCR analysis for mRNA quantitation of leukocyte chemoattractants. Rats were euthanized 24 h postinjury and RNA was extracted from the RCA and LCA, reverse transcribed to cDNA, and amplified by real-time qPCR for the neutrophil attractant CINC-2β (equivalent to IL8 in human) and the macrophage attractant MCP-1. The PCR primers for CINC-2β were forward 5′-TCAGGGACTGT-TGTGG-3′ and reverse 3′-TGACTTCTGTCTGGGTG-5′; and for MCP-1 were forward 5′-ATGCAGGTCTCTGTCACGCT-3′ and reverse 3′-GGTGCTGAAGTCCCTTAGGGT-5′ (20). Levels of specific mRNA were normalized using 18S RNA, forward 5′-GAAACGCTACCACATCC-3′ and reverse 3′-CACCAGACTTGC-CCTCCA-5′ (24).

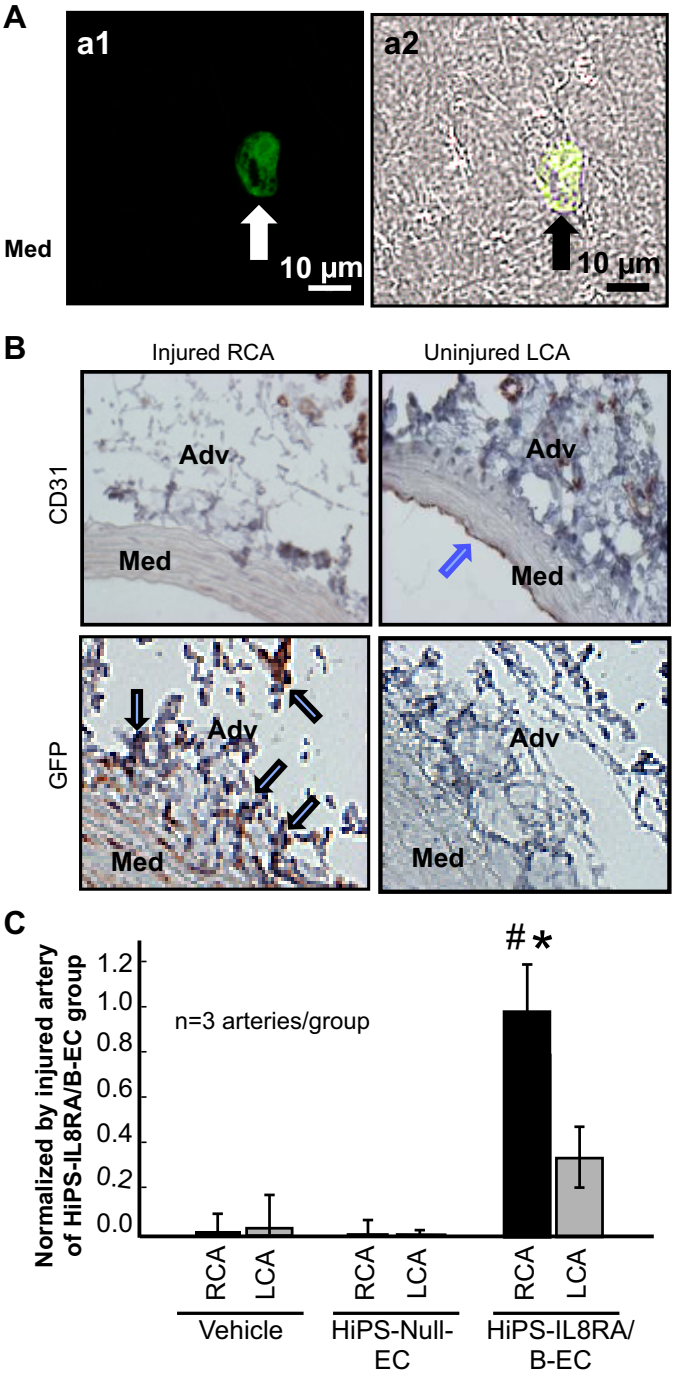

Fig. 4. *Aa1*: confocal en face microscopic imaging of an injured right carotid artery (RCA) 30 min after a HiPS-IL8RA/B-EC transfusion, showing the GFP+ cell attached to the injured endoluminal surface of RCA. *Aa2*: phase contrast micrograph showing the location of the GFP+ cell in *Aa1* on the endoluminal surface. *B*, top panels: CD31 (EC marker) immunostaining for ECs in the injured RCA and uninjured LCA 24 h postinjury. Blue arrow indicates intact endothelium (brown stain) on uninjured left carotid artery (LCA). *B*, bottom panels: GFP immunostaining of HiPS-IL8RA/B-ECs in the injured RCA and uninjured LCA 24 h postinjury. Black arrows indicate the GFP+ cells (brown stain) in the adventitia of injured RCA. Med, media; Adv, adventitia. *C*: in vivo tracking of HiPS-IL8RA/B-ECs or HiPS-Null-ECs 24 h after iv transfusion into rats with balloon injury of the RCA, using the real-time qPCR of GFP DNA technique. LCA was used as an uninjured control. Level of GFP DNA was used as an indicator of accumulated HiPS-Null-ECs or HiPS-IL8RA/B-ECs in injured RCA or uninjured LCA. Vehicle control group (no EC transfusion) was used as background signal control. GFP and GAPDH DNA levels were measured in the same real-time qPCR. The GFP DNA levels were normalized by GAPDH DNA, then standardized to mean DNA level of injured carotid arteries. Results are means ± SE. #$P < 0.05$ compared with respective HiPS-Null-EC group. *$P < 0.05$ compared with respective LCA control group.

Measurement of inflammatory mediator protein levels. Inflammatory cytokine levels were measured from protein homogenates of the injured RCA and uninjured LCA at 24 h postinjury using a multiplexed rat-specific magnetic beads-based sandwich immunoassay kit (Milliplex Rat Cytokine/Chemokine Panel, Millipore) with the Luminex xMap analyzer as described previously (5).

Morphometric analysis of neointima formation. Neointima and media areas were determined using 5-μm cross sections of the injured RCA and uninjured LCA at 28 days postinjury, as described previously (20). The computer-based Bioquant II Morphometric system was used for morphometric analysis. More than 3 elastin-stained sections from the middle third of each vessel were used for quantitation, and the measurements were averaged for statistical analysis. The cross-sectional areas of the vessel within the external elastic lamina (EEL area), within the internal elastic lamina (IEL area), and within the lumen (lumen area) were measured. Neointima formation in the injured RCA was determined by calculating the neointima-to-media area ratio.

Evans blue staining for reendothelialization. Balloon-injured carotid arteries were collected and analyzed for reendothelialization at 14 days postinjury, as described previously (20). To assess reendothelialization, rats received an intravenous injection of Evans blue dye (500 μl of a 0.1% Evans blue in saline) and then were perfused with 500 ml of 1 × PBS. Thirty min later, carotid arteries were harvested and photographed for analysis. The area of denuded endothelium was identified by blue staining. The ratio of reendothelialized region to total common carotid artery length (from aortic arch to bifurcation of internal and external carotid arteries) was calculated using ImageJ.

Statistical analysis. In vivo experiments utilized age-matched rats for all groups. All statistical analysis was completed using the SigmaStat statistical package (version 3.5). A Student's *t*-test was used for comparisons between individual groups, and one-way ANOVA analyses were used for comparison across multiple groups. When the *F* test ANOVA was statistically significant, a Tukey test was applied for further analysis. Results are presented as means ± SE. All statistically significant differences are reported when $P < 0.05$.

RESULTS

Both the Ad-Null and Ad-IL8RA/B adenoviruses have high transduction efficiency in HiPS-ECs and do not change the EC phenotype. Bright field and fluorescence (for GFP) microscopic analyses of HiPS-ECs at 48 h post transduction with Ad-Null or Ad-IL8RA/B adenoviruses showed greater than 90% transduction efficiency (Fig. 1*A*).

The Boyden chamber migratory activity assay showed a baseline level migration of the HiPS-ECs and that HiPS-IL8RA/B-ECs which overexpress IL8RA/B migrated >2.5 times faster toward IL8 than either HiPS-ECs or HiPS-Null-ECs. The HiPS-Null-ECs migrated toward IL8 only at supraphysiological concentrations of IL8 (≤500 ng/ml) (4, 9) while the nontransduced cells did not migrate toward IL8 at this concentration (Fig. 1*B*). We do not know whether HiPS-Null-ECs or HiPS-ECs can migrate toward IL8 at higher IL8 concentrations or longer incubation time conditions.

Confocal microscopic analysis of immunostaining using anti-IL8RA and anti-IL8RB primary antibodies showed that the expression of IL8RA and RB was increased in HiPS-ECs at 48 h post transduction with IL8RA/B adenoviruses (Fig. 2, *A* and *B*). HiPS-IL8RA/B-ECs exhibited increased staining of both IL8RA and RB compared with HiPS-Null-ECs and nontransduced HiPS-ECs. Low levels of IL8RA and RB were

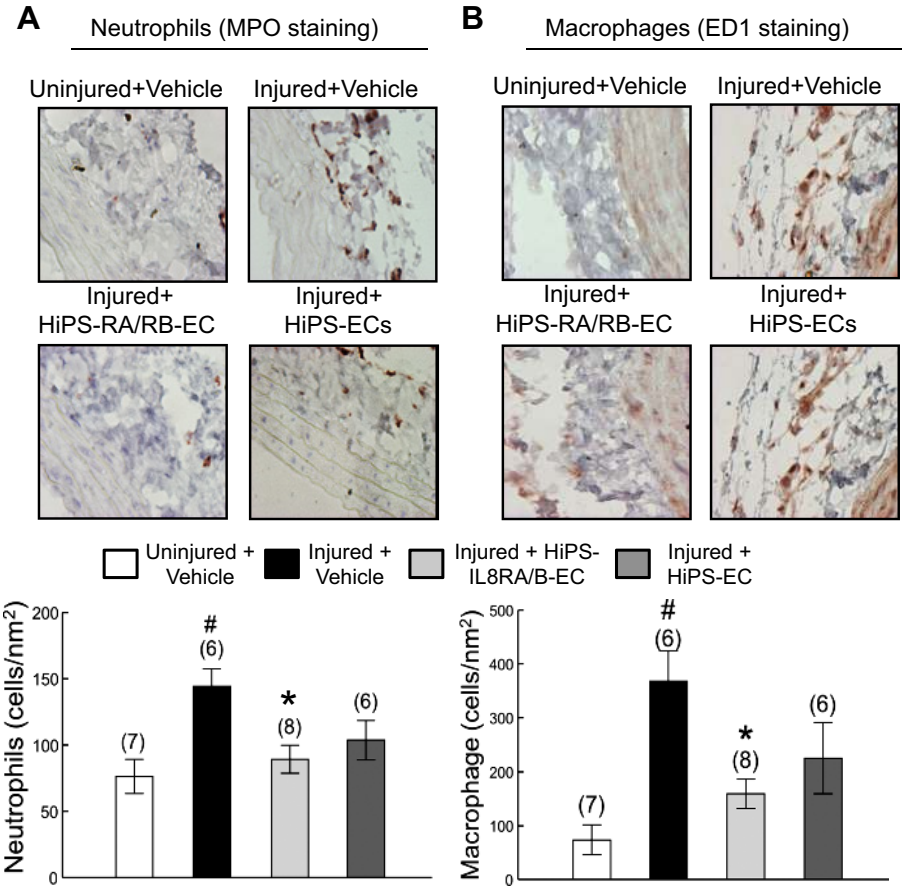

Fig. 5. Effects of HiPS-IL8RA/B-EC or HiPS-EC transfusion on neutrophils or monocytes/macrophages infiltration into injured RCA or uninjured LCA 24 h post injury and cell transfusion. Densities of neutrophils (*A*) and monocytes/macrophages (*B*) in adventitia of injured RCA or uninjured LCA 24 h postinjury. *B*, top panels: representative micrographs of neutrophils [immunostained with myeloperoxidase (MPO) antibody] or monocytes/macrophages (immunostained with ED1 antibody) in adventitia of 4 different groups of rats. *B*, bottom: numbers of MPO or ED1 positive cells were counted in 6 fields of cross sections of each vessel. Uninjured + Vehicle group was contralateral LCA without balloon injury. Injured + Vehicle group was RCA of rats that underwent balloon injury of the RCA but did not receive cell transfusion. Injured + HiPS-IL8RA/B-EC group received iv transfusion of ECs (1.5×10^6 cells/rat) overexpressing IL8RA/B immediately after injury. Injured + HiPS-EC group received iv transfusion of ECs without adenoviral transduction. Results are means ± SE; *n* = no. of vessels. #$P < 0.05$ compared with Uninjured + Vehicle control group. *$P < 0.05$ compared with Injured + Vehicle control group.

detectable in HiPS-Null-EC and HiPS-ECs (Fig. 2, *A* and *B*). Additional immunostaining demonstrated that HiPS-IL8RA/B-ECs and HiPS-Null-ECs expressed the same levels of the EC markers von Willebrand Factor (vWF) (Fig. 3*A*) and CD31 (Fig. 3*B*) as nontransduced HiPS-ECs. These findings indicated that HiPS-IL8RA/B-ECs and HiPS-Null-ECs retained the EC phenotype (Figs. 1*A* and 3, *A* and *B*) after adenoviral transduction.

HiPS-IL8RA/B-ECs localizes in the injured carotid artery. Confocal en face imaging of injured RCA of rats transfused with HiPS-IL8RA/B-ECs immediately after injury confirmed that cells overexpressing IL8RA/B bound to the endoluminal surface of injured RCA (Fig. 4*A*). CD31 immunostaining (to show all ECs) of injured RCA and uninjured LCA at 24 h after injury confirmed that there was no EC layer in the injured RCA (Fig. 4*B*, top left) but the endothelium was intact in the uninjured LCA (Fig. 4*B*, top right). CD31 was also expressed in vasa vasorum in the adventitia. GFP immunostaining (to show only the transfused cells) of injured RCA and uninjured LCA of rats transfused with HiPS-IL8RA/B-ECs showed GFP positive cells in the adventitia of the injured RCA (Fig. 4, bottom left), but not in the uninjured LCA at 24 h after injury (Fig. 4*B*, bottom right). These results indicate that transfused HiPS-IL8RA/B-ECs accumulated in the adventitia of the injured, but not the uninjured arteries.

In vivo tracking using levels of GFP DNA as an index of transfused HiPS-IL8RA/B-ECs at 24 h postinjury by the real-time qPCR analysis showed a >3-fold higher level of GFP DNA in the injured RCA compared with that in the uninjured LCA (Fig. 4*C*). GFP positive cells were virtually undetectable in the RCA and LCA of rats transfused with HiPS-Null-EC or saline vehicle control rats, or the other organs including dorsal aorta, lung, liver, spleen, kidney, brain, and heart of rats transfused with HiPS-IL8RA/B-ECs (data not shown). GFP DNA levels in injured RCA were undetectable at 14 days after the injury (data not shown).

Transfusion of HiPS-IL8RA/B-ECs decreased neutrophil and monocyte/macrophage infiltration into the injured carotid artery. A significant decrease in neutrophil and macrophage infiltration was seen in injured RCA of rats transfused with HiPS-IL8RA/B-ECs, as shown by immunohistochemical analysis of MPO and ED1$^+$ staining, compared with rats transfused

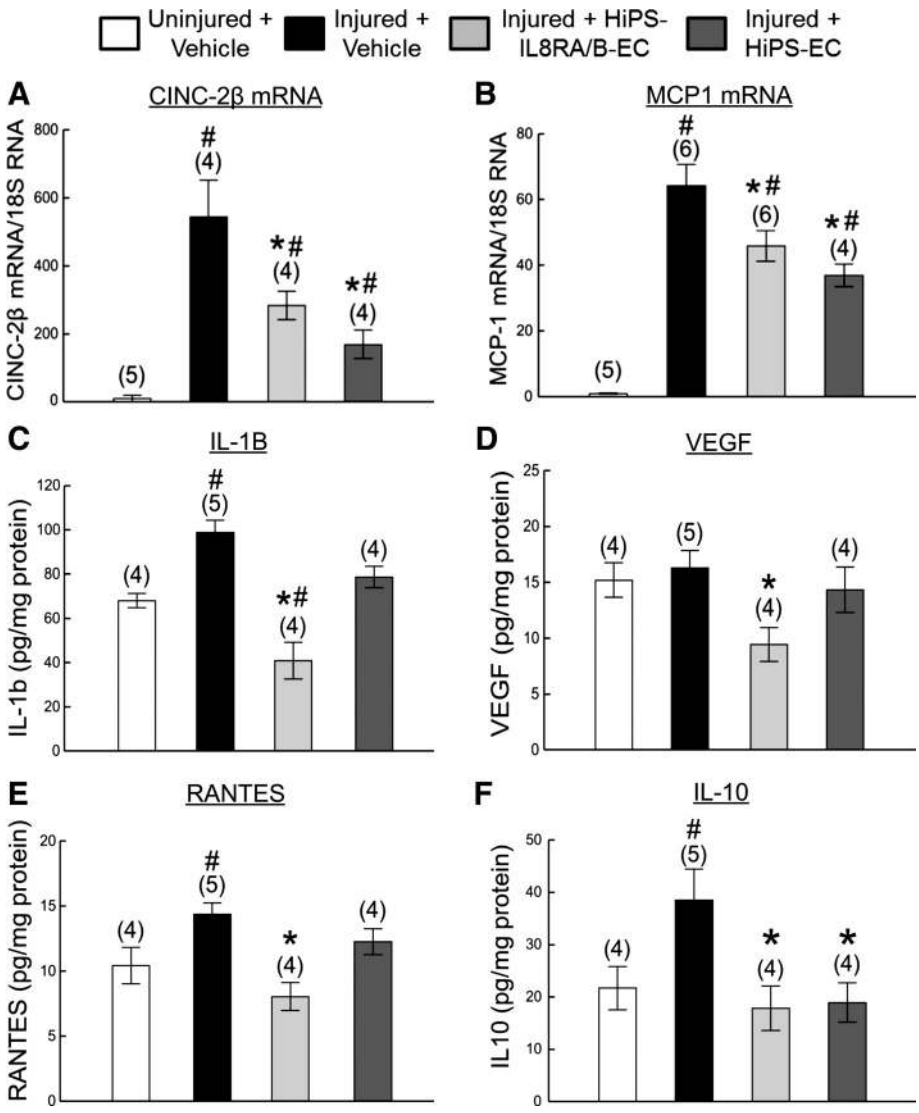

Fig. 6. Effects of HiPS-IL8RA/B-ECs or HiPS-ECs transfusion on mRNA or protein levels of chemokines and adhesion molecules in injured RCA and uninjured LCA 24 h post injury and cell transfusion. *A* and *B*: mRNA levels of CINC-2β (cytokine induced neutrophil chemoattractant-2-β, equivalent to human IL8) and MCP-1 (monocyte chemotactic protein-1) in injured RCA or uninjured LCA 24 h after injury and cell transfusion. CINC-2β and MCP1 mRNA levels in the whole vessel were measured by real-time qRT-PCR and normalized using 18S RNA levels to correct for differences in total RNA loading. In CINC-2β mRNA RT-qPCR, two samples of Injured + Vehicle group and two of Injured + HiPS-IL8RA/B-EC group were randomly excluded. Interleukin 1-β (IL-1β; *C*), vascular endothelial growth factor (VEGF; *D*), regulated on activation, normal T cell expressed and secreted (RANTES; *E*), and interleukin 10 (IL-10; *F*) protein levels in the whole vessel were measured using a Multiplex ELISA kit. Results are means ± SE; n = no. of vessels. #$P < 0.05$ compared with Uninjured + Vehicle control group. *$P < 0.05$ compared with Injured + Vehicle control group.

with vehicle control (no cell transfusion) 24 h postinjury (Fig. 5, *A* and *B*). Concurrent decreases in neutrophil chemoattractant CINC-2β (the rat homolog of IL8) and the macrophage chemoattractant MCP-1 mRNAs were seen in injured arteries of rats transfused with HiPS-IL8RA/B-ECs (Fig. 6, *A* and *B*). Nontransduced HiPS-ECs also caused a significant decrease in CINC2β and MCP-1 mRNA.

Transfusion of HiPS-IL8RA/B-ECs decreased levels of inflammatory cytokines in the injured carotid artery. Increases in inflammatory cytokine proteins interleukin-1B (IL-1B), vascular endothelial growth factor (VEGF), RANTES, and interleukin-10 (IL-10) were seen in vehicle-treated injured RCA compared with the uninjured LCA at 24 h postinjury (Fig. 6, *C–F*). The injured arteries of rats transfused with HiPS-IL8RA/B-ECs had decreased levels of IL-1B, VEGF, RANTES, and IL-10 compared with injured arteries of vehicle-treated animals at 24 h postinjury. In contrast, HiPS-EC transfusion had no significant effect on IL-1B, VEGF, or RANTES expression, but was associated with decreased IL-10 levels in injured arteries.

Neointima formation in injured carotid artery is attenuated by transfusion with HiPS-IL8RA/B-EC. Representative cross-sectional images of the uninjured LCA and injured RCA of vehicle control rats and the injured RCA of HiPS-IL8RA/B-ECs and HiPS-ECs transfused rats at 28 days postinjury are shown in Fig. 7*A*. Morphometric analysis of the images showed that the neointima-to-media area ratio of injured RCA rats treated with HiPS-IL8RA/B-ECs was significantly less than those of injured RCA of rats treated with nontransduced HiPS-ECs or vehicle (Fig. 7*B*). The area of the media remained unchanged for all groups (Fig. 7*C*), while the area of the neointima was significantly attenuated by treatment with HiPS-IL8RA/B-EC (Fig. 7*D*).

HiPS-ECs transduced with IL8RA/B accelerate reendothelialization of injured carotid artery. To elucidate the effects of HiPS-IL8RA/B-EC transfusion on reendothelialization, Evans blue staining of 14-day-injured RCA of rats transfused with HiPS-IL8RA/RB-ECs, vehicle (no EC transfusion), HiPS-ECs (cells without adenoviral transduction), or HiPS-Null-ECs was performed. Arteries from rats treated with HiPS-IL8RA/RB-ECs had smaller areas of blue staining relative to arteries from rats that received HiPS-Null-ECs, HiPS-ECs, or vehicle transfusion (Fig. 8*A*). Uninjured contralateral LCAs were virtually negative for Evans blue staining (data not shown). Quantitative assessment showed that transfusion with HiPS-IL8RA/B-ECs resulted in an ~2-fold reduction in the denuded (blue) area at 14 days after the injury, indicating a 2-fold increase in reendothelialization at this time point (Fig. 8*B*).

DISCUSSION

This study has shown for the first time that targeted cell therapy utilizing ECs derived from human induced pluripotent stem cells overexpressing IL8RA/B (HiPS-IL8RA/B-EC) attenuates the inflammatory response to and accelerated the reendothelialization after acute endovascular injury in the rat. We demonstrated that adenoviral transduction with IL8RA and RB genes did not alter the EC phenotype and that HiPS-IL8RA/B-ECs migrated toward increasing concentrations of IL8 both in vitro and localized to the balloon injured carotid artery in vivo. Femoral vein transfusion of HiPS-IL8RA/B-ECs was associated with significant attenuation of leukocyte

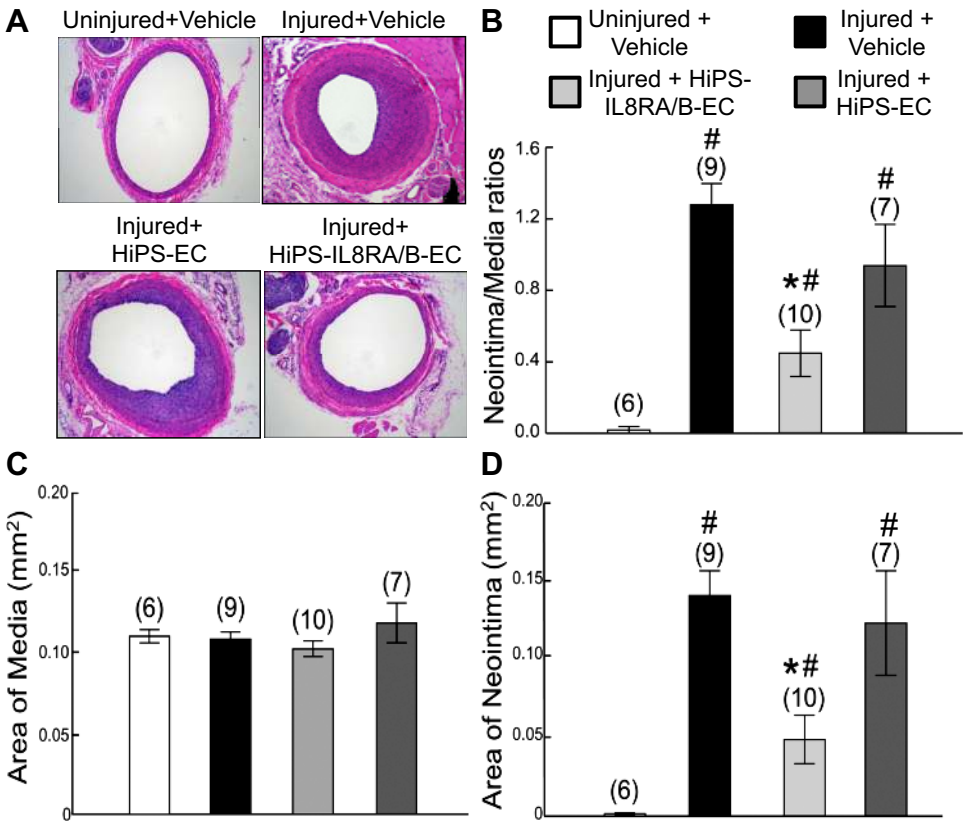

Fig. 7. Neointima formation in injured RCA or uninjured LCA at 28 days post carotid arterial injury. *A*: representative H/E-stained cross sections of injured RCA and uninjured LCA in rats with or without HiPS-ILRA/B-EC or HiPS-EC transfusion (immediately after the injury) at 28 days post balloon injury. Rats received transfusion of HiPS-EC were used as control. *B*, *C*, and *D*: quantification of neointima to media ratios, medial area, and neointimal area at 28 days postinjury of injured RCA, respectively. Results are means ± SE; n = no. of rats. #$P < 0.05$ compared with Uninjured + Vehicle control group; *$P < 0.05$ compared with Injured + Vehicle control group.

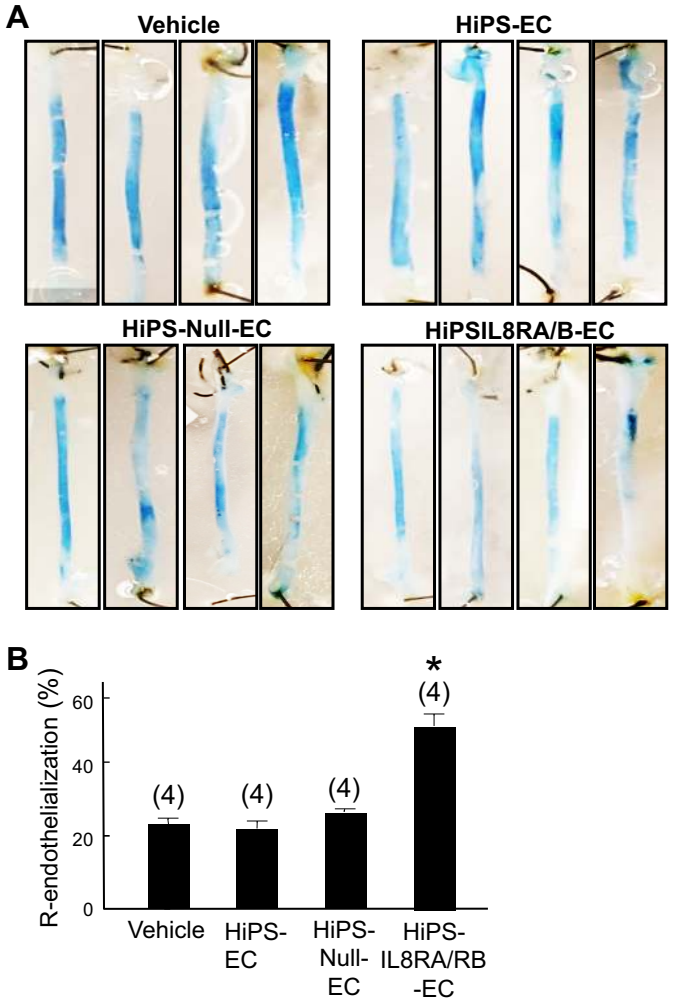

Fig. 8. Acceleration of reendothelialization in injured carotid arteries of rats with HiPS-IL8RA/B-EC transfusion. *A*: isolated common carotid arteries from rats 14 days after balloon injury. Rats were perfused with Evans blue (EB) dye before dissection. Injured right common carotid arteries were cut from the aortic arch to the bifurcation of the internal and external carotid arteries. Intact endothelium excludes the Evans blue dye. *B*: percentage of reendothelialization of injured RCA assessed by the Evans blue dye technique. % of reendothelialization = (length of common carotid artery minus length of blue region)/total length of common carotid artery × 100. Results are means ± SE; n = no. of rats. One-way ANOVA was used to analyze the data. *$P < 0.05$ vs. the other 3 control groups. Vehicle control group was rats with RCA injury but without EC transfusion.

infiltration and inflammatory cytokine expression at 24 h postinjury. There was a significant increase in reendothelialization concomitant with reduced neointima formation in injured carotid arteries of HiPS-IL8RA/B-EC treated rat compared with that of untreated control rat RCAs at 14 days postinjury. Using PCR detection of the GFP gene as a sensitive marker of transduced (IL8RA/B or Null) HiPS-EC localization, we demonstrated that HiPS-IL8RA/B-ECs homed to the injured carotid artery and were sparsely distributed in other organs. We have also shown that HiPS-IL8RA/B-ECs bound to the injured endoluminal surface of the RCA as early as 30 min after injury. These data support the concept of xenotransplantation of HiPS-ECs in an acute vascular injury model in the rat is an effective treatment and answer important questions regarding the tracking of transfused HiPS-ECs in this model (16).

Previous studies by the Cooke laboratory and others have shown that HiPS-ECs have the immunohistochemical and physiological characteristics of ECs (7, 8, 17, 18, 23). These HiPS-ECs also have been shown to express EC markers, including CD31, KDR, vWF, and VE cadherin, and to take up acetylated LDL (17). Further, HiPS-ECs can generate angiogenic cytokines and form tubular networks in matrigel in vitro and, when injected into the ischemic hindlimb of SCID mice [peripheral arterial disease (PAD) model], HiPS-ECs increase capillary density and improve perfusion, probably by secreting angiogenic cytokines (17). Together, these data demonstrate the therapeutic potential of direct injection of HiPS-ECs into ischemic tissues of immunocompromised mice.

Since direct injection of cells to the site of injury can be challenging for clinical application, the ability of intravenously transfused HiPS-ECs to home to the injured ischemic limb was tested (7). Previous studies transfusing ECs derived from mouse embryonic stem cells (ESC-ECs) via the femoral artery or femoral vein showed homing of cells to the ischemic hindlimb (23). In contrast, intravenously transfused (external jugular vein) HiPS-ECs did not home to the injured limb of the PAD model and were found to be lodged in the lung (7). In vitro microfluid and scratch migration assays confirmed that HiPS-ECs did not migrate toward stromal derived factor (SDF), an important chemokine produced under ischemic conditions which recruits stem/progenitor cells to ischemic tissues (1, 2). Although the HiPS-ECs express the CXCR4 receptor, which binds SDF, its presence was not sufficient to home the HiPS-ECs to the ischemic hindlimb. These findings provide evidence that systemically administered HiPS-ECs, without the ability to target to sites of injury, may be unsuitable for cell therapy.

To overcome the inability of HiPS-ECs to target to sites of injury, our laboratory has developed adenoviral vectors containing genes of IL8RA/B to cause transduced ECs to overexpress IL8RA/B on their cell surface. Our previous studies have shown that adult RAECs overexpressing IL8RA/B are effective in inhibiting the inflammatory response to acute vascular injury in a variety of rodent (rat) models, including the monocrotaline (MCT) model of pulmonary hypertension, the coronary artery ligation model of myocardial infarction (MI), and the carotid artery balloon injury model (5, 20, 24).

We have previously demonstrated transfusion with IL8RA/B-RAEC 1–5 h after balloon injury decreased inflammatory mediator expression and leukocyte infiltration into the injured artery at 24 h post injury increased reendothelialization of the injured vessel at 2 wk and decreased neointima formation at 28 days postinjury compared with injured arteries of rats treated with vehicle, Null-RAEC, or vascular smooth muscle cells (VSMCs) overexpressing IL8RA/B (20). Further, the overexpression of both IL8RA and RB together was more effective than either receptor subtype alone in decreasing chemokine and adhesion molecule expression and neutrophil infiltration at 24 h and at decreasing neointima formation at 2 wk postinjury. These effects were nearly identical to those seen in response to intravenous transfusion of HiPS-IL8RA/B-ECs in the current study.

In the current study, HiPS-ECs were successfully transduced with IL8RA/B-GFP or Null-GFP adenoviruses, and expression

levels of IL8RA/B in HiPS-IL8RA/B-ECs were comparable to those in adult IL8RA/B-RAECs used in previous studies (20). The adenoviral transduced HiPS-ECs maintained their EC phenotype after transduction with GFP and IL8RA/B genes, as assessed by immunocytochemical staining of EC markers CD31 and vWF. These results were similar to adenoviral transduced adult RAECs as previously reported (20). HiPS-IL8RA/B-ECs behaved similarly to adult IL8RA/B-RAECs in inhibiting the acute inflammatory response at 24 h postinjury, in decreasing neointima formation, and in increasing reendothelialization at 14 days after injury. Our previous study further demonstrated that the IL8RA/B-RAEC-induced attenuation of the vascular injury response was specific to ECs (adult IL8RA/B-rat arterial ECs) since VSMCs overexpressing IL8RA/B did not decrease neointima formation in injured arteries. These results suggest that the effects of IL8RA/B-ECs on neointima formation and reendothelialization may be independent from their competition with neutrophils for binding to IL8. We speculate that once IL8RA/B-ECs home to IL8 and seed on the injured endoluminal surface, the attached ECs can release paracrine factors that stimulate the growth and proliferation of resident ECs and accelerate reendothelialization. The latter part of the actions of transfused IL8RA/B-ECs is independent from their chemotaxic response to IL8.

In the current study, there were decreases in inflammatory cytokine expression and a trend toward decreases in neutrophils and monocytes/macrophages infiltration in injured arteries of the HiPS-ECs and the HiPS-Null-ECs transfused animals. The attenuation of the early inflammatory response in the injured RCA of rats treated with untargeted HiPS-ECs and HiPS-Null-ECs was not reflected in a reduction in neointima formation or increase in reendothelialization. We interpret this finding as an indication that both decreasing proinflammatory responses and accelerating reendothelialization contribute independently to inhibition of neointima formation in the balloon-injured rat carotid arteries. These data suggest that transfusion of IL8 receptor-targeted ECs is more effective than transfusion of equal numbers of untargeted ECs in inhibiting the later phases of the inflammatory response, e.g., cell migration, proliferation, remodeling of matrix proteins, and neointima formation.

These results demonstrate, for the first time, homing of cells overexpressing IL8RA/B to sites of vascular injury in vivo using GFP expression as a marker of HiPS-IL8RA/B-EC. The increased GFP signal seen in the injured carotid artery of the HiPS-IL8RA/B-EC group, compared with the uninjured contralateral artery, reflects the presence of larger numbers of HiPS-IL8RA/B-ECs in the injured vessel. Levels of GFP signal that were well above background were also observed in the uninjured artery of the HiPS-IL8RA/B-EC transfused animals, and likely reflect migration of the cells toward IL8 (CINC-2β in the rat) in the contralateral artery in the setting of acute vascular injury. This interpretation is consistent with the observation that HiPS-IL8RA/B-ECs migrate toward IL8 more readily than HiPS-Null-ECs and HiPS-ECs in the Boyden Chamber assay in vitro. Confocal microscopy studies show that HiPS-IL8RA/RB-ECs can bind to the injured endoluminal surface of an artery after 30 min of transfusion. Further, immunohistochemical analysis of cross sections of the injured carotid artery confirmed HiPS-IL8RA/B-EC via GFP positive cells in the vasa vasorum at 24 h after injury and cell transfusion. In the current study we found that untransduced HiPS-EC also expressed detectable amounts of IL8RA and IL8RB that might equip them to partially mimic the inhibitory action of HiPS-IL8RA/B-ECs on infiltration of neutrophils and macrophages into injured arteries (Fig. 5) and reduce neointima formation (Fig. 7). Results in Fig. 6 showed that the potency of HiPS-EC to inhibit IL-1B, VEGF, and RANTES expression was weaker than that of transduced HiPS-IL8RA/B-ECs. Together, these data show homing of the intravenous administration of IL8RA/B overexpressing HiPS-ECs to the injured carotid artery.

The current study extends our previous investigations by utilizing ECs derived from HiPS cells, thus providing proof of concept for translational studies and eventual clinical application. HiPS-ECs can be patient specific and are derived from plentiful and easily accessible tissues (8, 17, 23). By utilizing HiPS-EC we have shown that human cells can be transduced to overexpress IL8RA/B and can therefore be utilized in future translational research for treatment of vascular injury. We are aware of potential pitfalls with the utilization of HiPS-ECs, including the potential for genetic mutations from HiPS production and for teratoma formation in vivo from potential iPS contamination, cells not differentiated into iPS-ECs (23). In the current study, we did not see teratoma formation or abnormal ECs or VSMS growth in vivo at 14 days post ECs transfusion, but longer studies are necessary to test this outcome. Exhaustive characterization of cells intended for cell based therapy will be essential before use in clinical trials, as discussed in Volz et al. (23).

In the current study we have shown, for the first time, that HiPS-ECs can be transduced with IL8 receptors and act as an effective cross species cell therapy for vascular injury. The use of HiPS cells provides proof of concept for eventual autologous cell-based therapy, which would not cause an immune response, and thus increase the likelihood of future clinical application (7). Targeting of HiPS-IL8RA/B-ECs to the area of injury, as shown by the qPCR of GFP, demonstrates the potential of cell therapy through intravenous transfusion, a relatively benign, noninvasive procedure. In conclusion, these studies support the potential of targeted cell-based therapy utilizing systemic administration of ECs that overexpress IL8 receptors as a future treatment for inflammatory vascular disease.

GRANTS

This work was supported, in part, by UAB CCVC William W. Featheringill Innovative Award (Y. F. Chen); by National Institutes of Health Grants RO1-HL-116727 (Y. F. Chen), RO1-HL-087980 (S. Oparil), T32-HL-07457 (S. Oparil, S. Giordano); U01-HL-100397 and RC2-HL-103400 (J. P. Cooke), and R00-HL-098688 (N. F. Huang); by American Heart Association Grant AHA-SDG-0930098N (F. G. Hage); and by Veterans Affairs Biomedical Laboratory Research & Development Service Merit Award OMB 4040-0001 (F. G. Hage).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.G., X.Z., D.X., and Y.-F.C. conception and design of research; S.G., X.Z., D.X., J.L., and Y.-F.C. performed experiments; S.G., D.X., and Y.-F.C. analyzed data; S.G. and Y.-F.C. interpreted results of experiments; S.G. and Y.-F.C. drafted manuscript; S.G., F.G.H., S.O., J.P.C., J.L., K.N., N.F.H., and Y.-F.C. edited and revised manuscript; S.G., X.Z.,

D.X., F.G.H., S.O., J.P.C., J.L., K.N., N.F.H., and Y.-F.C. approved final version of manuscript; Y.-F.C. prepared figures.

REFERENCES

1. **Abbot JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ.** Stromal cell-derived factor 1α plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. *Circulation* 110: 3300–3305, 2004.
2. **Chao TH, Tseng SY, Chen IC, Tsai YS, Huang YY, Liu PY, Ou HY, Li YH, Wu HL, Cho CL, Tsai LM, Chen JH.** Cilostazol enhances mobilization and proliferation of endothelial progenitor cells and collateral formation by modifying vasculo-angiogenic biomarkers in peripheral arterial disease. *Int J Cardiol* 172: e371–e374, 2014.
3. **Chen SJ, Li H, Durand J, Oparil S, Chen YF.** Estrogen reduced myointimal proliferation after balloon injury of the rat carotid artery. *Circulation* 93: 577–584, 1996.
4. **Cree RTJ, Warnell I, Staunton M, Shaw I, Bullock R, Griffin SM, Baudouin SV.** Alveolar and plasma concentrations of interleukin 8 and vascular endothelial growth factor following oesophagectomy. *Anesthesia* 59: 867–871, 2004.
5. **Fu J, Chen YF, Zhao X, Creighton JR, Guo Y, Hage FG, Oparil S, Xing DD.** Targeted delivery of pulmonary arterial endothelial cells overexpressing interleukin-8 receptors attenuated monocrotaline-induced pulmonary vascular remodeling. *Arterioscler Thromb Vasc Biol* 34: 1539–1647, 2014.
6. **He TC, Zhou S, da Costa LT, Yu J, Kinzler KW, Vogelstein B.** A simplified system for generating recombinant adenoviruses. *Proc Natl Acad Sci USA* 95: 2509–2514, 1998.
7. **Huang NF, Dewi RE, Okgbaa J, Lee JC, Jalilrufaihah A, Heilshorn SC, Cooke JP.** Chemotaxis of human induced pluripotent stem cell derived endothelial cells. *Am J Transl Res* 5: 510–520, 2013.
8. **Huang NF, Niiyama H, Peter C, De A, Natkunam Y, Fleissner F, Li Z, Rollins MD, Wu JC, Gambhir SS, Cooke JP.** Embryonic stem cell-derived endothelial cells engraft into the ischemic hindlimb and restore perfusion. *Arterioscler Thromb Vasc Biol* 30: 984–991, 2010.
9. **Lee SH, Jeong MH, Bae HR, Jeong SJ, Jang JY, Lim YJ, Kim SH, Kim JW, Cha JK.** Circulating levels of interleukin 8 and vascular endothelial growth factor in patients with carotid stenosis. *J Korean Med Sci* 16: 198–203, 2001.
10. **Leeper NJ, Hunter AL, Cooke JP.** Stem cell therapy for vascular regeneration adult, embryonic, and induced pluripotent stem cells. *Circulation* 122: 517–526, 2010.
11. **Li G, Chen SJ, Oparil S, Chen YF, Thompson JA.** Direct in vivo evidence demonstrating neointimal migration of adventitial fibroblasts after balloon injury of rat carotid arteries. *Circulation* 101: 1362–1365, 2000.
12. **Luster AD.** Chemokines-chemotactic cytokines that mediate inflammation. *N Engl J Med* 338: 436–445, 1999.
13. **Miller AP, Feng W, Xing D, Weathington NM, Blalock JE, Chen YF, Oparil S.** Estrogen modulates inflammatory mediator expression and neutrophil chemotaxis in injured arteries. *Circulation* 110: 1164–1169, 2004.
14. **Oparil S, Chen SJ, Chen YF, Durand JN, Allen L, Thompson JA.** Estrogen attenuates adventitial contribution to neointima formation in injured rat carotid arteries. *Cardiovasc Res* 44: 608–614, 1999.
15. **Oparil S, Wyss JM, Yang RH, Jin HK, Chen YF.** Dietary Ca^{2+} prevents NaCl-sensitive hypertension in spontaneously hypertensive rats by sympatholytic mechanism. *Am J Hypertens* 3: 179S–188S, 1990.
16. **Rogers NM, Isengerg JS.** Endothelial cell global positioning system for pulmonary arterial hypertension: Homing in on vascular repair. *Arterioscler Thromb Vasc Biol* 34: 1336–1338, 2014.
17. **Rufaihah AJ, Huang NF, Jame S, Lee JC, Nguyen HN, Byers B, De A, Okogbaa J, Rollins M, Reijo-Pera R, Gambhir SS, Cooke JP.** Endothelial cells derived from human iPSCs increases capillary density and improve perfusion in a mouse model of peripheral arterial disease. *Arterioscler Thromb Vasc Biol* 31: e72–e79, 2011.
18. **Rufaihah AJ, Huang NF, Kim J, Herold J, Volz KS, Park TS, Lee JC, Zambidis ET, Reijo-Pera R, Cooke JP.** Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity. *Am J Transl Res* 5: 21–35, 2013.
19. **Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S.** Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861–872, 2007.
20. **Xing D, Li P, Gong K, Yang Z, Yu H, Hage FG, Oparil S, Chen YF.** Endothelial cells overexpressing interleukin-8 receptors reduce inflammatory and neointimal responses to arterial injury. *Circulation* 125: 1533–1541, 2012.
21. **Xing D, Miller AP, Novak L, Novak L, Rocha R, Chen YF, Oparil S.** Estradiol and progesterins differentially modulate leukocyte infiltration after vascular injury. *Circulation* 109: 234–241, 2004.
22. **Xing D, Nozell S, Chen YF, Hage F, Oparil S.** Estrogen and mechanisms of vascular protection. *Arterioscler Thromb Vasc Biol* 29: 289–295, 2009.
23. **Volz KS, Miljan E, Khoo A, Cooke JP.** Development of pluripotent stem cells for vascular therapy. *Vascul Pharmacol* 56: 288–296, 2012.
24. **Zhao X, Zhang W, Xing D, Li P, Fu J, Gong K, Hage FG, Oparil S, Chen YF.** Endothelial cells overexpressing IL-8 receptor reduce cardiac remodeling and dysfunction following myocardial infarction. *Am J Physiol Heart Circ Physiol* 305: H590–H598, 2013.