Apurinic/apyrimidinic endonuclease 1 maintains adhesion of endothelial progenitor cells and reduces neointima formation

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Circulating endothelial progenitor cells (EPCs), a subset of bone marrow (BM)-derived progenitor cells, play important roles in endothelial maintenance and vascular regeneration in ischemic tissues (5, 41). Therapeutic angiogenesis with EPCs is a highly promising strategy to ensure vascular repair and revascularization in ischemic cardiovascular diseases (30, 34, 43). However, the number of circulating EPCs and the cellular functions of EPCs are reduced in patients with risk factors for ischemic cardiovascular diseases and are negatively correlated with the Framingham cardiovascular risk factor score (13). Thus the beneficial effects of EPC transplantation may not always be observed in patients with cardiovascular disease (22, 32, 44).

To overcome this problem, a number of investigators have attempted to mobilize endogenous EPCs from BM to peripheral blood (8) or to treat extracted autologous EPCs before transplantation. To improve their proliferative, migratory, and angiogenic capabilities, certain specific target genes such as telomerase reverse transcriptase, VEGF, and hepatocyte growth factor have been expressed in EPCs (24, 31, 39). However, these strategies to functionally improve EPCs are not sufficient for in vivo therapeutic outcomes. One general problem with cellular therapy is the limited survival rate of transplanted cells within tissues. Unless transplanted EPCs can home to and survive at ischemic/inflammatory damaged sites, EPCs will not show any therapeutic effects in vivo, even though their cellular functions have been improved. Therefore, enhanced recruitment and specific homing of EPCs to target sites may be crucial for effective therapeutic outcomes.

Reactive oxygen species (ROS), including superoxide anions ($\text{O}_2^{-}$) and hydrogen peroxide ($\text{H}_2\text{O}_2$), are produced not only by activated inflammatory cells but also by other cells exposed to oxidative stimuli such as hypoxia/reperfusion and inflammatory cytokines (20). Elevated ROS induce an imbalance in the cellular redox state, resulting in cell death and genetic instability. For EPCs to have beneficial effects in ischemic/injured vascular tissues, EPCs should exhibit survival under unfavorable oxidative stress conditions. EPCs have been reported to have the unique property of resisting oxidative injury. Defense against oxidative stress in EPCs appears to be mediated by higher expression of anti-oxidative enzymes, including manganese SOD (MnSOD), catalase, and glutathione peroxidase (12, 17). However, whether these ROS elimination systems are sufficient for the anti-oxidative capacity of EPCs is not clear.

Apurinic/apyrimidinic endonuclease/redox factor-1 (APE1/ ref-1) is essential for the repair pathway of oxidatively damaged DNA, and APE1 also has reducing properties that promote the binding of many redox-sensitive transcription factors such as activator protein-1 and hypoxia-induced factor-1α to their cognate DNA sequences (6). In addition to a nuclear role for APE1, an extranuclear role for APE1 has been elucidated in vascular cell biology. APE1 reduces oxidative stress by modulating cytoplasmic ROS generated by rac1 GTPase (4, 33) and increases the nitric oxide bioavailability (25). APE1 also inhibits platelet-derived growth factor-mediated migration of vascular smooth muscle cells (18). Thus APE1 plays an important role in vascular cells to maintain the vascular structure and functional homeostasis.
ROLE OF APE1 IN EPC-MEDIATED VASCULAR REPAIR

In the present study, we found that EPCs expressed APE1 gene at a relatively high level among BM cells. Therefore, we further examined whether APE1 is an important molecule for EPCs to endure the oxidative stress and perform their vascular repairing effect.

MATERIALS AND METHODS

Animals. All animal experiments were performed according to procedures approved by the Animal Care and Use Committee of Asahikawa Medical University. Male mice (C57BL/6, 12- to 16-wk-old) were maintained on a standard rodent chow diet with 12-h:12-h light:dark cycles. For cell tracking experiments in vivo, green fluorescent protein (GFP) transgenic mice (C57BL/6) donated by M. Okabe (Osaka University, Japan) were used.

EPC isolation and culture. EPCs were isolated from BM-mono nuclear cells (MNCs) as described previously (27). Lineage negative (lin−) and c-kit+ Fk1+ cells were isolated from BM-MNCs with magnetic sorting system (Miltenyi Biotec, Auburn, CA). These lin− c-kit+ Fk1+ cells were incubated on fibronectin-coated dishes in complete medium [α-MEM (Gibco) supplemented with 10% FBS, 100 ng/ml mouse recombinant VEGF (Peprotech), 10 U/ml heparin sulfate (Sigma), 100 U/ml penicillin, and 100 µg/ml streptomycin]. These cells were used as EPCs for in vitro cellular function assays and in vivo cellular transplantation experiments.

Peripheral capillary endothelial cell lines. Immortalized peripheral capillary endothelial cell lines (CECs) were established from transgenic mice harboring the temperature sensitive simian virus 40 large T-antigen gene (Ts-SV40T) as previously described (21). Briefly, the femoral artery of Ts-SV40T mice was injured with a wire, and growing adventitial microvessels of injured arterial walls in these mice were digested with 0.1% dispase (Gibco) for 30 min at 37°C. CD146-positive cells were then isolated using magnetic sorting system (Miltenyi Biotec). Immortalized CECs were cultured in endothelial basal medium (EBM2; Lonza) containing 20 ng/ml VEGF at 33°C. CECs showed the typical endothelial genomic profile, i.e., von Willebrand factor, Flk1, and CD31. They formed a cobblestone-like monolayer in fibronectin-coated dishes and formed tubular structure in 3D-matrigel.

Determination of intracellular ROS production. Intracellular production of ROS was measured with a ROS/superoxide detection kit according to the manufacturer’s instructions (Enzo Life Sciences, Farmingdale, NY). Briefly, the cells were labeled with oxidative stress detection and superoxide detection reagents. Upon staining, positive cells were those in which the fluorescent products generated by these reagents were detected with a flow cytometer (Beckman Coulter, FC500). Total ROS-producing cells were calculated as the percentage of total cells.

EPC treatments. To overexpress APE1 in EPCs, the human APE1 gene was cloned from human mononuclear cells, and adenovirus harboring the APE1 gene (Ad-APE1) was created using the adeno-X expression system (Clontech). Full sequences of cloned APE1 were confirmed by direct DNA sequencing. Adenovirus harboring LacZ (Ad-LacZ) was also prepared as a control. Subconfluent EPCs in fibronectin-coated 35-mm dishes were incubated with adenovirus (500 moi)-containing opti-MEM (Gibco) for 1 h, followed by incubation in complete medium for 48 h. To knockdown endogenous APE1 in EPCs, APE1-specific small interfering (si) RNA was used. The targeted sequence for APE1 was 5'-GGCGCAAGGTTTTGGGGAA-3'. A scrambled siRNA sequence was used as a control. Subconfluent EPCs were transfected with siRNAs [50 nmol/ml: APE1 or control using the Lipo-mag kit (Oz Biosciences)] according to manufacturer’s instructions. Forty-eight hours after transfection, the cells were analyzed for gene expression and used for further experiments.

RT-PCR and quantitative real-time PCR. Expression at the RNA level was determined with RT followed by quantitative real-time PCR (qPCR). RNA was prepared with an RNeasy kit (Qiagen). RT-PCR was performed using a superscript one-step RT-PCR kit (Invitrogen) in the presence of 10 µM sense and anti-sense primers. The primers were human APE1, sense, 5'-CAAGGCGCCAACCAAATCTTT-3', anti-sense, 5'-GTGCCCCCTCAAAGTGTTT-3' and mouse APE1, sense, 5'-CCGCGCAACCAAACATTCTT-3', anti-sense, 5'-GGATTGGGTAAAGGAAGAAGCA-3'. Complimentary DNA was synthesized using SuperScript (Invitrogen), and qPCR was carried out with a SYBR Green PCR kit (Life Technologies) using an AB7300 real-time PCR cycler (Applied Biosystems). qPCR results were normalized to levels of small ribosomal protein and expressed as the change relative to the control.

In vitro EPC functional assay. The adhesion of EPCs was determined with an EPC culture assay as described (27). Briefly, EPCs were incubated on fibronectin (FN)-coated plates in complete medium (α-MEM supplemented with 10% FBS, 100 ng/ml mouse recombinant VEGF, 10 U/ml heparin sulfate, 100 U/ml penicillin, and 100 µg/ml streptomycin). The change in adhesion relative to the control was determined with the WST assay.

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were seeded on a fibronectin-coated slide in complete medium. After culture for 18 h, the number of adhesive EPCs, which take up 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-labeled acetylated low-density lipoprotein (AcLDL-DiI; Biogenesis) and bind FITC-labeled BS-1 lectin (Sigma), were counted in five randomly selected fields (×100) using fluorescence microscopy. The viability of EPCs was estimated using a WST cell viability assay (ScienCell). Briefly, subconfluent cells were incubated with fresh phenol-red free medium containing 2-(4-indophenyl)-3-(4-nitrophényl)-5-(2,4-disulfophenyl)-2H-tetrazolium for 2 h, and formazan formation was measured with optical density at 570 nm.

Expression of adhesion molecules in EPCs. The genomic profile of extracellular matrix (ECM) and adhesion molecules was estimated with an RT2 profiler PCR array (Qiagen). In brief, after EPCs were treated with \( \text{H}_2\text{O}_2 \), complementary DNA was prepared from total RNA that was extracted from treated EPCs and used for quantitative PCR analysis (Applied Biosystems) according to the manufacturer’s instructions. Data were analyzed using the comparative cycle threshold method with normalization of the data to \( \beta\)-actin expression levels.

Wire-mediated vascular injury and EPC transplantation studies. To reproducibly induce vascular remodeling, we used a wire-mediated endovascular injury model (27, 37). A spring wire (0.38 mm in diameter; Cook, Bloomington) was inserted into the left femoral artery and left in place for 1 min to induce transluminal arterial injury. To estimate the incorporation or recruitment of EPCs to the injured vascular walls, GFP-expressing EPCs were prepared from GFP transgenic mice. EPCs (1 × 10⁴) were infused via tail vein 1 day after vascular injury. At the indicated times, the femoral arteries were fixed in 4% paraformaldehyde, and their cryostat sections (7-μm thickness) were prepared for histological analyses (3). Nuclei were counterstained with Hoechst 33258 (Sigma). For histological examination of vascular architecture, the sections were observed with three-dimensional-deconvolution fluorescence microscopy (Leica; AF6000).

Statistical analysis. Results are presented as means ± SE. Comparisons between two groups were carried out using an independent Student’s \( t \)-test. Multiple comparisons among groups were carried out by one-way ANOVA with Fisher’s test. A value of \( P < 0.05 \) was considered statistically significant.

![Fig. 2. Effects of H2O2 or TNF-α on intracellular reactive oxygen species (ROS) levels in EPCs and CECs. A: CECs or EPCs were treated with H2O2 (500 μM) or TNF-α (50 ng/ml). Intracellular superoxide and other ROS were labeled with oxidative stress detection reagents and detected with flow cytometry (gray areas). B: the same value was expressed as the ratio of total ROS-producing cells (superoxide + other ROS-positive cells) to total cells. Data are presented as means ± SE. * \( P < 0.05 \). NS, not significant (n = 4).](http://ajpheart.physiology.org/doi/10.1152/ajpheart.00965.2012)
RESISTANT AGAINST OXIDATIVE STRESS IS DUE TO THE RELATIVELY HIGH EXPRESSION OF ROS SCAVENGERS IN EPCs. WHEN THE RATE OF ROS FORMATION EXCEEDS THE CAPACITY OF THE ANTI-OXIDATIVE DEFENSE SYSTEM, OXIDATIVE STRESS LEADS TO CELL DEATH AND GENETIC INSTABILITY (42). ROS PRODUCTION IN EPCs WAS NOT SIGNIFICANTLY INCREASED IN THE PRESENCE OF H2O2 (500 µM) OR TNF-α (50 ng/ml), WHEREAS INTRACELLULAR ROS IN CECs WAS SIGNIFICANTLY INCREASED (Fig. 2). NEXT, WE CONFIRMED THAT THE mRNA EXPRESSION LEVELS OF ROS-SCAVENGING ENZYMES SUCH AS MnSOD, CATALASE, AND GLUTATHIONE PEROXIDASE WERE SIGNIFICANTLY HIGHER IN EPCs COMPARED WITH CECs (Fig. 3). THEREFORE, THE RELATIVELY HIGH LEVEL OF EXPRESSION OF ROS-SCAVENGING ENZYMES IN EPCs MAY PARCUTLY CONTRIBUTE TO THE REDUCED SENSITIVITY TO ROS-INDUCED CELL DAMAGE/DEATH.

INHIBITION OF APE1 DECREASES THE ADHESION PROPERTY BUT NOT CELL VIABILITY OF EPCs. WE ESTIMATED THE EXPRESSION LEVEL OF APE1 IN EACH CELLULAR FRACTION DURING EPC-PURIFICATION FROM BM, BM-MNCs, LINEAGE-NEGATIVE CELLS (Lin−), AND cKit+ Flk+ EPC fractions (Fig. 4A). AS SHOWN IN Fig. 4B, THE EXPRESSION LEVEL OF APE1 WAS ENHANCED DURING PURIFICATION OF EPCs FROM BM-MNCs. Thus EPCs REPRESENT BM-DERIVED CELLS IN WHICH THE APE1 GENE WAS EXPRESSED AT A RELATIVELY HIGH LEVEL AMONG BM-MNCs.

THE SMALL MOLECULE E3330 IS A SELECTIVE ANTAGONIST OF THE APE1 REDOX DOMAIN FUNCTION (49). TO EXPLORE WHETHER APE1 IS AN IMPORTANT FACTOR IN PROTECTING EPCs FROM OXIDATIVE STRESS-INDUCED FUNCTIONAL DISORDERS, WE EXAMINED THE EFFECTS OF
E3330 on the cellular functions, i.e., viability and adhesion of EPCs. E3330 (40 µM) did not affect the cellular viability alone or in the presence of H2O2 (500 µM; Fig. 5A). In contrast, the adhesion property of EPCs was significantly reduced by E3330 in the presence of H2O2 (100 µM; Fig. 5B). Therefore, APE1 may contribute to maintenance of the adhesion function of EPCs during oxidative stress.

APE1 does not decrease ROS level during oxidative stress. ROS scavenging system in EPCs is one of the factors to have resistance to the oxidative stress (12, 17). Therefore, it is interesting if APE1 affects the expression of ROS scavengers and also intracellular ROS level in the presence of ROS inducers. H2O2 or TNF-α did not affect the ROS level in EPCs (Fig. 2) and mRNA level of ROS scavenger enzymes (Fig. 6). In addition, the expression of ROS scavengers and the ratio of ROS-positive cells were not affected by APE1 selective inhibitor E3330 with or without H2O2 or TNF-α (Fig. 6).

APE1 attenuates the adhesion disorder of EPCs during oxidative stress. We further explored the role of APE1 in EPCs using genetic gain- and loss-of function approaches. We first prepared the APE-EPCs in which exogenous APE1 was overexpressed using APE1-gene harboring recombinant adenovirus (Ad-APE1) and examined the cellular adhesion of EPCs in the presence of H2O2. Overexpression of exogenous APE1 did not affect the expression level of endogenous APE1 in EPCs (Fig. 7A). In this study, a modest dose of H2O2 (100 µM) was used so as to not affect the cellular viability. As shown in Fig. 7B, in the presence of H2O2, the adhesion ability of control EPCs was reduced by ~50%. When APE1 was overexpressed in EPCs, the reduced adhesion ability was significantly improved. In contrast, the opposite effects were observed in EPCs depleted of APE1 with RNA interference. When endogenous APE1 was specifically knocked down by APE1-specific siRNA, the adhesion ability of EPCs was reduced compared with that of control EPCs transfected with scrambled siRNA (Fig. 7B).

Gene expression profile of adhesion molecules in APE1-EPCs. To explore the mechanisms by which APE1 maintains adhesion property of EPCs during oxidative stress, expression of genes encoding adhesion molecules and the ECM was estimated with RT-PCR. After EPCs were pretreated with H2O2 for 24 h, total RNA was isolated from APE-EPCs and control-EPCs and used for PCR array system. The expression level of tested genes was mostly increased or not altered in APE-EPCs compared with control-EPCs. The genes that were increased by greater than twofold in APE-EPCs are listed in Table 1. Interestingly, among these genes, VCAM1 and connective tissue growth factor (CTGF) are important factors known to mediate the adhesion of EPCs (15, 38, 47). We further confirmed the mRNA level of CTGF in EPCs with or without H2O2. As shown in Fig. 7C, the expression of CTGF was decreased up to 35% by H2O2 (100 µM). In addition, E3330 further reduced the expression level of CTGF in the presence of H2O2 (Fig. 7C).

APE1 enhances the homing of EPCs to injured vascular walls. To test whether the function of APE1 in EPCs is important in pathophysiological in vivo conditions, we ob-
served the adhesion of EPCs within the injured vascular walls, where oxidative stress is abundant due to accumulation of inflammatory cells. GFP\(^+\) EPCs were transfected with adenovirus (Adeno) harboring βgal (control) on APE1 genes. Selective knockdown of endogenous APE1 was induced by transfection of APE1-small interfering (si)RNA. For a control, cells were treated with scrambled control siRNA. B: at 2-day incubation after transfection to allow the expression of APE1 or knockdown of endogenous APE1, the transfected cells were detached and used in an adhesion assay in the presence of H\(_2\)O\(_2\) (100 μM). C: EPCs were treated with or without E3330 (40 μM) in the presence of H\(_2\)O\(_2\), and the mRNA expression level of connective tissue growth factor (CTGF) was estimated by RT-PCR. Each expression level was calculated as the ratio to the internal control GAPDH. The values are expressed as percentage on the non-H\(_2\)O\(_2\)-treated group. Data are presented as means ± SE. \(*P < 0.05\) compared with control groups (\(n = 8\)). Adeno, adenovirus.

**DISCUSSION**

Atherosclerotic vascular diseases are characterized by impaired vascular cellular functions in accordance with oxidative stress in plaque lesions. Thus, to induce the vascular repair effects of EPCs, maintenance of survival and/or retention of EPCs in an unfavorable oxidative stress environment are important. We demonstrated that EPCs expressed high levels of
and the extracellular matrix

Table 1. Expression of genes related to adhesion molecules and the extracellular matrix

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<th>Gene</th>
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<tr>
<td>Ctgf</td>
<td>14.52</td>
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<tr>
<td>Lam1</td>
<td>14.50</td>
</tr>
<tr>
<td>Col4a3</td>
<td>6.72</td>
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<tr>
<td>Col6a1</td>
<td>4.36</td>
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<tr>
<td>Adams2</td>
<td>4.24</td>
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<tr>
<td>Col1a1</td>
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<td>Col3a1</td>
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<td>Postn</td>
<td>2.35</td>
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Apurinic/apyrimidinic endonuclease 1-endothelial progenitor cells (EPCs) and control-EPCs were pretreated with H$_2$O$_2$ (500 μM) for 16 h, and the mRNA level of genes related to adhesion molecules and the extracellular matrix was estimated with RT-PCR. The genes for which the mRNA expression was increased by > twofold in APE-EPCs compared with that in control-EPCs are listed.

ROS-scavenging enzymes resulting in reduced sensitivity toward ROS-mediated cell death (Figs. 2 and 3). This finding is consistent with previous studies (12, 17). Interestingly, among the cellular functions of EPCs, the adhesion property was relatively sensitive to oxidative stress. In this study, we found that APE1 was a crucial factor to maintain the adhesion of EPCs in part through the keeping expression of adhesion molecules (Fig. 7). Importantly, we also demonstrated that forced expression of APE1 enhanced the homing of EPCs to injured vascular walls and improved their vascular repair effects (Figs. 8 and 9).

The adhesion ability of EPCs was reduced by H$_2$O$_2$ and synergistically decreased by an APE1-specific redox inhibitor E3330 (Fig. 5). In addition, reduction of endogenous APE1 with siRNA enhanced the H$_2$O$_2$-mediated inhibition of EPC-adhesion without reducing cellular viability (Fig. 7). These data suggest that the redox function of APE1 is involved in maintaining the adhesion property of EPCs during oxidative stress. Several adhesion molecules mediate the interaction of EPCs with the ECM or other cell types including endothelial cells. For example, knock down of integrins in EPCs induces a significant reduction in the adhesion of EPCs and subsequent in vivo functions of EPCs (7, 26). We previously reported that integrin family members such as integrin β1 are crucial for the adhesion property of EPCs and the in vivo functions (2, 27).

However, in the present study, the expression of members of the integrin family including integrin β1 was not significantly changed by H$_2$O$_2$ or the expression of APE1 (data not shown). We showed that some adhesion molecules such as CTGF and VCAM1 were increased in EPCs by APE1 overexpression (Table 1) and conversely decreased by APE1 inhibitor E3330 (Fig. 7C) in the presence of H$_2$O$_2$. These molecules mediate the function of EPCs including the adhesion property (15, 38, 47). Thus we suggest that APE1 mediates adhesion property of EPCs under the oxidative stress in part through the keeping expression of adhesion molecules.

We showed that APE1 did not affect the expression of ROS scavengers and also the intracellular ROS level (Fig. 6). Indeed, APE1 maintained the adhesion property of EPCs even though intracellular ROS was kept at low level (Figs. 1 and 2). Therefore, this effect of APE1 would not be mediated by the ROS scavenging system. At present, we have not clarified the exact mechanisms of which APE1 maintains the adhesion molecules and maintains the adhesion property. It is known that APE1 protects the cells from oxidative stress through a variety of mechanisms beside ROS-reducing action (6). APE1 may prevent the reduction in adhesion molecules during oxidative stress, presumably through the action of transcriptional factors. Indeed, APE1 activates transcription factors such as hypoxia-induced factor-1α, which mediate adhesion molecules, such as integrins and chemokine stromal-derived factor1 receptor (29).

It is well established that a relatively low level of H$_2$O$_2$ (1 μM) in endothelial cells induced adhesion VCAM signals to mediate leukocytes trans-endothelial adhesion (9). However, this is in contrast with the 50–200 μM H$_2$O$_2$ released by neutrophils and macrophages in disease state for oxidative damage such as atherosclerosis (11). As demonstrated in the previous and the present studies, these relatively high levels of H$_2$O$_2$ (>50 μM) have opposing effects in the adhesion signals and cellular functions, inducing oxidative damage of endothelial cells (1, 9, 35). Thus it can be anticipated that antioxidative enzyme APE1 keeps the adhesion property of EPCs under the certain pathophysiological setting (e.g., injured vas-
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Fig. 9. Role of APE1 in EPCs-mediated vascular repair. One day after wire-mediated femoral artery injury, EPCs (1 × 10⁴) were injected into the tail vein. APE-overexpressing EPCs (APE-EPCs) were prepared by infection with Ad-APE1. For controls, the vehicle PBS only (none) and Ct-EPCs were used. Four weeks after vascular injury/cellular transplantation, histological analysis was performed. A: representative images of hematoxylin and eosin staining of a short-axis view of the femoral artery in each group are shown. Bar = 100 μm. B: intimal area to the medial area of arterial walls (I/M ratio). Data are presented as means ± SE. *P < 0.05, **P < 0.01 (n = 12).

We have confirmed that adhesion ability, with cytokines, including a combination of IL-1β, interferon-γ, and TNF-α. We have confirmed that cellular damage/apoptosis can be induced in vascular smooth muscle cells, and CECs can be induced by these cytokines (3). Cytokines such as TNF-α have been reported to have both beneficial and detrimental effects on certain cell types including mesenchymal stem cells and EPCs (40, 46). The divergent effects of cytokines have been attributed to their concentration, duration of exposure, and the specific receptor types (14). Furthermore, the effects of cytokines are mediated not only by ROS production but also other ROS-independent signal pathways.

Stem cells are characterized by the expression of stemness-related genes, which provide resistance to stress via DNA repair and detoxification effects (23, 36). Recently, APE1 has been reported to play an important role in several stem cell types. APE1 suppresses intracellular superoxide levels, reduces senescence of mesenchymal stem cells (19), and regulates hematopoietic differentiation of embryonic stem cells through its redox function domain (49). In this study, we demonstrated that EPCs express high levels of APE1 compared with BM cells, and APE1 in EPCs is a crucial protective factor against oxidative stress in vivo. E3330 has been reported to block the in vitro growth of human EPCs, an effect that is recapitulated by stable expression of a dominant negative redox domain mutant of APE1 (48). Therefore, APE1 may also maintain the function of EPCs as stem/progenitor cells including proliferation (reduced senescence) or differentiation in addition to adhesion ability, and contribute to their potent vascular repair effects.

Recently, forced expression of APE1 in vascular walls has been reported to attenuate balloon-injured neointima formation (28). The effect of APE1 on vascular remodeling may be mediated through the action on vascular cells, e.g., endothelial protection against oxidative stress (16, 25), and may also control the migration of vascular smooth muscle cells (18). In the present study, we showed for the first time that APE1 contributed to the regulation of vascular remodeling by improving the function of EPCs. We used relatively few (∼10⁴ cells/mouse) EPCs for cell transplantation, and thus, as expected, the effects of control EPCs on vascular remodeling were insufficient to induce vascular repair (Fig. 9). Importantly, a small amount of APE1-expressing EPCs showed in vivo effects compared with control EPCs (Fig. 9). The number and cellular function of EPCs have been reported to be reduced in patients with risk factors for ischemic cardiovascular diseases, and this is one of the critical reasons for the insufficient therapeutic effects of cellular transplantation (13, 44). Thus APE1 may be an attractive target gene to maintain/enhance the function of EPCs in patients and to provide effective outcomes following cellular therapy.

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DISCLOSURES

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