Prostaglandin I_2 Promotes Recruitment of Endothelial Progenitor Cells and Limits Vascular Remodeling

Jun-ichi Kawabe, Koh-ichi Yuhki, Motoi Okada, Takayasu Kanno, Atsushi Yamauchi, Naohiko Tashiro, Takaaki Sasaki, Shunsuke Okumura, Naoki Nakagawa, Youko Aburakawa, Naofumi Takehara, Takayuki Fujino, Naoyuki Hasebe, Shuh Narumiya, Fumitaka Ushikubi

Objective—Endothelial progenitor cells (EPCs) play an important role in the self-healing of a vascular injury by participating in the reendothelialization that limits vascular remodeling. We evaluated whether prostaglandin I_2 plays a role in the regulation of the function of EPCs to limit vascular remodeling.

Methods and Results—EPCs ($Lin^-cKit^+Flk\text{-}1^+$ cells) were isolated from the bone marrow (BM) of wild-type (WT) mice or mice lacking the prostaglandin I_2 receptor IP ($IP^{-/-}$ mice). Reverse transcription–polymerase chain reaction analysis showed that EPCs among BM cells specifically express IP. The cellular properties of EPCs, adhesion, migration, and proliferation on fibronectin were significantly attenuated in IP-deficient EPCs compared with WT EPCs. In contrast, IP agonists facilitated these functions in WT EPCs, but not in IP-deficient EPCs. The specific deletion of IP in BM cells, which was performed by transplanting BM cells of $IP^{-/-}$ mice to WT mice, accelerated wire injury–mediated neointimal hyperplasia in the femoral artery. Notably, transfused WT EPCs, but not IP-deficient EPCs, were recruited to the injured vessels, participated in reendothelialization, and efficiently rescued the accelerated vascular remodeling.

Conclusion—These findings clearly indicate that the prostaglandin I_2-IP system is essential for EPCs to accomplish their function and plays a critical role in the regulation of vascular remodeling. **(*Arterioscler Thromb Vasc Biol*. 2010;30:464-470.)**

Key Words: vascular remodeling ■ prostaglandin I_2 ■ endothelial progenitor cells ■ fibronectin ■ adhesion

Vascular remodeling characterized by neointimal hyperplasia frequently accompanies angioplasty and atherosclerosis.[1] The development of vascular remodeling is limited by a process of reendothelialization,[2] which is accomplished by covering the neointimal surface with a functional endothelial monolayer. Recently, endothelial progenitor cells (EPCs) have been established as the cells participating in reendothelialization.[3,4] EPCs, which originate in bone marrow (BM), are mobilized into peripheral circulation in response to vascular injury.[5,6] After mobilization, they are recruited to the injured vessels, differentiate into mature endothelial cells, and contribute to reendothelialization to a variable extent depending on the nature of the vascular injury.[7–9] Accordingly, the infusion of exogenous EPCs[8,9] or EPC-mobilizing factors,[7,10] which increases circulatory EPCs, facilitated reendothelialization of the injured vessels and thereby suppressed neointimal formation.

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Prostaglandin (PG) I_2 (prostacyclin), a potent antiatherogenic lipid mediator, is the major prostanoid in the cardiovascular system and is produced mainly by vascular endothelial cells. PGI_2 exerts a variety of actions via binding to the specific receptor IP and, thus, induces vascular relaxation, inhibits proliferation of vascular smooth muscle cells, and potently inhibits platelet activation.[11] Accordingly, mice lacking the IP ($IP^{-/-}$ mice) have shown the phenotypes characterized by enhanced vascular remodeling with augmented neointimal hyperplasia after a vascular injury[12] and by facilitated atherosclerosis when having a concomitant loss of apoprotein E or low-density lipoprotein receptor gene.[13,14] However, the precise mechanisms leading to the augmented vascular remodeling and facilitated atherosclerosis observed in $IP^{-/-}$ mice remain to be determined.

Herein, we show a novel role of the PGI_2-IP system in vascular remodeling. Mice lacking the IP specifically in BM have EPCs defective in functions, resulting in reduced recruitment of EPCs to the injured vessels, attenuated reendothelialization, and augmented neointimal hyperplasia in a model of wire injury–mediated vascular remodeling. The transfusion of wild-type (WT) EPCs, but not $IP^{-/-}$ EPCs, rescues this enhanced vascular remodeling.

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From the Department of Pharmacology, Asahikawa Medical College, Asahikawa, Japan (J.K., K.Y., T.K., and F.U.); the Department of Cardiovascular Regeneration, Asahikawa Medical College, Asahikawa, Japan (J.K., N.T., and N.H.); the Department of First Internal Medicine, Asahikawa Medical College, Asahikawa, Japan (J.K., M.O., A.Y., N.T., T.S., S.O., N.N., Y.A., T.F., and N.H.); and the Department of Pharmacology, Kyoto University Faculty of Medicine, Kyoto, Japan (S.N.).

Correspondence to Fumitaka Ushikubi, MD, PhD, Department of Pharmacology, Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa 078-8510, Japan. E-mail ushikubi@asahikawa-med.ac.jp

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Methods

For detailed methods, please see the supplemental materials (available at: http://atvb.ahajournals.org).

Animal Models

Wire-mediated vascular injury was induced in BM-transplanted mice[15]: WT mice with WT or $IP^{-/-}$ BM (WT/WT-BM or WT/$IP^{-/-}$-BM mice) and $IP^{-/-}$ mice with WT or $IP^{-/-}$ BM ($IP^{-/-}$/WT-BM or $IP^{-/-}$/$IP^{-/-}$-BM).

The Function of EPCs in Vivo

EPCs ($Lin^{-}cKit^{+}Flk\text{-}1^{+}$ cells) were isolated from BM mononuclear cells (MNCs) using a magnetic cell sorting system. EPCs were infused via a tail vein one day after the vascular injury. In the experiment examining incorporation or recruitment of EPCs to the injured vessels, EPCs were prepared from BM specimens of green fluorescent protein (GFP)–expressing WT and/or $IP^{-/-}$ mice. The sections were observed with 3-dimensional deconvolution fluorescence microscopy (model AF6000; Leica, Deerfield, Ill; Wetzlar, Germany) and confocal microscopy (model LSM 5 PASCAL; Zeiss, Oberkochen, Germany).

The Function of EPCs in Vitro

For the adhesion assay, isolated EPCs were plated on a fibronectin-coated plate and the adherent EPCs were estimated as adherent cells, taking up acetyl low-density lipoprotein and binding lectin. For the migration assay, EPCs were incubated on the Boyden chamber for 18 hours. The number of cells migrating into the lower compartment was counted. To examine the proliferation of EPCs, the cells were incubated on a fibronectin-coated plate for 7 days. The number of cells adsorbing acetyl low-density lipoprotein and binding lectin within each cell colony was counted. To estimate fibronectin-independent proliferation, EPCs were grown in 1% methylcellulose gel and the cell colonies were counted as colony-forming units.

Measurement of 6-Keto Prostaglandin $F_1\alpha$ Content

Subconfluent EPCs were incubated in fresh-complete medium for 24 hours. The prostanoids in the conditioned medium were preextracted using an SPE column (Cayman Chemical, Ann Arbor, Mich), then 6-keto prostaglandin $F_1\alpha$ (6-keto $PGF_1\alpha$), a stable PGI_2 metabolite, was measured by an enzyme-linked immunosorbent assay kit (Cayman Chemical).

Statistical Analysis

Data are presented as mean±SEM. The significance in multiple comparisons was determined using an analysis of variance, followed by the Fisher test. We compared the mean of two groups by an unpaired Student *t* test. $P<0.05$ was considered statistically significant.

Results

BM Reconstitution of WT Mice With $IP^{-/-}$ BM Cells Results in Augmentation of Neointimal Hyperplasia

To determine whether BM cells (BMCs) contribute to augmented neointimal hyperplasia observed in $IP^{-/-}$ mice, we reconstituted the BM of WT mice by transplanting $IP^{-/-}$ BMCs (WT/$IP^{-/-}$-BM mice) and examined vascular remodeling induced by wire injury. As reported previously in $IP^{-/-}$ mice subjected to an endovascular injury,[12] neointimal hyperplasia was significantly enhanced in mice lacking the IP in the whole body (ie, $IP^{-/-}$ mice transplanted with $IP^{-/-}$ BMCs [$IP^{-/-}$/$IP^{-/-}$-BM mice]), compared with that in WT mice transplanted with WT BMCs (WT/WT-BM mice), indicating an important role for the IP in vascular remodeling (Figure 1A and B). Interestingly, neointimal hyperplasia was also significantly enhanced in WT/$IP^{-/-}$-BM mice compared with that in WT/WT-BM mice (Figure 1A and B). Notably, the degree of this enhancement was almost similar to that in $IP^{-/-}$/$IP^{-/-}$-BM mice, indicating that the IP expressed in BMCs contributes critically to enhanced neointimal hyperplasia observed in $IP^{-/-}$/$IP^{-/-}$-BM mice. On the other hand, neointimal hyperplasia was also significantly enhanced in $IP^{-/-}$ mice transplanted with WT BMCs ($IP^{-/-}$/WT-BM mice), whereas the degree of enhancement was moderate (Figure 1A and B), indicating some roles for the IP expressed in vascular tissues. There were no significant differences in the medial areas among 4 groups of mice (Figure 1C). These results indicate that the depletion of the PGI_2-IP signaling in BMCs is almost sufficient to reproduce an atherogenic phenotype of $IP^{-/-}$ mice found in vascular remodeling.

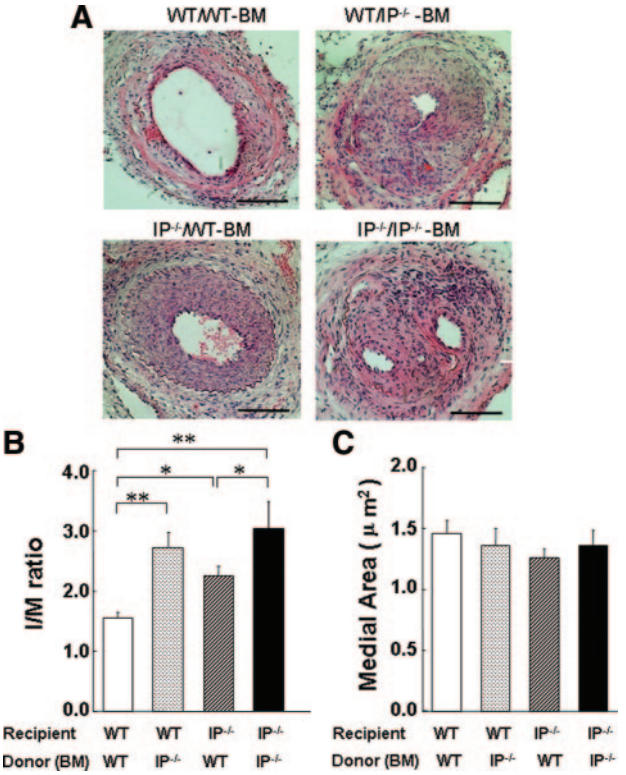

Figure 1. Bone marrow (BM)–specific deletion of IP augments neointimal hyperplasia induced by wire injury. A, Representative histological manifestation of femoral arteries stained with hematoxylin and eosin at 4 weeks after the vascular injury. The bar indicates 100 μm. B and C, Morphometric analysis of the femoral arteries at 4 weeks after the vascular injury. The degrees of neointimal hyperplasia were expressed as a ratio of intimal to medial area (I/M ratio). WT indicates wild type. *$P<0.05$ and **$P<0.01$ (n=9–14 per group).

Decreased Number of Adhesive EPCs in Mice With $IP^{-/-}$ BMCs Before and After the Vascular Injury

Regarding the vascular injury, the number of EPCs in peripheral blood increased significantly and reached a similar peak level at 14 days after the injury in WT/WT-BM and $IP^{-/-}$/WT-BM mice (Supplemental Figure I), indicating mobilization of EPCs from BM to peripheral circulation. Unexpectedly, the increases in the numbers of circulating EPCs in WT/$IP^{-/-}$-BM and $IP^{-/-}$/$IP^{-/-}$-BM mice were

augmented significantly compared with those in WT/WT-BM mice (Supplemental Figure I). The adhesive EPCs were identified as EPCs having the property of fibronectin adhesiveness in addition to acetyl low-density lipoprotein absorption and lectin binding capacities. In accordance with mobilization of EPCs, the number of adhesive EPCs also increased in peripheral blood after the vascular injury in WT/WT-BM mice. In WT/IP$^{-/-}$-BM mice, however, there was no significant increase in the number of adhesive EPCs in response to the vascular injury (Supplemental Figure II). These results suggest that the defective response in the number of adhesive EPCs, despite the augmented mobilization of EPCs, resulted in the enhanced neointimal hyperplasia in WT/IP$^{-/-}$-BM mice.

Enhanced Neointimal Hyperplasia Observed in WT/IP$^{-/-}$-BM Mice Was Rescued by Transfusion of WT EPCs Through Facilitated Reendothelialization

To further evaluate the role of EPCs in neointimal hyperplasia, EPCs isolated from the BM of WT and IP$^{-/-}$ mice were infused intravenously into WT/IP$^{-/-}$-BM mice one day after the vascular injury. The enhanced neointimal hyperplasia observed in WT/IP$^{-/-}$-BM mice was suppressed to a comparable degree with that in WT/WT-BM mice by transfusion of WT EPCs (Figure 2A). However, EPCs isolated from IP$^{-/-}$ mice failed to present such a suppressive effect (Figure 2A). This result clearly indicates that EPCs play a critical role in the development of vascular remodeling, suppressing neointimal hyperplasia, and that IP$^{-/-}$ EPCs are defective in this in vivo function. Notably, other kinds of BM-derived cells, such as platelets and leukocytes, would not participate in the present effect of EPCs, because infused EPCs essentially did not contain these kinds of cells.

Reendothelialization over the neointimal layer occurred at 1 to 4 weeks after the vascular injury; this was accompanied by termination of vascular remodeling. To determine whether transfused EPCs were incorporated into the regenerated endothelial layer, EPCs isolated from WT mice expressing GFP (GFP-WT mice) were infused into WT/IP$^{-/-}$-BM mice one day after the vascular injury. At 4 weeks after the injury, some GFP-positive WT EPCs were observed within the CD31-positive endothelial layer (Figure 2B), indicating that transfused EPCs were actually incorporated into the regenerated endothelial layer. To further test whether transfused EPCs participate in reendothelialization, WT or IP$^{-/-}$ EPCs were infused into WT/IP$^{-/-}$-BM mice after the vascular injury and reendothelialization was examined using Evans blue staining of the injured vessels. At 10 days after the injury, the degree of reendothelialization was significantly higher in mice transfused with WT EPCs compared with that in nontransfused control mice (Figure 2C and D). However, transfused IP$^{-/-}$ EPCs failed again to facilitate reendothelialization (Figure 2C and D).

BM-derived cells were also detected in the α-smooth muscle actin-positive neointimal area after the vascular injury (Supplemental Figure III), indicating the participation of BM-derived cells in neointimal hyperplasia. However, the ratio of BM-derived cells to total cells within the neointimal area in WT/IP$^{-/-}$-BM mice did not differ from that in WT/WT-BM mice. This result indicates that EPCs contributed rather critically compared with BM-derived cells, corresponding to the possible progenitor cells for vascular smooth muscle cells to the enhanced neointimal hyperplasia observed in WT/IP$^{-/-}$-BM mice.

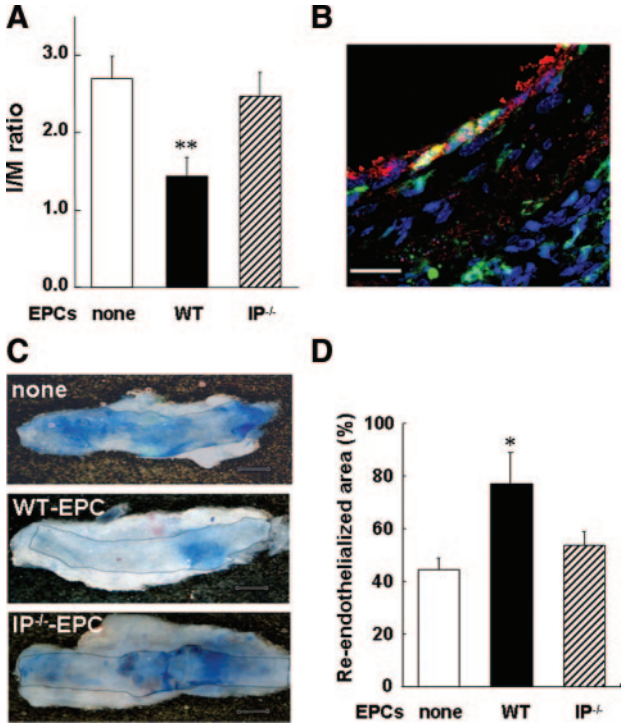

Figure 2. Transfused wild-type (WT) endothelial progenitor cells (EPCs), but not IP$^{-/-}$ EPCs, are recruited to the injured vessels, participate in reendothelialization, and rescue the augmented neointimal hyperplasia in WT/IP$^{-/-}$–bone marrow (BM) mice. A, Morphometric analysis of the femoral arteries at 4 weeks after the vascular injury. I/M ratio indicates the ratio of intimal to medial area. $^{**}P<0.01$ (n=6). B, Incorporation of EPCs into the endothelium. Green fluorescent protein (GFP)–expressing WT EPCs (green) were detected within the endothelial layer stained by anti-CD31 antibody with TRITC (red). Nuclei were counterstained with Hoechst 33258 (blue). The bar indicates 25 μm. C, Representative photomicrographs of femoral arteries stained by Evans blue dye at 10 days after the vascular injury. The area stained blue corresponds to the area not yet reendothelialized. D, Quantification of the reendothelialized area assessed by the percentage of Evans blue nonstaining area for the entire injured area. $^{*}P<0.05$ (n=6).

EPCs Express IP and Produce PGI_2

Our data suggest that the PGI_2-IP system is crucial for EPCs to perform their innate function (ie, suppressing neointimal hyperplasia). The IP messenger RNA (mRNA) was detected in the MNCs and Lin$^+$ cells of BM, but the expression levels were relatively low. However, the IP mRNA expression was strengthened in EPCs versus MNCs or Lin$^+$ cells (Figure 3A). Considering a small population of EPCs (3%–5% of MNCs), this result suggests that EPCs (and megakaryocytes) are the representative IP-expressing cells among BM MNCs. Next, we measured the concentrations of 6-keto-$PGF_1\alpha$, a stable metabolite of PGI_2, in the conditioned medium of EPCs. EPCs produced significant amounts of PGI_2, whose production was inhibited significantly by indomethacin (a nonselective cyclooxygenase [COX] inhibitor) and SC560 (a COX-1–selective inhibitor), but not by

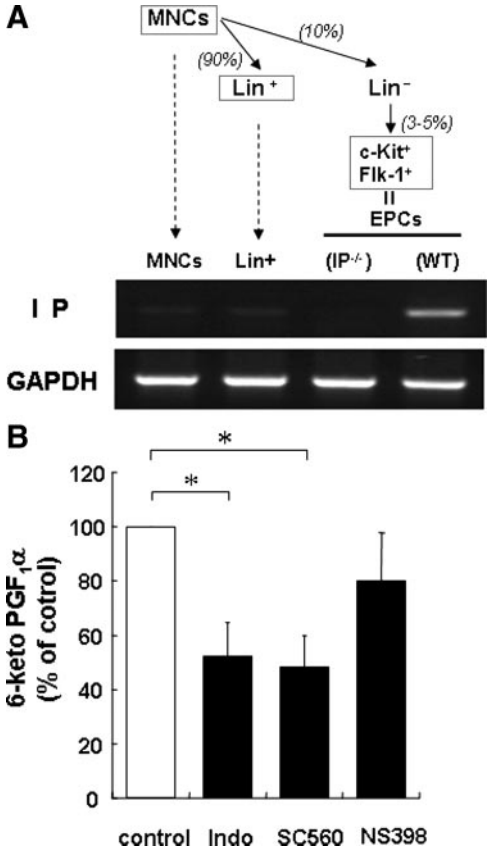

Figure 3. Endothelial progenitor cells (EPCs) express the IP and produce prostaglandin (PG) I_2. A, Reverse transcription–polymerase chain reaction analysis of the IP messenger RNA in each fraction of mononuclear cells (MNCs), Lin^+ cells, and EPCs ($Lin^-cKit^+Flk\text{-}1^+$) in bone marrow. MNCs and Lin^+ cells were prepared from WT mice. B, The concentrations of 6-keto $PGF_1\alpha$, a stable PGI_2 metabolite, in the conditioned medium of EPCs cultured in the absence or the presence of cyclooxygenase inhibitors, indomethacin (Indo; 10 μmol/L), SC560 (1 μmol/L), and NS398 (5 μmol/L). Data are expressed as a percentage of those in the control group. GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase. *$P<0.05$ (n=6–8).

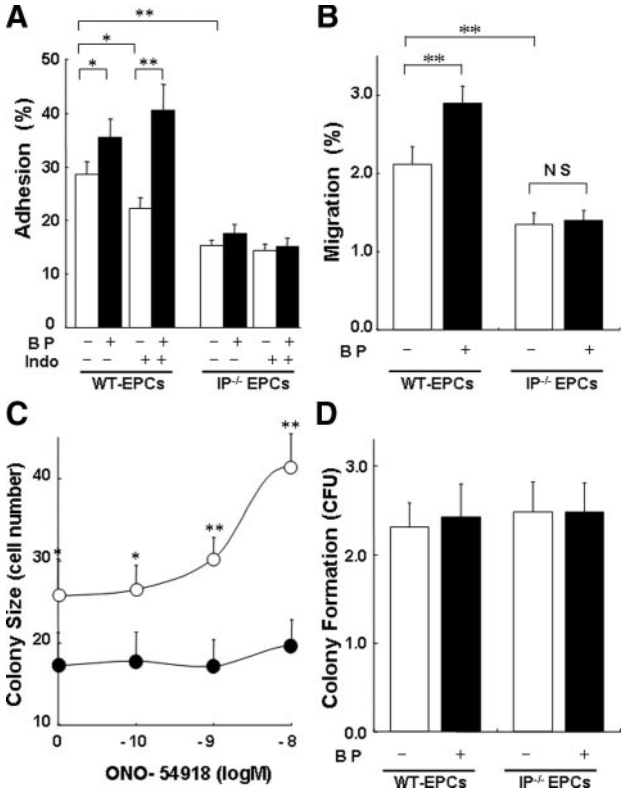

Figure 4. The IP-mediated signaling facilitates the fibronectin-dependent adhesion, migration, and proliferation of endothelial progenitor cells (EPCs). A, EPCs isolated from the bone marrow of wild-type (WT) and $IP^{-/-}$ mice were incubated in the absence or the presence of beraprost (BP; 1 nmol/L) and indomethacin (Indo; 10 μmol/L). B, The number of EPCs migrating through the fibronectin-coated filter to the lower compartment was counted. The value was presented as a percentage of the number of the applied cells. C, WT (unshaded circle) and $IP^{-/-}$ EPCs (shaded circle) were cultured on fibronectin-coated plates in the presence of indicated concentrations of ONO-54918. The number of EPCs in each colony was counted. D, EPCs were cultured in methylcellulose gel for 7 days in the absence or the presence of BP (1 nmol/L). The number of cell colonies formed was counted. CFU indicates colony-forming units; NS, nonsignificant. *$P<0.05$ and **$P<0.01$ (vs $IP^{-/-}$ EPCs at each dose) (n=6–10 per group).

NS398 (a COX-2–selective inhibitor) (Figure 3B); this indicated COX-1–dependent production of PGI_2 by EPCs. These results indicate that the PGI_2-IP system is imminent in EPCs.

PGI_2-IP Signaling Facilitates Fibronectin-Dependent Adhesion, Migration, and Proliferation of EPCs

The numbers of adhesive EPCs in both peripheral blood and BM were significantly lower in $IP^{-/-}$ mice compared with those in WT mice (Supplemental Figure II). To clarify the mechanisms leading to this phenotype of $IP^{-/-}$ mice, we examined the role of PGI_2-IP signaling in the cellular functions of EPCs. Indomethacin attenuated the adhesion of WT EPCs, but not of $IP^{-/-}$ EPCs (Figure 4A), indicating that endogenous PGI_2 could enhance the adhesion of EPCs in an IP-dependent manner. In contrast, beraprost, an IP agonist, enhanced the adhesion to a similar extent between indomethacin-treated and indomethacin-nontreated WT EPCs (Figure 4A). In $IP^{-/-}$ EPCs, however, the adhesion was significantly impaired compared with that in WT EPCs, and beraprost could not enhance the adhesion (Figure 4A), indicating that both endogenous PGI_2 and the IP agonist could enhance the adhesion of WT EPCs via the IP. In accordance with this result, the migration of WT EPCs through the pores of a fibronectin-coated membrane in the Boyden chamber system was also enhanced by beraprost in an IP-dependent manner (Figure 4B).

In both WT and $IP^{-/-}$ EPCs, the fibronectin-adherent EPCs formed colonies, and more than 90% of the cells in each colony adsorbed acetyl low-density lipoprotein and bound lectin after 7 days of culture. However, the number of colonies was significantly smaller in $IP^{-/-}$ EPCs than in WT EPCs; the numbers were 204.6 ± 22.5 and 291.3 ± 27.8 per 8-cm^2 dish, respectively (n=8 per group). In addition, the colony size, which was assessed by the cell number of each colony, was also significantly smaller in $IP^{-/-}$ EPCs compared with that in WT EPCs (Figure 4C). Furthermore, the proliferation of EPCs within the developing colonies was facilitated by ONO-54918, an IP agonist, and beraprost (data not shown) in a concentration-dependent manner in WT EPCs; no such effect was observed in

$IP^{-/-}$ EPCs (Figure 4C). To further examine whether defective proliferation of $IP^{-/-}$ EPCs originates from their impaired adhesion to fibronectin, EPCs were cultured in a methylcellulose gel. As was expected, there were no significant differences in the number of colonies between WT and $IP^{-/-}$ EPCs (Figure 4D). In addition, beraprost did not affect the colony number in both WT and $IP^{-/-}$ EPCs (Figure 4D), indicating that the effects of IP agonists on the proliferation of EPCs depend on cellular adhesion to fibronectin.

IP-Deficient EPCs Fail to Be Recruited Into the Injured Vessels

The contribution of EPCs to reendothelialization depends on their recruitment to the injured vessels, which was mediated by the extracellular matrices exposed on the luminal surface of the injured vessels. To examine whether IP deficiency affects the recruitment of EPCs in vivo, EPCs isolated from GFP-WT or GFP-$IP^{-/-}$ mice were transfused into WT/$IP^{-/-}$-BM mice one day after the vascular injury. At one week after the vascular injury, when recruitment of EPCs was readily evaluated, inflammatory cells accumulated on the luminal surface of the injured vessels. The transfused GFP-WT EPCs were frequently observed within the accumulated inflammatory cells, whereas the number of recruited $IP^{-/-}$ EPCs was significantly lower compared with that of WT EPCs (Figure 5A and B), indicating that PGI_2-IP signaling in EPCs would be critical for recruitment of EPCs to the injured vessels in vivo. Neither infused WT nor $IP^{-/-}$ EPCs were detected on the surface of intact vascular lumen (data not shown).

PGI_2-IP Signaling Upregulates the Expression of Fibronectin-Related Integrins in EPCs

Finally, we examined the expression of integrins in EPCs to clarify the molecular basis determining the phenotype of $IP^{-/-}$ EPCs, a defective adhesion to fibronectin. Integrins bind with the extracellular matrix, including fibronectin, whose interaction is required for cellular adhesion, migration, and subsequent proliferation.[16,17] In accordance with previous studies,[16] WT EPCs expressed mRNAs for integrins $\alpha4$, $\alpha5$, $\beta1$, and $\beta3$ (Figure 5C and D). In $IP^{-/-}$ EPCs, however, the expressions of integrin $\alpha5$, $\beta1$, and $\beta3$ were significantly attenuated compared with those in WT EPCs; such a difference was not found in the expression of integrin $\alpha4$ (Figure 5C and D). In addition, beraprost significantly upregulated the expressions of integrins $\alpha5$, $\beta1$, and $\beta3$ in WT EPCs in an IP-dependent manner without an effect on the expression of integrin $\alpha4$ (Figure 5C and D). Because $\beta1$- and $\beta3$-related integrin heterodimers, such as $\alpha4\beta1$, $\alpha5\beta1$, and $\alpha V\beta3$, work as major fibronectin receptors, the PGI_2-IP signaling might facilitate recruitment of EPCs to the injured vessels by upregulating the expressions of these fibronectin receptors in EPCs.

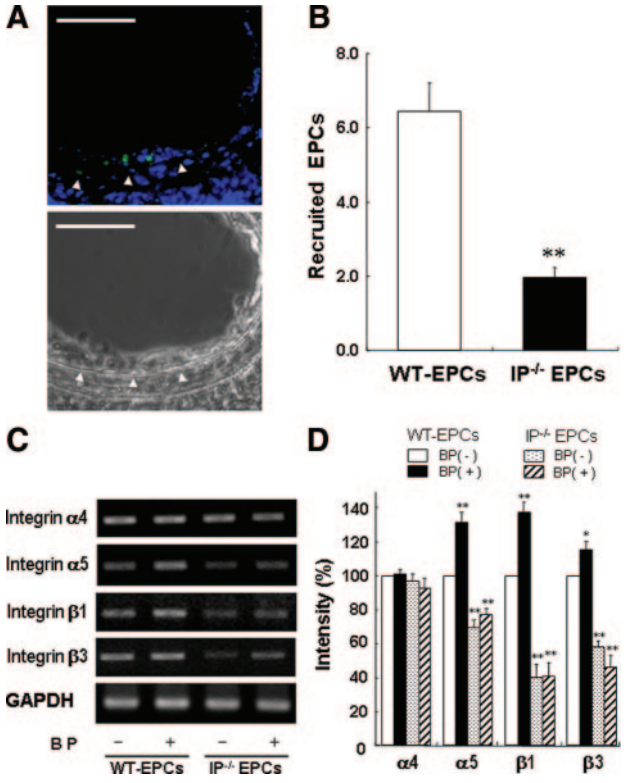

Figure 5. $IP^{-/-}$ endothelial progenitor cells (EPCs) are defective in their recruitment to the injured vessels and have attenuated expression of fibronectin-related integrins. A, A representative arterial section from a mouse transfused with green fluorescent protein (GFP)–expressing wild-type (WT) EPCs. Nuclei were counterstained with Hoechst 33258 (upper panel). Phase-contrast microscopy shows internal elastic lamina (arrowheads) (lower panel). The bar indicates 100 μm. B, The number of recruited EPCs in each section was presented. $^{**}P<0.01$ vs WT EPCs (n=6). C and D, WT and $IP^{-/-}$ EPCs were cultured for one day with or without beraprost (BP; 1 nmol/L), and the expression of integrin messenger RNAs was estimated by reverse transcription–polymerase chain reaction. Densitometric analysis of integrin levels normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). $^{*}P<0.05$ and $^{**}P<0.01$ vs the WT control (unshaded square) (n=4).

Discussion

Recent studies[12–14] have established the role for PGI_2 as a potent negative regulator of vascular remodeling and atherosclerosis. In these studies, enhanced neointimal formation and augmented atherosclerosis observed in $IP^{-/-}$ mice were attributed to the increased activity of platelets and the facilitated interaction of leukocytes with vascular endothelial cells, respectively. Our study clearly shows that IP deficiency only in BMCs reproduces a large part of the $IP^{-/-}$ mouse phenotype, the enhanced neointimal hyperplasia after the vascular injury. Furthermore, transfusion of isolated WT EPCs, but not of $IP^{-/-}$ EPCs, rescues the enhanced neointimal hyperplasia observed in WT/$IP^{-/-}$-BM mice, in association with the participation of transfused EPCs in reendothelialization, indicating an important role of PGI_2 action on EPCs distinct from those on platelets and leukocytes in vascular remodeling. We identify impaired function of $IP^{-/-}$ EPCs in fibronectin-dependent adhesion, migration, and proliferation. These functional impairments of $IP^{-/-}$ EPCs accord with the decreased expression of fibronectin-related integrins in $IP^{-/-}$ EPCs and result in defective recruitment of $IP^{-/-}$ EPCs to the injured vessels. Thus, we demonstrate a novel role of the PGI_2-IP system played in vascular remodeling by facilitating recruitment of EPCs to the injured vessels.

Recently, an important role of EPCs in vascular remodeling has been established.[6,18] After the vascular injury, EPCs were mobilized from BM to peripheral blood in mice having either

WT or $IP^{-/-}$ BM (Supplemental Figure I). Accordingly, the number of adhesive EPCs increased significantly in WT/WT-BM mice after the vascular injury. However, the number of adhesive EPCs did not increase significantly in WT/$IP^{-/-}$-BM mice, indicating that adhesive EPCs would play a critical role in vascular remodeling and that the IP regulates the function of EPCs. Although the number of circulating EPCs is inversely correlated with risk factors of cardiovascular diseases,[19–21] the function of EPCs has emerged as another critical factor affecting the progression of cardiovascular diseases.[22] In fact, dysfunctions of EPCs have been reported in diabetic patients with a high risk of cardiovascular diseases.[23,24] Interestingly, decreased production of PGI_2 in the cardiovascular system has long been known in diabetic patients,[25] whereas a relation between decreased PGI_2 synthesis and the dysfunction of EPCs in these patients remains to be clarified.

For the first time to our knowledge, we found the expression of IP mRNA in EPCs (Figure 3A) and showed that IP expressed in EPCs is functional, facilitating adhesion, migration, and proliferation of EPCs (Figure 4). In addition, EPCs produced PGI_2 in a COX-1–dependent manner (Figure 3B), as is the case of endothelial cells.[26] This result is consistent with a recent report[27] presenting a role of the COX-1/PGI_2 pathway in the angiogenic function of EPCs. Furthermore, circulatory EPCs stimulated PGI_2 production by endothelial cells in a paracrine fashion.[28] Taken together, the PGI_2-IP system inherent in EPCs would work in an autocrine-paracrine fashion, modulating the function of EPCs themselves. However, COX-2 is induced in activated endothelial and inflammatory cells,[26] and much of systemic PGI_2 is produced in a COX-2–dependent manner,[29] indicating that PGI_2 produced through multiple pathways would affect the function of EPCs. Recently, it was widely noticed that COX-2–selective inhibitors significantly increase the incidence of cardiovascular thromboembolic events, such as myocardial infarction and stroke.[30] Because the consequences are thought to be attributable to a deletion of COX-2–derived PGI_2,[31] a novel action of PGI_2 on EPCs presented herein might explain partly the adverse effects of selective COX-2 inhibitors.

The recruitment of EPCs to the injured vessels is a critical step for EPCs to participate in reendothelialization. In the present study, dysfunction of $IP^{-/-}$ EPCs was symbolized by impaired adhesion to fibronectin and thereby decreased migration and proliferation on fibronectin (Figure 4). Fibronectin is a representative component of the extracellular matrices that are exposed on the vascular injury, working as a multifunctional adhesion molecule.[32] Therefore, we speculated that impaired recruitment of $IP^{-/-}$ EPCs to the injured vessels results primarily from defective adhesion of $IP^{-/-}$ EPCs to fibronectin (Figure 5A and B). Recently, it has been reported that integrins are essential as an element of the homing mechanism of EPCs to the tumor neovasculature.[16] EPCs reportedly express integrin $\alpha4\beta1$, $\alpha5\beta1$, and $\alpha V\beta3$; $\alpha4\beta1$ dimer specifically mediates the homing of circulating EPCs to the neovasculatures via their ligands, fibronectin, and epithelial vascular cell adhesion molecule 1.[16] In addition, the migration of EPCs on fibronectin was blocked by anti-integrin $\beta1$ antibody.[17] As was speculated, the mRNA expressions of integrins $\beta1$, $\beta3$, and $\alpha5$ were significantly attenuated in $IP^{-/-}$ EPCs, and beraprost increased the expressions of these integrins in WT EPCs (Figure 5C and D). These results indicate that PGI_2 critically regulates the expression of integrins in EPCs and thereby controls the function of EPCs.

Notably, the regulatory action of PGI_2 on integrin expression may also explain the apparently increased mobilization of $IP^{-/-}$ EPCs on the vascular injury (Supplemental Figure I), because antibodies against $\beta1$ integrin, whose effect reproduces the condition of decreased $\beta1$ integrin observed in $IP^{-/-}$ EPCs, have been reported to promote mobilization of the progenitor cells from BM.[33,34] Alternatively, enhanced mobilization of $IP^{-/-}$ EPCs might be caused by EPC-mobilizing chemokines and cytokines,[7,35] which would be released for a longer time from the injured vascular wall as the result of delayed reendothelialization in mice with $IP^{-/-}$ BM (Figure 1A and B).

Considering the main signaling pathway of the PGI_2-IP system through G_s, adenosine 3≪,5≪-cyclic monophosphate–dependent signaling is anticipated to be involved in the functional regulation of EPCs. Indeed, some previous studies[36,37] have indicated that migration and adhesion of vascular endothelial cells are tightly regulated by adhesion molecules and adenosine 3≪,5≪-cyclic monophosphate or protein kinase A signaling. In addition, PGs and COX have been reported to be involved in integrin-mediated endothelial migration and angiogenesis.[38,39] These results suggest that adenosine 3≪,5≪-cyclic monophosphate–protein kinase A signaling would also be important for PGI_2 to functionally regulate adhesion molecules in EPCs. However, the precise mechanisms (and origin) of PGI_2 in the functional regulation of integrins in EPCs remain to be clarified.

There are two major aspects in the regenerative function of EPCs: proangiogenic function (angiogenesis) and repair of injured endothelium (reendothelialization). Although the role of PGI_2 in the proangiogenic function of EPCs has been reported by He et al,[27] the role of PGI_2 in reendothelialization remains to be determined. In the present study, we clarified a novel role of PGI_2 in the endothelium-repairing function of EPCs. In addition, the present study has shown the in vivo role of PGI_2 in the mobilization and recruitment of EPCs; these phenomena are important for EPCs to participate in reendothelialization and angiogenesis. He et al suggested the involvement of peroxysome proliferator–activated receptor δ, but not of IP, as a key molecule mediating the effect of PGI_2 on EPCs.[27] However, we presented a quite different mechanism in reendothelialization by EPCs; PGI_2 acts on the IP expressed on EPCs.

In summary, we demonstrate a novel role of the IP expressed on EPCs in vascular remodeling and gain a new mechanistic insight into PGI_2 action; PGI_2 facilitates the recruitment of EPCs partly by upregulating the expression of integrins. Our findings that PGI_2 works as such a mediator would contribute not only to a better understanding of the mechanisms maintaining the function of EPCs but also to the development of novel approaches in the expanding field of regeneration therapy using EPCs.

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Disclosures

None.

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