Angiotensin II Induces Premature Senescence of Vascular Smooth Muscle Cells and Accelerates the Development of Atherosclerosis via a p21-Dependent Pathway

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Background—Angiotensin II (Ang II) has been reported to contribute to the pathogenesis of various human diseases including atherosclerosis, and inhibition of Ang II activity has been shown to reduce the morbidity and mortality of cardiovascular diseases. We have previously demonstrated that vascular cell senescence contributes to the pathogenesis of atherosclerosis; however, the effects of Ang II on vascular cell senescence have not been examined.

Methods and Results—Ang II significantly induced premature senescence of human vascular smooth muscle cells (VSMCs) via the p53/p21-dependent pathway in vitro. Inhibition of this pathway effectively suppressed induction of proinflammatory cytokines and premature senescence of VSMCs by Ang II. Ang II also significantly increased the number of senescent VSMCs and induced the expression of proinflammatory molecules and of p21 in a mouse model of atherosclerosis. Loss of p21 markedly ameliorated the induction of proinflammatory molecules by Ang II, thereby preventing the development of atherosclerosis. Replacement of p21-deficient bone marrow cells with wild-type cells had little influence on the protective effect of p21 deficiency against the progression of atherogenesis induced by Ang II.

Conclusions—We demonstrated that Ang II promotes vascular inflammation by inducing premature senescence of VSMCs both in vitro and in vivo. Our results suggest a critical role of p21-dependent premature senescence of VSMCs in the pathogenesis of atherosclerosis. (Circulation. 2006;114:953-960.)

Key Words: aging ■ muscle, smooth ■ senescence

Aging is a physiological process associated with an increase in cardiovascular mobility and mortality even in the absence of known cardiovascular risk factors.1 Age-associated changes in the blood vessels include a decrease in compliance and an increase in the inflammatory responses that promote atherogenesis.2 It has been suggested that these alterations are attributable to age-related functional changes in vascular cells.3–5 For example, endothelium-dependent vasodilation is impaired with age owing to decreased endothelial production of vasodilators such as nitric oxide (NO) and prostacyclin and to reduced responsiveness of vascular smooth muscle cells (VSMCs) to these vasodilators.6 Adrenergic, endothelium-independent VSMC vasodilation also declines with age.3 Moreover, increased expression of proinflammatory and prothrombogenic molecules was observed in vascular cells of aged arteries.7 It is noteworthy that similar functional changes have been reported in senescent vascular cells in vitro.8–11

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Cellular senescence is the limited ability of primary human cells to divide when cultured in vitro; it is accompanied by a specific set of phenotypic changes in vitro, expression, and function. These phenotypic changes have been suggested to have a role in human aging and age-associated diseases.12 This hypothesis of cellular aging was established by Hayflick13 and is supported by evidence that the replicative potential of primary cultured human cells is dependent on donor age and that the growth potential of cultured cells is well correlated with the mean maximum lifespan of the species of origin. The histology of the lesions of human atherosclerosis has been studied extensively, and these studies have demonstrated that there are vascular cells that exhibit the morphological features of cellular senescence. These suggest the occurrence of cellular senes-
cence in vivo. Recently, this notion has been confirmed by cytochemical analysis in vivo with senescence-associated β-galactosidase (SA β-gal) activity, a biomarker for cellular senescence. SA β-gal–positive vascular cells were detected in rabbit carotid arteries subjected to vascular injury.16 We and others have demonstrated SA β-gal–positive vascular cells in human atherosclerotic plaque of coronary arteries obtained from patients who had ischemic heart disease.17,18 SA β-gal–positive cells were predominantly localized on atherosclerotic plaque, but no positive cells were observed in the internal mammary arteries from the same patients, in which atherosclerotic changes were minimally observed. In advanced plaque, SA β-gal–positive VSMCs were detected in the intima and, to a lesser extent, in the media.19 SA β-gal–positive cells exhibit increased expression of p53 and p21
\(^{\text{Waf1/Cip1}}\), alternative markers for cellular senescence, in human atheroma, which suggests the further evidence of in vivo senescence. These cells also show impaired function, such as the decreased expression of endothelial NO synthase and the increased expression of proinflammatory molecules.19 There is also evidence indicating that progressive telomere shortening, a biomarker of cellular aging, occurs in human blood vessels, which may be related to age-associated vascular diseases.20,21 Thus, cellular senescence in vivo may contribute to the pathogenesis of vascular aging.

Arterial components of the angiotensin II (Ang II) signaling cascade increase with aging and contribute to the pathogenesis of atherosclerosis, whereas inhibition of Ang II activity has been shown to reduce the morbidity and mortality of cardiovascular disease.7 Ang II signaling appears to play a critical role in regulating many of the stimuli and signals that govern vascular aging and atherogenesis; however, the mechanism underlying the deleterious effects of Ang II on the cardiovascular system is not yet fully understood. We previously reported that Ras activation induced vascular cell senescence and inflammation, and we suggested that vascular cell senescence might contribute to human atherosclerosis.19

Because Ang II is known to activate the Ras signaling pathway,22,23 we hypothesized that Ang II may promote the development of atherosclerosis by inducing vascular cell senescence. In the present study, we demonstrate that Ang II promotes vascular inflammation by inducing premature senescence of VSMCs. Ang II induces premature senescence via the p53/p21-dependent pathway. Inhibition of this pathway effectively suppresses induction of the production of proinflammatory cytokines, as well as cellular senescence, and thereby prevents the development of atherosclerosis. These results disclose a novel role of Ang II in cardiovascular diseases and will provide insights into a novel treatment for atherosclerosis.

Methods

Cell Culture and Treatment

Primary cultured human aortic VSMCs were purchased from Cambrex (Walkersville, Md) and were grown according to the manufacturer’s instructions. After cultures reached confluence in growth medium (SmGM-2, Cambrex), the cells were transferred to serum-free DMEM (Sigma-Aldrich, St. Louis, Mo) and incubated with Ang II (Sigma). Staining for SA β-gal was performed as described previously.18 Briefly, the samples were incubated for 24 hours at 37°C in freshly prepared β-gal staining solutions (pH 6.0) containing 1 mg/mL 5-bromo-4-chloro-3-indyl β-1-galactopyranoside (X-gal), 5 mMol/L potassium ferrocyanide, 5 mMol/L potassium ferricyanide, 150 mMol/L NaCl, 2 mMol/L MgCl₂, 0.01% sodium deoxycholate, and 0.02% Nonidet-40. In some experiments, VSMCs were treated with olmesartan (Sankyo Co, Ltd, Tokyo, Japan), PD123319 (Sigma), or N-acetyl cysteine (NAC; Sigma).

Luciferase Assay

The reporter gene plasmid (1 μg) was transfected into VSMCs at 24 hours before the luciferase assay. The control vector encoding Renilla luciferase (0.1 μg) was co-transfected as an internal control. Then the luciferase assay was performed with a dual luciferase reporter assay system (Promega, Madison, Wis) according to the manufacturer’s instructions. The expression vector encoding p21 cDNA was a gift from Dr B Vogelstein (Johns Hopkins University, Baltimore, Md). pPG13-Luc, the luciferase reporter gene containing the p53 binding sites, was also a gift from Dr Vogelstein. p55-A2-Luc, the luciferase reporter gene containing the κB binding sites, was a gift from Dr T Fujita (The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan).

Experimental Animals

The animal experiments were approved by our institutional review board. ApoE-deficient mice (C57BL/6 background) and p21-deficient mice were obtained from the Jackson Laboratory (Bar Harbor, Me). The p21-deficient mice were backcrossed with wild-type C57BL/6 mice for 6 generations, whereas apoE/p21-deficient mice (C57BL/6 background) were generated by crossing apoE-deficient mice and p21-deficient mice. Animals were housed under a 12-hour light/dark cycle and fed a normal chow diet. Blood pressure was measured with a noninvasive tail-cuff system. Blood samples were obtained from the mice at the time of euthanasia. Mice (4 to 6 months old) were anesthetized by intraperitoneal injection of a mixture of ketamine 100 mg/kg and xylazine 5 mg/kg, and an osmotic minipump (Alzet model 2004, Direx Corp, Cupertino, Calif) was implanted to deliver Ang II subcutaneously at a dose of 1.44 mg · kg⁻¹ · d⁻¹ for 4 weeks. After Ang II treatment, the mice were killed by cervical dislocation. The heart and aorta were removed after systemic perfusion with phosphate-buffered saline (PBS) for histological examination, zymography, and RNA analysis.

Statistical Analysis

Results are expressed as mean±SEM unless otherwise stated. Comparison of results between different groups was performed by 1-way ANOVA or 2-way ANOVA followed by the Scheffé post hoc test, or by a nonparametric Kruskal-Wallis test followed by a Dunn multiple comparison test, as indicated in the online Data Supplement. An unpaired Student t test was performed for single comparisons between groups. Survival was estimated with the Kaplan-Meier method. A log-rank test was used to compare survival between groups. Statistical significance was accepted at a value of P<0.05.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Ang II Induces Premature Senescence In Vitro

To determine whether Ang II induces cellular senescence in vitro, we treated human VSMCs with Ang II for 3 days and then examined markers of cellular senescence. SA β-gal activity was significantly increased in Ang II–treated VSMCs compared with control cells (Figure 1A). Ang II also significantly increased the transcriptional activity of p53 compared with that in vehicle-treated cells, and its effect was dose dependent (Figure 1B). Moreover, expression of p21 and p53 was elevated in Ang II–treated VSMCs (Figure 1C). Because Ang II type 1 (AT₁)–specific inhibitor but not Ang II type 2
Ang II (indicated as AII) induces vascular cell senescence in vitro. A, After cultures reached confluence in growth medium, human VSMCs were transferred to serum-free medium and incubated with Ang II at a pathological concentration ($10^{-7}$ mol/L) for 3 days. SA β-gal staining was performed as described in Methods, and the number of SA β-gal-positive cells was counted. *P<0.05 (unpaired t-test, n=4). B, The luciferase reporter gene plasmid pPG13-Luc containing the p53-binding sequence was transfected into VSMCs, and the cells were treated with Ang II at the indicated concentration 24 hours before luciferase activity was measured. The activity of PG13-Luc in VSMCs treated with vehicle (Ang II=0) was designated as 1, and relative values were plotted. *P<0.05 vs Ang II=0 (1-way ANOVA, n=4). C, Whole-cell lysates (20 μg) were prepared from VSMCs treated with Ang II (+; $10^{-7}$ mol/L) or vehicle (-) for 3 days and were examined to detect expression of p53, p21, and actin (control) by Western blot analysis. Expression of p53 and p21 was standardized on the basis of actin expression, and the relative levels of expression are plotted in the graphs. The corrected value in vehicle-treated cells was designated as 1. *P<0.05 vs vehicle-treated cells (unpaired t-test, n=5). D, VSMCs were infected with pBabe (Mock) or pBabe E6 (E6) and purified by culture with puromycin (0.8 μg/mL) for 3 days. The purified infected cells were then treated with Ang II (+) or vehicle (-) for 5 days, after which expression of p53, p21, and actin (control) was examined by Western blot analysis. Expression of p53 and p21 was standardized on the basis of actin expression, and the relative levels of expression are plotted in the graphs. *P<0.05 vs mock-infected cells (unpaired t-test, n=5). E, VSMCs were transfected with siRNA targeting p21 (p21) or random sequence (Mock) and treated with Ang II (+) or vehicle (-) for 3 days. SA β-gal–positive cells were counted. *P<0.05 vs Mock/Ang II (+; 2-way ANOVA, n=3). F, VSMCs were transfected with siRNA targeting p21 (p21) or random sequence (Mock) and treated with Ang II (+) or vehicle (-) for 3 days. Genomic DNA was extracted, and telomere length was evaluated by Southern blot analysis as described in the Data Supplement (Methods). TRF indicates terminal restriction fragment.

It has been reported that Ang II causes vascular inflammation by upregulating the expression of various proinflammatory cytokines. Because cellular senescence is also associated with the inflammatory response, it is possible that Ang II promotes vascular inflammation via the p53/p21-dependent pathway. To test this notion, we examined the effects of E6 on Ang II–induced expression of proinflammatory cytokines. Expression of interleukin (IL)-1β by mock-infected VSMCs was increased after treatment with Ang II (Figure 2A), whereas this increase was effectively inhibited by introducing (AT2)–specific inhibitor significantly suppressed induction of p21 expression by Ang II treatment (Data Supplement, Figure I), it is likely that AT2, mediates Ang II–induced senescence. To further investigate the relationship between Ang II and p53 transcription, we tested whether ablation of p53 could prevent Ang II–induced senescence. To do this, we infected VSMCs with a retroviral vector encoding the E6 oncprotein of HPV16, which binds p53 and facilitates its destruction by ubiquitin-mediated proteolysis, or with an empty vector (mock infection). Western blot analysis revealed that introduction of E6 effectively reduced the level of p53 protein and also markedly reduced the expression of p21, its target protein (Figure 1D). Treatment of mock-infected VSMCs with Ang II significantly increased the number of SA β-gal–positive cells (Figure 1E). In contrast, the effect of Ang II on senescence was reduced in E6-infected VSMCs. We next examined the effect of p21 expression on Ang II–induced senescence. Overexpression of p21 significantly increased the number of SA β-gal–positive cells (Figure 1F), whereas p21 knockdown with a small interfering RNA (siRNA) system effectively blunted the effect of Ang II on senescence (Figure 1G). These results indicate a critical role of the p53/p21 pathway in Ang II–induced senescence.

Signals other than extended proliferation have been shown to result in cells developing a phenotype indistinguishable from that of senescent cells at the end of their replicative life span. For example, the constitutive activation of mitogenic stimuli or oxidative stress induces a senescent phenotype. Cellular senescence triggered by such stimuli is independent of replicative age, and these signals act before the replicative limits of cells. Hence, it is apparently telomere-independent and thus is termed “stress-induced premature senescence.” Ang II treatment induced cellular senescence within 3 days without telomere shortening (Figure 1H), which suggests that Ang II provokes stress-induced premature senescence.
tion of E6 (Figure 2A). Overexpression of p21 markedly increased the expression of IL-1β compared with that in mock-infected cells (Figure 2B). Nuclear factor κB (NF-κB) is known to be one of the important transactivators of inflammatory cytokines. To investigate whether overexpression of p21 led to an increase in NF-κB activity, a luciferase reporter gene containing NF-κB binding sites was introduced into p21-infected or mock-infected VSMCs. NF-κB activity was found to be significantly higher in p21-infected VSMCs than in mock-infected VSMCs (Figure 2C). Conversely, knockdown of p21 significantly inhibited NF-κB activity by inducing p21 expression. It has been shown that p21 causes an increase of reactive oxygen species (ROS), which can activate NF-κB. Therefore, we also examined the effect of an ROS scavenger, N-acetyl cysteine (NAC), on NF-κB activity. Enhancement of luciferase activity by p21 was significantly weaker in the presence of NAC (Figure 2C), which suggests that an increase of ROS mediated via p21 may be involved in Ang II–induced vascular inflammation. Moreover, NAC treatment effectively inhibited Ang II–induced senescence (Figure 2E).

**Ang II Induces Cellular Senescence In Vivo**

Next, we investigated whether Ang II could induce cellular senescence in vivo. We treated apoE-deficient mice with Ang II for 4 weeks and examined markers of cellular senescence in the vasculature. Consistent with our in vitro data, treatment with Ang II enhanced SA β-gal activity in the aortas of apoE-deficient mice (Figures 3A and 3B). Treatment of wild-type mice with Ang II did not markedly increase SA β-gal activity in the aortas (data not shown), and therefore, we focused on the effects of Ang II in apoE-deficient mice for further experiments. In apoE-deficient mice, most of the vascular cells that were positive for SA β-gal activity concomitantly exhibited immunoreactivity for α-smooth muscle actin (Figure 3A), which indicates that these were probably senescent VSMCs. Northern blot analysis revealed that aortic expression of p21 was markedly elevated by treatment with Ang II (Figure 3C). To further define the role of p21 in Ang II–induced senescence, we established apoE/p21-deficient mice and treated these animals with Ang II for 4 weeks. There were no differences in the blood pressure and lipid profiles between apoE/p21-deficient mice and apoE-deficient mice after Ang II treatment (Table). Disruption of p21 did not significantly decrease SA β-gal–positive cells in the aorta of control apoE-deficient mice. In the Ang II–treated group, however, apoE/p21-deficient mice had significantly fewer SA β-gal–positive cells in the aorta than apoE-deficient mice (Figure 3B). To ascertain whether Ang II induces vascular inflammation via a p21–dependent pathway, we examined the aortic expression of various proinflammatory molecules. Ang II treatment strikingly induced the expression of proinflammatory cytokines, such as IL-1β and IL-6, as well as intercellular adhesion molecule-1 in the aortas of apoE-deficient mice, whereas p21 deficiency effectively blocked the induction of these molecules by Ang II treatment (Figure 4A; Data Supplement, Figures III and IV). Likewise, Ang II markedly increased the aortic activity of matrix metalloproteinase-2 in apoE-deficient mice but not in apoE/p21-deficient mice (Figure 4B).

These results suggested that inhibition of vascular cell senescence might prevent the induction of atherosclerosis by
Ang II. It has already been reported that treatment with Ang II exacerbates atherogenesis in apoE-deficient mice and thereby promotes the formation of aortic aneurysms.\(^{32,33}\) Consistent with such findings, we showed that Ang II treatment significantly increased the area of intimal thickening and the extent of aneurysm formation in the aortas of apoE-deficient mice (Figures 5A and 5B). The development of aortic aneurysms and atheroma after Ang II treatment was significantly less prominent in apoE/p21-deficient mice than in apoE-deficient mice (Figures 5A and 5B). Suppression of the induction of p21 by Ang II significantly increased the survival of these mice by preventing the rupture of aortic aneurysms (Figure 5C). Histological analysis revealed that the number of proliferating cell nuclear antigen–positive VSMCs in the intima of apoE/p21-deficient mice was greater than that of apoE-deficient mice (Data Supplement Figure V), which suggests that absence of p21 stabilizes atherosclerotic plaque. p21 has been reported to play a critical role in regulating the survival, proliferation, and differentiation of hematopoietic cells.\(^{34–36}\) Because hematopoietic cells, especially macrophages, are involved in the process of atherosclerosis,\(^{37}\) p21 deficiency may affect macrophage proliferation

Figure 3. Ang II (indicated as AII) induces vascular cell senescence in vivo. A, ApoE knockout mice were treated with Ang II (1.44 mg · kg\(^{-1} · \text{d}^{-1}\), ApoE KO+All) or vehicle (ApoE KO) for 4 weeks. Then, the aortas were excised and subjected to SA β-gal staining (arrow, upper panel). Scale bar=1 mm. After the stained arteries were photographed, frozen sections were also prepared and stained with an antibody for α-smooth muscle actin (lower panel). Scale bar=50 μm. B, ApoE knockout mice (ApoE KO) and apoE/p21 double-knockout mice (DKO) were treated with Ang II (+All) or vehicle (+Vehicle) for 4 weeks. Untreated wild-type mice (WT) served as a control. The number of SA β-gal–positive VSMCs is shown relative to the total number of VSMCs. *P<0.01 vs ApoE KO+Vehicle, †P<0.01 vs ApoE KO+All, ‡P<0.01 vs DKO+Vehicle (1-way ANOVA, n=10). C, Total RNA (30 μg) was extracted from the aortas of apoE-deficient mice treated with Ang II (ApoE KO+All) or vehicle (ApoE KO) for 4 weeks, after which expression of p21 and 36B4 (control) was examined by Northern blot analysis. Similar results were obtained from 3 independent experiments.

Figure 4. Ang II (indicated as AII) induces vascular inflammation in vivo. A, Ribonuclease protection assay for expression of IL-1β and IL-6 in the aortas of apoE-deficient mice (ApoE KO) or apoE/p21-deficient mice (DKO) treated with Ang II (+All) or vehicle (+Vehicle) for 4 weeks, or in the aortas of wild-type mice (WT). Expression of IL-1β and IL-6 was standardized on the basis of GAPDH expression, and the relative levels of gene expression are plotted in the graph. The corrected value in wild-type mice was designated as 1. *P<0.05 vs ApoE KO+vehicle, †P<0.05 vs ApoE KO+All (1-way ANOVA, n=5). B, Gelatin zymography for matrix metalloproteinase-2 (MMP-2) activity in the aortas of the same types of mice as in Figure 4A. The value in wild-type mice was designated as 1, and the relative levels of enzyme activity in the aortas of the same types of mice as in Figure 4A. The relative levels of enzyme activity are plotted in the graph. *P<0.05 vs ApoE KO+vehicle, †P<0.05 vs ApoE KO+All (1-way ANOVA, n=5).
and thus protect against the promotion of atherogenesis by Ang II. To test this possibility, apoE<sup>−/−</sup> p21<sup>+/+</sup> bone marrow cells were transplanted into apoE/p21−deficient or apoE-deficient mice that were then treated with Ang II for 4 weeks. Despite the transplantation of apoE<sup>−/−</sup> p21<sup>+/+</sup> bone marrow, the number of SA β-gal–positive cells was still significantly lower in the aortas of apoE/p21−deficient mice than in apoE-deficient mice (Figure 6A). Likewise, the formation of aortic plaque was significantly inhibited in marrow-transplanted apoE/p21−deficient mice compared with marrow-transplanted apoE-deficient mice (Figure 6B), which suggests that p21 expression by vascular cells has a critical role in the development of atherosclerosis.

### Discussion

Various mechanisms for the promotion of atherogenesis by Ang II have been suggested. Ang II is thought to increase the production of ROS and thereby activate several proinflammatory transcription factors such as NF-κB, thus leading to the onset of vascular inflammation and atherogenesis. The present study showed that Ang II significantly induced vascular cell senescence and inflammation both in vitro and in vivo, with this induction being inhibited by the suppression of p21 expression. Ang II treatment prematurely provoked cellular senescence within 3 days without telomere shortening in vitro, which suggests that Ang II encourages stress-induced premature senescence. The protective effects of p21 deficiency against Ang II–induced atherogenesis were preserved in apoE/p21−deficient mice even after transplantation with p21<sup>+/+</sup> bone marrow cells. Given these findings, we propose that Ang II induces vascular cell senescence and inflammation via a p21-dependent pathway, which may be one of the mechanisms underlying the promotion of atherogenesis by Ang II. Several enzymes have been implicated in the mechanism of vascular ROS formation mediated by Ang II, such as xanthine oxidase, cytochrome P450, uncoupled NO synthase, and nicotinamide adenine dinucleotide (NADH) oxidase. The present in vitro data suggest that p21 activates proinflammatory transcription factors by increasing the production of ROS in vascular cells. It has been shown that cyclin-dependent kinase inhibitors, including p21, induce the expression of PIG3, a human homolog of the oxidoreductase genes of several species that increases the production of ROS. Moreover, the signaling pathways of cyclin-dependent kinase inhibitors, it has been suggested, have a role in the activation of small G proteins that positively regulate NADH oxidase activity. Collectively, these results suggest a critical role of p21 in the increase of vascular cell ROS formation provoked by Ang II.

We noted that the number of SA β-gal–positive cells was increased in the aortas of apoE-deficient mice compared with wild-type mice (Figures 3A and 3B). A small induction of proinflammatory molecules was also observed in the aortas of...
apoE-deficient mice compared with wild-type mice, which likely contributes to the development of plaque formation (Figures 4A and 4B and Figure 5A). These results suggest that dyslipidemia also promotes vascular cell senescence and inflammation. Although p21 deficiency significantly ameliorated Ang II–induced vascular cell senescence and inflammation, only a small effect of p21 deficiency was observed in the vehicle-treated apoE-deficient mice (Figures 3A and 3B and Figures 4A and 4B), which suggests that dyslipidemia induces cellular senescence mainly via p21-independent pathways. Because loss of p21 did not completely suppress the acceleration of atherogenesis by Ang II (Figures 5A and 5B), p21-independent pathways are also activated by Ang II treatment. We found that expression of p16Ink4a in the aortas of apoE-deficient mice was induced by Ang II treatment (Kunieda et al, unpublished data, 2006). Thus, both p53/p21- and p16-dependent pathways may be involved in Ang II–induced senescence and atherogenesis.

Merched and Chan have recently reported that the absence of p21 protects against atherosclerosis in fat-fed apoE-deficient mice. Transplantation of p21-deficient bone marrow cells to wild-type mouse partially prevented the progression of atherosclerosis (32% reduction) compared with global p21 inactivation (52% reduction), which suggests that bone marrow–derived cells partially mediate the beneficial effects of p21 deficiency. Alternatively, other types of cells, such as vascular cells, likely mediate its effects to some extent. Consistent with the present study results, Merched and Chan’s histological analyses of apoE/p21-deficient mice revealed a thicker and better-formed fibrous cap that consisted of VSMCs in atherosclerotic plaque than that found in apoE-deficient mice. Another recent study has demonstrated that the transcription factor Ets-1 is a critical regulator of Ang II–mediated vascular inflammation and remodeling in wild-type mice. Although that study showed that Ang II treatment induced p21 expression via an Ets-1–dependent pathway, it remains unclear whether upregulation of p21 mediates Ang II–induced vascular inflammation and remodeling.

p53 immunoreactivity is present in vascular cells at sites of chronic inflammation in human arteries affected by atheroma, whereas only a few cells positive for p53 are found in control normal arteries. p21 immunoreactivity is also detected in human atheroma but not in normal vessels, and it colocalizes with p53 immunoreactivity. These observations suggest a pathological role of both p53 and p21 in human atherogenesis. It has been reported that atherosclerosis is aggravated in p53-/apoE-deficient mice and that macrophage p53 deficiency plays a critical role in the progression of atherosclerosis. In contrast, a study using the perivascular collar model in apoE-deficient mice showed that overexpression of p53 results in a marked decrease of cell number and the extracellular matrix in cap lesions, leading to spontaneous plaque rupture. In the present study, we demonstrated that suppression of p21 expression in the vasculature significantly reduced cellular senescence and the progression of atherosclerosis induced by Ang II. p21 deficiency stabilized atherosclerotic plaque by inhibiting vascular inflammation and inducing VSMC growth and thereby prevented plaque rupture. Thus, overexpression of p53 and p21 by vascular cells may have a deleterious effect in human atherosclerosis. Further studies on vascular cell senescence may provide novel insights into the clinical treatment of atherosclerosis.

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Disclosures
None.

References
Vascular cells have a finite lifespan when cultured in vitro and eventually enter an irreversible growth arrest called “cellular senescence.” We previously demonstrated the presence of senescent vascular cells in human atherosclerotic lesions but not in nonatherosclerotic lesions. Moreover, these cells express increased levels of proinflammatory molecules and decreased levels of endothelial nitric oxide synthase, which suggests that cellular senescence contributes to the pathogenesis of human atherosclerosis. Angiotensin II (Ang II) has been reported to contribute to the pathogenesis of various human diseases, including atherosclerosis, and inhibition of Ang II activity has been shown to reduce the morbidity and mortality of cardiovascular diseases. Various mechanisms for the promotion of atherogenesis by Ang II have been suggested. Here, we report exciting new findings to show that Ang II promotes vascular inflammation by inducing cellular senescence. Ang II induces cellular senescence via a p53/p21-dependent pathway. Inhibition of this pathway effectively suppresses induction of the production of proinflammatory cytokines, as well as cellular senescence, by Ang II and thereby prevents the development of atherosclerosis. Our findings will provide insights into a novel treatment for atherosclerosis. Antisenescence would be a useful strategy for protection against age-associated vascular diseases.
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In the article, “Angiotensin II Induces Premature Senescence of Vascular Smooth Muscle Cells and Accelerates the Development of Atherosclerosis via a p21-Dependent Pathway” by Kunieda et al that was published in the August 29, 2006, issue of the journal (Circulation. 2006;114:953–960), Figure 2C and Figure 2D were reversed. The corrected figure is in the current online version. The authors regret this error.

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