



Review Article

Chronic inflammation and atherosclerosis : A critical role for renin angiotensin system that is activated by lifestyle-related diseases

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It is generally believed that atherosclerosis is a chronic inflammatory disease that is promoted by lifestyle-related diseases, such as hypertension, dyslipidemia, and diabetes. The renin-angiotensin system (RAS) has been demonstrated to play a critical role in the initiation and progression of atherosclerosis, thereby contributing to development of cardiovascular diseases. Angiotensin II (Ang II), a major substrate in RAS, stimulates atherosclerosis through various deleterious effects such as endothelial dysfunction, cellular proliferation and inflammation. Reactive oxygen species (ROS) play a major role in the athero-promoting actions of Ang II. In fact, recent basic and clinical studies demonstrated that pharmacological inhibition of renin-angiotensin system is effective in prevention of atherosclerotic diseases. Elucidation of molecular mechanism of chronic inflammation should lead to development of effective strategies against lifestyle-related diseases.

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Renin-angiotensin system

The renin-angiotensin system (RAS) has been considered as a circulating hormonal system that regulates blood pressure, blood flow, fluid volume and electrolyte balance¹. Angiotensinogen produced in the liver is cleaved to angiotensin (Ang) I in circulation by renin that is secreted from the kidney. Ang I is cleaved to Ang II by angiotensin converting enzyme (ACE) that is mainly distributed in pulmonary circulation. Ang II plays a main role in the RAS by interacting with its specific receptor, Ang II type 1 receptor (AT1R). Ang II-AT1R interaction causes vasoconstriction and aldosterone release from the adrenal gland. This classical view of the RAS has been expanded by recent findings that RAS is activated locally, particularly in the heart², the vessel wall³, the kidney⁴ and the brain⁵. There are RAS components in these tissues, allowing local synthesis of Ang peptides. Recent reports also identified other receptors⁶ and angiotensin-related peptides such as Ang (1-7)⁷. Ang II was also reported to be generated by other enzymes such as chymase⁸. These findings indicate that RAS could be activated locally and regulated by the complicated crosstalk of the RAS components in each organ.

Local effects of an activated RAS in vasculature

The RAS serves as a key player in the pathogenesis of atherosclerosis by simulating a series of coordinated cellular and molecular events observed in the lesions⁹. It is now well established that Ang II has significant pro-inflammatory actions on the vessel wall, leading to progression of atherosclerosis¹⁰. There are two different types of Ang II receptors, AT1R and AT2R, in mammals. Both AT1R and AT2R have been identified in the vessel wall, although AT1R is believed to mediate most of the atherogenic actions of Ang II¹¹. The greatest AT1R density has been found on vascular smooth muscle cells and endothelial cells. In the vascular wall, ACE is readily detectable on endothelial cells and smooth muscle cells¹². Most of the components of RAS could be detected in vasculature¹³. RAS is activated locally in the atherosclerotic lesions¹⁴ and in the damaged vessels¹⁵. Thus, these results suggest that not only systemic but also local Ang II-AT1R pathway could contribute to initiation and progression of atherosclerosis.

Effects of RAS on vascular cells

Ang II up-regulates expression of adhesion molecules¹⁶, chemokines¹⁷ and cytokines¹⁸. These molecules induce endothelial cell dysfunction¹⁹, oxidation and uptake of LDL²⁰, and proliferation of smooth muscle cells²¹. In advanced atherosclerotic lesions, Ang II stimulates expression of matrix metalloproteinases (MMPs)²² and plasminogen activator inhibitor-1²³, leading to destabilization of atherosclerotic plaque and alteration of fibrinolytic balance. Ang II also up-regulates expression of VEGF that promotes adventitial angiogenesis²⁴ (**Fig. 1**).

Conversely, previous reports demonstrated that inhibition of the Ang II-AT1R pathway reduces atherosclerosis^{25,26}. It is generally assumed that the beneficial effects obtained by Ang II-AT1R blocking are mediated by reduction of oxidative stress, inhibition of inflammation and improvement of endothelial cell function²⁷. We generated apolipoprotein E (ApoE)-/-AT1aR-/- double knockout mice by cross-breeding ApoE-/-AT1aR+/+ mice and ApoE+/+AT1aR-/- mice²⁸. Moreover, genetic disruption of AT1R resulted in reduced lipid deposition and increased collagen contents in the atheroma. These results demonstrated that blockade of Ang II-AT1R pathway not only reduces atherosclerotic lesions but also stabilizes the plaque²⁸.

It should be noted that the production of Ang II could be increased and may act on the AT2R, when AT1R is genetically disrupted or pharmacologically blocked²⁹. Previous reports suggested an anti-atherogenic effect of AT2R, although its function and distribution are still under debate³⁰. AT2R stimulation interacts with AT1R stimulation at intracellular signaling molecules, such as through activation of phosphatase³¹. In fact, Iwai et al. demonstrated that AT2R stimulation attenuates atherosclerosis through inhibition of oxidative stress and that the anti-atherosclerotic effect of an ARB could be at least partly due to AT2R stimulation by analyzing AT2R/ApoE-double-knockout mice³².

Pharmacological inhibition of renin-angiotensin system

AT1R blockers (ARBs) specifically block Ang II binding to AT1R. Eventually, Ang II is directed to stimulate AT2R. On the other hand, ACE inhibitors (ACEIs) suppress angiotensin II production. ACEIs also inhibit break down of bradykinin, leading to

increase in nitric oxide production. It has been reported that ARBs or ACEIs exert various favorable effects on endothelial function^{25,26,33}, cardiac function³⁴, cerebral vascular function³⁵ and renal function³⁶ other than blood pressure lowering. These findings suggest that blockade of RAS is an effective

strategy for organ protection^{25,26,33}. In fact, many clinical studies demonstrated that AT1R blockers (ARBs) or ACE inhibitors are effective for patients with cardiovascular, cerebrovascular and renal diseases³⁷.

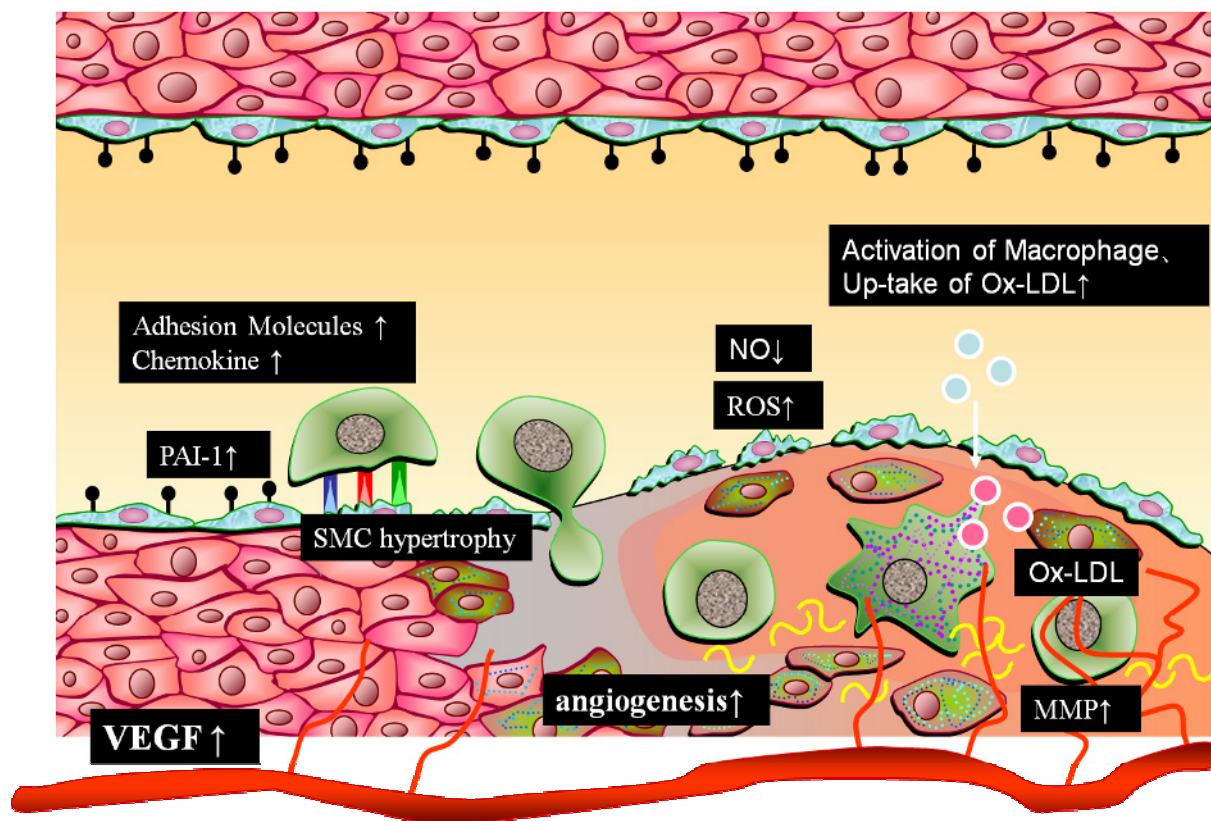


Fig. 1 Athero-promoting effects of angiotensin II

Angiotensin II (Ang II) impairs NO synthesis and promotes reactive oxygen species production by endothelial cells, causing endothelial dysfunction. Ang II also promotes adhesion and infiltration of monocytes/macrophages by up-regulating adhesion molecules and chemokines such as MCP-1. Ang II promotes oxidation of LDL and foam cell formation of macrophages. Ang II functions to destabilize atherosclerotic plaques by activating macrophages, which induce apoptosis of smooth muscle cells and proteolysis of collagen by MMPs. Ang II promotes perivascular angiogenesis by up-regulating VEGF expression.

Atherosclerosis is an inflammatory disease

Atherosclerosis occurs in whole arteries and results in various organ damages, including myocardial infarction, cerebral infarction, and peripheral arterial diseases, the main cause of death in Western countries³⁸. Atherosclerosis is considered to be one of the chronic inflammatory diseases¹⁰. Although multifactorial in etiology, continuous recruitment of circulating leukocytes into the vessel wall plays crucial roles in the pathogenesis of atherosclerosis. In-

flammatory cells detected in atherosclerotic lesions are derived from bone marrow (**Fig. 2**). Recent advances in immunology have identified several molecular pathways that induce and promote inflammatory responses in atherosclerotic lesions.

Roles of reactive oxygen species in atherogenesis

Accumulating evidence indicates that vascular reactive oxygen species (ROS) play a crucial role in atherogenesis. Among many ROS generators, nicotinamide dinucleotide phosphate (NAD(P)H) oxi-



dase-dependent pathway is important in vascular system³⁹). Barry-Lane et al. demonstrated that NAD(P)H oxidase is important in the pathogenesis of atherosclerosis by analyzing the genetically modified mice that are deficient for both ApoE and p47phox, one subunits of NAD(P)H oxidase⁴⁰). In this study, the double knockout mice showed significant reduction in atherosclerotic lesion compared with that of ApoE-deficient mice. ROS acts not only

as a modulator of vascular tonus but also as a second messenger to alter the vascular cell phenotypes. ROS activates mitogen-activated protein kinase⁴¹), Akt⁴²), and JAK (janus kinase)/STAT (signal transducers and activators of transcription)⁴³) pathways. These signals play a crucial role in cell proliferation, apoptosis and phenotypic modification that are observed in atherosclerotic lesions.

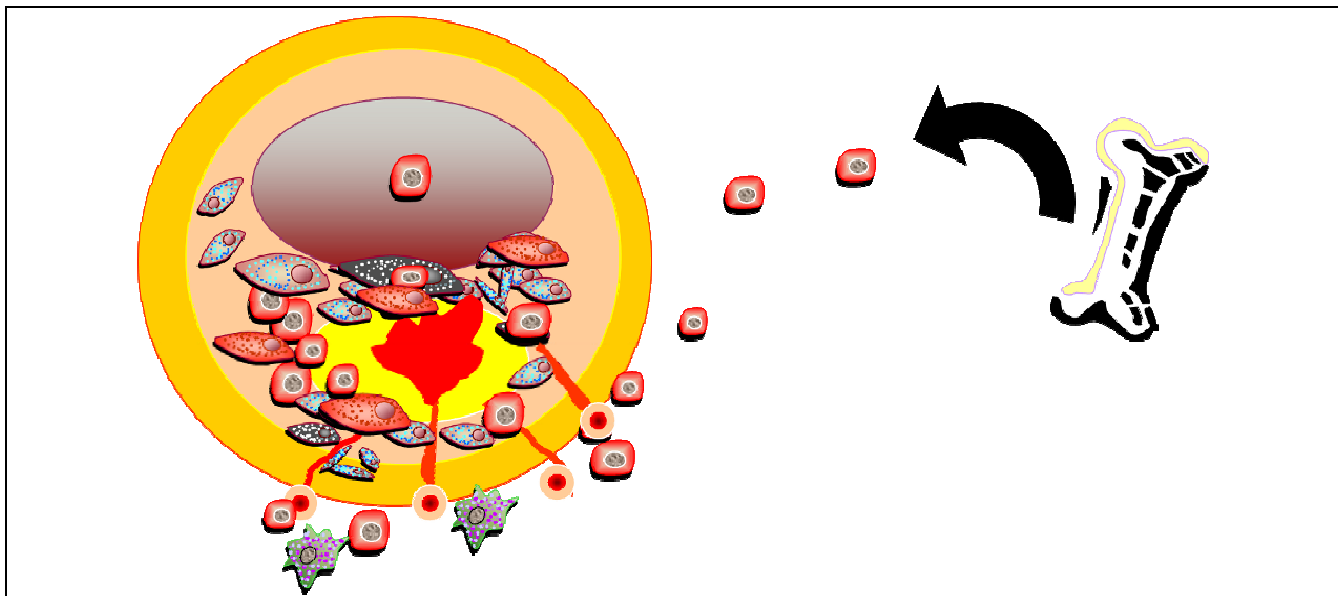


Fig. 2 Atherosclerosis is an inflammatory disease

Ang II has significant pro-inflammatory actions on the vessel wall, leading to progression and destabilization of atherosclerotic lesions^{10, 86}). Although multifactorial in etiology, continuous recruitment of circulating leukocytes into the vessel wall plays crucial roles in the pathogenesis of atherosclerosis. Inflammatory cells detected in atherosclerotic lesions are derived from bone marrow.

Association between RAS and ROS has been investigated extensively³). Ang II induces production of ROS, one of the most important mediators of the atherogenic actions of RAS⁴⁴). Although Ang II up-regulates expression of cytokines such as interleukin-6 and tumor necrosis factor- α , pharmacological blockade of AT1R with ARBs would not so effective to inhibit cytokine production completely. It was demonstrated that cytokines such as TNF- α , IL-1 β and IFN- γ increase mitochondrial- and NADPH oxidase-generated ROS⁴⁵). Thus, the *in vivo* inhibition of intracellular ROS production by blocking vascular AT1R may play an adjunct rather than a major role to prevent or reduce atherogenesis. The above suggestion could be also compatible with the accumulating findings that AT1R blocker could only have a modest effect on atherosclerosis diseases in patients⁴⁶).

Roles of inflammatory cells in atherogenesis

In initiation and progression of atherosclerotic lesions, RAS is activated locally and stimulates expression of vascular cellular adhesion molecule-1, intracellular adhesion molecule-1 and monocyte chemoattractant protein-1 (MCP-1)¹⁶). These molecules accelerate recruitment of inflammatory cells into the vessel walls. It is generally believed that the vascular endothelium serves as an inflammatory barrier by providing a nonadherent surface to leukocytes. However, upon Ang II stimulation, endothelium turns to promote infiltration of inflammatory cells by expressing adhesion molecules and chemokines. After migrating into the vessel wall, monocytes transform into macrophages and contribute to lipid deposition in the plaque⁴⁷). Monocytes/macrophages se-



cret chemokines⁴⁸⁾ and MMPs⁴⁹⁾, leading to acceleration of atherosclerotic lesion development. Moreover, recruited leukocytes themselves have NAD(P)H oxidase subunits and serve as a source of ROS⁵⁰⁾. Thus, activated RAS promotes interaction between circulating leukocytes and vascular cells, an important step in the pathogenesis of atherosclerosis⁹⁾. High levels of ACE expression and Ang II have been shown in experimental and human atherosclerotic lesions⁵¹⁾. In human atherosclerotic lesions, ACE, Ang II, and its receptor are co-localized at the areas of inflammation³⁾. Taken together, these results suggest that local effects of an activated RAS in vessel walls promote infiltration of inflammatory cells into the vessel walls, a key feature of atherosclerosis.

Local effects of an activated RAS in bone marrow

Bone marrow is a highly organized organ. All blood cells derive from hematopoietic stem cells through complex steps of division and maturation. Previous reports elucidated the surface receptors, cytokines, and growth factors that potentially regulate hematopoiesis⁵²⁾. However, the precise mechanism by which the proliferation and differentiation of hematopoietic stem cells are regulated is not fully understood.

A locally activated RAS has been suggested to contribute to differentiation and proliferation of bone marrow-derived cells⁵³⁾. Recently, we proposed a hypothesis that the local RAS in bone marrow plays crucial roles in atherosclerosis^{25,28)}. We demonstrated that Ang II-AT1R pathway in bone marrow contributes to atherosclerotic development in the hypercholesterolemic mice.

Randomized clinical trials have proved beneficial effects of ACE inhibitors or ARBs in the treatment of cardiovascular diseases⁵⁴⁾. However, it was reported that ACE inhibitors or ARBs may have suppressive effects on hematological processes. It is reported that ACE inhibitors induced anemia and leukocytopenia⁵⁵⁾. ACE inhibitors and ARBs have been shown to effectively reduce hematocrit values in patient with renal transplantation⁵⁶⁾. Haznedaroglu et al. proposed the existence of a locally activated RAS in bone marrow that contributes to hematological processes⁵⁷⁾. Others also demonstrated the presence of RAS components in bone marrow and circulating blood cells. Rodgers et al. showed the presence of AT1R in CD34⁺CD38⁺ cells, CD34⁺CD38⁻ cells and lymphocytes⁵⁸⁾. The authors demonstrated that Ang

II accelerated colony formation of hematopoietic progenitor cells from murine lineage negative bone marrow cells in a dose dependent manner. Ang II also stimulated differentiation of human CD34⁺ hematopoietic progenitors from cord blood. The effects of Ang II on hematopoietic progenitors were clearly inhibited by an ARB, losartan. It was also reported that Ang II and Ang (1-7) accelerated recovery of circulating leukocytes and the myeloid lineage cells in bone marrow after chemotherapy and irradiation^{59,60)}. Similarly, other reports demonstrated that RAS components in bone marrow contribute to hematopoiesis⁶¹⁾. On the other hand, several papers reported that a local RAS in bone marrow plays a role in the pathological hematopoiesis⁶²⁾. Bone marrow stromal cells also express AT1R, whose activation possibly causes secretion of growth factors or cytokines that increase hematopoietic progenitor cells⁶³⁾. Thus, it is likely that angiotensin peptides are potential stimulators of proliferation and differentiation of multiple hematopoietic lineages under physiological and pathological conditions.

Ang II stimulates contribution of bone marrow-derived cells to the pathogenesis of atherosclerosis

Recently, we proposed that bone marrow-derived cells significantly contribute to pathogenesis of atherosclerosis^{25,28,64-71)}. This phenomenon was confirmed not only in animal models of vascular diseases, but also in human samples^{72,73)}. Ang II is supposed to promote contribution of bone marrow-derived cells to atherosclerosis by enhancing their mobilization, recruitment, differentiation, and proliferation^{25,28)}. To confirm this notion, we performed bone marrow transplantation from GFP (Green Fluorescent Proteins)+/+ApoE^{-/-} mice to GFP^{-/-}ApoE^{-/-} mice. Administration of Ang II to these bone marrow chimeric mice promoted atherosclerosis lesion formation, which was associated with increased infiltration of bone marrow-derived GFP-positive cells to the lesion^{25,28)} (**Fig. 3A**). We also observed that Ang II infusion increased the number of smooth muscle progenitor cells, which are peripheral blood cells that turn to α -smooth muscle actin-positive cells after culture in the presence of PDGF-BB⁷⁰⁾ (**Fig. 3B**). These smooth muscle-like cells expressed abundant matrix metalloproteinase-9 (MMP-9), which substantially contribute to destabilization of atherosclerotic plaques.

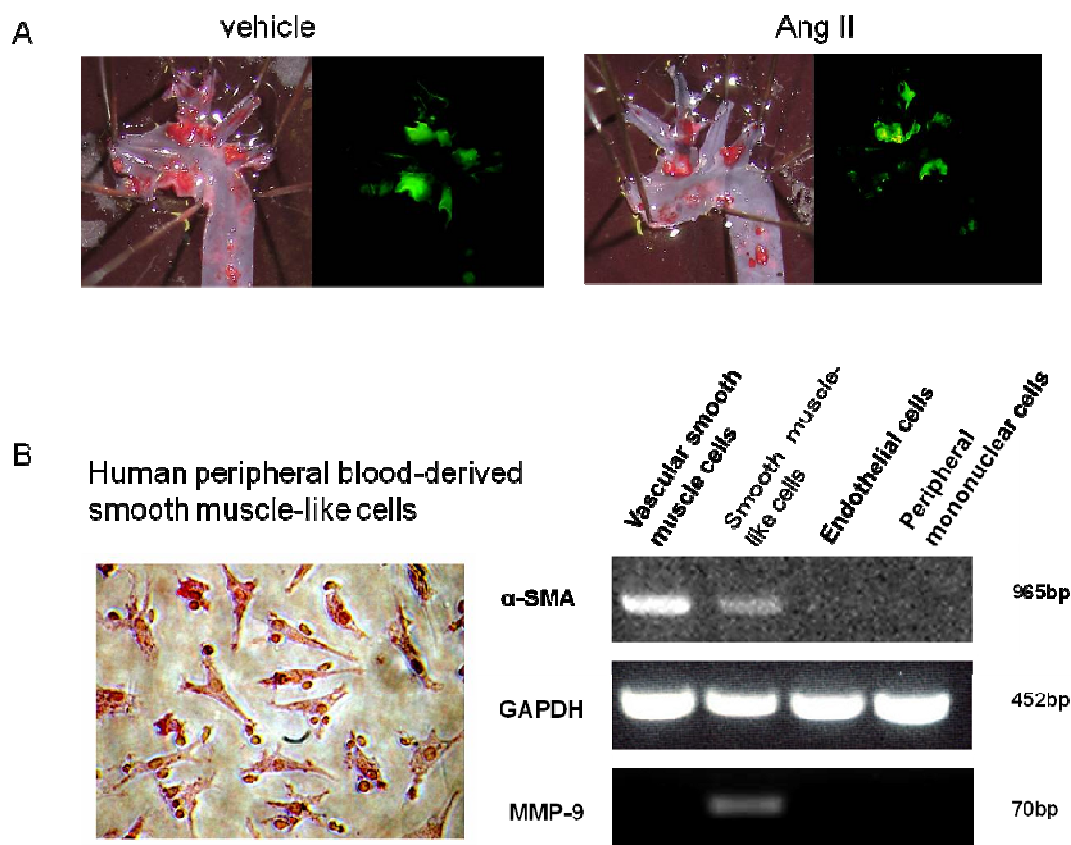


Fig. 3 Ang II promotes accumulation of macrophages in atherosclerotic plaque

- A) Ang II infusion into the bone marrow-chimeric mice promoted atherosclerotic lesion formation as determined by en face Sudan IV staining. Bone marrow-derived GFP-positive cells accumulated at the sites of atherosclerosis.
- B) α -smooth muscle actin-positive cells could be obtained from the culture of human peripheral mononuclear cells. Those smooth muscle-like cells expressed MMP-9.

RAS and endothelial progenitor cells

It is a generally accepted view that atherosclerotic lesions are initiated by endothelial cell damage, followed by monocyte/macrophage adhesion and invasion as well as smooth muscle cell migration and proliferation^{74, 75}. Although there are a number of cellular and molecular differences, restenosis after angioplasty shares an important pathophysiological process with atherosclerosis, where injuries to the endothelium are followed by impaired re-endothelialization^{76,77}. It has been believed that re-endothelialization is caused only by migration and proliferation of adjacent endothelial cells in the vessel wall⁷⁸. However, accumulating evidence indicates that bone marrow derived endothelial progenitor cells (EPCs) also participate in this process⁷⁹. EPC-dependent neovascularization has been implicated in collateral development in occlusive vascular diseases⁸⁰. Bone

marrow cells including stem cells express AT1R. Thus, it is possible that a local RAS in bone marrow has a role in EPC biology leading to neovascularization. Actually, it was demonstrated that activation of RAS stimulates EPC proliferation and neovascularization⁸¹. These studies suggest that ROS may be involved in the balance between self-renewal and differentiation of progenitors and that anti-oxidant may play a role in preservation of stemness of progenitors⁸². Murohara and his colleagues showed that the Ang II-AT1R pathway plays an important role in ischemia-induced angiogenesis by supporting inflammatory cell infiltration and angiogenic cytokine expression⁸³. On the other hand, it was reported that blockade of RAS increase the number of EPC and neovascularization in animals models of metabolic diseases⁸⁴. These studies suggested that Ang II accelerates the onset of EPC senescence by a gp91phox-mediated increase of oxidative stress



leading to impairment of EPC proliferation. Under pathological conditions, RAS may be over-activated and the excess production of Ang II might accelerate EPC senescence, resulting in the impairment of EPC function. Future study is required to confirm that RAS is essential for EPC proliferation and neovascularization but excessive activation of RAS may turn to enhance senescence and dysfunction of EPCs⁷¹⁾.

Closing remarks

Our findings demonstrate that RAS not only in vessel wall but also in bone marrow-derived cells plays a role in the pathogenesis of atherosclerosis, at least in part, by accelerating infiltration of bone marrow-derived inflammatory cells in the vessel wall^{28,67,85)}. Therefore, blockade of AT1 receptor not only in vascular cells but also in bone marrow could be an important strategy to prevent progression and destabilization of atherosclerotic plaques. Elucidation of molecular mechanism of chronic inflammation should lead to development of effective strategies against lifestyle-related diseases.

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