

To mark the 60th birthday of *Circulation Research* (1953–2013), the editors have commissioned *Circulation Research Classics*, a series of commentaries highlighting seminal articles published in the journal over the past 6 decades that have importantly shaped cardiovascular research. Written by leading experts, *Circulation Research Classics* are intended to describe the impact of these articles on the field by putting them in an historical perspective. The concept of classic is inextricably linked to time—a classic is something that maintains its value regardless of its age. Thus, an important consideration in selecting the articles to be highlighted is that they have stood the test of time, which is the most reliable indicator of the value of scientific work. By looking back at the illustrious past of *Circulation Research*, we hope to promote a deeper appreciation of the contributions of this journal to the advancement of knowledge.

Reactive Oxygen Species Promote Vascular Smooth Muscle Cell Proliferation

Hiroaki Shimokawa

Active Oxygen Species Stimulate Vascular Smooth Muscle Cell Growth and Proto-Oncogene Expression

Rao GN, Berk BC
Circ Res. 1992;70:593–599.

Do reactive oxygen species play a role in the pathogenesis of cardiovascular diseases? In 1992, Dr Berk and his group published an article that began to answer this question. During the following years, they performed a series of research projects that established the role of reactive oxygen species in vascular smooth muscle cell proliferation and cardiovascular diseases.

It is now widely recognized that production of intracellular reactive oxygen species (ROS) is substantially involved in the pathogenesis of cardiovascular diseases, in part by promoting vascular smooth muscle cell (VSMC) proliferation. However, 20 years ago, it was elusive why VSMCs proliferate in response to arterial injury. Thus, Dr Berk et al hypothesized that ROS generated during arterial injury could be a common mechanism involved. Using xanthine/xanthine oxidase to generate ROS, they were able to demonstrate that ROS stimulate VSMC proliferation in vitro and that H₂O₂ was primarily responsible for xanthine/xanthine oxidase-induced VSMC DNA synthesis.¹ This *Circulation Research* article was the first milestone article that directly demonstrated the role of ROS in VSMC proliferation, triggering the studies that followed targeting the role of ROS in the pathogenesis of cardiovascular diseases. Until now, this article has been referenced by more than 450 articles. Three years later, they further

reported that both O₂⁻ and H₂O₂ stimulate VSMC growth but only O₂⁻ rapidly activates MAP kinase, suggesting that additional signal events are required for the mitogenic effects of H₂O₂.² Based on these reports, it has been elucidated that the excess amount of ROS (oxidative stress) promotes VSMC proliferation and potentially develops cardiovascular diseases. In the subsequent 5 years, they focused on the interesting finding that ROS stimulates ERK1/2 in a biphasic manner in VSMCs. Finally, they demonstrated that one explanation for the delayed ERK1/2 activation was the response to the secreted oxidative stress-induced factors.³ They analyzed the proteins released into the medium in response to ROS and found that cyclophilin A (CyPA) is one of the major secreted oxidative stress-induced factors.⁴ Importantly, they demonstrated that human recombinant CyPA stimulates ERK1/2 and DNA synthesis in VSMCs in a concentration-dependent manner.⁴ Thus, extracellular CyPA turned out to be a novel growth factor that contributes to ROS effects in VSMCs by promoting growth.

ROS Promote the Development of Cardiovascular Diseases

Based on these reports, Dr Berk et al performed a series of studies demonstrating that changes in vascular redox state and extracellular CyPA are a common pathway involved in the pathogenesis of vascular restenosis,⁵ aortic aneurysms,⁶ atherosclerosis,⁷ and cardiac hypertrophy.⁸ Originally, intracellular CyPA was identified as the main target for the immunosuppressive drug cyclosporine 30 years ago.⁹ Now, it is known that CyPA is secreted from VSMCs via a highly regulated pathway that involves vesicle transport and plasma membrane binding.¹⁰ Additionally, the expression of the dominant-negative mutants of RhoA and Cdc42 and a Rho-kinase inhibitor blocked ROS-induced CyPA secretion.^{6,10} These results suggest that CyPA is secreted from VSMCs through a process that requires ROS production, RhoA/Rho-kinase activation, and vesicle formation.¹¹ In VSMCs, extracellular CyPA stimulates ERK1/2, Akt, and JAK, which contribute to ROS production.^{12,13} In endothelial cells, extracellular CyPA augments proinflammatory pathways, including increased expression of adhesion molecules,

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From the Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan.

Correspondence to Hiroaki Shimokawa, MD, PhD, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai 980–8574, Japan. E-mail shimo@cardio.med.tohoku.ac.jp
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and promotes atherosclerosis.^{7,14} In inflammatory cells, extracellular CyPA works as a chemoattractant in cooperation with other cytokines and chemokines. Although the protein Basigin has been proposed to serve as an extracellular receptor for CyPA in inflammatory cells,¹⁵ the identity of CyPA receptors remains to be elucidated in endothelial cells and VSMC. Further knowledge of the extracellular CyPA receptors on vascular cell will contribute to the development of novel therapies for cardiovascular diseases.

Nox enzymes contribute to VSMC proliferation and the development of cardiovascular diseases.¹⁶ Importantly, it has been demonstrated that CyPA plays a crucial role in the translocation of Nox enzymes such as p47phox.¹⁷ Because ROS production by Nox enzymes activates other oxidase systems, CyPA and Nox enzymes amplify oxidative stress through synergistic cooperation.

Physiological Levels of ROS Are Important for Vascular Homeostasis

In the vascular wall, ROS are generated by several mechanisms, including NADPH oxidases, xanthine oxidase, the mitochondrial respiratory chain, lipoxygenases, and nitric oxide synthases.¹⁶ Vascular ROS formation is stimulated by mechanical stretch, pressure, shear stress, hypoxia, and humoral factors such as angiotensin II.¹⁶ Excessive ROS (oxidative stress) targets several biomolecules and causes severe damage, such as lipid peroxidation, protein oxidation/inactivation, and DNA damage/mutations.¹⁶ Thus, high levels of ROS are hazardous to cells and their content. In contrast, physiological levels of ROS are important to regulate cell functions, proliferation, and death. For example, at a low concentration, H₂O₂ plays an important role for endothelial cell function and vascular relaxation.^{18–20} We have demonstrated that H₂O₂ plays an important role as an endothelium-derived hyperpolarizing factor and contributes to the vascular homeostasis.^{18–20} Thus, H₂O₂ has physiological and pathological roles and contributes to vascular homeostasis and diseases depending on its concentrations and the source of synthesis.^{20,21}

Clinical Application of Oxidative Stress Research

Numerous basic and clinical studies have demonstrated that ROS play a major role in the pathogenesis of endothelial dysfunction and atherosclerosis. However, we still have no therapeutic strategy for clinical use of antioxidant agents. One of the reasons for this dilemma could be that ROS play an important role in intracellular signaling pathways that are crucial for vascular functions at their physiological low concentration.

The identification of CyPA as a mediator of tissue damage associated with inflammation and oxidative stress provides insight into the mechanisms of several therapies. We have recently demonstrated that plasma levels of CyPA are significantly increased in patients with coronary artery disease.²² In this study, we showed that plasma levels of CyPA were elevated in patients with angiographically proven coronary artery disease.²² Importantly, CyPA levels were also elevated in patients with hypertension, diabetes mellitus, smoking, dyslipidemia, and advanced age,²² all of which are atherosclerotic

risk factors as well as ROS inducers. Additionally, we demonstrated that CyPA is a prognostic marker for cardiovascular intervention such as percutaneous coronary intervention and coronary artery bypass graft.²² These results suggest that circulating CyPA is a novel biomarker for coronary artery disease and plays a crucial role for ROS augmentation. Several risk factors, such as hypertension, diabetes mellitus, smoking, and aging, induce the generation of ROS and promote the secretion of CyPA.²² Thus, based on the proinflammatory role of extracellular CyPA and circulating CyPA (plasma CyPA), they may synergistically augment ROS production and, therefore, contribute to the progression of atherosclerosis.¹³

Based on the role of extracellular CyPA, it is logical to propose that agents that prevent CyPA binding to its receptors and those that reduce circulating CyPA may have therapeutic potentials. By blocking this vicious cycle that augments ROS production through CyPA autocrine/paracrine signaling pathway, we may have a novel therapeutic tool for controlling cardiovascular diseases. However, the regulation of CyPA expression and the identity of its extracellular receptors still remain to be fully elucidated. Thus, further basic and clinical studies are needed to identify CyPA-related therapeutic targets in the near future.²³

“There’s Nothing Better Than Achieving Your Goals”

Four years ago, Dr Berk had a bike accident that injured his spinal cord. He spent 10 days in intensive care, 21 days on a ventilator, and 120 days in acute rehabilitation. After intense and endurable continuous ambulatory, he showed unimaginable recovery. In June 2013, he took part in his first big ride since the accident. Dr Berk rode in the Tour de Cure, a charity event to raise funds for diabetes mellitus research, with more than 2000 people participating in 5 different rides starting at Monroe Community College in Rochester, New York. It is exciting to know that Dr Berk rode in the Tour’s 15-mile ride, and he said, “I’m all about positive and setting goals, and there’s nothing better than achieving your goals.” We strongly expect his further recovery and continuous contribution to cardiovascular science and medicine.

Disclosures

None.

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