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Invited Review

Cyclophilin A in Cardiovascular Homeostasis and Diseases

Kimio Satoh[1]

[1]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Vascular homeostasis is regulated by complex interactions between many vascular cell components, including endothelial cells, vascular smooth muscle cells (VSMCs), adventitial inflammatory cells, and autonomic nervous system. The balance between oxidant and antioxidant systems determines intracellular redox status, and their imbalance can cause oxidative stress. Excessive oxidative stress is one of the important stimuli that induce cellular damage and dysregulation of vascular cell components, leading to vascular diseases through multiple pathways. Cyclophilin A (CyPA) is one of the causative proteins that mediate oxidative stress-induced cardiovascular dysfunction. CyPA was initially discovered as the intracellular receptor of the immunosuppressive drug cyclosporine 30 years ago. However, recent studies have established that CyPA is secreted from vascular cell components, such as endothelial cells and VSMCs. Extracellular CyPA augments the development of cardiovascular diseases. CyPA secretion is regulated by Rho-kinase, which contributes to the pathogenesis of vasospasm, arteriosclerosis, ischemia/reperfusion injury, hypertension, pulmonary hypertension, and heart failure. We recently reported that plasma CyPA levels are significantly higher in patients with coronary artery disease, which is associated with increased numbers of stenotic coronary arteries and the need for coronary intervention in such patients. Furthermore, we showed that the vascular erythropoietin (Epo)/Epo receptor system plays an important role in production of nitric oxide and maintenance of vascular redox state and homeostasis, with a potential mechanistic link to the Rho-kinase-CyPA pathway. In this article, I review the data on the protective role of the vascular Epo/Epo receptor system and discuss the roles of the CyPA/Rho-kinase system in cardiovascular diseases.

Keywords: biomarker; cardiovascular diseases; cyclophilin A; oxidative stress; vascular smooth muscle cells
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Introduction

The interactions among endothelial cells (ECs), vascular smooth muscle cells (VSMCs), adventitial inflammatory cells, and autonomic nervous system play an important role in regulating vascular function. ECs secrete a variety of vasoactive substances including nitric oxide (NO) and prostacyclin, which protect against vascular remodeling (Shimokawa et al. 1983; Shimokawa 1999). It has now become clear that VSMCs secrete a variety of growth factors that elicit autocrine/paracrine growth pathways. Many other stimuli that modulate VSMC function, including reactive oxygen species (ROS), promote cell proliferation by inducing autocrine/paracrine growth mechanisms (Shimokawa and Satoh 2014a, b). ROS includes superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH). VSMCs contain numerous sources of ROS such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, the mitochondrial respiratory chain, lipoxygenases, and NO synthases (Berk 2001). During the last 20 years, the precise mechanism of how ROS damage vascular function and promote vascular remodeling has been extensively studied.

Almost all cardiovascular diseases are initiated by endothelial activation that leads to expression of adhesion molecules for inflammatory cells (Berk 2008). Migrating inflammatory cells produce large amounts of ROS and secrete cytokines/chemokines and growth factors that promote cardiovascular diseases. Thus, the oxidizing environment induced by vascular inflammation creates a vicious cycle of disease progression. Importantly, cyclophilin A (CyPA) has emerged as a key player in the molecular mechanism of this vicious cycle of ROS production in cardiovascular tissues (Fig. 1) (Satoh et al. 2010b). CyPA was initially discovered as the intracellular receptor of the immunosuppressive drug cyclosporine 30 years ago (Handschumacher et al. 1984). Intracellular CyPA plays an important role in protein folding and trafficking of extracel-

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Correspondence: Kimio Satoh, M.D., Ph.D., Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980-8574, Japan.
e-mail: satoh-k@cardio.med.tohoku.ac.jp
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lular signal regulated kinase (ERK)1/2 (Pan et al. 2008) and apoptosis-inducing factor (Zhu et al. 2007). Although CyPA was initially thought to function primarily inside the cell, recent studies have revealed that it can be secreted in response to ROS (Jin et al. 2000; Liao et al. 2000). Extracellular CyPA initiates expression of adhesion molecules in ECs (Jin et al. 2004), induces apoptosis (Nigro et al. 2011), and serves as a chemoattractant for inflammatory cells (Khromykh et al. 2007; Satoh et al. 2008). We found that intracellular and extracellular CyPA promotes intimal thickening, abdominal aortic aneurysms, atherosclerosis, and cardiac hypertrophy in mice (Satoh et al. 2008, 2009b, 2011d; Nigro et al. 2011). The secretion of CyPA is regulated by activation of Rho-kinase (Suzuki et al. 2006), which plays a crucial role in inflammation, vascular contraction, and the development of cardiovascular diseases (Shimokawa and Takeshita 2005; Satoh et al. 2011c). In a recent clinical study, we have demonstrated that plasma levels of CyPA are significantly increased in patients with coronary artery disease (CAD) (Satoh et al. 2013). Basigin (Bsg, a protein encoded by the *Bsg* gene also known as CD147 or EMMPRIN) is an extracellular CyPA receptor (Yurchenko et al. 2002). Importantly, Bsg is an essential receptor for merozoites, the malaria-causing invasive form of *Plasmodium falciparum*, which disrupts the NO metabolism and induces harmful endothelial activation, including the activation of Rho/Rho-kinase (Miller et al. 2013). The interaction between extracellular CyPA and Bsg contributes to several cardiovascular diseases (Seizer et al. 2010, 2011, 2012, 2013).

In this article, I will discuss the roles of endothelial function, VSMC proliferation, and inflammation in the development of cardiovascular diseases. Furthermore, I will focus on the recent progress in understanding of the pathogenesis of cardiovascular diseases as well as current challenges on the way to developing clinical applications and conducting translational research.

Redox Status Equilibrium and Cardiovascular Function

Among the vascular cell components, ECs substantially contribute to vascular function and homeostasis (Shimokawa et al. 1983; Shimokawa 1999; Satoh 2013b, 2014). Strictly controlled ROS, including O_2^-, H_2O_2, and ·OH, mediate many important intracellular signaling and physiological vascular functions. For example, H_2O_2, one of the endothelium-derived hyperpolarizing factors, at very low concentrations plays an important role (Vanhoutte 2001) by modulating vascular tone in microvessels (Matoba et al. 2000; Morikawa et al. 2003; Takaki et al. 2008; Enkhjargal et al. 2014). In contrast, excessive amounts of ROS impair vascular function by promoting secretion of inflammatory cytokines/chemokines and growth factors that induce inflammation in an autocrine/paracrine manner (Satoh et al. 2011a, c, 2014a). ROS excess further induces DNA damage and harmful protein oxidation, ultimately promoting vascular diseases (Sandow 2004; Satoh 2013a). Several enzymes generate intracellular ROS, including NADPH oxidases (Nox) that produce O_2^- and H_2O_2. Moreover, NO produced by endothelial nitric oxide synthase (eNOS) for the synthesis of cyclic guanosine monophosphate can also react with O_2^- to form peroxynitrite ($ONOO^-$) (Cohen and Adachi 2006). Oxidative stress damages mitochondrial proteins and further increases the levels intracellular ROS, thus creating a vicious cycle of ROS augmentation (Shimokawa and Satoh 2014a, b). Therefore, the balance between oxidants and antioxidants (Shao et al. 2012) and the dual role of ROS, in particular, H_2O_2, as both protective and hazardous agents (Vanhoutte et al. 2009; Shimokawa 2010) are important for the redox status equilibrium and normal cardiovascular functions. Although our understanding of vascular-derived oxidants is continuously expanding, further studies are needed to clarify the regulatory mechanisms of redox status equilibrium (Satoh et al. 2014a).

Rho-kinase and Vascular ROS Formation

Rho-kinase enhances myosin light chain (MLC) phosphorylation and mediates agonists-induced VSMC contraction (Amano et al. 1996; Kimura et al. 1996). In addition to MLC, the identified substrates of Rho-kinase include ezrin, radixin, and moesin (ERM) family, adducing, phosphatase and tensin homolog on chromosome ten (PTEN), and LIM-kinases (Satoh et al. 2011c). Both endothelial NO production and NO-mediated signaling in VSMCs are targets and effectors of the RhoA/Rho-kinase pathway. In ECs, the RhoA/Rho-kinase pathway negatively regulates NO production. In VSMCs, this pathway activates gene expression and secretion of growth factors, which promotes VSMC proliferation and vascular remodeling. Rho-kinase is also important for formation of NADPH oxidase complex (Higashi et al. 2003), which promotes VSMCs growth by inducing auto/paracrine growth mechanisms (Taniyama and Griendling 2003). Among the auto/paracrine factors, CyPA has been identified as a ROS-responsive protein that is secreted by vascular cell components upon Rho-kinase activation (Satoh et al. 2009a; Suzuki et al. 2006). Extracellular CyPA reduces eNOS expression and impairs endothelial function (Nigro et al. 2011), which represents a mechanism of Rho-kinase-mediated vascular dysfunction.

Rho-kinase is substantially involved in the vascular effects of various vasoactive factors, including angiotensin II, thrombin, platelet-derived growth factor, extracellular nucleotides, and urotensin (Shimokawa 2002; Shimokawa and Takeshita 2005; Satoh et al. 2011c; Ikeda et al. 2014). Vascular ROS formation can be stimulated by mechanical stretch, pressure, shear stress, hypoxia, and growth factors (Griendling et al. 1994), all of which activate the RhoA/Rho-kinase system. Importantly, statins and selective Rho-kinase inhibitors block the secretion of CyPA by VSMCs (Suzuki et al. 2006; Satoh et al. 2008). Thus, Rho-kinase plays an important role in mediating various cellular func-

tions besides the VSMC contraction, such as actin cytoskeleton organization, cytokinesis, and ROS augmentation. In summary, the RhoA/Rho-kinase system plays a crucial role in the development of cardiovascular disease through endothelial dysfunction, VSMC contraction/proliferation, and inflammation.

Intrinsic Protective System for Endothelial Function

Hypoxia has been considered to increase plasma levels of erythropoietin (Epo) and hematocrit, resulting in enhanced blood viscosity and pulmonary hypertension (PH). However, exogenous Epo did not promote hypoxia-induced PH in rats (Petit et al. 1993). Instead, Epo has been shown to exert direct protective effects on ECs (Ghezzi and Brines 2004; Noguchi et al. 2008). Pulmonary ECs are important in the maintenance of pulmonary vasculature (Voelkel and Tuder 2000) while EC dysfunction accelerates hypoxia-induced PH (Steudel et al. 1998). Using EpoR$^{-/-}$-rescued mice that express Epo receptor (EpoR) only in the erythroid lineage but not in the cardiovascular system (Suzuki et al. 2002), we demonstrated the protective effects of the endogenous Epo/EpoR system in hypoxia-induced PH (Satoh et al. 2006).

Hypoxia induces vascular endothelial growth factor (VEGF) and Epo (Semenza 2004) expression, promoting ECs turnover and angiogenesis in the ischemic tissue. Hypoxia-inducible factor is one of the important factors inducing angiogenesis, which upregulates both Epo and VEGF. These angiogenic cytokines play a crucial role in enhancing EC proliferation and migration, synthesis of extracellular matrix, and resultant angiogenesis. Epo is a hypoxia-induced hormone that stimulates proliferation and differentiation of erythroid progenitor cells and ECs (Beleslin-Cokic et al. 2004). EpoR is known to be expressed in the bone marrow but also in a variety of organs, including the cardiovascular tissue (Anagnostou et al. 1994; Suzuki et al. 2002). Our experiments on EpoR$^{-/-}$-rescued mice suggested a crucial role of endogenous Epo/EpoR system in ischemia-induced angiogenesis (Nakano et al. 2007). Importantly, we found that ischemia significantly enhanced the expression of VEGFR-2 in the skeletal muscle of wild-type mice but not in EpoR$^{-/-}$-rescued mice, revealing the interaction between the Epo/EpoR and VEGF/VEGFR systems. Additionally, we found that the intrinsic Epo/EpoR system is important for ischemic preconditioning in the heart (Tada et al. 2006). Altogether, we have demonstrated that the intrinsic Epo/EpoR system is important for the maintenance of EC function under hypoxic and ischemic conditions.

Erythropoietin and Cyclophilins

Exogenous Epo reduces oxidative stress by regulating the subunits of the mitochondrial permeability transition pore and cyclophilin D (CyPD) (Nishihara et al. 2007). However, exogenous administration of Epo sometimes leads to unexpected physiological results, raising caution over the use of this agent and warranting further investigation. It has been shown that inadequate Epo production in Epo-producing cells is due to the inhibitory effect of an immunosuppressive drug cyclosporine A (Vannucchi et al. 1993). Cyclosporine A binds to several immunophilins, including CyPA and CyPD (Handschumacher et al. 1984; Harding et al. 1986; Siekierka et al. 1989; Bierer 1994). The promoter activity and expression of CyPA are upregulated by hypoxia-inducible factor transcription factor (Choi et al. 2007), which is also important for Epo production. However, the precise mechanisms for controlling the level of oxygen and oxidative stress have not been elucidated. Here, I focus on the roles of Epo and CyPA, which may have a close mechanistic interaction and contribute to the regulation of oxygen supply/consumption, resulting in changes in oxidative stress levels in vivo.

Intracellular CyPA as a Multifunctional Chaperone

Cyclophilins are a family of highly conserved and ubiquitous proteins (Marks 1996). The most abundant cyclophilin, CyPA, is widely distributed in almost all tissues (Galat and Metcalfe 1995). Cyclophilin B (CyPB) (Price et al. 1991), Cyclophilin C (CyPC) (Schneider et al. 1994), and CyPD (Bergsma et al. 1991) are less abundant and localize not only to the cytosol but also to membranes and subcellular organelles. Human CyPB and murine CyPC are localized to the endoplasmic reticulum (Bergsma et al. 1991). CyPD is found in mitochondria where it serves as an integral part of the mitochondrial permeability transition complex and plays crucial roles in apoptosis (Baines et al. 2005), Alzheimer's disease (Du et al. 2008), and heart failure (Elrod et al. 2010). Intracellular CyPA was identified as the main target of an immunosuppressive drug, cyclosporine (Handschumacher et al. 1984). Based on its enzymatic properties, cellular localization, and role in protein folding, CyPA is classified into a diverse family of proteins termed foldases (Theuerkorn et al. 2011). CyPA catalyzes cis-trans isomerization of peptidyl-prolyl bonds in certain proteins and accelerates protein folding and assembly. In addition, intracellular CyPA has a variety of other functions, including intracellular trafficking, signal transduction, and transcriptional regulation (Zhu et al. 2007). Importantly, intracellular CyPA plays a crucial role in the translocation of Nox enzymes such as p47phox (Soe et al. 2013), which are known to contribute to VSMC proliferation (Lassegue et al. 2012). Since ROS production by Nox enzymes activates other oxidase systems, CyPA and Nox enzymes amplify ROS formation in a synergistic manner, leading to increased oxidative stress.

Extracellular CyPA as a Promoter of Cardiovascular Diseases

CyPA is secreted from ECs, VSMCs, cardiac fibroblasts, activated macrophages, and platelets via a highly regulated pathway, which involves vesicle transport and plasma membrane binding (Suzuki et al. 2006). Many vas-

cular cell components secrete CyPA via a process that requires ROS production, RhoA/Rho-kinase activation, and vesicle formation (Satoh et al. 2011c). In ECs, extracellular CyPA augments proinflammatory pathways, including enhanced expression of adhesion molecules, and promotes atherosclerosis (Jin et al. 2004; Nigro et al. 2011). In VSMCs, extracellular CyPA stimulates ERK1/2, Akt, and Janus protein tyrosine kinase (JAK), which contribute to ROS formation (Satoh et al. 2010a, b). In inflammatory cells, extracellular CyPA works as a chemoattractant in cooperation with other cytokines and chemokines. Bsg has been proposed to serve as an extracellular receptor for CyPA in inflammatory cells (Pushkarsky et al. 2001). Recently, we have demonstrated that Bsg is a receptor in VSMCs (Satoh et al. 2014b). Elucidation of the extracellular CyPA receptors of vascular cell components will contribute to the development of novel therapies for cardiovascular diseases.

Mechanism of CyPA-induced VSMC Proliferation

In ECs, CyPA mostly activates proinflammatory pathways, including increased expression of vascular cell adhesion molecule (VCAM)-1 and E-selectin (Jin et al. 2004). In VSMCs, ROS such as superoxide activate a pathway that involves vesicles and results in secretion of CyPA (Suzuki et al. 2006). Secreted extracellular CyPA stimulates ERK1/2, Akt, and JAK in VSMCs, which, in turn, contribute to ROS production (Fig. 1) (Satoh et al. 2010b). CyPA is secreted from VSMCs via a highly regulated pathway that involves vesicle transport and plasma membrane binding (Suzuki et al. 2006). Despite the mounting evidence that cyclophilins serve multiple intracellular and extracellular functions, surprisingly little is known regarding their effect on specific receptors.

Rho GTPases such as RhoA are key regulators in signaling pathways linked to actin cytoskeletal rearrangement (Mackay and Hall 1998). RhoA plays a central role in vesicular trafficking pathways by controlling organization of actin cytoskeleton. Active participation of Rho GTPases is required for secretion. Consistently, dominant-negative mutants of RhoA inhibited ROS-induced CyPA secretion, suggesting that it is regulated by RhoA-dependent signaling events (Suzuki et al. 2006). Myosin II is involved in secretory mechanisms as a motor for vesicle transport (Neco et al. 2004). Rho-kinases, downstream effectors of RhoA, mediate myosin II activation via phosphorylation and inactivation of myosin II light chain phosphatase (Kimura et al. 1996). Rho-kinase inhibitor reduces ROS-induced CyPA secretion (Suzuki et al. 2006). These results suggest that Rho-kinase-mediated vesicle transport is required for CyPA secretion from VSMCs. Thus, CyPA may be a key mediator of Rho-kinase that generates a vicious cycle of ROS augmentation affecting ECs, VSMCs, and inflammatory cells.

Animal Models of Cardiovascular Diseases

Since extracellular CyPA stimulates endothelial inflammation (Jin et al. 2004) and proliferation and migra-

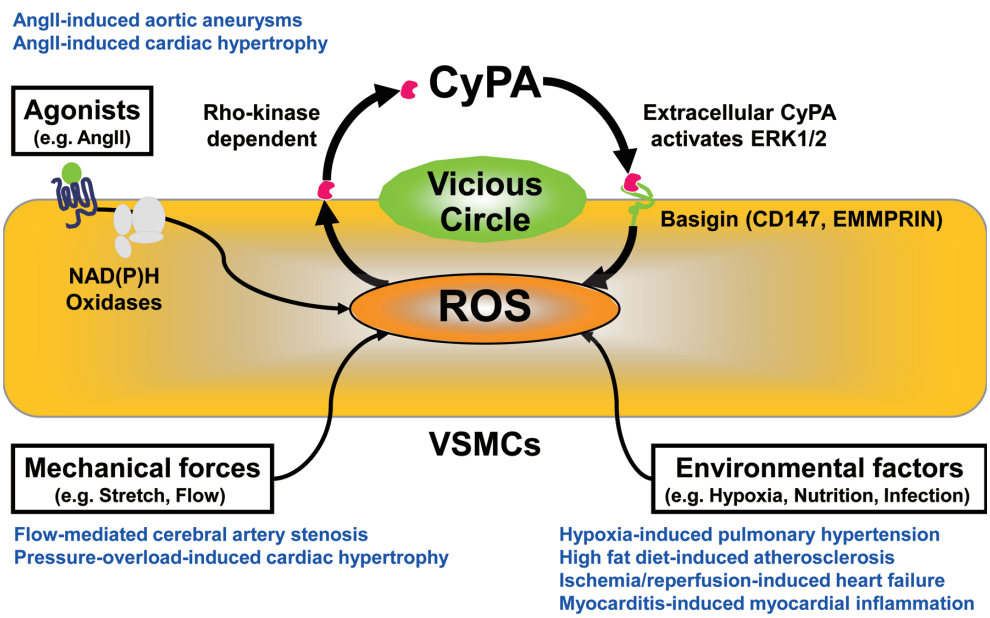

Fig. 1. Reactive Oxygen Species (ROS)-Induced Secretion of Cyclophilin A (CyPA) Synergistically Augments ROS Production.
ROS inducers such as angiotensin II (AngII), mechanical stress, and environmental factors promote (CyPA) secretion from cardiovascular tissues. Secreted CyPA activates ERK1/2 and promotes further ROS production. The data are from (Satoh et al. 2010b), reproduced with permission from the publisher. VSMCs, vascular smooth muscle cells.

tion of VSMCs (Jin et al. 2000; Liao et al. 2000), we decided to analyze several cardiovascular disease models using CyPA-deficient ($CyPA^{-/-}$) mice (Satoh et al. 2008, 2009b, 2011d; Nigro et al. 2011). First, we performed carotid ligation to confirm the role of CyPA in intimal thickening and the development of vascular stenosis (Satoh et al. 2008). CyPA expression increased with a time course that paralleled neointima formation after carotids ligation, suggesting an important role of CyPA in the cell response to oxidative stress induced by vascular injury (Satoh et al. 2008). In parallel with CyPA expression, carotid ligation induced phosphorylation of ERK1/2 in wild-type carotids, which was significantly less intensive in $CyPA^{-/-}$ carotids. Co-localization of CyPA, α-smooth muscle actin (SMA), and Masson-Trichrome stain revealed that CyPA expression was especially elevated in VSMCs. VSMC-specific CyPA overexpression (VSMC-Tg mice) resulted in increased medial and intimal areas in the ligated arteries, suggesting that VSMC-derived CyPA promotes vascular restenosis. In addition, VSMC-Tg mice exhibited enhanced accumulation of inflammatory cells in the ligated carotids, which supports the important role of CyPA in mediating the recruitment of inflammatory cells (Satoh et al. 2008).

Because angiotensin II (AngII) is well known as a strong ROS inducer, we next created a mouse model of AngII-induced abdominal aortic aneurysms (AAA). AAA formation is a consequence of chronic inflammation of the aortic wall associated with decreased numbers of medial VSMCs and progressive degeneration of structural components, particularly the elastic lamina. The key mechanisms include VSMC senescence, oxidative stress, local production of proinflammatory cytokines, and increased activities of matrix metalloproteinases (MMPs) that degrade extracellular matrix. All of these characteristics suggested a potential profound contribution of CyPA to the development of AAA. As expected, AngII induced ROS production and MMP activation via a CyPA-dependent pathway and promoted aortic aneurysm formation (Satoh et al. 2009b). Of note, AngII-induced aortic aneurysm formation in apolipoprotein E-deficient mice ($Apoe^{-/-}$) was completely prevented on the $CyPA^{-/-}$ background (Satoh et al. 2009b). In particular, AngII-induced aortic rupture and sudden death, which occurred in 40% of $Apoe^{-/-}$ animals, were entirely blocked in $Apoe^{-/-}$ $CyPA^{-/-}$ mice (Satoh et al. 2009b). Chronic inhibition of Rho-kinase by fasudil was previously reported to reduce AngII-induced aortic aneurysm formation (Wang et al. 2005). CyPA secretion from VSMCs depends on Rho-kinase activation (Suzuki et al. 2006), and extracellular CyPA stimulates VSMC migration, proliferation, and MMPs activation (Jin et al. 2000; Liao et al. 2000). AngII induces Rho-kinase-mediated CyPA secretion, which augments oxidative stress in a synergistic manner (Satoh et al. 2010a). All these data prove the concept that both Rho-kinase and CyPA play crucial roles in VSMCs by augmenting ROS generation (Fig. 1). The role of CyPA as an essential protein for the development of AAA has been highlighted in the *New England Journal of Medicine* (Weintraub 2009). These data and reports suggest that the Rho-kinase/CyPA signaling pathway is a novel therapeutic target for the treatment of aortic aneurysms (Satoh et al. 2010b).

Since AngII proved to have a strong effect in the CyPA-mediated ROS formation and inflammation, we performed further analyses of the development of cardiac hypertrophy and fibrosis (Satoh et al. 2011d). Cardiac hypertrophy was not significantly different between $CyPA^{+/+}$ and $CyPA^{-/-}$ mice infused with AngII. Because CyPA is a proinflammatory cytokine secreted in response to oxidative stress, we hypothesized that increased ROS generation or inflammation is required for CyPA to play a role in cardiac hypertrophy. It had been demonstrated previously that the hearts of $Apoe^{-/-}$ mice exhibit increased ROS production. Therefore, we investigated the effect of CyPA under the conditions of high ROS levels and inflammation using this animal model. In contrast to $Apoe^{-/-}$ mice, $Apoe^{-/-}CyPA^{-/-}$ mice exhibited significantly less AngII-induced cardiac hypertrophy and fibrosis (Satoh et al. 2011d). Since ROS generation stimulates secretion of CyPA from VSMCs, we compared the secretion of CyPA from WT and $Apoe^{-/-}$ cardiac fibroblasts in response to AngII. Secretion of CyPA was barely detectible in conditioned media (CM) from WT fibroblasts. In contrast, there was abundant CyPA secretion from AngII-treated $Apoe^{-/-}$ cardiac fibroblasts. Because ROS are key mediators of AngII action, we next investigated whether CyPA altered the redox state of the heart after the AngII treatment by incubating heart sections with dihydroethidium. In saline-infused hearts, ROS production was low in both the $Apoe^{-/-}$ and $Apoe^{-/-}CyPA^{-/-}$ mice. In contrast, after the AngII treatment, oxyethidium fluorescence was 2-fold greater in the $Apoe^{-/-}$ mice than in the $Apoe^{-/-}CyPA^{-/-}$ animals. Furthermore, in the perivascular area of the $Apoe^{-/-}$ mice, ROS production was increased 4-fold after the AngII-treatment. In contrast, in the $Apoe^{-/-}$ $CyPA^{-/-}$ mice, the perivascular increase in ROS levels was markedly reduced. These data suggest that CyPA is a key determinant of AngII-mediated ROS production. Although the precise mechanism by which CyPA directly enhances cardiac hypertrophy remains to be elucidated, the mechanistic evidence of the synergy between CyPA and Rho-kinase that increases ROS generation (Satoh et al. 2010b, 2011c) suggests that Rho-kinase and CyPA may work together to promote AngII-induced cardiac hypertrophy. In fact, direct potentiation of ROS production, stimulation of proliferation and migration of cardiac fibroblasts, and promotion of cardiac myocyte hypertrophy by CyPA was required for AngII-mediated cardiac hypertrophy in mice (Satoh et al. 2011d). Therefore, inhibition of CyPA may be a useful therapeutic strategy to attenuate cardiac hypertrophy in patients with high oxidative stresses, for example, caused by smoking, hypertension, and hyperlipidemia.

Changes in vascular redox state and extracellular CyPA level are commonly involved in the pathogenesis of

vascular restenosis (Satoh et al. 2008), aortic aneurysms (Satoh et al. 2009b), and cardiac hypertrophy (Satoh et al. 2011d). Therefore, we hypothesized that CyPA (both intracellular and extracellular) contributes to atherosclerosis by promoting apoptosis, adhesion molecule expression, and inflammation in ECs (Nigro et al. 2011). Arteriosclerosis is a slowly progressing inflammatory process in the arterial wall that involves the intima, media, and adventitia (Shimokawa 2002). In the context of atherosclerosis, CyPA is considered a pro-inflammatory and pro-atherogenic molecule. The role of CyPA in inflammation was discovered using the $Apoe^{-/-}CyPA^{-/-}$ mice, which appeared to be protected from atherosclerosis development. The atheroprotection observed in these animals was due to the decreased levels of EC inflammation mediated by the absence of CyPA (Bell et al. 2012). The vascular endothelium expresses a large array of vital proteins that function in normal cellular processes, the loss of which leads to initiation of atherosclerosis. For example, eNOS function is critical for vascular homeostasis via generation of NO, and its loss is pro-atherogenic. Furthermore, the progression of atherosclerosis is associated with decreases in both eNOS expression and NO production. Aortic staining revealed significantly higher eNOS expression in the $Apoe^{-/-}CyPA^{-/-}$ mice compared with $Apoe^{-/-}$ mice (Nigro et al. 2011), indicating that CyPA plays a role in regulating the eNOS/NO levels. Moreover, shear stress-induced eNOS expression was significantly increased when CyPA siRNA was used to silence CyPA in human umbilical vein endothelial cells (HUVEC) (Nigro et al. 2011). In addition, CyPA knockdown in HUVEC increased eNOS promoter activity and eNOS mRNA levels, whereas overexpression of CyPA reduced eNOS protein and mRNA levels. Both the N-acetyl cysteine and Tiron antioxidants reversed this CyPA-mediated inhibition of eNOS promoter activity (Nigro et al. 2011). These findings suggest a novel mechanism by which CyPA promotes atherosclerosis through suppression of eNOS transcription. Furthermore, the overall ROS production was significantly higher in HUVEC overexpressing CyPA than in cells transfected with a vector control. This indicates that CyPA plays a critical role in ROS generation not only in VSMCs but also in ECs (Satoh et al. 2009a) and that CyPA likely induces inflammation through ROS-dependent mechanisms in these cell types (Satoh et al. 2010b). Based on these results, it is likely that CyPA is the primary mediator that augments ROS production, contributing to vascular inflammation and atherogenesis. Accumulating evidence indicates that the Rho-kinase-mediated pathway is substantially involved in EC dysfunction (Takemoto et al. 2002), VSMC contraction (Kandabashi et al. 2003), VSMC proliferation and migration in the media (Yamakawa et al. 2000), and accumulation of inflammatory cells in the adventitia (Miyata et al. 2000). The expression of Rho-kinase is enhanced in the inflammatory and arteriosclerotic arterial lesions in animals (Kandabashi et al. 2003) and humans (Kandabashi et al. 2002). Thus, Rho-kinase-mediated cellular responses lead to the development of vascular disease. In the context of atherosclerosis, Rho-kinase works in concert with CyPA as a pro-inflammatory and pro-atherogenic molecule.

Pulmonary arterial hypertension (PAH) is associated with hypoxic exposure, endothelial dysfunction (Nakano et al. 2007; Satoh et al. 2006, 2011b), VSMC hypercontraction and proliferation (Rabinovitch 2012), enhanced ROS production, and inflammatory cell migration in which Rho-kinase is substantially involved (Satoh et al. 2011c). Indeed, it has been shown that Rho-kinase activity is enhanced in patients with PAH (Do. e et al. 2009). Furthermore, intravenous infusion of fasudil significantly reduced pulmonary vascular resistance in such patients (Fukumoto et al. 2005), whereas its oral administration ameliorated the development of PAH, indicating involvement of Rho-kinase in the pathogenesis of PAH in humans (Fukumoto et al. 2013). In agreement, long-term treatment with fasudil suppressed the development of monocrotaline-induced PH in rats (Abe et al. 2004) and hypoxia-induced PH in mice (Abe et al. 2006). Moreover, we found that ROCK2 plays a crucial role in the development of hypoxia-induced PH in mice (Shimizu et al. 2013). Statins and a Rho-kinase inhibitor significantly reduced the secretion of CyPA from VSMCs (Satoh et al. 2009b), and pravastatin ameliorated hypoxia-induced PH in mice (Satoh et al. 2009a). Thus, it is possible that inhibition of CyPA secretion by statins (Satoh et al. 2009a) or Rho-kinase inhibitors (Abe et al. 2004; Elias-Al-Mamun et al. 2014) may have contributed to the therapeutic effects of these drugs on PH. Therefore, we tested the hypothesis that CyPA contributes to the development of PH in mice and in humans. As expected, CyPA/Bsg signaling turned out to be a novel promoter of PH (Satoh et al. 2014b). Extracellular CyPA and vascular Bsg played a crucial role in hypoxia-induced PH by inducing growth factor secretion, inflammatory cell recruitment, and VSMC proliferation. The cells residing in the vessel wall, specifically, VSMCs, appear to be the central players in the CyPA/Bsg-mediated PH development. The development of PH in $Bsg^{+/+}$ recipient mice did not differ, even after transplantation of $Bsg^{+/+}$ or $Bsg^{+/-}$ bone marrow. In addition, PH severity was exacerbated in $Bsg^{+/+}$ versus $Bsg^{+/-}$ recipient mice regardless of the bone marrow source ($Bsg^{+/+}$ or $Bsg^{+/-}$). Based on these findings, we proposed a novel mechanism for hypoxia-induced PH in which hypoxia induces growth-promoting genes in VSMCs through a CyPA/Bsg-dependent pathway (Satoh et al. 2014b). Our recent study suggests that extracellular CyPA and vascular Bsg are crucial for PH development. Thus, expression of NADPH oxidases (e.g. NOX2 and p47phox) was lower in $Bsg^{+/-}$ than in $Bsg^{+/+}$ VSMCs. In contrast, Nrf2 and its downstream target HO-1 were induced in $Bsg^{+/-}$ VSMCs compared with $Bsg^{+/+}$ VSMCs. Additionally, expression of BMPR2, which negatively regulates PDGF-BB and EGF in patients with PH, was induced in $Bsg^{+/-}$ VSMCs. Mechanistic studies demonstrated that

$Bsg^{+/-}$ VSMCs secreted less cytokines/chemokines and growth factors (e.g. PDGF-BB). VSMC proliferation was significantly reduced in $Bsg^{+/-}$ compared with $Bsg^{+/+}$ in response to 2% FBS, suggesting the crucial role of Bsg in VSMC proliferation (Satoh et al. 2014b). We further examined CyPA-induced secretion of growth factors by VSMCs harvested from the pulmonary arteries of patients with PAH. Extracellular CyPA induced secretion of growth factors and chemokines (e.g. PDGF-BB, SDF-1, and FGF-2) and inflammatory cytokines (e.g. IL-1β, IL-2, and TNF-α), and this effect was enhanced by hypoxia (2% O_2). Finally, in a clinical study, plasma CyPA levels in patients with PAH were increased in accordance with the severity of pulmonary vascular resistance. Furthermore, event-free curve analysis revealed that high plasma CyPA levels predicted poor outcome in patients with PAH (Satoh et al. 2014b).

Clinical Application of Oxidative Stress Research on CyPA

The identification of CyPA as a mediator of cardiovascular diseases associated with inflammation and oxidative stress provides new insight into the mechanisms of several therapies. We have demonstrated that plasma levels of CyPA are significantly increased in patients with angiographically-proven CAD (Satoh et al. 2013). Importantly, CyPA levels were elevated in patients with hypertension, diabetes, smoking, dyslipidemia, and advanced age (Satoh et al. 2013), 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 (PCI) and coronary artery bypass grafting (CABG) (Satoh et al. 2013). Taken together, these results suggest that circulating CyPA is a novel biomarker for CAD and plays a crucial and synergistic role in ROS augmentation, contributing to the progression of atherosclerosis (Satoh et al. 2010b).

Plasma Level of CyPA is a Novel Biomarker for Coronary Artery Disease

Fig. 2A shows the distribution of the plasma levels of CyPA in patients with and without coronary artery stenosis. The plasma levels of CyPA were significantly higher in patients with coronary organic stenosis than in those without stenosis (Fig. 2B). Moreover, the CyPA level increased with the angiographic severity of coronary disease (Fig. 2C).

All the cases were categorized into quartile groups based on the plasma level of CyPA to examine its correlation with the number of stenotic coronary arteries. The patients with CyPA in the upper quartile were older and more likely to have clinically significant CAD ($P < 0.001$). The prevalences of hypertension and diabetes were higher

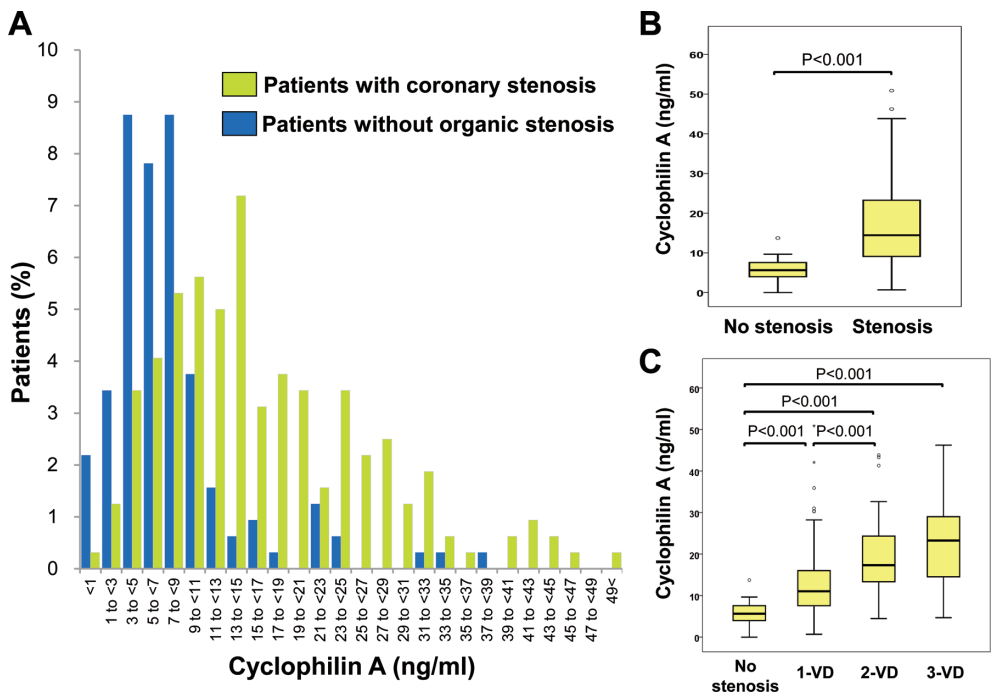

Fig. 2. Circulating Cyclophilin A (CyPA) Levels in Patients with 1-, 2-, and 3-Vessel Disease.
(A) Green and blue bars represent the number of patients with and without coronary artery stenosis, respectively. (B) The data are box-and-whisker plots of CyPA levels in patients with (> 51%, $n = 189$) and without ($n = 131$) organic stenosis. CyPA was elevated in patients with coronary stenosis ($P < 0.001$) compared to those without stenosis. (C) CyPA was elevated in patients with 1-, 2-, and 3-vessel disease (VD) (all P-values < 0.001) compared to the control group (no organic stenosis/no vasospastic angina). CyPA increased sequentially within the coronary stenosis group as the number of stenotic vessels increased (P value for trend < 0.001). The data are from (Satoh et al. 2013), reproduced with permission from the publisher.

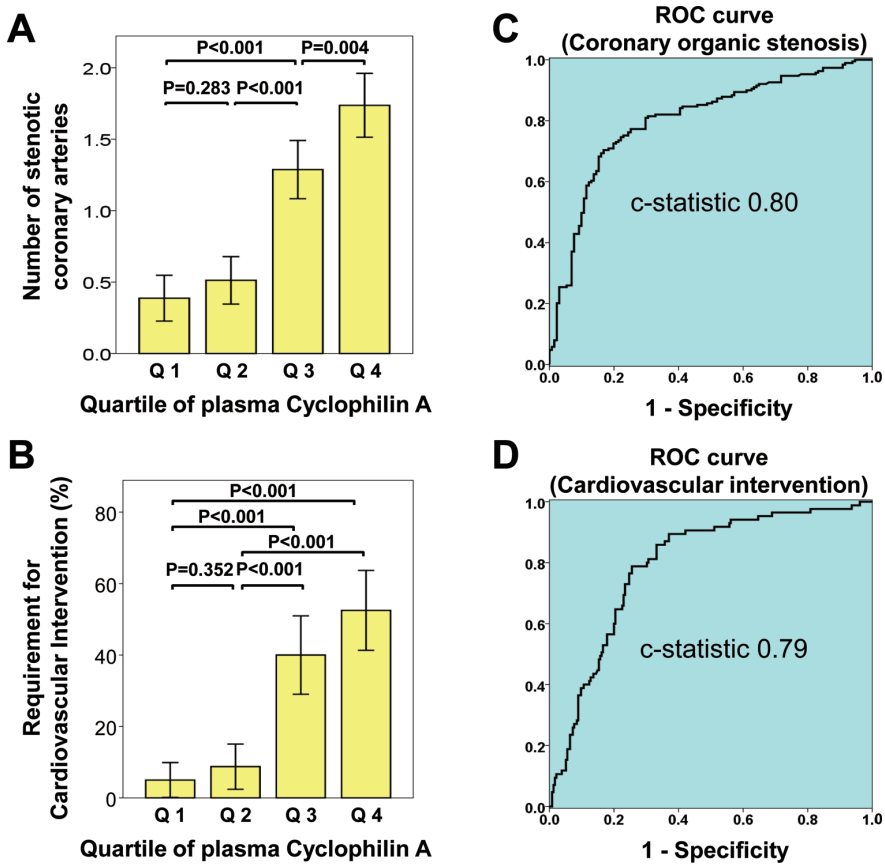

Fig. 3. Number of Stenotic Coronary Arteries and Requirement for Cardiovascular Intervention According to Quartiles of Cyclophilin A (CyPA).
The data from 320 patients with or without coronary stenosis are divided according to the quartiles of plasma CyPA levels. The CyPA levels were as follows: first quartile (Q1), < 6.1 ng/ml; second quartile (Q2), 6.2-9.6 ng/ml; third quartile (Q3), 9.7-17.4 ng/ml; and fourth quartile (Q4), > 17.5 ng/ml. The *P*-value obtained by the log-rank test for the overall comparison among the groups was < 0.001. (A) The number of stenotic coronary arteries according to quartiles of CyPA. CyPA was elevated in the patients of Q3 ($P < 0.001$) and Q4 ($P < 0.001$) compared to Q1. The number of stenotic coronary arteries increased sequentially as the quartile number increased. (B) The need for cardiovascular intervention according to quartiles of CyPA. The patients of Q4 required cardiovascular intervention more often than those of Q1 ($P < 0.001$) and Q2 ($P < 0.001$). Cardiovascular intervention included percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). (C and D) Receiver operator characteristic (ROC) curves and c-statistics for baseline measurements of CyPA levels along with corresponding values of sensitivity and specificity. (C) The ROC curve describing the performance of CyPA in diagnosing coronary organic stenosis of > 50% in at least 1 vessel compared with the reference standard of invasive quantitative coronary angiography (CAG). The c-statistic was 0.80 (95% confidence interval (CI), 0.75-0.85). (D) The ROC curve describing the performance of CyPA in predicting the need for future cardiovascular intervention compared with the reference standard of invasive quantitative CAG. The c-statistic was 0.79 (95% CI, 0.74-0.85). The data are from (Satoh et al. 2013), reproduced with permission from the publisher.

in the 4th quartile, whereas estimated glomerular filtration rate (eGFR) was slightly reduced. The number of stenotic coronary arteries was significantly increased in the higher CyPA quartiles ($P < 0.001$, Fig. 3A). Furthermore, the patients in the 4th quartile required cardiovascular interventions such as PCI and CABG significantly more often than those in the lower quartiles ($P < 0.001$, Fig. 3B). ROC curve analysis demonstrated that the plasma levels of CyPA is useful for the diagnosis of coronary organic stenosis (c-statistic = 0.802) and predicting future cardiovascular intervention (c-statistic = 0.793) (Fig. 3C, D).

CyPA was elevated in patients with traditional cardiovascular risk factors such as hypertension, diabetes, smoking, dyslipidemia, and advanced age (all $P < 0.001$). Division of the cohort into quartiles according to plasma CyPA levels provided additional evidence of the association between plasma CyPA and CAD. In an analysis with adjustment for age, sex, and traditional cardiovascular risk factors (smoking, diabetes, hypertension, dyslipidemia), the patients in quartiles 2, 3, and 4 of plasma CyPA had an increased risk of CAD compared to those in quartile 1 (odds ratios, 1.73, 9.94, and 10.29; *P*-value for trend < 0.001). This result remained significant after adjustment for traditional cardiovascular risk factors plus high-sensitivity C-reactive protein (hsCRP) levels (odds ratios, 1.84, 10.53, and 10.78; *P*-value for trend < 0.001). Several known car-

diovascular risk factors were associated with CAD in logistic-regression models adjusted for age, sex, and body mass index. Diabetes and hypertension were each linked to an increased risk of CAD. All the known risk factors, in addition to plasma CyPA, were then combined in a single logistic-regression analysis. In this model, which included the hsCRP level, plasma CyPA level > 15 ng/ml remained highly related to the disease status (odds ratio 6.20, $P < 0.001$). Multivariable analysis demonstrated that, in addition to the established risk factors (age, sex, smoking, hypertension, diabetes, and hsCRP), CyPA level > 15 ng/ml was significantly correlated with CAD (Satoh et al. 2013).

The addition of plasma CyPA to the known cardiovascular risk factors (age, sex, smoking, hypertension, diabetes, dyslipidemia) significantly improved the overall performance of the logistic-regression model, as reflected in the increase in the c-statistic from 0.807 to 0.870. The addition of plasma CyPA did not reduce model discrimination as assessed by goodness-of-fit statistics. Thus, CyPA added prognostic information above and beyond that provided by age, sex, family history of ischemic heart disease, presence of hypertension, diabetes, smoking status, body mass index, eGFR, and plasma lipid levels. When hsCRP level was included in the baseline model, the c-statistic increased from 0.807 to 0.873. Excluding the 141 patients with high hsCRP did not significantly change the results. In patients with hsCRP values < 1.000, the adjusted odds ratio for CAD in the fourth quartile of CyPA, as compared with the first quartile, was 13.2 (95% confidence interval, 3.2-53.9, $P < 0.001$). Additionally, CyPA level > 15 ng/ml remained a strong prognostic marker, with an adjusted odds ratio of 5.9 (95% confidence interval, 2.3-14.8, $P < 0.001$). Among the patients with hsCRP above 1.000, the same trend was observed, suggesting the potential usefulness of the combination of these biomarkers for diagnosing CAD (Satoh et al. 2013).

Plasma Levels of hsCRP and Angiographic Status

It has been demonstrated that hsCRP predicts future cardiovascular events and CAD (Ridker 2004). In particular, patients with hsCRP level < 1 mg/L are at low risk and those with hsCRP level > 1 mg/L are at moderate to high risk of future cardiovascular events (Ridker 2004). We found that, although the number of stenotic coronary arteries was slightly increased in the 4th hsCRP quartile, the *P*-value for the trend was insignificant ($P = 0.107$) compared to that in the CyPA quartiles ($P < 0.001$). Moreover, hsCRP quartiles did not show strong correlations with the need for future cardiovascular intervention. We thus evaluated the roles of CyPA in patients with high (> 1 mg/L, $n = 141$) and low (< 1 mg/L, $n = 179$) hsCRP and found no correlation between plasma CyPA and hsCRP levels ($P = 0.511$). Furthermore, there was no significant difference in the angiographic findings ($P = 0.602$) and the need for cardiovascular intervention ($P = 0.348$) between the high and low hsCRP groups. However, plasma levels of CyPA still revealed a strong correlation with the severity of CAD in each group (Fig. 4). In the subgroups of the population with both low and high hsCRP levels, the levels of CyPA were significantly higher in the patients with severe CAD than in those without CAD (Satoh et al. 2013).

Predictive Value of Plasma CyPA and hsCRP Levels

Ninety-five (53.1%) of the 179 patients with low hsCRP had CAD. The median CyPA level was elevated in the subjects with CAD (17.1 ng/ml with CAD vs. 7.7 ng/ml without CAD, $P < 0.001$). In contrast, 94 (66.7%) of the 141 patients with high hsCRP had CAD, and the median CyPA level was elevated in these subjects (16.1 ng/ml with CAD vs. 8.0 ng/ml without CAD, $P < 0.001$). Importantly, the CyPA quartiles did not correlate with the plasma levels of hsCRP ($P = 0.103$).

Therefore, we used the Gensini score (Gensini 1983), which reflects the severity of coronary atherosclerosis (Matsubara et al. 2012), to correlate CAD with levels of CyPA or hsCRP. The Gensini score was significantly increased in the higher CyPA quartiles ($P < 0.001$, Fig. 5A) and the 4th hsCRP quartile ($P = 0.007$, Fig. 5B). Overall, plasma levels of CyPA were superior to those of hsCRP in terms of evaluation of the severity of CAD.

Plasma CyPA and Atherosclerotic Risk Factors

We conducted translational studies in patients with stable CAD to examine the prognostic importance of CyPA. We found that CyPA is a prognostic marker for cardiovascular interventions such as PCI and CABG. The increased severity of CAD observed in patients with elevated CyPA may be a consequence of a higher frequency of risk factors for atherosclerosis, all of which promote ROS production and CyPA secretion. All these mechanisms create oxidative stress environment, thereby contributing to the increased plasma levels of CyPA in patients with severe CAD. As was mentioned above, vascular ROS formation can be stimulated by mechanical stretch, pressure, shear stress, environmental factors such as hypoxia, and secreted factors such as AngII (Rao and Berk 1992; Baas and Berk 1995; Griendling and FitzGerald 2003; Taniyama and Griendling 2003). In addition, extracellular CyPA induces ROS production in VSMCs and recruits inflammatory cells, resulting in the augmentation of vascular ROS and atherosclerosis (Nigro et al. 2011). All these data strongly suggest that circulating CyPA is a novel biomarker for CAD.

CyPA and Atherosclerotic Unstable Plaque

We hypothesized that large amounts of secreted CyPA are accumulated in atherosclerotic plaque in the coronary arteries. Indeed, we observed strong CyPA expression in the coronary arteries of patients with myocardial infarction (Satoh et al. 2013). Importantly, this expression was localized to the region just beneath the thin fibrous cap of the atherosclerotic plaque. CyPA may play an important role in several stages of atherosclerosis. During fatty streak for-

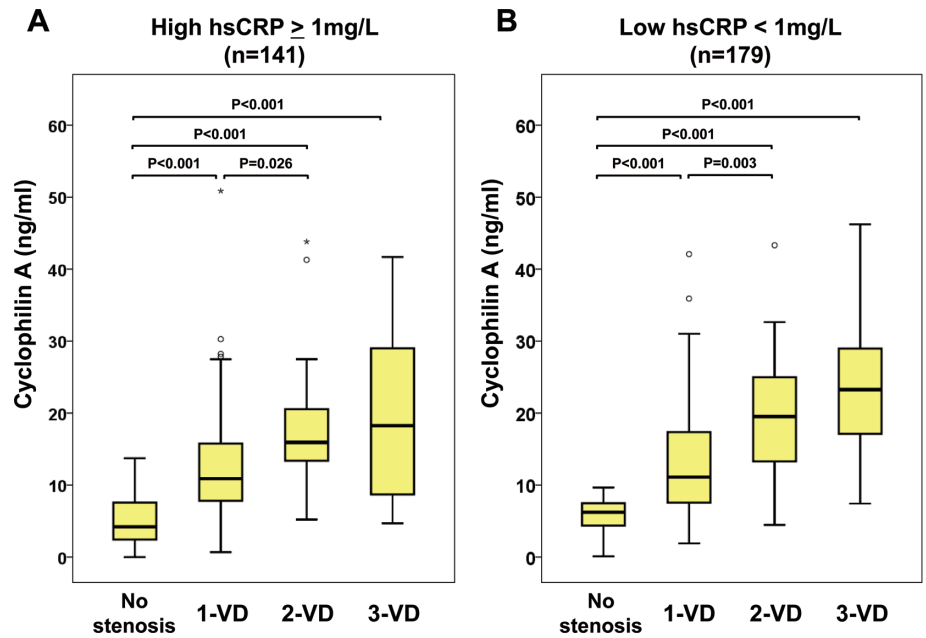

Fig. 4. Diagnostic Performance of Plasma Cyclophilin A (CyPA) for Coronary Diseases in Patients in Higher and Lower hsCRP Subgroups.
The data are box-and-whisker plots of CyPA levels in patients with higher (> 1 mg/L, n = 141) lower (< 1 mg/L, n = 179) high-sensitivity C-reactive protein (hsCRP) levels. (A) CyPA was elevated in patients with higher hsCRP levels who had 3-, 2-, and 1-vessel disease (VD) compared to the no-stenosis group ($P < 0.001$ in all cases). (B) In patients with lower hsCRP levels, CyPA was elevated in patients with 1-, 2-, and 3-VD compared to the no-stenosis group ($P < 0.001$ in all cases). CyPA levels increased sequentially within the coronary stenosis groups as the number of stenotic vessels increased irrespectively of the levels of hsCRP. The data are from (Satoh et al. 2013), reproduced with permission from the publisher.

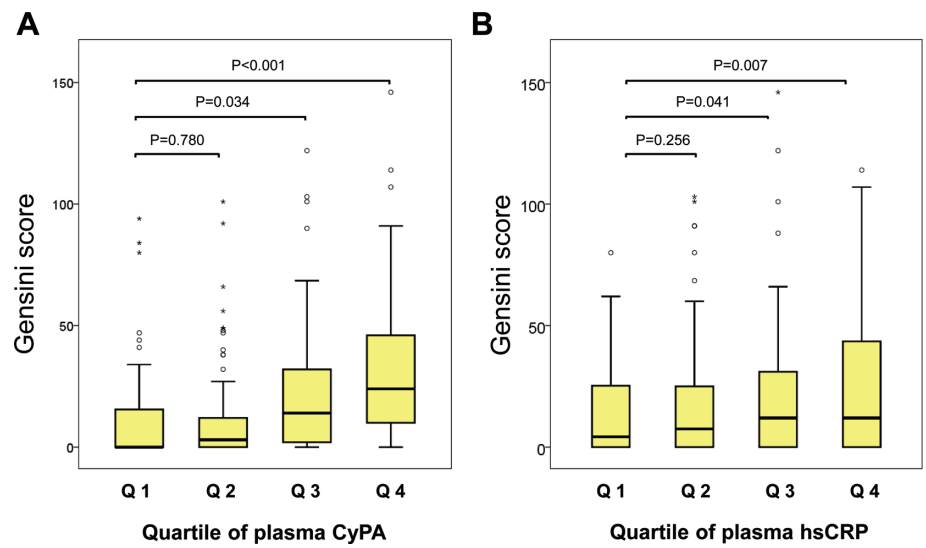

Fig. 5. Gensini Scores According to Quartiles of Cyclophilin A (CyPA) and High-Sensitivity C-reactive Protein (hsCRP) Levels.
The data from 320 patients with or without coronary stenosis are divided according to the quartiles of plasma CyPA levels. The CyPA levels were as follows: first quartile (Q1), < 6.1 ng/ml; second quartile (Q2), 6.2-9.6 ng/ml; third quartile (Q3), 9.7-17.4 ng/ml; and fourth quartile (Q4), > 17.5 ng/ml. The P-value obtained by the log-rank test for the overall comparison of the groups was < 0.001. (A) Gensini scores according to quartiles of CyPA levels. (B) Gensini scores according to quartiles of hsCRP levels. The hs-CRP levels were as follows: first quartile (Q1), < 0.35 mg/l; second quartile (Q2), 0.36-0.82 mg/l; third quartile (Q3), 0.82-2.0 mg/l; and fourth quartile (Q4), > 2.0 mg/l. The data are from (Satoh et al. 2013), reproduced with permission from the publisher.

mation, it may participate in lipid uptake via its effect on scavenger receptors (Nigro et al. 2011). In all stages, it may play a role in inflammation by promoting monocyte adhesion and recruitment as well as by contributing to the oxidative environment (Satoh and Berk 2008). The data from Seizer et al. (2010) that CyPA is secreted by foam cells suggest its important role in later stages of atherosclerosis. Altogether, our data suggest that the agents that inhibit CyPA secretion might suppress the development of atherosclerosis. Bsg has been identified as a tumor cell membrane protein that is expressed in VSMCs, activated by ROS, and stimulates MMP production (Guo et al. 1998). A recent study demonstrated ROS-dependent increases in Bsg (Haug et al. 2004), which may be activated by binding of extracellular CyPA (Yurchenko et al. 2002). Therefore, ROS-induced secretion of CyPA may also contribute to the development of unstable atherosclerotic plaque and plaque rupture. We believe that discovery of more selective and specific inhibitors of CyPA secretion may provide an effective therapeutic approach for the treatment of CAD.

CyPA as a Biomarker for Cardiovascular Diseases

Our study demonstrates that plasma levels of CyPA in patients with stable CAD provide prognostic information on the severity of CAD and the need for cardiovascular intervention (Satoh et al. 2013). These findings support the previous animal studies suggesting that CyPA augments the development of atherosclerosis in mice (Satoh et al. 2008; Nigro et al. 2011). As determined by angiography, patients with high CyPA levels had a significantly higher prevalence of CAD than those with low concentrations of CyPA. A possible role for CyPA in atherosclerosis is becoming increasingly apparent. We showed that knock-down of CyPA in ECs reduced TNFα-induced apoptosis *in vitro* and that CyPA deficiency was associated with a marked decrease in EC apoptosis in early stages of atherosclerosis (Nigro et al. 2011). The increase in the vascular oxidative stress requires CyPA (Satoh et al. 2010b) and thereby sensitizes ECs to apoptosis. In addition, CyPA secretion is regulated by Rho-kinase activation, which is important for VSMC contraction and atherosclerosis (Satoh et al. 2011c). Consistently, plasma levels of CyPA were significantly increased in patients with CAD.

We have previously demonstrated that ROS inducers, such as mechanical stress, angiotensin II, and dyslipidemia, promote the secretion of CyPA in a Rho-kinase-dependent manner (Satoh et al. 2011c). It is well known that Rho-kinase is associated with activation of the NADPH oxidases, with resultant ROS production (Higashi et al. 2003), which plays a crucial role in the development of several cardiovascular diseases (Shimokawa and Takeshita 2005). In support of this notion, CyPA was elevated in patients with hypertension, diabetes, smoking, dyslipidemia, and advanced age (Satoh et al. 2013). This is the first study that has examined the association between CyPA and ROS-inducers, all of which are atherosclerotic risk factors in humans. The number of patients in the study was relatively small (n = 320). However, even in this small population, plasma levels of CyPA were closely related to the severity of CAD. Future analysis in a large prospective cohort will further elucidate the importance of plasma CyPA in CAD. As for the plasma levels of CyPA in patients with myocardial infarction, we need to additionally consider two different mechanisms that increase plasma levels of CyPA. One mechanism is the oxidative stress-induced CyPA secretion from the vasculature. Another mechanism is the CyPA release from the necrotic tissue after myocardial infarction. Therefore, we excluded patients with unstable CAD or myocardial infarction and recruited patients with stable CAD. The plasma levels of CyPA in patients with unstable CAD or myocardial infarction need to be examined in future studies. In conclusion, plasma CyPA level is a novel biomarker of CAD. Further studies are needed to establish the clinical significance of CyPA in the pathogenesis of atherosclerotic cardiovascular diseases (Satoh and Shimokawa 2014).

CyPA as a Therapeutic Target for Cardiovascular Diseases

Numerous basic and clinical studies have demonstrated that ROS play a major role in the pathogenesis of endothelial dysfunction and atherosclerosis. However, no therapeutic strategies are yet available for clinical use of antioxidants. We hypothesize that one of the reasons for this is that low concentrations of ROS, particularly H_2O_2, play an important role in intracellular signaling pathways that are crucial for numerous vascular cell functions. In addition, the dual roles of ROS also correspond to the role of NO in cell signaling (Cohen and Adachi 2006). Furthermore, production of ROS in inflammatory cells plays a crucial role in cellular responses involved in immune response and infection (Kesarwani et al. 2013). Many strategies to control oxidative stress have been implemented in the treatment of various diseases. However, we need to consider the existence of a complex network of molecules that exogenously or endogenously contribute to the balance between oxidant and antioxidant systems. Thus, a promising clinical strategy may be to use antioxidants and/or drugs that can prevent oxidation of selected redox-sensitive targets under certain disease conditions while allowing ROS to continue to function in normal processes.

The identification of CyPA as a mediator of oxidative stress-induced tissue damage has provided some additional insight into the mechanisms of several therapies. For example, the Rho-kinase inhibitor and simvastatin significantly reduced CyPA secretion from VSMCs (Suzuki et al. 2006). Rho-kinase is an important therapeutic target in cardiovascular diseases (Shimokawa and Takeshita 2005), and its inhibition has been reported to reduce AngII-induced abdominal aortic aneurysm formation (Wang et al. 2005), atherosclerosis, pulmonary hypertension (Shimizu et al.

2013), and cardiac hypertrophy (Higashi et al. 2003). Moreover, angiotensin II type 1 (AT1) receptor blockers and angiotensin-converting enzyme inhibitors have been shown to prevent cardiovascular diseases (Cassis et al. 2007), and reduced CyPA secretion may partially contribute to the therapeutic effect of these drugs on aortic aneurysms, atherosclerosis, cardiac hypertrophy, and PAH (Satoh et al. 2010b). Therefore, it is logical to propose that agents which prevent CyPA binding to its receptors may have therapeutic potential. By blocking this vicious cycle that augments ROS production through CyPA autocrine/paracrine signaling pathway, we may obtain a novel therapeutic tool for controlling cardiovascular diseases in the near future. This warrants further investigation of the role of CyPA to identify potential CyPA-related therapeutic targets (Weintraub 2009).

Importantly, CyPA is highly expressed at sites with unstable atherosclerotic plaques, especially those associated with macrophages and foam cells (Satoh et al. 2013). Based on the role of extracellular CyPA in MMP activation, it is reasonable to assume that agents that prevent CyPA receptor binding and reduce circulating CyPA may have therapeutic potential for inhibiting atherosclerotic plaque rupture. Thus, further basic and clinical studies are needed to identify CyPA-related therapeutic targets.

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Conflict of Interest

The author declares no conflict of interest.

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