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Cyclophilin A: A Mediator of Cardiovascular Pathology

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ABSTRACT

Cyclophilin A (CyPA) is a 17 kDa, ubiquitously expressed multifunctional protein that possesses peptidylprolyl cis-trans isomerase activity and scaffold function. Its expression is increased in inflammatory conditions including rheumatoid arthritis, autoimmune disease and cancer. Intracellular CyPA regulates protein trafficking, signal transduction, transcription regulation and the activity of certain other proteins. Secreted CyPA activates cardiovascular cells resulting in a variety of cardiovascular diseases; including vascular remodeling, abdominal aortic aneurysms formation, atherosclerosis, cardiac hypertrophy and myocardial ischemic reperfusion injury. **(J Korean Soc Hypertens 2011;17(4):133-147)**

Key Words: Cyclophilin A; Oxidative stress; Cardiovascular diseases

Introduction

Oxidative stress resulting from increased reactive oxygen species (ROS) formation contributes to the pathogenesis of cardiovascular diseases. Changes in vascular redox state are a common pathway involved in the pathogenesis of atherosclerosis, aortic aneurysms, vascular restenosis and ischemic reperfusion injury. ROS promotes vascular smooth muscle (VSMC) growth in part by increasing cell proliferation, hypertrophy and also inducing apoptosis in a concentration dependent manner.[1,2] In addition, ROS modulates endothelial cells (EC) function by multiple mechanisms including increased inflammatory mediators and apoptosis to promote atherosclerosis.[3] Vascular ROS formation is stimulated by secreted factors such as Angiotensin II (AngII),[4] shear stress,[5] hypoxia,[6] mechanical stress.[7] In recent years Cyclophilin A (CyPA) has been described as having a key role in each of these cardiovascular pathologies. Understanding the mechanism(s) of CyPA in normal as well as diseased states is crucial for preventing cardiovascular disease progression.

Cyclophilins (CyPs) are members of the immunophilin family of proteins which possess peptidyl-prolyl cis-trans isomerase (PPIase) activity[8] that regulates cis-trans isomerization of Xaa-Pro peptide bonds and promote protein folding and assembly of multidomain proteins. In humans, there are at least 16 homologues of CyPs. Within the CyP family, CyPA is the most abundant and comprises approximately 0.1-0.6% of the total cytosolic proteins.[9] It was first purified from bovine thymocytes and described as the intracellular binding ligand of the immunosuppressant drug cyclosporine A (CsA).[10] CyPA

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regulates diverse cellular functions including protein folding,[11,12] intracellular trafficking,[13] signaling transduction[14,15] and transcription regulation[16] by its enzymatic activity as well as non-enzymatic scaffold function.

There have been several reports on the effects of CsA, a pharmacological inhibitor of CyPA PPIase activity, on neointima formation after balloon injury of rat or rabbit carotid.[17-20] However, the results from these studies are contradictory with investigators finding that VSMC growth and neointima formation in animals that received CsA were increased,[19] not changed,[20,21] or decreased.[18] Finally, a paper by Walter[17] showed that CsA protected EC from apoptosis. Clearly our data[22,23] suggest that CyPA stimulates VSMC growth and promotes EC apoptosis. Our new data using CyPA transgenic and knockout mice substantiate a role for CyPA in neointima formation.[24] The reasons for the conflicting data are unclear, but may be related to CsA pharmacokinetics because its excretion is highly regulated by renal function, and dosing varied from 5 to 50 mg/kg/day in the studies.

Despite mounting evidence that cyclophilins serve multiple intracellular and extracellular functions, surprisingly little is known regarding their mechanisms of extracellular action (Fig. 1). Several molecules have been proposed to serve as extracellular receptors for cyclophilins including CD147,[25-27] CD[14,28] syndecan-1 (for CyPB),[29] heparan sulfate proteoglycans (for CyPB)[30] and CD91.[31] To date none of these proteins have unequivocally been proven to mediate the cellular events associated with CyPA. CD147[26] or extracellular matrix metalloproteinase inducer (EMMPRIN)[25] is a 50-60 kD integral membrane glycoprotein that is widely expressed. CyPA has been shown to be incorporated into the virions of human immunodeficiency virus type 1 (HIV-1) and enhances HIV-1 infection via interactions with CD147.[26] We have obtained antibodies to CD147 and think CD147 is unlikely to be the relevant CyPA receptor in VSMC and EC, due to low level expression, failure of CD147 antibodies to block CyPA action, presence of CD147 on Chinese hamster ovary cells which do not increase extracellular signal-regulated kinases (ERK)1/2 in response to CyPA, and evidence that deleting the CD147 cytoplasmic tail does not inhibit signaling.[32]

Intracellular CyPA has numerous functions including a role as immunophilins that interact with calcineurin, components of a caveolin-cholesterol-cyclophilin complex, and components of the cell cycle.[8] Our model for CyPA action is cell type specific (Fig. 1). In VSMC, ROS such

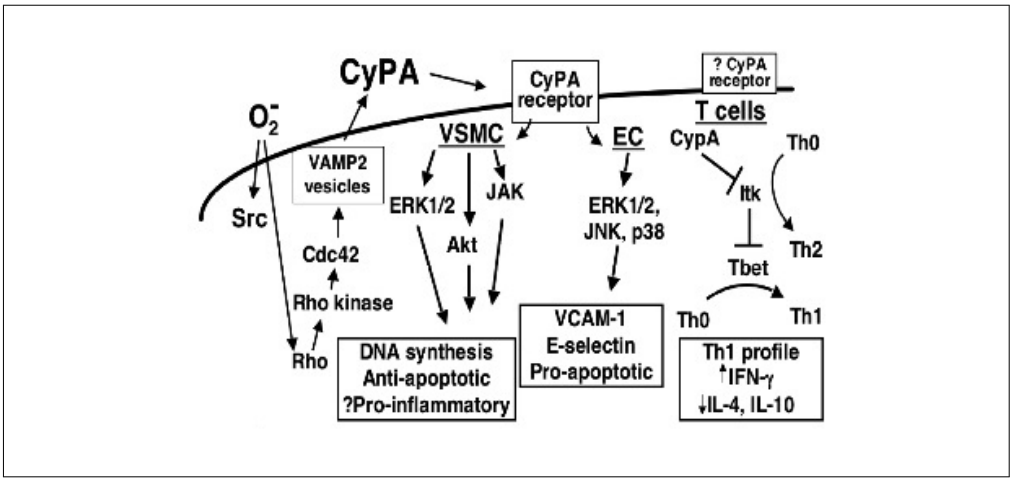

Fig. 1. Cyclophilin A (CyPA) effects on vascular smooth muscle (VSMC), endothelial cells (EC) and T cells. VCAM-1, vascular cell adhesion molecule-1; IFN, interferon; IL, interleukin.

as superoxide activates a pathway (involving Rho, Rho kinase, Cdc42 and VAMP2 containing vesicles) that results in secretion of CyPA.[33] CyPA stimulates at least 3 VSMC signaling pathways (ERK1/2, Akt, and JAK) that contribute to DNA synthesis and prevent apoptosis.[23] In EC, CyPA activates proinflammatory pathways including increased expression of vascular cell adhesion molecule-1 and E-selectin.[15,22,34] In T cells, CyPA has been shown to regulate calcineurin in the context of CsA treatment and to inhibit Itk, a Tec family tyrosine kinase (Figs. 1, 2). Since Itk normally inhibits T-bet, the T helper type 1 (Th1) specific transcription factor, CyPA acts as a positive regulator of Th1 profile promoting differentiation of Th0 cells into Th1 lymphocytes (increased IFN-g).[35] Conversely, CyPA relatively inhibits Th2 differentiation (less IL-4 and IL-10). In the absence of CyPA, Itk becomes fully active, T-bet is inhibited and there is decreased Th1 profile (less IFN- g). A T-cell infiltrate is always present in atherosclerotic lesions. Such infiltrates are predominantly CD4+ T cells, which recognize protein antigens presented to them as fragments bound to major-histocompatibility- complex class II molecules.[36,37] CD4+ T cells reactive to the disease-related antigens oxidized low-density lipoproteins (LDL), HSP60, and chlamydia proteins have been cloned from human atherosclerotic lesions.[37,38] When the antigen receptor of the T cell is ligated, an activation cascade results in the expression of a set of cytokines, cell-surface molecules, and enzymes.

Increased CyPA expression and secretion are observed in oxidative stress and inflammatory related conditions including cardiovascular diseases. However the precise mechanism of CyPA in cardiovascular diseases remains unclear. Therefore, better understanding of CyPA function may be promising therapeutic application in prevention, diagnosis and treatment in cardiovascular diseases. In this review, we will focus on the current understanding of the role of CyPA in cardiovascular diseases.

CyPA as a secreted protein

CyPA is present in both the cytoplasm and nucleus[13,39-41] but increasing evidence points to it also being secreted. Sherry and colleagues first descried CyPA as a secreted protein from macrophages.[42] Conditioned medium (CM) of lipopolysaccharide[43,44] (a bacterial cell

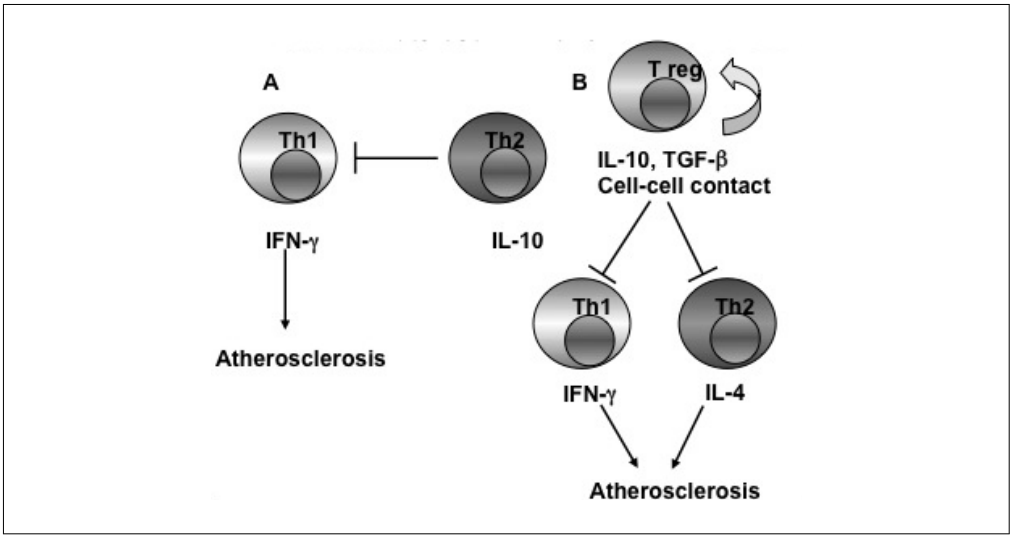

Fig. 2. Immune modulation of T cell function. (A) Th2 inhibits Th1 responses. (B) T regs regulate both Th1 and Th2 responses. IFN, interferon; IL, interleukin; TGF, transforming growth factor.

wall component known to activate inflammatory process) stimulated macrophages showed a significant amount of secreted CyPA and highly regulated migration of neutrophils and monocytes suggesting the important role of CyPA in inflammatory diseases. There is also a relationship between inflammation, ROS and cyclophilin released as shown by the high CyPA levels in serum from patients with HIV, rheumatoid arthritis and sepsis.[45-47] Because these diseases are usually accompanied by the generation of superoxide (O2-) by neutrophils, lymphocytes, and vessel wall cells, it is possible that O2- may stimulate CyPA secretion and expression *in vivo*.

Recently, we proved that CyPA was secreted from VSMC and fibroblasts in response to ROS. ERK1/2 activation by a ROS generator, napthoquinolinedione (LY83583), had a biphasic pattern of early (10 minutes) and late activation (120 minutes).[48] The first peak of activation was mediated by a protein kinase C dependent mechanism[49] and the second peak which is crucial for cell cycle progression and cell proliferation[50,51] occurred after sufficient time for *de novo* protein synthesis, secretion and resulting autocrine or paracrine action. Therefore we investigated the secreted factors induced by ROS using sequential column chromatography. CM purified from LY83583-induced VSMC and *Mox1* (a super generating homology of the phagocyte NADPH oxidase catalytic subunit) transfected fibroblast showed abundant secretion of CyPA. Immunodepletion of CM with CyPA antibody inhibited conditioned medium from LY83583-stimulated cells induced ERK1/2 activation suggesting secreted CyPA is important autocrine factor for the second peak of ERK1/2 activation.[23] CyPA secretion is an active process involving vesicle transport as well as docking and fusion at the plasma membrane[33] (Figs. 1, 3). In response to ROS, CyPA translocated to the plasma membrane and colocalized with membrane fusion protein VAMP2 for secretion. Rho kinase inhibitor Y27632, dominant negative Rho GTPase, myosin II light chain inhibitor blebbistatin, actin polymerization agent jasplakinolide and depolymerization agent cytochalasin D inhibited CyPA membrane translocalization and secretion suggesting that CyPA secretion required the Rho GTPase - myosin II - actin remodeling pathway. AngII increased ROS production by regulating NADPH oxidase in smooth muscle cell.[4,52,53] AngII is an important ROS inducer in cardiovascular diseases. We showed that AngII-induced

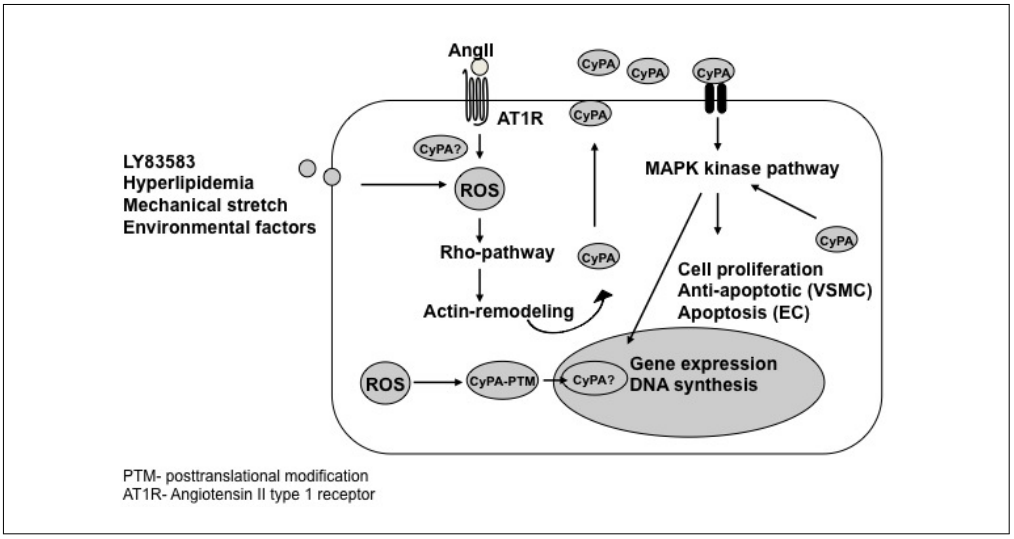

Fig. 3. Mechanism of cyclophilin A (CyPA) regulation on cardiovascular cells. ROS, reactive oxygen species; VSMC, vascular smooth muscle; EC, endothelial cells.

CyPA secretion is inhibited by Rho kinase inhibitor suggesting important role of Rho GTPase pathway in AngII-induced CyPA secretion.[54] Furthermore increased ROS production in glutathione peroxidase-deficient smooth muscle cells caused CyPA secretion providing further evidence that ROS is a mediator of CyPA secretion.[55]

Besides secretion from VSMC, CyPA is secreted by other cardiovascular cells under oxidative stress conditions. Lipopolysaccharide treated human endothelial cells secreted CyPA in a time and dose dependent manner without decreasing cell viability suggesting that CyPA is secreted by an active process.[56] Hypoxia followed by reoxygenation sequentially activated mitogen-activated protein kinase (MAPK) signaling pathway in cardiac myocytes.[57,58] This signaling cascade regulates gene expression for cytokines, growth factor and cell adhesion in cardiomyocytes. Interestingly CM from hypoxia- reoxygenation induced cardiomyocytes showed a significant amount of CyPA secretion.[58] Moreover recombinant human CyPA increased activation of ERK, p38MAPK, stress-activated protein kinases and Bcl-2 expression. Together, these data indicate the significant role of extracellular CyPA in the activation of cardiovascular cells.

Recently data from our lab using *ApoE*$^{-/-}$ mice showed CyPA was secreted from cardiac fibroblasts under oxidative stress conditions. AngII induced secretion of significant amounts of CyPA from *ApoE*$^{-/-}$ cardiac fibroblast,[59] further indicating that CyPA is secreted by an active mechanism under oxidative stress conditions.

CyPA and posttranslational modification

The wide tissue distribution of CyPA, together with its high degree of conservation throughout evolution, suggests an essential role in cellular function. There are many types of post-translational modification of proteins, which can affect a protein's function, stability, degradation and/or ability to interact with other proteins. CyPA is modified by several chemical groups in response to many different stimuli. Stimulation of chemokine receptor CXCR4 mediated phosphorylation of CyPA in HEK293T cells.[41] There is substantial data that ROS stimulates formation of acetylated CyPA (Acyl-CyPA). Following oxidative stress, CyPA underwent glutathionylation on Cys52 and Cys62 residues that induced structural changes resulting in regulation of T cell function.[60] Glutathionylated CyPA was also observed in oxidatively stressed hepatocytes and hepatoma cells.[61] Furthermore in the mouse model for amyotrophic lateral sclerosis, in which oxidative stress is induced (by mutating SOD1 to make it inactive), acyl-CyPA was highly expressed.[62] Most importantly Lammer et al.[63] demonstrated an important functional role for Acyl-CyPA in decreasing the pathogenicity of HIV. However, the role of post-translational modification of CyPA in cardiovascular pathology remains unclear and needs to be addressed.

CyPA and cardiovascular diseases

Many cardiovascular diseases initiate as increased oxidative stress and inflammation. The preceding sections have highlighted the importance of CyPA as an oxidative stress and inflammatory related protein. Using genetically modified mice deficient for CyPA expression, we and others have demonstrated its important role in vascular remodeling, abdominal aortic aneurysms (AAA) formation, atherosclerosis, cardiac hypertrophy and myocardial ischemic reperfusion injury.

1. CyPA and vascular remodeling

Vascular remodeling is a consequence of the interaction between endothelial cells and vascular smooth muscle cells in response to hemodynamic changes.[64-66] Smooth muscle cell proliferation, migration and collagen syn-

thesis are the key players in neointima formation which determines intima-media thickening of the vascular wall.[67-71] Accumulating evidence suggests that oxidative stress and inflammation are strongly correlated with neointima formation and vascular remodeling.[72-75] Alternation in blood flow, growth factors and cytokines are important factors regulating oxidative stress and inflammation in neointima formation.[76-80] Oxidative stress causes VSMC growth and proliferation by regulating intracellular second messengers and downstream signaling pathways such as mitogen activated protein kinase, protein tyrosine kinase and phosphatase.[49,81-86]

Interestingly, CyPA has been reported as an autocrine growth factor in VSMC.[23] Secreted CyPA from LY-induced conditioned medium and human recombinant CyPA stimulated activation of ERK1/2, Janus kinases/signal transducers and activators of transcription (JAK/STAT) as well as promoting DNA synthesis. These data suggest an important role for CyPA in MAPK kinase pathway signaling in rat aortic smooth muscle cell growth. Moreover Yang et al.[87] showed that recombinant CyPA increased the proliferation of human aortic smooth muscle cells (HAoSMC) and human lung microvascular endothelial cells (HMVECs-L) but not human coronary artery endothelial cells (HCAECs). Of note, CyPA significantly increased gene expression of CD147 (CyPA receptor) and vascular endothelial growth factor receptor-2 (VEGFR-2) in HAoSMC as well as endothelin-1 and vascular endothelial growth factor receptor-1 (VEGFR-1) in HMVECs-L.[87] Therefore CyPA plays a significant role in the regulation of cell proliferation and growth.

In balloon injured rat carotid artery, CyPA protein expression was dramatically increased with a time course that parallelled neointima formation.[23] We next investigated the finding of increased CyPA expression and its contribution in neointima formation by using genetically modified CyPA knockout (*Ppia*$^{-/-}$) and mice that over expressed CyPA specifically in VSMC (VSMC-Tg).[24] Obviously *Ppia*$^{-/-}$ mice prevented carotid ligation induced neointima formation whereas VSMC-specific over expressed CyPA dramatically enhanced neointima thickening. Additionally, CyPA expression was significantly increased in ligated carotid artery. CyPA secretion, VSMC proliferation and migration were correlated with CyPA expression level. These results suggested that chronic injury enhanced CyPA secretion and expression which promoted VSMC growth and neointima formation. ERK1/2 activation in WT-ligated artery was inhibited in *Ppia*$^{-/-}$ carotid artery suggesting intracellular CyPA can regulate cell growth and proliferation by regulating gene expression of mitogenic proteins. Moreover, CyPA induced ERK1/2 activation in monocytes/macrophages,[88] leukocytes[89] and cancer cells.[90-92] Additionally, in HEK293T cells, CXCL12 stimulated phosphorylation of CyPA which induced nuclear translocation of ERK1/2 where it activated many transcription factors.[41] Moreover the role of intracellular CyPA in regulation of protein expressions were described in somewhere as.[93,94] Taken together all these data indicate significant roles for both extracellular and intracellular CyPA in growth and proliferation of cells of the cardiovascular system.

Cell migration is a complex process of cytoskeletal reorganization, cell membrane protrusion and matrix adhesion.[95] Cytokines and growth factors such as monocyte chemoattract protein-1, platelet derived growth factor are important chemotactic factors for cell migration. It has been reported that CyPA has strong chemotactic activity for neutrophils, eosinophils and monocytes.[96,97] Surprisingly, AngII-induced secretion and expression of cytokines and ckemokines from VSMC were dramatically inhibited in *Ppia*$^{-/-}$ in compared with WT mice[54] suggesting CyPA may regulate cell migration by enhancing syn-

thesis and secretion of chemotactic factors. It is also possible that secreted CyPA directly binds with CyPA receptor on the target cells.

2. CyPA and AngII-induced abdominal aortic aneurysm formation

The weakening, dilation and occasionally rupturing of the vessel wall characterize AAA. The key mechanisms of AAA development include chronic inflammation of aortic wall,[98] oxidative stress,[99-101] increased local production of proinflammatory cytokines and increased activities of Matrix Metallloproteinases (MMPs).[102] AAA development and rupture depend on VSMC-derived MMP2[103] and macrophage derived-MMP9[104] which are activated by membrane type-1 MMP (MT1-MMP).[105] AngII is an important growth factor for the production of ROS,[53] generation of inflammatory cytokines,[106,107] and the secretion and activation of MMPs.[108] It is well documented that MMP expression and activation are strongly dependent on ROS[109,110] indicating the crucial role of oxidative stress in AngII-induced AAA development and progression. To understand the role of the proinflammatory mediator CyPA in AAA formation, ApoE and CyPA double knockout mice (DKO; *ApoE*$^{-/-}$*Ppia*$^{-/-}$) were infused with AngII (1,000 ng/min/kg for 28 days). We found that AngII-induced AAA formation was significantly reduced in DKO mice compared to *ApoE*$^{-/-}$ controls with a concomitant increase in survival rate. Deletion of CyPA prevented AngII-dependent ROS production and pro-MMP2 activation/secretion in VSMC suggesting that CyPA was crucial for ROS and MMP2 regulation in AAA development.[54]

3. CyPA and atherosclerosis

Atherosclerosis, chronic inflammation of medium and large arteries, leads to serious complications of cardiovascular diseases including acute myocardial infarction, aneurysm formation and stroke.[111-113] Atherosclerosis is initiated by the activation of EC leading to expression of adhesion molecules for inflammatory cells.[3] In addition, these activated EC facilitate the passage of lipid components in the plasma, such as LDL.[37] A critical element in the progression of atherosclerosis is the development of an oxidizing environment due to the activation of macrophages that become loaded with oxidized LDL and other lipids. These macrophages produce ROS and secrete cytokines and growth factors that contribute to the progression of atherosclerotic plaques and promote vulnerable lesions.[114] Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) causes activation of inflammatory and apoptosis signaling pathways resulting in endothelial cell apoptosis.[3,115,116] We have shown that extracellular CyPA activated the MAPK pathway and NF-KB, cell adhesion molecules expression as well as apoptosis in endothelial cells.[22] These results suggest that extracelluar CyPA is a cytokine that functions similar to TNF-α. Interestingly Kim et al.[56] showed that CyPA promoted both proliferative and apoptotic pathways in endothelial cells depending on its concentration. At low concentrations, CyPA increased EC proliferation and angiogenesis. In contrast high concentrations of CyPA decreased EC viability and increased Toll Like Receptor-4 expression. Under hypoxic conditions, CyPA expression was increased by a Hypoxia-inducible factor-1 regulated mechanism.[117] This suggests that CyPA is involved in different processes during atherosclerosis formation. Hypoxia-induced angiogenesis inside atherosclerotic lesion is caused by low concentrations of CyPA that are secreted in the early stages of atheroma formation. Further atheroma formation leads to increased hypoxic conditions resulting in more CyPA expression and secretion. This high concentration of secreted CyPA from

EC, VSMC and macrophages leads to endothelial cell apoptosis or death and ultimately thrombosis complication.

Substantial studies from our lab using high fat diet induced atherosclerosis formation in *ApoE*$^{-/-}$ versus *ApoE*$^{-/-}$ *Ppia*$^{-/-}$ mice revealed that CyPA regulates atherosclerosis in several ways.[118] Decreased lipid uptake as seen in *ApoE*$^{-/-}$ *Ppia*$^{-/-}$ aorta was the result of CyPA regulation on scavenger receptors including lectin-like oxidized low-density lipoprotein receptor, CD36 and scavenger receptor class B member 1 expression on the vessel wall. In addition CyPA inhibited eNOS expression, an important regulator of NO production for vascular homeostasis,[3] by suppression of the key transcription factor Kruppel-like factor 2 (KLF-2). This suggests that intracellular CyPA is also an important mediator of atherosclerosis by regulating gene transcription.

4. CyPA and cardiac hypertrophy

Cardiac hypertrophy is a fundamental response of cardiac cells to common clinical disorders such as arterial hypertension, valvular heart disease, myocardial infarction, cardiomyopathy, and congenital heart disease.[119] AngII plays a key role in many physiological and pathological processes in cardiac cells, including cardiac hypertrophy.[120] Therefore, understanding the molecular mechanisms responsible for AngII-mediated myocardial pathophysiology will be critical to developing new therapies for cardiac dysfunction.[121] One important mechanism now recognized to be involved in AngII-induced cardiac hypertrophy is ROS production,[122,123] but the precise mechanism by which ROS cause hypertrophy remains unknown. Our recent study provides strong mechanistic evidence of synergy between CyPA and AngII to increase ROS generation.[54] Since ROS stimulate myocardial hypertrophy, matrix remodeling, and cellular dysfunction,[124] we tested the hypothesis that CyPA enhances AngII-induced cardiac ROS production, and therefore cardiac hypertrophy. To examine the involvement of CyPA in the process of the cardiac hypertrophy, we used the AngII-infusion approach, a well-established mouse model to study cardiac hypertrophy. In contrast to *ApoE*$^{-/-}$ mice, *ApoE*$^{-/-}$ *Ppia*$^{-/-}$ mice exhibited significantly less AngII-induced cardiac hypertrophy. CyPA secretion from cardiac fibroblasts isolated from *ApoE*$^{-/-}$ *Ppia*$^{-/-}$ mice was dramatically less compared to *ApoE*$^{-/-}$ fibroblasts when stimulated with AngII.

CyPA has important roles in the immune system and it is a well described regulator of T lymphocyte functions.[15] It is relevant to note that the primary sources of CyPA responsible for cardiac hypertrophy were likely cells in the heart and not inflammatory cells, because transplantation with *Ppia*$^{+/+}$ bone marrow cells still caused less cardiac hypertrophy in *ApoE*$^{-/-}$ *Ppia*$^{-/-}$ compared to *ApoE*$^{-/-}$ mice. We demonstrated that AngII-induced fibrosis and bone marrow-derived cell migration were much more pronounced in the perivascular region than in the myocardial interstitial space, findings consistent with recent reports.[125] These data suggest the importance of cardiac CyPA for recruitment of bone marrow-derived cells to perivascular tissues to create an environment that is pro-hypertrophic.

5. CyPA and myocardial ischemic reperfusion injury

Reperfusion therapy by coronary angioplasty or thrombolysis for acute myocardial ischemia (AMI) patients causes serious complications called ischemic/reperfusion injury (I/R injury) in which reversible ischemic tissue changes to irreversible tissue injury.[126-128] It has been reported that increased ROS production in I/R injury by coronary EC and circulating phagocytes enhance degradation of NO and expression of adhesion molecules in EC, resulting in inflammatory cell recruitment to injuried

tissue.[129-132] CyPA has been recognized as a proinflammatory cytokine which activate EC[22,56,118] and recruits inflammatory cells suggesting it is an important mediator of cardiovascular diseases associated with EC dysfunction and inflammation such as IR injury. Seizer et al.[133] showed that CyPA and CD147 expression was increased in the heart tissues of AMI patients as well as in the left anterior descending artery ligation induced I/R mice model. Neutrophil and monocyte infiltration into cardiac tissues were significantly inhibited in CyPA-/- mice compared to the control group. Moreover monocyte migration induced by cardiac-derived CyPA and exogenous CyPA was inhibited by anti-CD147 pretreatment suggesting extracelluar CyPA was important for inflammatory cell recruitments in I/R injury. However the role of CyPA in EC dysfunction in I/R injury remains unclear and needs to be further elucidated.

Conclusion

This review has described numerous *in vivo* and *in vitro* studies that have revealed that CyPA is an important mediator of cardiovascular diseases. Importantly secreted CyPA is a proinflammatory cytokine which activates cardiovascular cells involved in different aspects of the disease process. Therefore inhibition of CyPA secretion and/or its binding to target receptor will be a promising therapy for prevention and treatment in cardiovascular diseases. Oxidative stress and inflammation are pivotal to cardiovascular dysfunction and CyPA is a key molecule in their formation. The better understanding of ROS dependent CyPA function (e.g., posttranslational modification of CyPA) as well as CyPA regulated ROS production will hopefully provide an increased number of specific therapeutic targets for controlling cardiovascular pathology in the future.

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