Cyclophilin A (CyPA) Is More Strongly Associated With Oxidative Stress and Inflammation Than C-Reactive Protein (CRP)

Atherosclerosis is a response by the vessel wall to chronic multifactorial injury and it leads to the formation of atheromatous or fibrous plaques. Endothelial dysfunction represents an initial stage of atherosclerosis, with the other stages including smooth muscle dysfunction, metabolic abnormalities of the vessel wall, inflammation, oxidative stress and alterations in the neurohumoral balance. A new clinical entity “vascular failure”, has been proposed to integrate these vascular abnormalities. An understanding of both the pathophysiology of atherosclerosis and the molecular events implicated in the progression of subclinical disease to overt disease has enabled the development of biomarkers of cardiovascular disease. Recently, a number of reports have shown that selected markers can be detected in the peripheral circulation during different phases of disease progression.

CyPA, an emerging biomarker of cardiovascular disease, is likely to play a crucial role in all stages of atherosclerosis. In this issue of the Journal, Satoh et al reported that CyPA has the potential to be a valuable biomarker of coronary artery disease (CAD) in humans. They found that plasma levels of CyPA correlated with the anatomical severity of stable CAD, and were independent of CRP levels. This finding is of particular significance in the clinical setting, where CRP is generally used as a marker of cardiovascular events. They also demonstrated robust CyPA expression just beneath the thin fibrous cap of atherosclerotic plaques in patients suffering from acute myocardial infarction, supporting their hypothesis that secreted CyPA accumulates in atherosclerotic plaques within coronary arteries.

CyPA is a 20-kD chaperone protein that was identified in 1984 as the main target for the immunosuppressive drug cyclosporine A. It is secreted from vascular smooth muscle cells (VSMCs) in response to reactive oxygen species (ROS), such as superoxide, and has been shown to stimulate VSMC proliferation and inflammatory cell migration in vitro and in vivo. Secretion of CyPA occurs via a highly regulated pathway that involves vesicle transport and plasma membrane binding, and is suppressed by inhibition of Rho-kinase. Once transported to the plasma membrane, CyPA colocalizes with vesicle-associated membrane proteins in response to ROS stimulation. Extracellular CyPA stimulates proinflammatory signals in endothelial cells (ECs), including expression of E-selectin and VCAM-1. In VSMCs, extracellular CyPA stimulates ERK1/2, Akt and JAK, and contributes to further ROS production. In addition to its effects on vascular cells, CyPA has been shown to be a direct chemoattractant for inflammatory cells and to promote matrix metalloproteinase (MMP) activation. Extracellular CyPA induces interleukin (IL)-6 secretion from inflammatory cells and, in monocyte/macrophage cell lines, induces the expression of cytokines/chemokines such as tumor-necrosis factor α, monocyte chemotactic protein-1, IL-8, IL-1β and MMP-9 through a nuclear factor-κB-dependent pathway. Because of these findings, it has been postulated that CyPA is associated with the initiation, progression, and destabilization of atherosclerotic plaques.

Why is CyPA expression, rather than CRP expression, more closely correlated with CAD severity? Perhaps because CyPA is a marker of both oxidative stress and inflammation within the vessel wall, whereas CRP is merely a marker of inflammation. It has been reported that plasma CRP levels do not correlate with plaque volume or plaque composition as measured by virtual histology intravascular ultrasound. CyPA is secreted in response to oxidative stress, and stimulates ERK1/2 activity and DNA synthesis in VSMCs, while also promoting ROS production. This vicious cycle of both response to, and amplification of, oxidative stress may augment the progression of atherosclerosis, thereby explaining the high correlation between CyPA level and CAD severity.

Clinical Perspectives and Residual Issues

Patients with chest pain are often encountered in the clinical setting. Upon presentation, most of the patients with a history of chest pain are asymptomatic, do not demonstrate ST-segment or T-wave changes on ECG, and have undetectable plasma levels of cardiac troponin T. Under these circumstances, it can be difficult to determine whether the patient’s symptoms are cardiac (angina) or non-cardiac in origin. It is thus helpful to consider whether such patients are at high risk for atherosclerosis. At present, however, there are no biomarkers for CAD detection, and the diagnosis of CAD cannot be solely based on results of imaging studies such as coronary angiography or coronary CT angiography. Because of these restrictions, plasma levels of CyPA could significantly help identify patients with CAD. In addition, CyPA may be a use-
ful marker for evaluating the therapeutic effects of cardiovascular medications; Satoh et al demonstrated that medical treatments intended to control atherosclerotic risk factors also decreased plasma CyPA levels in patients with stable CAD. 3

Several issues remain regarding the role of CyPA as a biomarker of CAD. First, several basic science studies have demonstrated an association between CyPA and ROS, but studies showing a direct relationship between CyPA and ROS in the clinical setting are lacking. Second, CyPA may be used with other biomarkers to predict and treat acute coronary syndrome (ACS) before myocardial damage occurs. Unstable coronary plaque formation results from chronic inflammation of vessel walls, and is associated with decreased medial VSMCs and progressive destruction of structural components, particularly the elastic lamina. 8 Key mechanisms include VSMC senescence, oxidative stress, increased local production of proinflammatory cytokines and increased activity of MMPs, which degrade the extracellular matrix. CyPA (both intracellular and extracellular) contributes to atherosclerosis by promoting EC apoptosis, increasing EC expression of leukocyte adhesion molecules, stimulating inflammatory cell migration, enhancing ROS production, increasing the proliferation of macrophages and VSMCs, and increasing proinflammatory signal transduction in VSMCs. 9 CyPA is highly expressed at sites of unstable atherosclerotic plaque, especially those associated with macrophages and foam cells. Satoh et al demonstrated robust CyPA expression just beneath the thin fibrous cap of atherosclerotic plaques in a patient suffering from acute myocardial infarction, and suggested that plasma CyPA levels might be a predictor of plaque vulnerability and/or rupture. 9 Therefore, plasma CyPA levels should be extensively assessed in patients with ACS. Because MMPs play a key role in the mechanism of plaque vulnerability and/or rupture, plasma levels of MMPs have been measured as a marker for ACS. We previously observed that, in plasma derived from coronary sinus blood, levels of MMP-1 and MMP-3 were higher in patients with ACS relative to patients with chronic CAD or control subjects. Both the MMP-1 and MMP-3 level correlated with the level of high-sensitivity CRP in ACS patients. 10 Kai et al demonstrated that peripheral blood levels of MMP-2 and MMP-9 were elevated in ACS patients relative to patients with chronic CAD or control subjects. 11 Soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) is another promising biomarker of plaque vulnerability and/or rupture. 12 LOX-1 is involved in EC dysfunction, leukocyte adhesion, VSMC proliferation, migration and apoptosis, foam cell formation and platelet activation. Administration of anti-LOX-1 antibodies decreases these cellular events, thereby inhibiting atherosclerosis. Hence, LOX-1 is an attractive therapeutic target for human atherosclerotic diseases, including ACS. 13 It is intriguing to consider the future use of a multibiomarker strategy using CyPA, MMPs and LOX-1 to detect plaque vulnerability prior to the occurrence of myocardial necrosis in patients with ACS. Finally, CyPA may be a novel therapeutic target for ACS prevention.

ROS induce CyPA secretion in a Rho-kinase-dependent manner. It has been suggested that suppression of CyPA signaling by inhibition of its extracellular receptors, or suppression of CyPA secretion by Rho-kinase inhibition, may reduce ROS-induced progression of atherosclerosis. 14 However, the extracellular receptors for CyPA remain unknown. Several molecules have been proposed, including extracellular MMP inducer (EMMPRIN). Thus, blocking Rho-kinase or EMMPRIN has the potential to inhibit CyPA autocrine/paracrine signaling pathways, thereby blocking the vicious cycle that augments ROS production. These molecules represent novel therapeutic targets for treating cardiovascular diseases.

References