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Using Plasma Matrix Metalloproteinase-9 and Monocyte Chemoattractant Protein-1 to Predict Future Cardiovascular Events in Subjects with Carotid Atherosclerosis

Yonggang Ma[1], **Andriy Yabluchanskiy**[1], **Michael E. Hall**[1,2], and **Merry L. Lindsey**[1,2,3]

[1]San Antonio Cardiovascular Proteomics Center and Jackson Center for Heart Research, Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA

[2]Cardiology Division, University of Mississippi Medical Center, Jackson, MS, USA

[3]Research and Medicine Services, G.V. (Sonny) Montgomery Veterans Affairs Medical Center, Jackson, MS, USA

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Atherosclerosis is characterized by the gradual deposition of fat and cholesterol in the arterial wall, which over time can progress to yield inflammation and extracellular matrix-rich lesions that occlude vessels and restrict blood flow. During the formation of atherosclerotic lesions, monocytes are recruited through a mechanism that involves upregulation of the monocyte chemoattractant protein (MCP)-1/chemokine receptor CCR2 signaling pathway [1]. In addition to uptake of lipids, macrophages secrete high concentrations of inflammatory mediators and proteinases such as matrix metalloproteinases (MMPs), which contribute to atherosclerotic plaque progression [2]. Collagen, a key component in the fibrous cap of atherosclerotic plaque, helps sustain the plaque tension and stability. MMPs degrade collagen and lead to increased plaque instability [3]. As such, inflammation and MMPs are implicated in all phases of atherosclerosis, from disease initiation through progression and eventually to the onset of symptoms.

In this issue of *Atherosclerosis*, Tan and colleagues investigated the connection between MMP-9 and MCP-1 concentrations with the severity of carotid atherosclerosis in patients [4]. They measured serum concentrations of MMP-9 and MCP-1, and evaluated plaque score, plaque stability (assessed by surface characteristics, echogenicity, and texture of atherosclerotic plaque), and intima-media thickness (IMT). Using multinomial logistic regression model, the authors showed that MMP-9 was highly associated with plaque score

Address for Correspondence: Merry L. Lindsey, Ph.D., Department of Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216-4505, USA. (phone) 601-815-1329, (fax) 601-984-1817, mllindsey@umc.edu.

Conflict of interest

All authors declare that there is no conflict of interest associated with this manuscript.

and instability in a concentration-dependent manner; while MCP-1 correlated with IMT but not plaque score or stability. The authors concluded that MMP-9 and MCP-1 may be useful biomarkers to distinguish stable and unstable plaques and predict future cardiovascular events.

MMP-9 proteolytically processes all major extracellular matrix (ECM) components of the atherosclerotic plaque, including collagen, elastin, and proteoglycans, and as such is a critical modulator of plaque stability [5, 6]. Increased MMP-9 was shown in advanced atherosclerotic lesions in the *Ldl4*$^{-/-}$*Apob*$^{100/100}$ mouse model [7, 8]. Gough and colleagues revealed that macrophage expression of active, but not pro-, MMP-9 induced plaque disruption [9]. MMP-9 is overexpressed in progressive atherosclerotic plaques obtained from humans undergoing endarterectomy and is especially prevalent in the cap regions where macrophages accumulate, implicating macrophages and MMP-9 in plaque rupture [10]. The current study provides translational support by demonstrating an increased risk of severe atherosclerosis and unstable plaques in patients with higher MMP-9 levels.

In addition to ECM substrates, MMP-9 also proteolytically cleaves a wide array of inflammatory mediators and growth factors, which implicate MMP-9 in plaque growth. Mice expressing human MMP-9 at pathological levels in macrophages show increased collagen deposition in atherosclerotic lesions, which occurs through increased MMP-9-mediated activation of transforming growth factor-β, suggesting pro-fibrotic and protective roles of MMP-9 in atherosclerosis [5]. Targeted disruption of the MMP-9 gene impairs smooth muscle cell migration and limits arterial remodeling, suggesting a growth role of MMP-9 in atherosclerosis [11]. Our lab and others have revealed dual roles of MMP-9 in left ventricular remodeling following myocardial infarction. Both MMP-9 deletion and macrophage MMP-9 overexpression attenuate cardiac remodeling in a mouse model of myocardial infarction [12, 13]. MMP-9 roles depend on the cellular source, time course, and surrounding microenvironment. Fig. 1 summarizes how MMP-9 modulates atherosclerosis by mediating both plaque growth and instability through ECM regulation.

MCP-1, a member of the CC chemokine family, is a potent monocyte attractant upregulated by oxidized lipids [14]. MCP-1 deletion prevents macrophage recruitment and the development of atherosclerotic lesions in *Apob* overexpressing mice, suggesting an early pro-atherosclerotic role for MCP-1 [15]. The lower expression of MCP-1 in carotid artery may account for its relative resistance to atherosclerosis compared to the more atherosclerosis-prone aorta [16]. In patients, plasma MCP-1 levels significantly correlated with peripheral artery disease, independent of traditional risk factors for coronary artery disease [17].

IMT, a measure of the arterial intima and media thickness, is used to monitor the extent of atherosclerosis in humans and experimental animal models. The MCP-1 gene A2518G polymorphism correlates with IMT in patients with type 2 diabetes, linking MCP-1 with increased smooth muscle proliferation [18]. Tan and colleagues determined that plasma MCP-1 levels were significantly associated with IMT, suggesting that mechanisms in addition to its regulation of macrophage recruitment may be important (Fig. 1).

The findings of this study are encouraging, but several aspects need to be taken into account when interpreting and translating these results. First, large-scale prospective trials are warranted to confirm the findings above. Whether MMP-9 and MCP-1 also predict future cardiovascular events (e.g. myocardial infarction or stroke) has not been unraveled.

Second, the molecular mechanisms whereby MMP-9 mediates atherosclerotic progression to instability and rupture are not totally understood. Emerging evidence has shown that ECM or non-ECM MMP-9 substrates modulate tissue remodeling by regulating inflammatory and fibrotic responses [19, 20]. Hence, a better understanding of the biological activity of MMP-9 proteolysis may provide new intervention opportunities to slow, delay, or even reverse the development and rupture of an unstable atherosclerotic lesion. A proteomics approach that targets inflammation and ECM would help identify the missing pieces of the puzzle by providing a more thorough and high throughput identification of novel ECM and non-ECM substrates [21, 22].

Third, atherosclerosis is pathologically complicated and no single biomarker will be the perfect indicator. It will likely be necessary to utilize several biomarkers (e.g. C-reactive protein, MMP-9, MCP-1, and uric acid) in combination to diagnose and monitor atherosclerosis progression or treatment efficacy. In order for the optimal biomarkers to be defined, a computational approach will likely be needed to refine the list of most informative indicators [23, 24].

In summary, inflammation plays a key role in the initiation and progression of atherosclerosis, and Tan and colleagues have identified MCP-1 and MMP-9 as key mediators. Identification and stabilization of vulnerable plaques is highly important for clinicians, as these plaques are the ones that cause acute syndromes (e.g. myocardial infarction and stroke). Therefore, a strategy evaluating these biomarkers (specifically MMP-9) seems appealing for helping identify which patients may benefit from more aggressive medical therapies (e.g. statins, antiplatelet agents, etc) to prevent these acute unstable plaque ruptures and subsequent complications.

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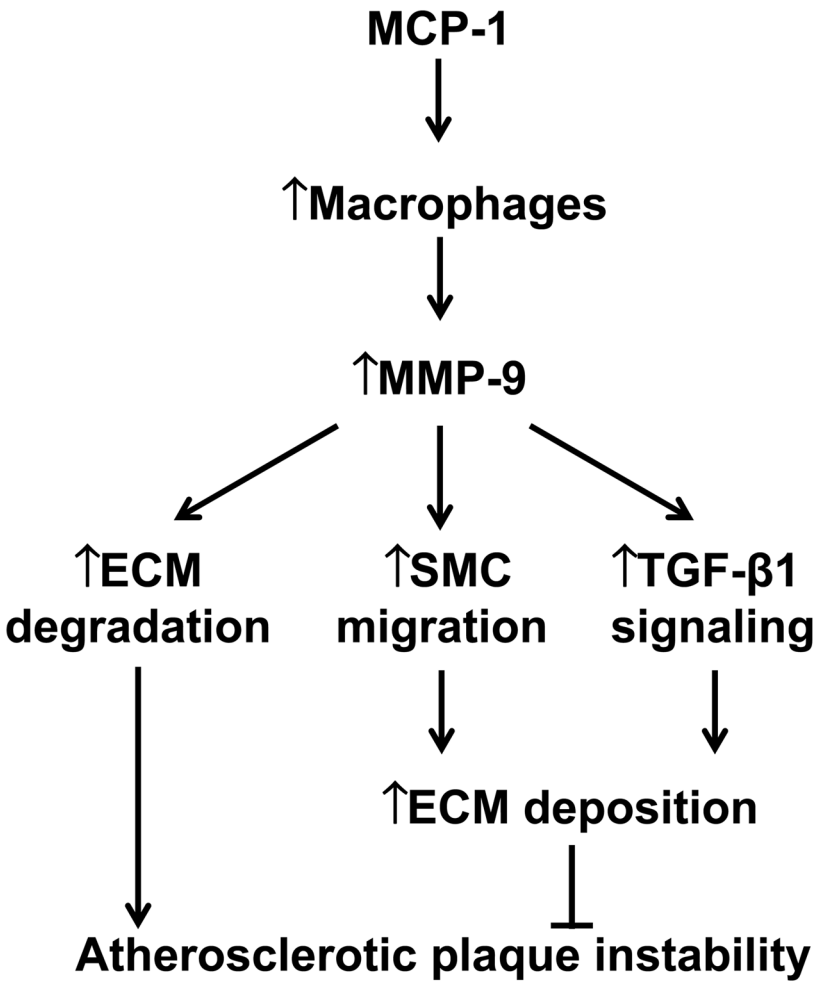

Fig. 1.

A diagram of the mechanisms by which MCP-1 and MMP-9 regulate the development and progression of atherosclerosis. MCP-1 recruits monocytes to the site of atherosclerotic lesion, where they mature into macrophages. MMP-9, derived mainly from macrophages, exerts dual roles in regulating atherosclerosis. On the one hand, MMP-9 cleaves ECM substrates (especially collagen) in the fibrous cap to increase plaque vulnerability. On the other hand, MMP-9 stimulates smooth muscle cell (SMC) migration and transforming growth factor (TGF)-β1 signaling to facilitate ECM deposition. To what extent both effects occur *in vivo* remain to be elucidated.