Review

Extracellular Matrix and Matrix Metalloproteinases in Atherosclerosis

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Abstract: This review focuses on recent works of matrix metalloproteinases (MMPs) and extracellular matrix macromolecules (ECMs) in relation to atherosclerosis. Many kinds of ECMs are mainly produced by smooth muscle cells (SMC) in both normal arterial wall and atherosclerotic lesions. In particular, type I, III, IV, V, VIII and elastin are high level expressed in atherosclerotic lesions. MMPs mainly produced by SMC and macrophages are MMP-I, 2, 3, 7, 9 and 12 in arterial wall. MMP-1, 2 and 3 highly expressed in the media of fetuses and neonates but during the aging, the MMP-1 and 3 expression is decreased. Platelet-derived growth factors can stimulate SMC to produce MMP-1, 3 and 12 and collagen type I. MMP9 and 3 expression is related to aneurysm formation in aortas. Elevation of the blood level of MMP-2 and 9 is detected in acute coronary syndrome due to the plaque rupture but no direct causal relationship between MMP activity and plaque rupture as yet to be demonstrated. Since the multi-potential roles in many pathological, events in arterial wall including atherosclerosis, aneurysm and plaque instability, MMPs would become a potential therapeutic target. In addition to regulation of the MMP expression in arterial wall, recent genetic work should be focused to a better understanding of the pathological events in arterial wall, their protection and the prognosis.

Key words: matrix metalloproteinases, atherosclerosis, extracellular matrix, polymorphism, lipid peroxides

INTRODUCTION

Since we had described the matrix metalloproteinase I (MMP-I) production by human endothelial and aortic smooth muscle cells (SMC) 2, a vast of knowledge on roles of MMPs in relation to atherosclerosis have been accumulated. At present over 20 enzymes are members of the MMP family, which cleaves various extracellular matrix in many organs 3. Recently, many studies suggest that MMPs also relate to immune response 4, cell mobility and growth via digestion of matrices and non-matrix substrates 5. On the other hand, the pathogenesis of atherosclerosis includes many aspects such as matrix metabolism, inflammation, and immune response exerted by vascular constituents 6-7. This review focuses on recent works of MMPs and extracellular matrix in relation to atherogenesis, and includes four sections: 1) Extracellular matrix macromolecules (ECMs) and 2) MMPs in developing and atherosclerotic artery. 3) Plaque instability and MMPs, 4) MMPs as a potential therapeutic target, and 5) Polymorphism of the MMP gene and arterial disease.

Especially we would like to describe about the function of arterial SMC, which secrete both matrices and MMPs in atherosclerotic lesion.

1 Extracellular Matrixes in Developing and Atherosclerotic Artery

It is well known that the synthesis and degradation of ECMs are essential for many physiological conditions, such as reproduction 8-9 and morphogenesis 10,11. The ECM is a major component of normal artery 12,13 and its turnover is critical for arterial development 14,15. Mouse with mutated collagen type III (Col3a1/-/-) gene developed a rupture of blood vessels and elastin knockout (ELN/-/-) mouse caused obstructive arterial disease by subendothelial SMC proliferation. These two studies from knockout mouse models indicate that the vascular wall matrixes have only a structural role but also a functional role on cardiovascular development.

The ECMs, mainly produced by SMCs in the vascular wall, include major collagens types I and III, and minor types IV, V, VI, and VIII 16,17. In the aortic media, type I, III and fibronectin localize in the interstitial space 17. In atherosclerotic lesion, both types of collagen increase and type I is more than type III 18,19. The type I and type III collagen synthesis localizes in the intima 19,20 and fibrous caps and shoulder regions of the plaques are rich in type I procollagen-synthesizing cells 21. Type IV, V collagens and laminin are present at the subendothelial region 17. Type V collagen is not detected in early fatty streaks or mild intimal thickening of children, but...
increases with advancing age and lesion progression, while type IV collagen deposition is seen in the fibrous cap. Type VIII collagen is found to be present more in neonatal aorta than adult, and is expressed at high levels in atherosclerotic lesions. The pathogenesis of atherosclerosis includes abnormal production of ECMs mainly by synthetic phenotype of SMC. In fact, decreased SMC/ECM ratio is reported in advanced atherosclerotic lesion in cases of progeria and hyperhomocystinemia.

Elastin is the main component of the media and musculoelastic layer of the intima. The elastin production by SMC increase in advanced atherosclerotic lesions, but integration of the protein into a functional elastic fiber may be impaired and the fibers are often split or frayed in the lesions.

2 MMPs in Developing and Atherosclerotic Artery

Our previous report described development-related changes of MMP expression profiles in human aorta by immunohistochemistry. In fetuses and neonates, the medial SMCs highly express of MMP-1, 2, and 3. During the aging, only the MMP-2 is constitutively expressed and the MMP-1 and 3 expression is decreased (Fig. 1), while the SMCs in the thickened intima produce all three types of MMPs (Fig. 2). This change of MMP profile in the intima well coincides with the age-related appearance of collagen types V as well as I and III in the intima.

Many reports indicate an increased expression of MMPs (MMP-1, 2, 3, 9, and 12 or more) (Table 1) in the atherosclerotic lesion. Both SMC and macrophages are responsible to the MMPs production in the atherosclerotic lesion. The balance between the MMP activity and matrix synthesis affects the net matrix turnover and modulates a matrix environment of the arterial wall. Interestingly, platelet-derived growth factor-BB stimulates SMC to produce both MMP-1, 2, 3, and MMP-12 (Fig. 3) and collagen type I, which is the major matricial component in the wall and enzymatic substrate for MMP-1.

Active MMP-2 is found in the actively remodeling areas of atherosclerotic plaques, although both normal and atherosclerotic arteries include SMC expressing proMMP-2. Endogenous co-expression TIMP in the media of the uninvolved arteries may regulate the matrix degrading potential. In contrast to normal arteries, MMP-1, MMP-3 and MMP-9 are expressed in the SMC and macrophages in the fibrous areas around the lipid cores. On the other hand, MMP-7 and MMP-12, which have a broad spectrum of substrate specificity including proteoglycan, insoluble elastin, and fibronectin, are prominently expressed in the lipid-laden macrophages at the border of the fibrous cap and the cellular core.
Table 1. The family of matrix metalloproteinases.

<table>
<thead>
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<th>Group name</th>
<th>MMP number</th>
<th>Mr latent/active</th>
<th>Substrates</th>
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<td>Collagenase collagenase 1</td>
<td>MMP-1</td>
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<td>MMP-8</td>
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<td>MMP-18</td>
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<td>42,000</td>
</tr>
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<td>Gelatinase Gelatinase A</td>
<td>MMP-2</td>
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<td>Gelatinase B</td>
<td>MMP-9</td>
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<td>84,000</td>
</tr>
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<td>Stromelysin Stromelysin 1</td>
<td>MMP-3</td>
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<td>Stromelysin 2</td>
<td>MMP-10</td>
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<td>MT2-MMP</td>
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<td>MT3-MMP</td>
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Abbreviations: PLP, proteoglycan link protein; a2M, a2 macroglobulin; a1Pl, a1proteinase inhibitor.

Elastin is a critical matrix of the aortic media to maintain the wall structure by its elastic nature. Therefore, elastolytic activity and pathogenesis of aortic aneurysms, which are characterized by disruption and degradation of the elastic media, were first discussed with regards to MMP-9 expression in the aneurysms[42]. In addition to MMP-9, three more members of MMP family are known to degrade insoluble elastic fibers: MMP-2, MMP-7, and MMP-12.[43,44]. In the hypocellular and amorphous media with absence of elastic lamellae observed in abdominal aortic aneurysms contain macrophages positive for MMP-2, MMP-9 and MMP-12. In the sites of ongoing elastolysis in the amorphous media, MMP-12 localizes to the residual elastin fiber fragments and macrophages, which are lack of MMP-2 and MMP-9 expression. The elastolytic MMPs, especially MMP-12, may contribute to matrix degradation in aortic aneurysms[45]. However, there is a conflicting report that MMP-7 and MMP-12 expression are not detected in the aortic aneurysm, but MMP-9[46]. Recent report indicates that only MMP-3 is highly expressed in aneurysm than in aortic occlusive disease and that a possible involvement of MMP-3 expression in the pathogenesis of the aortic aneurysm[47]. Supporting this observation, a study using knockout mouse lacking MMP-3 and TIMP-1 genes shows a reduced and enhanced aneurysm formation by inactivated MMP-3 and TIMP-1 genes expression, respectively[48,49].

3 Plaque instability and Matrix

Rupture of the atherosclerotic plaques causes myocardial infarction due to thrombi from the unstable plaques[50]. The stability is depends on the structural integrity of the fibrous cap, which covers the fragile lipid-rich cores. The fibrous cap consists of the extracellular matrix rich in collagen, especially rich in type I collagen[51]. So the content of fibrillar interstitial collagen is one of the determinants of plaque stability[52]. The thinning of the fibrous cap of plaques is a characteristic morphology of the vulnerable, rupture-prone plaque[53]. Thus, expression of various matrix-degrading MMPs in the plaque is described in relation to its
Extracellular Matrix and Matrix Metalloproteinases in Atherosclerosis

Elevation of the blood levels of MMP-2 and MMP-9 is also reported in acute coronary syndrome due to the plaque rupture\(^{31}\). MMP-1, MMP-8, and MMP-13 are known to degrade intact triple-helical collagens\(^{53,54}\), by which the fragmented collagens become susceptible to further digestion by other MMPs such as MMP-2, MMP-3, and MMP-9 expressed in the plaques. However, MMP-2 and MMP-9, which are classified into the gelatinase group, also digest intact fibrillar collagens\(^{55}\). Both collagenases and gelatinases would participate in degradation of the plaque’s interstitial collagens that may control plaque stability, although no direct causal relationship between MMP activity and plaque rupture has yet to be demonstrated\(^{50}\).

In the context of the atherosclerosis, SMC are one of the major sources of MMPs in the lesion environment. However, macrophages can also be the source of MMPs\(^{56}\). The vulnerable plaques have common histological features: thin fibrous cap, prominent collection of extracellular lipids, and abundant macrophages\(^{57-59}\). Indeed, increased expression of MMPs in the macrophages in the shoulder region of the vulnerable plaques is reported\(^{31,32,50,51,53,54}\). It is suggested that there is a direct link between MMPs expressed in the macrophages and fibrous cap weakening\(^{60}\).

In our previous reports\(^{61,62}\), we reported that increase in lipid peroxide provoke injury to endothelial and smooth muscle cells and macrophages, which injury is considered to initiate and progress atherosclerosis and that lipid peroxide stimulates SMC or endothelial cells to induce MMP-1 and -3 or MMP-1 alone (Fig. 4). Since the amount of lipid peroxides should increase in accumulated lipid in the atheroma and in blood stream during progression of atherosclerosis, we postulate that the MMPs induction by lipid peroxide participate in the process of plaque rupture.

Another factor that controls plaque stability is a content of matrix in the fibrous cap, since collagen degradation and synthesis can take place in the same plaque\(^{19}\). Net collagen loss and thinning of fibrous cap may occur when collagen production is impaired in the lesion. Apoptotic cell depletion of SMC may contribute to the collagen loss\(^{63}\), because SMC are main source of collagen production the atherosclerotic lesion. In fact, the shoulder region of the vulnerable plaque contains less.

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Fig. 3. MMP-12 protein expression in SMC.
Western blot analysis (A) and casein zymography analysis (B) reveal production of MMP-12 in condition medium form U937-derived macrophages and SMC stimulated by PDGF. MS: human aortic medial SMC, IS: human aortic intimal SMC, ISS10: immortalized human aortic intimal SMC.

Fig. 4. MMP-1 and 3 production in SMC injured by lipid peroxide.
Western blot analysis shows production of MMP-1 (A) and MMP-3 (C) but not MMP-2 in condition medium from SMC treated with 0, 0.5, 1, 2.0 nmol/ml of linoleic acid hydroperoxide (1, 2, 3, and 4, respectively).
SMC and much macrophages than fibrous cap region\textsuperscript{57-59}. A rabbit balloon injury model for atherosclerotic plaque rupture well represents the relation between regional cellular composition and matrix content\textsuperscript{64}. In this rabbit model, hypercholesterolemia induces plaque rupture at the shoulder region where includes much macrophages less SMC and collagens. The expression of MMPs in arterial wall is summarized in Fig. 5.

### 4 MMPs as a Therapeutic Target

Because of the multi-functional roles of MMPs including cell proliferation, cell growth, cell death, cell migration, and cell-cell communication\textsuperscript{50}, which are all involved in the process of atherogenesis, MMPs would become a potential therapeutic target. Recently some synthetic inhibitors are used to reduce aneurysmal growth and associated MMP-9 expression in rat model\textsuperscript{65,66}. Doxycycline is also reported to have an inhibitory effect on MMP-9 expression and aneurysmal growth\textsuperscript{67}. Furthermore, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) is known to inhibit MMP-9 secretion from macrophages\textsuperscript{68,69}.

Another approach to modulate the MMP activity in the atherosclerotic lesions is to deliver tissue inhibitor of MMPs\textsuperscript{30} by gene transfer. Using replication-defective recombinant adenovirus to deliver and overexpress TIMP-1, 2, and 3 in human saphenous vein in organ culture system succeeded to inhibit the neointimal formation and gelatinolytic activity\textsuperscript{70-72}.

### 5 Polymorphism of MMP Gene and Arterial Disease

Sequence variation in the promoter region may result in different levels of transcriptional activity and expression of the MMPs, which would control the susceptibility of atherosclerotic arterial disease. Recent accumulating genetic works in relation to clinical observation suggest some genotypes of MMPs closely related to the atherosclerosis, aneurysm and its complications\textsuperscript{73}.

Our previous work revealed that human MMP-9 promoter region contains a variation of length of d(CA) from 14 to 23 repeats at -90 relative to the start of transcription\textsuperscript{74}. This length variation of the d(CA)-repeat, which should be bound with a protein(s), is functional related and the longer d(CA) exhibited the higher transcriptional activity. We also reported that most Japanese have two alleles with 20, 21 or 22 d(CA) repeats (79%, \(n=223\)) (Fig. 6). However, studies from Pittsburgh and French groups showed the allele frequency of d(CA)14 counted more than 50% of populations\textsuperscript{75,76}. Racial factor may exist in the difference of the length variation of the d(CA) repeat of MMP-9 gene. One report indicates that the longer length of the d(CA) repeat relates to an occurrence of intracranial aneurysm\textsuperscript{75}, while another study indicates no relation to coronary aneurysm\textsuperscript{76}. On the other hand, C to T transi-
Extracellular Matrix and Matrix Metalloproteinases in Atherosclerosis

A

\[
\begin{array}{cccccccc}
-600 & -591 & -563 & -558 & -553 & -527 & -474 & -463 \\
-131 &       &       &       &       &       &       &       \\
\end{array}
\]

\[\text{d(CA)21 repeat}\]

B

Numbers of d(CA) repeats in 223 Japanese subjects

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<td>11.8</td>
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Fig. 6. Sequence of promoter region in the MMP-1 and polymorphism in Japanese.

Schematic presentation of cis-acting elements and d(CA) 21 repeat sequence in the promoter region of the MMP-9 gene (A) and numbers of d(CA) repeats in 223 Japanese subjects.

Concerning MMP-12, a common A/G polymorphism within the promoter region at −82 relative to the start of transcription is identified. The A allele has a higher promoter activity of MMP-12 by a higher binding affinity of AP-1, which locates one base downstream of the A/G site and is critical for basal and inducible promoter activity of MMP-12. The A allele is associated with a smaller luminal diameter of the coronary artery in diabetic patients, but no association is detected in the cases of coronary aneurysm. Similar polymorphism involving binding affinity of transcriptional factors is reported in the promoter region of MMP-7.

Genetic analyses of MMP-3 gene are well studied by many groups. There is a common polymorphism in the promoter of the human MMP-3 gene located 1171 bp upstream from the start of transcription in which one allele has a run of 6A and another has 5A. The promoter containing the 6A is found to be less active than that containing the 5A, and an oligonucleotide probe corresponding to 6A allele has higher binding affinity to nuclear proteins than that corresponding to 5A.

Fig. 7. Electrophoretic mobility shift assay using synthetic d(CA) repeat probes.

The numbers represent probes of each d(CA) repeat length [14: d(CA) 14; 18 : d(CA) 18; 20 : d(CA) 20; 21 : d(CA) 21; 22 : d(CA) 22]. A: Each probe was incubated with increased amounts of the nuclear extracts (0, 2, 5, 10 and 20 mg/lane from left to right, shown as triangles. B: In competition studies. Homologous and heterologous cold competitors (Comp.) were mixed in the reaction in 100-fold excess.
Binding and increases the promoter activity. MMP-3 expression phenotype is related to rapid progression of coronary stenoses, increased carotid artery intima-media thickness, and increased risk for restenosis after balloon angioplasty. On the other hand, the 5A allele is associated with occurrence of aneurysms in the aorta, intracranial and coronary arteries, and coronary artery calcification. Furthermore, the 5A genotype is frequent in the patients with acute myocardial infarction than in control population. The 5A/6A polymorphism in the MMP-3 promoter region would be an important risk factor for arterial diseases.

References

Extracellular Matrix and Matrix Metalloproteinases in Atherosclerosis

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