# Role of Metalloproteinases in Plaque Rupture

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SUMMARY

Rupture of the fibrous cap over an atherosclerotic plaque is the main cause of myocardial infarctions and strokes. Plaques vulnerable to rupture have a relatively thin fibrous cap, are highly inflamed and contain less structural collagen. This suggests that increased production of proteases, including metalloproteinases (MMPs), in response to inflammation is responsible for weakening the plaque cap. If so, then MMPs or the inflammatory mediators that lead to their overexpression are attractive targets for plaque stabilizing therapy. On the other hand, remodeling of extracellular matrix and cell surface proteins promotes migration and proliferation of endothelial and smooth muscle cells which could promote vascular repair and therefore plaque stability. Greater understanding of the role of individual MMPs and the regulation of their production is therefore needed to refine therapeutic approaches. [International Journal of Gerontology 2007; 1(3): 103–111]

**Key Words:** atherosclerotic plaque, fibrous cap, metalloproteinases

## Vascular Biology of the Metalloproteinases (MMPs)

The MMPs are a family of at least 24 proteins with diverse substrate specificities that include extracellular matrix (ECM) and cell surface proteins[1]. While most MMPs are secreted, the six membrane-type MMPs (MT-MMPs) are integral membrane proteins with catalytic domains on the cell surface (Figure 1). MMP activity is increased by transcription and translation of MMP genes and by proform activation; it is kept in check by complex formation with four tissue inhibitors of MMPs (TIMPs) (Figure 1)[1].

Degradation of ECM components by MMPs could reduce plaque size and promote plaque instability[2]. For example, collagenases (MMP-1, MMP-2, MMP-8, MMP-13 and MMP-14) cleave fibrillar type I and III collagens, which provide most tensile strength (Figure 2). MMP-9 and MMP-12 degrade elastin, while stromelysins and matrilysins (e.g., MMP-3 and MMP-7) have a broad specificity that includes cleaved collagens and the core proteins of proteoglycans (Figure 2). Apart from removing excess ECM, MMPs also promote infiltration of immune-inflammatory cells[3] and the sprouting of endothelial cells that underlies angiogenesis[4]; both of these are associated with plaque growth and increased vulnerability to rupture[5]. On the other hand, some MMPs (particularly MMPs-2, MMP-9, MMP-12 and MMP-14) promote migration and proliferation of vascular smooth muscle cells, which although they promote plaque growth increase stability of the fibrous cap[6]. A key mechanism mediating increased migration is simply relieving the constraints caused by adhesion of cells to their basement membranes[7]. Other more subtle mechanisms promote proliferation. These include shedding cadherins, which causes loss of cell–cell contacts and translocation of the transcriptional regulator, β-catenin, to the cell nucleus (Figure 2). The result is altered transcription of key cell cycle genes including cyclin D1 and p21 cyclin dependent kinase inhibitor[8,9]. Remodeling of the ECM also permits binding to cell surface integrin receptors and activation of signaling from focal adhesions (Figure 2). This ultimately mediates downregulation of both p21 and p27 cyclin dependent kinase inhibitors[10,11]. Both pathways stimulate cell

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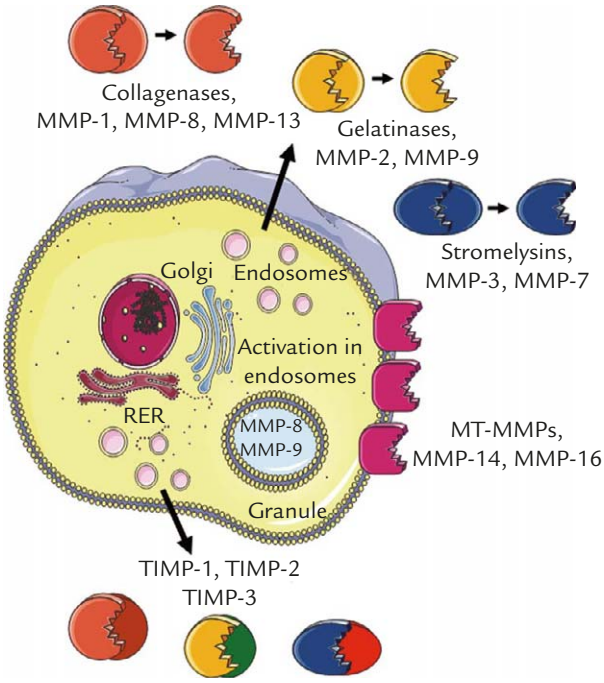


Figure 1. MMP production and activation. MMPs are synthesized as proforms in the rough endoplasmic reticulum (RER) and mostly secreted via the endosomal pathway. In leukocytes, MMP-8 and MMP-9 are sometimes stored in granules. Secreted MMPs are activated by removal of a propeptide in the extracellular compartment. Membrane-type MMPs (MT-MMPs) are unusual for two reasons: they are expressed on the cell surface and are activated by furins in the endosomes. Tissue inhibitors of MMPs (TIMPs) are secreted and inactivate MMPs by blocking their catalytic sites.

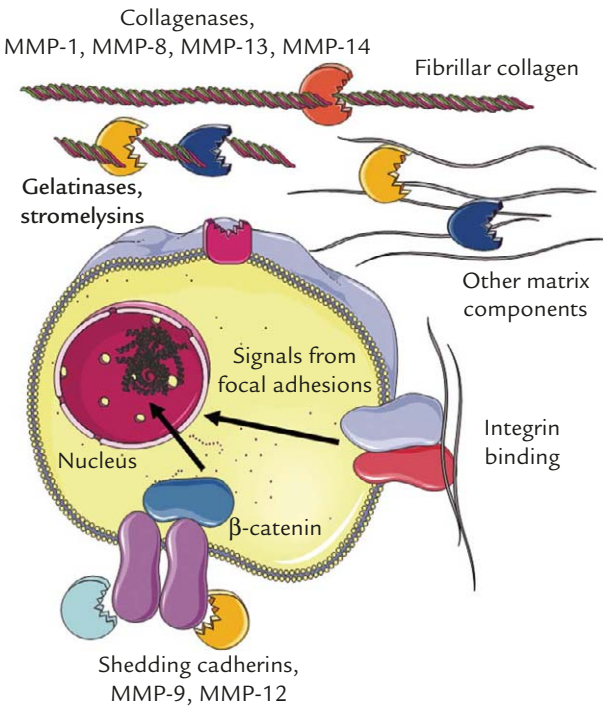


Figure 2. Function of MMPs. As shown, only a few MMPs have the ability to make the initial ¼, ¾ cleavages of fibrillar collagens. However, other MMPs cooperate in the destruction of cleaved collagen and other matrix components. MMPs affect plaque cell migration by removing matrix barriers. Another important effect of matrix turnover is promoting interactions with integrins, which triggers signaling to the nucleus from focal adhesions. MMPs also remodel cell surface proteins including cadherins. Shedding of cadherins frees β-catenin to translocate the nucleus and modulate gene expression. These signaling pathways control migration, proliferation and apoptosis of vascular cells.

cycle progression and smooth muscle cell proliferation. Hence individual MMPs could in theory either promote or impair plaque stability, and the predominant effect needs to be established by careful experimentation.

## Localization of MMPs in Human Atherosclerotic Plaques

The presence and localization of MMP proteins and activity have been used to establish "guilt by association" for their involvement in plaque vulnerability. Normal arteries express only pro-MMP-2, TIMP-1 and TIMP-2 and show no MMP activity by *in situ* zymography[12–14]. Increased levels of MMP-1, MMP-3 and MMP-9 are detected in rabbit[15,16] and mouse[17] atherosclerotic plaques and at the rupture-prone shoulder regions of human atherosclerotic plaques[12,18–20]. MMP-1 is localized at sites of high circumferential tensile stress[21]. MMPs are prominent in macrophage-derived foam cells, but are also found in lymphocytes, smooth muscle and endothelial cells. MMPs -8, -11, -14, and -16 are also overexpressed at rupture-prone regions of human plaques[22–28], while MMP-7 and -12 seem to localize more specifically to macrophages at the borders between the lipid core of human[22] and rabbit[29] plaques. *In situ* zymography detects MMP activity at the shoulder regions of human plaques[12–14], while 3D imaging of MMP activity towards a synthetic substrate confirms the association of MMP activity in highly inflamed plaques[30]. The collagenases MMP-1 and MMP-13 co-localize with markers for cleaved collagen at shoulder regions in plaque caps[27]. All of this data supports an association between high levels of MMP activity and matrix turnover in inflamed plaques, and at sites vulnerable to rupture. Furthermore, Sluijter et al. recently used a large biobank to show that levels of MMP-8 and MMP-9 are significantly increased in vulnerable compared to stable carotid plaques[31]. On the other hand, levels of

MMP-2 were increased in stable plaques[31]. Hence increased levels of some MMPs could be markers of vascular repair rather than net matrix destruction.

## Genetic Epidemiology

Many groups have investigated the association between polymorphisms that influence the production of MMPs and the incidence of cardiovascular disease[32]. For example the 5A/6A promoter polymorphism in the MMP-3 gene causes greater transcription of MMP-3. Several but not all studies suggest that increased transcription of MMP-3 is associated with less advanced coronary[33–35] and carotid[36] atherosclerosis but with greater incidence of MI[33,37,38] and strokes[39]. High levels of MMP-1 promoter activity also appear to worsen symptomatic coronary heart disease[40] and carotid artery stenosis[36] but favor plaque instability and precipitate MI[41]. Perhaps MMP-1 and MMP-3 decrease ECM accumulation and this leads to smaller but less stable plaques, although this remains to be shown directly. By contrast, greater MMP-9 promoter activity appears associated with increased disease severity[42,43], contrary to the results with MMP-1 and MMP-3.

## Pharmacologic Studies and Genetic Manipulation in Animals

Most studies used mice in which increased lesion size, abundance of macrophages and decreased content of collagen have been used as surrogate markers for plaque instability. Acute and healed plaque ruptures have also been quantified in mice[44]. However, the anatomical differences between mouse and human plaques[45] caution against over eager extrapolation.

TIMP-1 gene deletion in apolipoprotein (ApoE) knockout mice increased vessel wall MMP activity and elastin degradation in two studies[46,47]. One study reported a decrease[47], the other found no effect[46] on aortic plaque size. Overall, the studies suggest a modest deleterious effect of unchecked MMP activity on plaque stability. Consistent with this, systemic, adenovirus-mediated TIMP-1 overexpression decreased aortic sinus lesion size and macrophage content in one study[48]. Similar overexpression of TIMP-1 in a later study had no effect on brachiocephalic artery plaques, although TIMP-2 did decrease plaque size and markers of plaque rupture[49]. Moreover, delayed TIMP-2 administration arrested the development of established plaques[49]. Given these promising findings, it is disappointing that oral administration of broad-spectrum, synthetic MMP inhibitors that should mimic the action of TIMP-2 had no effect on lesion size or stability in hypercholesterolemic mice[49,50] or atherosclerotic primates[51]. Tetracyclines are a family of antibiotic drugs that also inhibit MMP expression and activity. However, doxycycline treatment of hyperlipidemic mice had no effect on the extent of atherosclerosis[52]. One explanation for these findings is that broad-spectrum MMP inhibitors prevent the beneficial effects of some MMPs as well as the harmful effects of others. Hence, the likely clinical benefits of MMP inhibition are hard to predict. The narrow therapeutic window of available MMP inhibitors is also a hindrance to clinical application[53].

The role of individual MMPs has been investigated by overexpression or deletion. Overall, the results show clear deleterious effects of MMPs on plaque instability but also some beneficial effects, consistent with the actions noted in the section above on "vascular biology".

### *Collagenases*

Expressing collagenase-resistant collagen-I in ApoE null mice produces more stable plaques owing, as expected, to collagen accumulation[54]. MMP-13 replaces MMP-1 as the main interstitial collagenase in mice. MMP-13 deletion also increases collagen accumulation but does not affect lesion size or inflammation[55]. These two studies suggest that collagenases mainly affect plaque stability through direct effects on collagen levels. However, overexpressing human MMP-1 in the macrophages of ApoE null mice unexpectedly produced smaller plaques with a stable phenotype[56].

### *Stromelysins*

MMP-3 deficiency leads to larger, more stable aortic atherosclerotic plaques in ApoE null mice[57] but larger less stable plaques in the brachiocephalic artery[58]. MMP-7 deletion had no effect on plaque growth or stability[58]. The role of stromelysins is therefore unclear.

### *Gelatinases*

A variety of studies have been conducted in ApoE null mice. MMP-2 knockout produced smaller lesions with fewer smooth muscle cells compared to macrophages[59], implying a role for MMP-2 in smooth muscle accumulation and fibrous cap formation. MMP-9 deficiency

in one study did not affect the size of early lesions in the descending aorta and aortic root but reduced the size of advanced plaques[60]. Fibrillar collagen was reduced, implying less stability, but macrophage content was decreased, implying greater stability[60]. In another study[58], MMP-9 knockout increased plaque size in the brachiocephalic artery with less collagen and more macrophages, implying less stability. The conflicting results can be explained by the ability of MMP-9 to facilitate migration of vascular smooth muscle cells and macrophages, which would have opposite effects on plaque stability. In other studies, local overexpression of pro-MMP-9 had no effect on the size of early or advanced carotid lesions but promoted intraplaque hemorrhage in advanced lesions[61]. Macrophage-specific overexpression of active MMP-9 also induced plaque disruption, without significantly affecting lesion size or macrophage content[62]. Hence, raising active MMP levels to unphysiologic levels clearly promotes plaque instability.

### *Metalloelastase*

MMP-12 deletion promotes smaller, more stable lesions in the brachiocephalic artery of ApoE null mice[58]. It reduces elastin degradation but does not alter the size or cellular composition of early or advanced aortic plaques[60]. Overexpression of active MMP-12 promotes inflammation and reduces collagen content of atherosclerotic plaques in rabbits fed an extremely cholesterol-rich diet[63]. All studies, therefore, show plaque destabilizing effects of MMP-12, although the details differ. MMP-12 may therefore be a favorable target for selective pharmacotherapy.

## What Switches MMPs On?

Given the limitations of MMP inhibitor strategies (see above), perhaps it would be more fruitful to identify and use drugs to target the causes of MMP upregulation. In this context, inflammation seems to be a key factor. The inflammatory mediators (e.g., interleukin [IL]-1 or tumor necrosis factor [TNF]-α) increase MMP-1, MMP-3 and MMP-9, but not TIMP-1 or TIMP-2 secretion from endothelial[64] and smooth muscle cells[65,66]; growth factors (e.g., platelet-derived growth factor or fibroblast growth factor-2) act synergistically with inflammatory mediators[65,67]. Ingestion of oxidized low-density lipoprotein or treatment with TNF-α or prostaglandin E2 upregulates several MMPs in macrophages. Indeed,

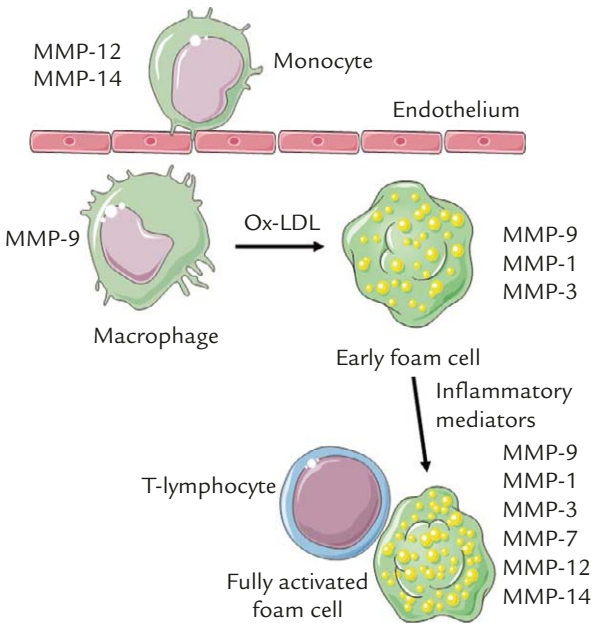


**Figure 3.** Upregulation of MMPs in plaque macrophages. Monocytes express low levels of MMP-12 and MMP-14, which are probably required for migration across the endothelium. Differentiation to macrophages upregulates MMP-9. Ingestion of oxidized low density lipoprotein (Ox-LDL) by foam cells upregulates MMP-1 and MMP-13, which are, therefore, found in early fatty streaks. Action of cytokines and contact with T-lymphocytes further increases the spectrum of MMPs expressed.

foam cell macrophages isolated from rabbits overexpress MMP-1, MMP-3 and MMP-12 compared to non-foamy macrophages[29,68,69]. CD40L, a component of activated T-lymphocyte membranes, induces MMP expression in endothelial, smooth muscle and macrophages[26,70–75]. Hence, foam cell formation and inflammation progressively increases the spectrum of MMPs that would be upregulated in plaques (Figure 3). The combined effect could be total destruction of the ECM leading to plaque rupture.

## Can We Switch MMPs Off?

Physiologic mechanisms for suppressing MMP activity provide valuable approaches. Nitric oxide, for example, inhibits MMP-9 expression by reducing superoxide generation and subsequent ERK activation[76]. Heparin and heparan sulfate proteoglycans inhibit induction of several MMPs from smooth muscle cells[77]. Transforming growth factor (TGF)-β inhibits MMP-1, MMP-3 and MMP-7 induction in fibroblasts[78], MMP-9[79] secretion in mast cells, and MMP-7[80] and MMP-9[81] in macrophages. The

T helper 2 cytokines IL-4 and IL-10 inhibit MMP secretion from macrophages[82,83]. TGF-β and IL-10 have established atheroprotective roles that could be partly explained by effects on MMPs. However, IL-4 and the T helper 1 cytokine, interferon-γ, which inhibits the CD40 ligation-induced secretion of MMPs from smooth muscle cells[84] and macrophages[70,75], are on balance proatherogenic. More encouragingly, direct inhibition of CD40L leads to smaller and more stable plaque phenotypes in atherosclerosis prone mice[85–87].

Statins are potent lipid-lowering drugs that prevent atherosclerosis progression and coronary events. Statins reduce the expression and secretion of MMP-1, MMP-2, MMP-3 and MMP-9 from macrophages and smooth muscle cells *in vitro*, and in rabbit and human atheroma[16,88–90], but increase TIMP-1 expression[89], in part by posttranslational mechanisms[90]. Hence, statin treatment may render plaques more stable, in part, by inhibiting MMP secretion. Peroxisome proliferator-activated receptor α and γ ligands, which inhibit MMP-9 secretion from smooth muscle cells[91], and macrophages[92,93] also have established therapeutic potential against atherosclerosis.

## Summary

Histologic studies provide strong "guilt by association" evidence that MMPs promote plaque vulnerability in man. Genetic epidemiologic studies show, in particular, that MMP-1 and MMP-3 overactivity results in smaller but less stable plaques. Knockout and transgenic studies provide evidence that MMPs promote plaque instability but also clear protective effects, consistent with their ability to promote smooth muscle cell migration and proliferation. Possibly for this reason, broad-spectrum MMP inhibitors have little net effect on plaque progression or vulnerability in animal models at clinically tolerable concentrations. Successful interventions are likely therefore to come from selectively targeting individual MMPs or from intervening in the mainly inflammatory pathways that lead to MMP over production.

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