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The role of matrix metalloproteinases in coronary atherosclerotic plaque instability

Coronary atherosclerosis is a complex process characterized by the accumulation of lipid, macrophages and smooth muscle cells in intimal plaques in the large and medium sized epicardial coronary arteries (13). The vascular endothelium plays a critical role in maintaining vascular integrity and homeostasis. Mechanical shear stress, biochemical abnormalities and immunological factors may contribute to the endothelial injury, which is believed to trigger atherogenesis. The resultant endothelial dysfunction allows accumulation of oxidized lipoproteins which are taken up by macrophages to produce lipid-laden foam cells. The release of host of cytokines, such as platelet-derived growth factor and transforming growth factor-β promote further accumulation of macrophages as well as smooth muscle cell migration and proliferation. Coronary atherosclerosis is a progressive disease. Disease progression occurs by two basic mechanisms: gradual increase in plaque size by incorporation of lipid, and the more unpredictable changes in lumen encroachment as a result of plaque rupture (13, 15). Recent studies have supported the hypothesis that the depletion of matrix components, specifically fibrillar collagens, from the fibrous cap caused by an imbalance between synthesis and breakdown leads to thinning of the cap, predisposing the cap to spontaneous rupture or rupture in response to extrinsic triggers. The inflammatory cells may play a critical role in plaque rupture by contributing to depletion of extracellular matrix in the fibrous cap through increased matrix breakdown or reduced matrix synthesis (15).

Breakdown of extracellular matrix is attributed to a family of matrix-degrading, zinc--requiring matrix metalloproteinases (MMPs) that are expressed in atherosclerotic plaques by inflammatory cells (macrophages, foam cells, T lymphocytes) and, to a lesser extent, by smooth muscle cells and endothelial cells (13, 15). The activity of MMPs is tightly regulated at the level of gene transcription and by their secretion in an inactive zymogenic form requiring extracellular activation and cosecretion of tissue inhibitor of metalloproteinases (TIMPs) (3). Thus, increased gene transcription or enhanced activation of matrix metalloproteinases or reduced activity of their tissue inhibitors may result in increased matrix breakdown (2). Activation of latent MMPs (zymogenic form) can be induced by plasmin produced by urokinase-type plasminogen activator (uPA) from plasminogen (by macrophages) or by trypsin and chymase (derived from degranulating mast cells) or other MMPs (3). Increased MMP production can be induced by oxidized lipids, reactive oxygen species, chlamydial heat shock protein, CD-40 ligation, inflammatory cytokines, tenascin-C derived from macrophages, and hemodynamic stress (6, 13, 15). Thus all components necessary for the activation of MMP pathway have been shown to exist in the atherosclerotic plaques. In addition to MMPs' increased expression of cysteine and aspartate proteases of the cathepsin family and reduced expression of their inhibitor cystatin-C in human atherosclerotic lesions may also contribute to matrix breakdown in plaques (6, 13, 15). The MMPs are categorized into three major functional groups, in part based on substrate specificity. The collagenases (MMP-1, -8 and –13), that preferentially have affinities toward collagen types I, II and III, the stromelysins (MMP-3, -10, and –11) with specificity for laminin, fibronectin and proteoglycans, and the gelatinases (MMP-2

and MMP-9), which most effectively cleave gelatin as well as type IV and V collagen (3). The proteolytic targets of MMP also include many other proteinases, proteinase inhibitors, clotting factors, chemotactic molecules, latent growth factors, growth factor binding proteins, cell surface receptors, as well as cell-cell and cell-matrix adhesion molecules (3). MMPs cooperate to degrade substrates (2). Fibrillar collagens I and III are partially degraded by MMP-1, and the released fragments by MMP-2 and MMP-9 (2). MMP-1 and MMP-2 have different binding sites on collagen. Hence both enzymes can be present at the same time at the site of their biological action, and the degradation of the extracellular matrix might be achieved by the sequential and coordinated action of different proteolytic activities (3).

In adult organisms under physiological conditions, arteries exhibit a low rate of extracellular matrix and cell turnover. Smooth muscle cells from the intima and from the media express MMP-2, TIMP-1 and TIMP-2 but little MMP-1, MMP-3 and MMP-9 (6, 15). Luminal endothelial cells stain for MMP-2 and TIMP-2 (5). MMP-2 is constitutively expressed and is present in extracts from normal arteries (5).

MMPs overexpression might be responsible for the rupture of the fibrous cap separating the lipid core of the plaque from the blood (15). In the coronary arteries, rupture leads to thrombosis and myocardial infarction (13). Moreover, MMP overexpression might be associated with intra plaque hemorrhage resulting from the rupture of neocapillaries present within the plaque. Such hemorrhage could produce a rapid increase in plaque size and a decrease in the vessel lumen (6, 13, 15) (Table 1).

Table 1. Matrix metalloproteinases (MMPs) in atherosclerotic plaque

MMP	Alternative names	Enzyme classification number	Substrates
MMP-1	collagenase (type I, interstitial)	EC 3.4.24.7	collagens (I, II, III, VII, X); gelatin; aggrecan; L-selectin; IL-1 beta, proteoglycans; entactin; ovostatin; MMP-2; MMP-9
MMP-2	gelatinase A	EC 3.4.24.24	collagens (I, IV, V, VII, X, XI, XIV); gelatin; elastin; fibronectin; aggrecan; MBP; osteonectin; laminin-1, -5; MMP-1; MMP-9; MMP-13
MMP-3	stromelysin 1	EC 3.4.24.17	collagens (III, IV, V, IX); gelatin; aggrecan; perlecan; decorin; laminin; elastin; casein; osteonectin; ovostatin; entactin; plasminogen; MBP; IL-1 beta; MMP-2/TIMP-2; MMP-7; MMP-8; MMP-9; MMP-13
MMP-9	gelatinase B	EC 3.4.24.35	collagens (IV, V, VII, X, XIV); gelatin; entactin; aggrecan; elastin; fibronectin; osteonectin; plasminogen; MBP; IL-1 beta

In coronary atherosclerotic lesions MMP-9 (gelatinase B) was found to be highly expressed. MMP-9 as well as MMP-3 (stromelysin 1) and MMP-1 (interstitial collagenase) were overexpressed in regions of foam cell accumulation, whereas normal arteries stained uniformly for MMP-2 (gelatinase A) and TIMPs (5, 6), the latter of which may play an important regulatory role in arterial wall homeostasis. Indeed, in porcine coronary arteries, a higher intrinsic gelatinolytic activity and a rapid cell outgrowth was seen in the adventitia, whereas preferential expression of TIMPs was present in the media that exhibited slower cell outgrowth (6). Impairment of TIMP synthesis may thus contribute to the pathogenesis of coronary lesion formation (6, 15). Nevertheless, the intracellular localization of MMP-9 was most frequently documented in coronary atherectomy specimens from patients with atherosclerosis and angina with acute ischemia, when compared with those without acute ischemia. This suggests that active synthesis of MMP-9 by macro-

phages and smooth muscle cells is strongly associated with the clinical syndrome of unstable angina, possibly by metalloproteinase-induced matrix degradation, which promotes plaque rupture (4).

Several studies suggest that the interactions of smooth muscle cells, macrophages, T lymphocytes and mast cells within the atherosclerotic plaque contribute to the disruption of the extracellular matrix by decreasing the synthesis of collagen, and shifting the proteolytic balance towards an increase of MMP activity (13, 15). The principal source of MMPs in the atherosclerotic plaque is represented by macrophages (6). Resident macrophage-derived foam cells, characteristic of unstable plaques, have been identified as a major source of MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, and MMP-associated activity in human and experimental atherosclerotic lesions (7). In addition they are a source of plazminogen activators and hence provide potential activators of MMPs (13). Futhermore, macrophages at sites of plaque rupture are associated with mast cells which can contribute to MMP activation (13). MMP-1 expression has been detected by *in situ* hybridization and immunohistochemistry in carotid atherosclerotic plaques containing a lipid core (7). MMP-1 mRNA is mostly present in the outer edge of the lipidic core, a region containing a high density of macrophages. MMP-1 protein is colocalized with lipid laden macrophages. Stromelysin (MMP-3) mRNA is present in lipid laden macrophages at the base of the plaque. Macrophages were also found to be stained by antibodies against MMP-2, MMP-3, MMP-9, and TIMP-1 and TIMP-2 (5). The staining was mostly localized to the fibrous cap, the shoulder region, and the base of the lipid core (5). Macrophages and T lymphocytes from coronary stenotic lesions obtained by directional atherectomy stained for MMP-9. Moreover, MMP-2 and MMP-9 extracted from atherosclerotic lesions are present in their activated form (5, 6). Furthermore, upon stimulation by macrophage-derived TNF-α and IL-1 β, smooth muscle cells synthesize increased amounts of MMP-1, MMP-3 and MMP-9, but do not synthesize increased amounts of tissue inhibitors of metalloproteinases (6, 15). Hence these cytokines shift smooth muscle cell proteolytic balance towards proteolysis *in vitro*. There is evidence that smooth muscle cells are actually stimulated by cytokines within the plaque (6, 15). Smooth muscle cells expressing MMP-1, MMP-3 and MMP-9 have been noticed in plaques (10). These findings demonstrate that smooth muscle cells contribute in structural events in the plaque by increased synthesis of MMPs. Furthermore, endothelial cells on the lumianal surface of the plaque and microvascular endothelial cells in the plaque express MMP-1 (5). Mast cells might contribute to MMP-mediated plaque instability: mast cells stimulate endothelial cell proliferation, induce ICAM-1 expression on endothelial cells and activate zymogenic forms of MMP-1, MMP-2 and MMP-3 via mast cell-derived tryptase. Hence, endothelial cells could contribute to atherosclerotic plaque instability through mechanisms dependent upon MMP activity: firstly, endothelial cell spreading into the plaque might be mediated by MMPs; secondly, endothelial cells in the plaque might facilitate the influx of inflammatory cells resulting in local increase of MMP activity (6, 13, 15).

In the absence of an experimental model, it is difficult to assess the role of MMPs evidenced in atherosclerotic lesions in regard to plaque rupture. Nevertheless, a few studies have established a correlation between the expression of MMPs and the structural events in the plaque, such as rupture and disease progression. Gelatinase B-deficient mice were partially protected against ventricular enlargement, collagen accumulatuion and cardiac rupture, which are complications of acute myocardial infarction. Temporary TIMP-1 gene transfer in these mice prevented cardiac rupture completely and did not abort infarct healing (8). In addition, ischemia-and reperfusion-induced expression of both latent and active gelatinase B were significantly reduced in mice lacking one gelatinase B allele. Less neutrophils were detected in the infarction area after ischemia-reperfusion in knock-out vs wild–type mice. These data indicate that gelatinase B might be a target for treatment of acute myocardial infarction (14).

After balloon catheter injury of the carotid artery of the rat, the production of 88-kDa gelatinase was induced and continued during the period of migration of smooth muscle cells from the media to the intima. This suggests that gelatinase expression directly facilitates smooth muscle cell migration within the media and into the intima and plays a role in neointimal formation that characterizes arterial tissue remodelling after injury (1, 12). Nevertheless, the inhibition of smooth

muscle cell migration seems not to be sufficient to inhibit lesion growth. Lesion size eventually reaches control levels via increased smooth muscle cell replication (1). Gelatinase B was also detected after perivascular injury in mice, and this is mainly in macrophages in the adventitia (11).

The specific factors that exactly induce the expression of MMPs in the cells within the plaque remain undefined. It is unknown whether all or most of the cells in a lesion respond to the same stimuli, or whether a select population of cells respond. MMPs are the potential terminal effector of extracellular matrix degradation, because their activity is tightly regulated by a wide range of molecules (cytokines, growth factors, adhesion molecules) also upregulated in the arterial wall. This position at the end of chain of biological responses to arterial injury makes MMP inhibition a promising therapeutic approach to pathologic responses of arteries. Furthermore, peripheral blood levels of MMP-2 and MMP-9 may be increased in patients with unstable atherosclerotic plaque and acute coronary syndrome, raising the interesting question of the possibility to develop noninvasive tests for detection of plaque vulnerability.

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SUMMARY

The principal role in the acute coronary syndromes is played by instability and rupture of the atherosclerotic plaque, followed by platelet activation, thrombosis and vasoconstriction. The plaque that is vulnerable to rupture is characterized by: a large, soft, acellular lipid-rich core, a thin fibrous cap with reduced collagen content, reduced smooth muscle cell density and increased macrophage density and activity. The inflammatory cells may play a critical role in plaque rupture by contributing to depletion of extracellular matrix in the fibrous cap through secretion of proteolytic enzymes. Depletion of extracellular matrix components, especially fibrillar collagens, from the fibrous cap is caused by an imbalance between synthesis and breakdown accomplished by matrix metalloproteinases (MMPs). The principal sources of MMP-1, -2, -3, and -9 in the atherosclerotic plaque are macrophages, foam cells, T lymphocytes, smooth muscle cells and endothelial cells. An inadequate expression of matrix metalloproteinases along with altered expression of tissue inhibitors of metalloproteinases (TIMP-1, -2) is one of the suggested factors that contribute to thinning of the cap, and predispose atherosclerotic plaque to rupture.

Rola metaloproteaz macierzy w niestabilności blaszki miażdżycowej tętnic wieńcowych

Główną rolę w powstawaniu ostrych zespołów wieńcowych odgrywa niestabilność i pęknięcie blaszki miażdżycowej z następczą aktywacją płytek krwi, tworzeniem zakrzepu i wazokonstrykcją. Czynnikami decydującymi o podatności blaszki miażdżycowej na pęknięcie są: duży rdzeń lipidowy, zmniejszenie grubości pokrywy włóknistej, zmniejszona liczba komórek mięśni gładkich oraz zwiększona liczba i aktywność makrofagów. W procesie uszkodzenia blaszki miażdżycowej istotną rolę przypisuje się komórkom zapalnym, które wydzielając enzymy proteolityczne przyczyniają się do osłabienia włóknistej pokrywy. Zmniejszenie zawartości składników macierzy zewnątrzkomórkowej, a zwłaszcza włókien kolagenu w obrębie pokrywy włóknistej jest powodowane zaburzeniem równowagi pomiędzy ich wytwarzaniem a degradowaniem przez metaloproteazy macierzy (MMP). Makrofagi, komórki piankowate, limfocyty T, komórki mięśni gładkich i komórki śródbłonka naczyń stanowią główne źródło MMP-1, -2, -3 i -9 w blaszce miażdżycowej. Niewspółmierna ekspresja metaloproteaz macierzy łącznie ze zmienioną ekspresją tkankowych inhibitorów metaloproteaz (TIMP-1, -2) stanowią jeden z czynników przyczyniających się do zmniejszenia grubości pokrywy włóknistej i usposabiają do pęknięcia blaszki miażdżycowej.