Metalloproteinases in Development and Progression of Vascular Disease

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Key Words

Restenosis · Neointima · Atherosclerosis · Aneurysm · Metalloproteinase

Abstract

Remodeling of the vascular wall plays a role in many physiological processes, but also in the pathogenesis of major cardiovascular diseases such as restenosis and atherosclerosis. Remodeling requires proteolytic activity to degrade components of the extracellular matrix; this can be generated by the matrix metalloproteinase (MMP) system alone or in concert with the fibrinolytic (plasminogen/plasmin) system. Several lines of evidence suggest that the MMP system plays a role in vascular smooth muscle cell migration and neointima formation after vascular injury. In atherosclerotic lesions, active MMPs may contribute to plaque destabilisation by degrading extracellular matrix components, but may also promote aneurysm formation by proteolytic degradation of the elastic lamina. The MMP system may therefore represent a potential therapeutic target for treatment of restenosis or atherosclerosis.

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Introduction

Remodeling of the vascular wall in response to changes in its hemodynamic environment may occur through a variety of structural adaptations, that may result in vessel enlargement or diminution. The direction and extent of vascular remodeling is coordinated via endothelial production of growth factors, cellular adhesion molecules and proteinases [1]. Inappropriate remodeling underlies the pathogenesis of major cardiovascular diseases such as restenosis and atherosclerosis. Cell migration and tissue remodeling require degradation of extracellular matrix (ECM). Two proteolytic systems, the fibrinolytic (plasminogen/plasmin) and matrix metalloproteinase (MMP) systems in concert can degrade most ECM components. Plasmin can only degrade some components of the ECM directly, such as laminin and fibronectin, whereas other components such as elastin and collagen are degraded by MMPs. The plasminogen/plasmin system can, however, play a role in the activation of several proMMPs. The role of both systems in vascular remodeling and atherogenesis has recently been reviewed [2,3].

Vascular remodeling does not only play a role in disease states; it also contributes to physiological processes such as blood vessel growth and regression during fetal development and growth, exercise training and pregnancy [4]. In this contribution we will focus on the potential role of the MMP system in arterial restenosis and atherosclerosis.

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Vascular interventions for the treatment of atherothrombosis induce restenosis of the vessel within 3-6 months in 30-50% of treated patients. Arterial stenosis may result from remodeling of the vessel wall (such as occurs predominantly after balloon angioplasty) and/or from accumulation of cells and ECM in the intimal layer (such as occurs predominantly after intraluminal stent application).

Atherosclerotic lesions initially consist of fatty streaks that develop into fibroproliferative lesions. A mature lesion consists mainly of foam cells, smooth muscle cells (SMC), a necrotic core and a fibrous cap containing extracellular matrix components. Clinical complications of atherosclerosis are often triggered by rupture of unstable plaques, whereas thinning of the atherosclerotic vessel wall due to elastin and collagen degradation and media necrosis may result in aneurysm formation and bleeding. Proteolysis may contribute to neovascularization and rupture of plaques, or to ulceration and rupture of aneurysms.

MMP Family

MMPs constitute a tightly regulated family of zinc dependent endopeptidases that degrade most components of the ECM and basement membrane, and can also function in many other proteolytic processes. Most MMPs are synthesized and secreted as inactive proenzymes. MMPs comprise several domains. The signal peptide (17-29 amino acids) is followed by the propeptide domain (77-87 amino acids) that constitutes the amino-terminus of the enzyme. It contains a unique conserved sequence with a cysteine residue that ligates the catalytic zinc to maintain the latency of the proenzymes. MMP-23 lacks this sequence but contains a conserved cysteine, while some others, e.g. MMP-11, MMP-14, MMP-15, MMP-16 and MMP-17 have a furin recognition site, and *Xenopus* MMP has an additional vitronectin-like domain [5,6]. The catalytic domain (about 170 amino acids) contains the catalytic machinery including the zinc-binding site and a conserved methionine [7]. This domain contains additional zinc and calcium ions which maintain the three dimensional structure of MMPs, required for their stability and enzymatic activity. The two gelatinases (MMP-2 and MMP-9) contain additional repeats of fibronectin-like domains which interact with collagen and gelatins. The carboxy-terminal hemopexin-like domain (about 210 amino acids) has an ellipsoidal disc shape and plays a key role in substrate specificity [8]. The hemopexin domain of proMMP-2 is also required for its cell surface activation by MT1-MMP [9]. MMP-7 and MMP-26 lack this domain entirely and in some members of the MMP family, e.g., MMP-23, the hemopexin domain is substituted by a cysteine-rich, proline-rich and interleukin-1 receptor-like motif.

The catalytic and the hemopexin domains are linked by a proline-rich linker domain (the hinge region). A transmembrane domain occurs in MT-MMPs, which anchors them to the cell surface [10].

The members of the MMP family (23 identified at present) are divided into five classes based on their structure and substrate specificities. These are collagenases, gelatinases, stromelysins, membrane type MMPs (MT-MMPs) and others. Although each MMP is encoded by a different gene, there is a high degree of sequence homology and structural domain conservation. MMPs are synthesized, and mostly also secreted as zymogens that are extracellularly activated by several proteinases (including plasmin, trypsin, chymotrypsin, kallikrein, cathepsin, or neutrophil elastase and other MMPs) or in vitro by non-proteolytic agents such as 4-aminophenyl mercuric acetate, hypochlorous acid, oxidized glutathione, and denaturants [11].

Active MMPs are inhibited by tissue inhibitors (TIMPs); four homologous TIMPs (TIMP-1 to -4) have been identified. They form noncovalent complexes with the active forms of MMPs as well as with some proMMPs, thereby inhibiting active MMPs or impairing the activation of proMMPs [12].

Arterial Restenosis

Expression of MMP Components

Proteinases from the MMP system participate in the proliferation and migration of SMC, and in matrix remodeling during arterial wound healing [3,13-17]. Only MMP-2 appears to be expressed in the quiescent SMC, whereas expression of MMP-3, -7, -9, -12 and -13 is induced in injured, transplanted, or atherosclerotic arteries [18-23]. In a balloon-injured rat carotid artery model, a correlation was observed between changes in MMPs and SMC migration [24,25]. Increased levels of TIMP-2 in this model suggested that changes in the proteolytic balance play a role in SMC migration after arterial injury [26].

Furthermore, it was demonstrated that SMC migration in baboon aortic explants requires plasminogen activators, MMP-2, MMP-9, basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) [27,28]. PDGF-induced migration of SMC depended on MMP-2, whereas bFGF-induced migration depended on both MMP-2 and MMP-9 [28]. Studies in balloon-injured rabbit carotid arteries suggested that MMP-2 facilitates migration of SMC from the media to the intima and does not affect replication [29]. In patients with ischemic heart disease coronary angioplasty induces MMP-2 activity in the coronary circulation, which may contribute to vascular remodeling and late restenosis [30]. Serum MMP-9 levels are also elevated in

patients with severe coronary stenosis [31] and may constitute a predictor of cardiovascular mortality in patients with coronary artery disease. At present, the association between polymorphisms in MMP genes (MMP-2, -3, -9 and -12) and cardiovascular disease is also under investigation [32,33]. To assess the role of the MMP system in SMC migration and neointima formation experimentally, a perivascular electric injury model in the mouse has been used [34]. In this model, surgically exposed femoral arteries are injured perivascularly via delivery of an electric current, which destroys all medial SMC, denudes the injured segment of intact endothelium, and transiently induces platelet-rich mural thrombosis. A vascular wound healing response results characterized by repopulation of the media and accumulation in the neointima of SMC originating from the uninjured borders.

The temporal and topographic expression pattern of MMPs (MMP-2, -3, -9, -12 and -13) after vascular injury in this model is compatible with a role in ECM degradation and SMC migration [35]. Two weeks after injury, MMP-2 and -3 are detected in α -actin stained SMC. MMP-9, -12 and -13 are found in macrophages located mainly in the adventitia and only occasionally in subintimal areas. Total MMP-9 levels (active plus latent) are also markedly increased after injury. MMP-3 expression after injury gradually increases to reach a maximum at about 2 weeks. These data are compatible with findings in the rat carotid artery model, showing constitutive expression of latent MMP-2 and markedly enhanced levels of active MMP-2 up to 7 days after balloon injury, whereas also active and latent MMP-9 are induced after injury [24,36]. In this rat model, MMP-3 is detectable as early as 2 hours after balloon injury and reaches a maximal 2-fold increase at 7 days [36].

Studies in Gene-Deficient Mice

A role of MMP-9 in arterial remodeling by promoting migration and replication of SMC is confirmed by injury models in MMP-9 deficient mice [37,38]. Deficiency of MMP-2 in mice suppresses SMC invasion and development of experimental intimal hyperplasia [39]. Mice with MMP-11 (stromelysin-3) deficiency show accelerated neointima formation after vascular injury [40]. Intima/media ratio's are significantly enhanced in MMP-11^{-/-} mice as a result of enhanced neointima formation 2 to 3 weeks after injury. In mice with deficiency of TIMP 1, the intimal areas at 1 to 3 weeks after injury are significantly larger than in wild-type mice, and contain abundant SMC, whereas the medial areas are comparable, resulting in significantly higher intima/media ratio's in the TIMP-1 deficient mice. These data thus support a physiological role of TIMP-1 in vascular remodeling, most likely via monitoring of MMP activity. This is further supported by the finding that stromelysin

mRNA antisense oligonucleotides inhibit phenotypic modulation of rat arterial SMC and thereby cause a decrease in migration and proliferation and in neointima formation after vessel wall injury [42].

Pharmacological Modulation of MMP Activity

Non-specific MMP inhibitors have been studied in several animal models in vivo and ex vivo. Marimastat inhibits gelatinase activity and reduces neointimal thickening in cultured human internal mammary arteries, a model of arterial intimal hyperplasia [43]. Metalloproteinase inhibition by Batimastat reduces late lumen loss after balloon angioplasty in atherosclerotic micropigs by inhibition of constrictive arterial remodeling, without effect on neointima formation [44]. MMP inhibition by GM6001 blocks in-stent intimal hyperplasia in a rabbit model [45]. In vivo SMC migration in the rat is also inhibited by administration of MMP inhibitors [24,46].

Alternative approaches include overexpression of TIMP-1 [47]. Adenoviral gene transfer of TIMP-1 reduces neointimal hyperplasia in balloon-injured rat carotid arteries [48] and intimal thickening in a rabbit restenosis model [49]. Adenovirus-mediated gene transfer of the human TIMP-1 gene inhibits SMC migration and neointimal formation in the human saphenous vein [50]. A hybrid protein consisting of TIMP-1 linked to the receptor-binding amino-terminal fragment of u-PA strongly enhances the inhibitory effect of TIMP-1 on neointima formation, presumably by blocking binding of u-PA to u-PAR and impairing cellular plasminogen activation [51].

Taken together, these data are compatible with a role of MMPs in ECM degradation and SMC migration after vascular injury.

Atherosclerosis

Expression of MMP System Components

A potential role for increased proteolysis in atherosclerosis is supported by the enhanced expression of t-PA, u-PA and several MMPs in plaques [19,22,52,53]. Several MMP system components (MMP-1, -2, -3 and -9) are expressed in atherosclerotic tissue [20,54,55]; in their active form they may contribute to vascular remodeling and plaque disruption. Stromelysin-3 (MMP-11), an unusual MMP that does not degrade any of the major ECM components, is also expressed in human atherosclerotic lesions and is regulated via CD40-CD40 ligand signaling [56]. Expression of MT1-MMP in human atherosclerotic plaques is upregulated by proinflammatory mediators [57]. Coexpression of cyclooxygenase-2 with MMP-9 and MT1-MMP by macrophages and SMC in atherosclerotic lesions was reported [58]. SMCs

and macrophages in human atherosclerotic plaques also express MT3-MMP; its increased expression by macrophages induced by inflammatory molecules may promote ECM degradation and contribute to plaque destabilization [59]. Expression of MMP-9 is upregulated and that of TIMP-1 downregulated in human monocyte-derived macrophages by oxidized low-density lipoprotein, suggesting that these may contribute to matrix degradation in the atherosclerotic plaque, predisposing to plaque rupture and/or vascular remodeling [60]. Also interleukin-8 downregulates TIMP-1 expression in cholesterol-loaded human macrophages and may affect the stability of atherosclerotic plaques and thus play a potential atherogenic role [61]. Regulated expression of TIMP-3, in addition to TIMP-1 and TIMP-2, was shown to counteract MMP activity in atheroma and hence to influence plaque stability [62].

Differences in expression of MMPs/TIMPs may determine the evolution of advanced atherosclerotic plaques and contribute to their vulnerability. Thus, MMP-1 and -3 are more expressed in human aneurysm lesions than in occlusive plaques, whereas MMP-9 is mainly detected in carotid as compared with femoral arteries, and TIMP-1 was associated with arterial calcification [63]. Leukocyte-derived MMP-9 is associated with aortic wall degeneration and aneurysm formation [64]. Serum levels of MMP-3 and -9 and of TIMP-1 are elevated in asymptomatic hyperlipidemic subjects at high cardiovascular risk, and MMP-3 and TIMP-1 levels are strongly positively associated with the presence of carotid lesions [65]. Measurement of serum MMP-9 levels may represent a novel marker of inflammation in patients with coronary artery disease and might provide an index of plaque activity [66].

It should also be kept in mind that several members of other classes of proteases are expressed and regulated in atherosclerotic lesions (e.g. serine, cysteine and aspartic proteases) and may contribute to ECM remodeling [67,68].

Studies in Gene-deficient Mice

In the atherosclerosis susceptible ApoE^{-/-} mice, expression of MMPs in atherosclerotic lesions depends on the size of the lesion but also on the genetic background. Thus, C3H/HeJ Apo E-deficient mice show increased MMP-2 and -12 activity in aortas and macrophages compared with those of C57Bl/6 ApoE^{-/-} mice. MMP-9 activity is comparable in aortic tissues of the 2 strains but is higher in macrophages from C3H/HeJ ApoE^{-/-} mice [69].

The hypothesis that MMP-3 may play a role in development and progression of atherosclerotic lesions and in aneurysm formation, was tested with the use of mice with deficiency of ApoE (ApoE^{-/-}:MMP-3^{+/+}) or with combined deficiency of ApoE and MMP-3 (ApoE^{-/-}:MMP-3^{-/-}), kept on a cholesterol-rich diet [70]. Atherosclerotic lesions

throughout the thoracic aorta are significantly larger in ApoE^{-/-}:MMP-3^{-/-} than in ApoE^{-/-}:MMP-3^{+/+} mice, and contain more fibrillar collagen. Aneurysms in the thoracic and abdominal aorta are less frequent in ApoE^{-/-}:MMP-3^{-/-} than in ApoE^{-/-}:MMP-3^{+/+} mice. Immunocytochemistry reveals significantly enhanced accumulation of macrophages in atherosclerotic lesions of ApoE^{-/-}:MMP-3^{+/+} mice and expression of urokinase-type plasminogen activator (u-PA) and MMP-3, colocalizing with macrophages. Zymography confirms the presence of u-PA and MMP-3 activity in extracts of atherosclerotic aorta's. These data suggest that plasmin, generated via macrophage-secreted u-PA activates proMMP-3 produced by accumulated macrophages. MMP-3 may thus on the one hand contribute to plaque destabilization, possibly by degrading extracellular matrix components, and on the other hand promote aneurysm formation by degrading the elastic lamina. Using the same model, it was recently shown that ApoE^{-/-}:MMP-9^{-/-} plaques are smaller and contain fewer macrophages than ApoE^{-/-}:MMP-9^{+/+} plaques, whereas MMP-12 deficiency does not significantly affect lesion size or macrophage content. However, both MMP-9 and MMP-12 deficiency protect against atherosclerotic media destruction and subsequent aneurysm formation [71].

A study in mice with combined deficiency of ApoE and TIMP-1 (ApoE^{-/-}:TIMP-1^{-/-}) showed that enhanced MMP activity, as a result of TIMP-1 deficiency, contributes to a reduction of plaque size but promotes aneurysm formation [72]. A similar study confirmed that the atherosclerotic lesions of ApoE^{-/-}:TIMP-1^{-/-} mice develop more aortic medial ruptures in which the elastic lamellae of the media are degraded and infiltrated with macrophages forming pseudo-microaneurysms [73]. Overexpression of TIMP-1 reduces atherosclerotic lesions in ApoE^{-/-} mice [74] and prevents aortic aneurysm degeneration and rupture in a rat model [75], further substantiating a functional role of MMPs.

These studies implicate an important role of u-PA in the structural integrity of the atherosclerotic vessel wall, likely via triggering activation of MMPs, and suggest that increased u-PA levels are a risk factor for aneurysm formation [76].

Pharmacological Modulation of MMP Activity

Several studies have explored the potential of MMP inhibition in atherosclerosis. Avasimibe, an inhibitor of acyl-CoA: cholesterol 0-acyltransferase limits macrophage enrichment and thereby MMP expression resulting in stabilization of preestablished atherosclerotic lesions in rabbits [77]. Treatment of atherosclerotic cynomolgus monkeys with a broad spectrum MMP inhibitor after (stent)-angioplasty reveals inhibition of angiogenesis but no improve-

ment of artery wall remodeling or intimal hyperplasia [78]. In a porcine model of atherosclerosis, antioxidant vitamins C and E contribute to stabilize atherosclerotic plaques after angioplasty by reducing vascular MMP-1 expression [79]. In patients requiring carotid endarterectomy, the non-specific MMP inhibitor doxycycline reduces the expression of MMP-1 in carotid plaques, whereas MMP-2, -3, and -9 or TIMP-1 and -2 are not affected [80]. Thus, several lines of

evidence support a role of MMP system components in development and progression of atherosclerosis, but a causative role is difficult to establish conclusively. The identification of specific proteinases involved in these processes and the development of more specific inhibitors will be required before presently available MMP inhibition can be a therapeutic option. The therapeutic potential of presently available MMP inhibitors has been reviewed [81-83].

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