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The role of matrix metalloproteinases in pathological remodelling of arterial wall

The extracellular matrix (ECM) maintains arterial structure and stability and helps to regulate arterial wall homeostasis. Coordinated breakdown, synthesis and remodelling of the arterial extracellular matrix are crucial events in normal physiological situations such as embryonic development and angiogenesis (5, 10). Synthesis progresses via mesenchymal cells, degradation is controlled by the proteolytic effect of a group of extracellular matrix-degrading proteolytic enzymes (1). On the other hand, matrix degradation occurs in pathological conditions such as arterial aneurysms and arterial restenosis (5). Remodelling of the arterial extracellular matrix requires cooperation of many different protease systems. Extracellular matrix-degrading proteolytic enzymes can be divided into four subgroups according to their amino acid residue or cofactor required for catalytic activity: cysteine proteases, aspartic proteases, serine proteases, and metalloproteinases, which contain a metal ion in the catalytic site. Matrix metalloproteinases (MMPs) are the principal enzymes involved in matrix turnover. 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 the affinity 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 (1). The expression of most MMPs is transcriptionally regulated by growth factors, cytokines, hormones, cell-matrix and cell-cell interactions. MMPs act primarily on the cell surface or in the extracellular space and the activities are controlled by a combination of zymogen activation and inhibition by endogenous inhibitors like α2-macroglobulin and the tissue inhibitors of metalloproteinases (TIMPs) (1). While α2-macroglobulin and related inhibitors are primarily the regulators in the fluid phase, TIMPs are considered to be the key inhibitors in tissues. The expression of TIMPs in the tissue, like MMPs expression, is controlled during tissue remodelling under physiological conditions to maintain a balance in the metabolism of the extracellular matrix (1). Disruption of this balance may result in diseases associated with uncontrolled turnover of matrix in pathological vascular remodelling, observed in the pathogenesis of cardiovascular diseases, including abdominal aortic aneurysms and arterial restenosis (5).

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. Luminal endothelial cells stain for MMP-2 and TIMP-2. MMP-2 is constitutively expressed and is present in extracts from normal arteries (4).

Aneurysmal arterial dilatation may represent an extreme form of outward remodelling. The expression of MMPs has been studied in human abdominal aortic aneurysms, in comparison with normal abdominal aortas from organ donors and atherosclerotic aortas from patients with occlusive disease. The upregulation of MMP-9 mRNA and protein has been reported in aneurysmal human arteries as compared to normal arteries (6). MMP-9 protein has been localized in the lu-

minal portion of the media and in the adventitial macrophages and vasa vasorum. Adhesion molecules on the endothelium mediating the recruitment of inflammatory are increased in aortic aneurysms (5). Taken together, these results suggest that the influx of mononucleaer phagocytes into the aortic wall via neocapillaries is a mechanism leading to increased MMP activity in aortic aneurysms. Neverthless, only few studies have shown a difference in MMP-9 expression between aortic aneurysms and aortas with occlusive atherosclerotic disease, and many studies have not found this difference (5). However, an involvement of MMP-9 in the aneurysmal disease seems certain. MMP-9 has elastinolytic properties, hence can be an effector of extracellular matrix changes in aneurysms. In addition, there is more MMP-9 activity in the adventitia of large, rupture-prone aneurysms, then in small aneurysms, suggesting a relationship between MMP-9 activity and aneurysmal rupture (3). Lastly, although MMP-9 is upregulated in aortic aneurysms and aortas with occlusive atherosclerotic disease, the localization of MMP-9, macrophages, and neocapillaries is different in these two conditions. MMP-9 is preferentially localized in the outer media of aortic aneurysms, and in the inner media of aortas with stenotic lesions. There is a strong correlation between the presence of neocapillaries in the outer part of aortic wall and elastin destruction, and chronic inflammation in aortic aneurysms. Hence topographic rather than quantitative differences in extracts of the aortic wall might account for differences in MMP-9 expression between aortic aneurysms and aortas with occlusive atherosclerotic disease (5). MMP-1 and MMP-3 are increased in aortic aneurysms as compared to aortas with occlusive atherosclerotic disease (14). MMP-1 has been detected in the adventitia of aortic aneurysms, and the increase in collagenolytic activity is associated with the rupture of aneurysms. Hence MMP-1 is likely to be involved in the rapid collagen resorption leading to rupture, together with MMP-9, illustrating the possible cooperation of the two MMPs to degrade collagen and its subproducts (14). MMP-3 colocalizes with urokinase-type plasminogen activator in macrophages infiltrating aortic aneurysms (8). Increased amounts of the activators of plasminogen: tissue-type plasminogen activator and urokinase-type plasminogen activator and plasminogen, have been detected in non-ruptured aneurysms (8). Moreover, plasmin was shown to be increased in extracts from aneurysms. Although MMP-9 can be detected in the media of both abdominal aortic aneurysms and aortas with stenotic lesions, MMP-3 is present in the media of abdominal aortic aneurysms only (14). MMP-3 could be a trigger for the activation of the MMP pathway in the media, leading to the formation of aneurysms. MMP-2 (gelatinase A) is present at higher concentrations in small aneurysms (4–4.5 cm) than in large aneurysms (> 5 cm), and is the main gelatinase in small aneurysms (3). Hence, MMP-2 activity could be involved in the remodelling of small aneurysms leading to enlargement and increased risk of rupture. Immunoreactive MMP-2 is localized in the fibrous part of the luminal atherosclerotic plaque and in the acellular media of the aneurysmal wall (3). Increased MMP-2 and MMP-9 expression was detected in human abdominal aortic aneurysms (12). Medial smooth muscle cells isolated from abdominal aortic aneurysm tissue seem to produce significantly higher levels of MMP-2 and MMP-9 *in vitro* than cells obtained from control arterial tissues (9). However, the histological feature most clearly associated with enlarging human abdominal aortic aneurysms diameter is a higher density of mural inflammation, composed primarily of macrophages (3). Increased MMP-9 expression may account for the propensity of abdominal aortic aneurysms to continue to expand. Local TIMP-1 overexpression prevented aneurysmal degeneration and rupture in a rat model of aneurysm (5). Experimental models of aneurysmal destruction of arteries in genetically deficient mice showed protection in MMP-9–null animals, confirming an important role for MMP-9 (5).

Pathological vascular remodelling defined as any enduring change in the size and/or composition of an adult blood vessel, is not only associated with abdominal aortic aneurysms, but also underlies the pathogenesis of arterial restenosis (5, 14). A common occurrence after the treatment of coronary and peripheral atherosclerosis by balloon angioplasty–restenosis is a result of the concomitant contribution of intimal hyperplasia as well as constrictive remodelling. Recent studies have revealed that constrictive remodelling of human coronary arteries after angioplasty and atherectomy was the most important determinant of restenosis and of posttransplant vasculopathy (5). The reaction of various components of the vessel wall to direct vascular injury by balloon angio-

plasty has been investigated in many experimental models, including the rat, rabbit, pig, primates, and mouse arteries (5). This shares major features with the process of wound healing, including deposition of collagen and tissue contraction. The remodelling of matrix is a result of the interplay between increased degradation early after injury and subsequent matrix accumulation and contraction. Such a temporal sequence was suggested by studies of arterial remodelling after balloon injury in the rabbit, which showed a delay between the immediate increase in procollagen mRNA expression and a detectable increase in vessel mural collagen content (11).This pattern may be due to a post–balloon injury peak in MMP expression and activity, reported in numerous studies (15). The contribution of smooth muscle cell migration and thus the need for degradation of the internal elastic lamina remain to be discovered in pathogenesis of human lesions (5). Administration of a nonselective MMP inhibitor reduced smooth muscle cell migration and neointimal thickening in the rat carotid injury model, supporting MMP participation in the breakdown of vascular matrix and especially of the internal elastic lamina allowing migration of smooth muscle cells from outer layers. Similarly, synthetic MMP inhibitors and antibodies raised against MMPs dramatically reduce *in vitro* migration of rat smooth muscle cells through a reconstituted basement membrane (5). Conversely, overexpression of MMP-9 has been shown to enhance migration of rat smooth muscle cells in a collagen invasion assay (5). Proteolytic activators of latent MMPs, plasmin and thrombin, may cooperate to enhance smooth muscle cell migration. Inactivation of these proteases through use of specific antibodies inhibited *in vitro* migration of aortic smooth muscle cells, whereas genetic deficiency inhibited *in vivo* smooth muscle cell migration and neointima formation in mice (7). MMPs may also play a role in smooth muscle cell proliferation, as suggested by experiments where MMP inhibitors diminished rabbit vascular smooth muscle cell proliferation *in vitro* (5). The overexpression of various TIMPs in rat smooth muscle cells has been shown to result in multiple divergent effects including inhibition of smooth muscle cell proliferation and migration, induction of smooth muscle cell apoptosis, decreased intimal hyperplasia, and increased accumulation of matrix after arterial balloon injury (5).

Taken together, recent data suggest that several matrix metalloproteinases are induced and activated after balloon innjury with or without stenting and in vein grafts. The alteration of the synthesis of collagen, and shifting the proteolytic balance towards an increase of MMP activity may contribute to pathological remodelling of arterial wall. Moreover, several studies point to a role for MMPs in freeing vascular smooth muscle cells from their interaction with the extracellular matrix, which allows them to migrate to form a neointima. Remodelling of the extracellular matrix also promotes new cell-matrix interactions that facilitate phenotypic modulation and proliferation of vascular smooth muscle cells.

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SUMMARY

The extracellular matrix maintains structure and stability of tissues that form walls of blood vessels. There exists a balance between synthesis and degradation of extracellular matrix components. Disruption of this balance may result in pathological remodeling of arterial walls associated with dilatation of arteries, characteristic feature of aneurysms. On the other hand, pathological arterial remodelling may result in narrowing of the lumen, observed in restenosis after balloon angioplasty. While pathological remodelling of the arterial wall requires cooperation of many differenet protease systems, the matrix metalloproteinases (MMPs) are the prinicipal matrix proteinases involved in this process. Evidence points to a role for matrix metalloproteinases in freeing vacular smooth muscle cells from their interaction with the extracellular matrix, which allows them to migrate to form a neointima.

Rola metaloproteaz macierzy w patologicznej przebudowie ścian tętnic

Macierz zewnątrzkomórkowa utrzymuje strukturę i stabilność tkanek tworzących ściany naczyń krwionośnych. W warunkach fizjologicznych istnieje stan równowagi pomiędzy syntezą a degradacją składników macierzy zewnątrzkomórkowej. Zaburzenie tej równowagi może powodować patologiczną przebudowę ścian tętnic, związaną z poszerzeniem ścian tętnic, charakterystycznym dla tętniaków. Z drugiej jednak strony patologiczna przebudowa tętnic może doprowadzać do zwężenia ich światła, obserwowanego w restenozie po angioplastyce balonowej. Patologiczna przebudowa ścian tętnic wymaga udziału wielu różnych układów proteaz, jednak metaloproteazy macierzy (MMP) są głównymi proteazami zaangażowanymi w ten proces. Wiele danych wskazuje na rolę metaloproteaz macierzy w uwalnianiu komórek mięśni gładkich z macierzy zewnątrzkomórkowej, ich migracji oraz w wytwarzaniu neointimy.