Novel Transgenic Rabbit Model Sheds Light on the Puzzling Role of Matrix Metalloproteinase-12 in Atherosclerosis

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Matrix metalloproteinases (MMPs), also termed matrixins, are known to affect atherosclerosis in many diverse ways. Apart from a role in the progression of atherosclerotic lesions, MMPs have also been involved in plaque destabilization and rupture and in the development of aneurysms. Despite intense research efforts during the last decade, it has been challenging to formulate a unifying model on the specific role of MMPs in atherosclerosis. In fact, mouse genetic studies have yielded controversial insights, highly dependent on genetic background, dietary regimen, and arterial site of analysis, whereas human genetic association studies do not prove causality of MMPs in atherosclerosis. Moreover, the lack of specific inhibitors precluded pharmacological dissection of the role of MMPs in vivo. There is thus a need for alternative strategies to decipher the MMP puzzle in atherosclerosis. In this issue of Circulation, Liang et al used a novel approach: They generated transgenic rabbits to study the role of MMP-12, also known as macrophage elastase, in atherosclerosis. Their results indicate that MMP-12 causes media destruction and pseudoaneurysm formation and, more surprisingly, accelerates plaque growth in an animal model that more closely resembles atherosclerosis in humans.

MMPs belong to one of the most ancient families of enzymes that arose in evolution soon after the first signs of life to regulate, in general, interactions of cells with the extracellular matrix (ECM). To cope with this formidable challenge, more than 65 structurally related MMPs developed in bacteria, plants, nematodes, fruit flies, sea urchins, and vertebrates. One of their key activities is breaking down ECM substrates in the basement membrane and interstitial matrix. As such, MMPs facilitate migration of macrophages and smooth muscle cells (SMCs) in atherosclerotic plaques by degrading ECM barriers, exposing novel ECM adhesion sites, and relieving the binding between basement membrane components and cell surface integrins that restrains movement. In addition, detachment of SMCs from its basement membrane and generation of ECM degradation products may induce proliferation, migration, and a switch to a synthetic phenotype. Of all MMPs, MMP-12 evolved as the most active elastase; its hemopexin-like domain diverged into a separate subfamily, more specialized in the binding of elastin than the corresponding substrate binding domains of other MMPs.

Apart from degrading ECM proteins, MMPs also acquired many other functions during evolution by broadening their substrate specificity. Many of their nonmatrix substrates have been implicated as targets in atherosclerosis. For example, MMP-12 might promote macrophage recruitment by activating the latent growth factor tumor necrosis factor-α or by modulating levels of the proinflammatory cytokine monocyte chemotactic protein-1 (MCP-1); other MMPs have similar effects on interleukins. Furthermore, by cleaving membrane-bound Kit ligand, MMPs (including MMP-12; Tjwa et al, unpublished results, 2006) regulate the recruitment of proatherogenic, bone marrow–derived progenitors. Other MMPs are capable of regulating the recruitment of inflammatory cells and the growth of SMCs and endothelial cells in plaques by liberating growth factors sequestered in the ECM (fibroblast growth factor-2, transforming growth factor-β, vascular endothelial growth factor, and others).

By disrupting CD44 and cadherins and causing shedding of selectins, MMPs may modulate vascular and inflammatory cell migration. Apart from the fact that MMP-12 cleaves membrane-anchored CD14 on macrophages into a soluble form, other MMPs regulate immunity via different mechanisms and influence apoptosis, in part by releasing the apoptotic Fas ligand. Because many MMPs have overlapping functions (for instance, in addition to MMP-12, MMP-2, -3, -7, and -9 have elastase activity), deficiency of a single MMP rarely causes diseases; an exception is the causal role of MMP-2 in a rare inherited bone disorder, nodulosis-artropathy-osteolysis syndrome. Instead, MMPs more often modulate disease severity in a context-dependent manner. Thus, on the basis of its activity profile, MMP-12 would be expected to modulate atherosclerosis as well. Liang et al studied how MMP-12 affects this disease in transgenic rabbits.

MMP-12 is the most important enzyme responsible for the degradation of elastin, a stretchy rubberlike protein found in blood vessels, lungs, and skin. With the advent of a high-pressureized closed circulation in vertebrates, arteries acquired increasing amounts of elastic fibers, arranged in concentric elastic lamellae with interposed SMCs. This allowed vessels to absorb the energy in each pulse sent from the heart to keep peak blood pressure from rupturing delicate capillaries, and then to release it to maintain blood pressure between pulses.
In healthy vessels, MMP-12 expression and elastin remodeling are minimal, but once vessels become inflamed and infiltrated with macrophages, such as in response to atherosclerosis, transplantation, mechanical injury, or aneurysmal dilation, the elastolytic activity in arteries is significantly upregulated. In the study by Liang et al, overexpression of MMP-12 in macrophages caused breakdown of the elastic laminae in atherosclerotic vessels. Because aneurysmal bulging of atherosclerotic arteries only occurs when the arterial wall collagen network is also broken down, collagenolytic MMPs must have been induced as well. These results are consistent with previous findings that reduced elastolytic MMP-12 activity in mice lacking MMP-12 or the urokinase-type plasminogen activator protected apolipoprotein (apo) E−/− mice against aneurysmal dilation or chemically induced medial destruction. The destructive activity of MMP-12 may also explain why plaques in MMP-12/apoE−/− mice exhibited characteristics of stable plaques.

Liang et al also report that MMP-12 overexpression stimulates plaque growth in rabbits. Although some caution is warranted to extrapolate conclusions drawn from overexpression of a transgene to the presumed role of the endogenous gene itself, a previous study in apoE-deficient mice revealed that loss of MMP-12 reduced plaque size in brachiocephalic arteries. In addition, an MMP-12 gene variation that upregulates MMP-12 expression is associated with luminal narrowing after angioplasty and stenting in diabetics. All of these findings would suggest that MMP-12 is causally involved in atherosclerosis progression. However, an association of MMP-12 with atherosclerotic lesion growth in humans has not been formally reported to date. Moreover, another study in apoE-deficient mice reported that loss of MMP-12 did not affect lesion growth in the aorta. Thus, in contrast to the consistent role of MMP-12 in aneurysm formation, the role of this elastase in plaque growth appears to be more contextual and dependent on the species, genetic background, gender, diet composition, artery, and stage of the disease.

How can we explain the counterintuitive result that plaque size is increased by overexpression of a proteinase, if it breaks down ECM proteins and therefore would be expected to reduce the amount of ECM and, consequently, decrease plaque size? As paradoxical as this may seem, proteinases may indirectly promote lesion growth by facilitating infiltration of macrophages, SMCs, and fibroblasts. Foamy macrophages constitute a significant volume fraction of the lesion, whereas SMCs and fibroblasts deposit bulky ECM. In transgenic MMP-12 rabbits, larger numbers of macrophages and SMCs accumulated in plaques, which suggests that MMP-12 indeed accelerated atherosclerosis at least in part by facilitating cell migration. Because ECM deposition was not quantified, it remains unknown whether MMP-12 also regulates this mechanism. This seems, however, a reasonable hypothesis, as MMP-12 proteolytically inactivates coagulation inhibitors and thus may promote intraplaque fibrin deposition.

How solid is the evidence that MMP-12 stimulates the infiltration of macrophages and SMCs in atherosclerotic plaques? Genetic studies in mice revealed that MMP-12 facilitates infiltration of macrophages in the lungs, joints, and skin, not only because MMP-12 breaks down elastin barriers, but also because it degrades other ECM components in basement membranes (such as collagen type IV, fibronectin, and laminin) and generates chemotactic elastic peptides and cytokines for macrophages. The MMP-12 transgenic rabbit model offers the opportunity to study whether MMP-12 also regulates tumor necrosis factor-α, MCP-1, or KitL levels to recruit bone marrow–derived cells into plaques. The increased accumulation of macrophages in aortic plaques in transgenic MMP-12 rabbits is consistent with the reduced macrophage infiltration in plaques in brachiocephalic arteries of MMP-12/apoE−/− mice. However, the general relevance of this finding will need to be further confirmed, because in another study, loss of MMP-12 in apoE−/− mice did not affect macrophage infiltration in lesions in the aorta, and overexpression of MMP-12 did not influence macrophage accumulation in coronary plaques in transgenic rabbits.

Liang et al also report that overexpression of MMP-12 increased the accumulation of SMCs in transgenic rabbits. This is a puzzling observation, because loss of MMP-12 in apoE−/− mice either did not affect or even increased SMC infiltration in plaques. At first glance, a role for MMP-12 in SMCs in atherosclerosis might not be expected, because most genetic studies previously documented a predominant macrophage phenotype, and this elastase is primarily expressed in macrophages, SMCs, by contrast, predominantly express MMP-2 and MMP-9. In response to cytokines, MMP-12 may be upregulated by activated SMCs in vitro, but MMP-12 expression has thus far not been reported in SMCs in atherosclerotic vessels in vivo. Obviously, SMCs could also be a target of elastases. For instance, treatment of aortic SMCs with elastase is known to induce the release of macrophage-recruiting chemokines in vitro. Questions as to whether MMP-12, like its other MMP partners, also regulates SMC migration, growth, and a switch to a synthetic phenotype by affecting growth factors and junctional and adhesion molecules could be addressed in this novel MMP-12 transgenic rabbit model. Given that this elastase is known to impair endothelial cell growth via proteolytic cleavage of the membrane-anchored urokinase-type plasminogen activator receptor, another intriguing question is whether MMP-12 regulates angiogenesis in plaques. Because plaques are generally too small to become hypoxic (their size is <200 μm, the oxygen diffusion limit), angiogenesis is not a predominant feature in murine lesions. By contrast, because the size of atherosclerotic plaques exceeds the oxygen diffusion limit in rabbits, angiogenesis in plaques, and the role of MMP-12 in this process, can be better studied in this novel rabbit model. A similar controversy has been reported for the role of MMP-9 in atherosclerotic plaque growth. Studies in humans indicated that plasma MMP-9 levels are elevated in patients with acute coronary syndromes and that high-expressing MMP-9 gene variations accelerate atherosclerosis, thus suggesting that this proteinase generally stimulates plaque growth. Consistent herewith, gene knockout studies in mice also suggested that MMP-9 stimulates infiltration of macrophages and SMCs, resulting in accelerated lesion development in the aorta and carotid artery. These findings...
suggest that similar to MMP-12, MMP-9 also regulates plaque growth by facilitating cellular infiltration rather than via removal of bulk matrix. In contrast, another study reported that MMP-9 reduced macrophage accumulation and plaque growth while stimulating SMC ingrowth in the brachiocephalic artery. It was proposed that in this model, the predominant role of MMP-9 was to “heal” the plaque (and thus reduce its size) by facilitating the infiltration of wound fibroblasts.

Why do MMPs have so many different faces, and why do they induce such divergent activities in a context-dependent manner? It is probably because there are so many of them, each with a myriad of possible functions and a complex cell-specific expression. Slight differences in experimental conditions may thus elicit a different expression profile or activate a distinct set of biological functions of these MMPs. Depending on the relative expression and biological activity of the various MMPs in the different cell types and the biological activity at stake, a destructive attack may be generated (as is the case for the MMP-12–overexpressing macrophages when they destroy the elastic laminae), or alternatively, a healing response may be initiated (as is the case when MMP-12– or MMP-9–expressing SMCs and wound fibroblasts infiltrate the plaques). Depending on the amount of ECM deposited, the lesion may either regress and “heal” as a fibrotic scar or expand in size and become a bulky plaque.

Given the formidable advantage of genetically manipulating the mouse genome in a very specific and sophisticated manner, why would one even consider using the rabbit as a model to study atherosclerosis or cardiovascular disease? The cost of generating and maintaining a transgenic rabbit colony substantially exceeds that of maintaining a mouse colony, and the advantages of the transgenic rabbit, compared with the mouse, relate in part to its relatively larger size, which enables facile studies of vascular injury and in vivo noninvasive magnetic resonance imaging. However, the advantages of the transgenic rabbit, compared with the mouse, much less of the genome has been mosaicism in the founder population, as well as the 6-month period before a new generation can be bred, increases the difficulty of establishing stable experimental cohorts. Compared with the mouse, much less of the genome has been sequenced and fewer research reagents are available for the rabbit. However, the advantages of the transgenic rabbit, compared with the mouse, relate in part to its relatively larger size, which enables facile studies of vascular injury and restenosis, as well as improved clinical evaluation of cardiac function and in vivo noninvasive magnetic resonance imaging of vessel wall morphology, lipid-rich plaques, intraplaque angiogenesis, and plaque rupture-associated thrombosis.

In the study of heart failure, the choice of the rabbit as the transgenic model is also commendable because the rabbit heart has a sarcomeric protein composition more similar to that of the human heart and the length of the contractile cycle is significantly longer. In addition, whereas rabbits are similar to mice in lacking apo(a) and lipoprotein(a), their lipoprotein profile more closely mimics that of humans, with low-density lipoprotein as the predominant plasma lipoprotein. When fed a cholesterol-rich diet, rabbits develop well-characterized humanlike atherosclerotic lesions, which can be induced to rupture as well.

Because of their key roles in tissue remodeling and cell infiltration, MMP inhibitors have been considered attractive drug targets. However, most preclinical and clinical studies did not yield the expected result, in part because nonselective inhibitors were used, and several of these MMPs have pleiotropic, sometimes even opposite activities. These failures should not necessarily remove all hope that more selective MMP inhibitors (as have been developed recently for MMP-1233) might ever become clinically useful. Additional models, such as that developed by Liang et al, will help to sort out this important question in the future.

Disclosures
None.

References


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