Matrix metalloproteinases (MMPs) are a family of approximately 30 structurally related Zn$^{2+}$ endopeptidases that each degrades several extracellular matrix (ECM) proteins as well as nonmatrix substrates. Although membrane-type MMPs are integral membrane proteins, most MMPs are secreted or confined to the pericellular environment by binding to surface receptors. Their activity is tightly controlled by regulating transcription and translation of genes and, in a few cases, by packaging and secretion from vesicles, by activation of proforms, and by binding to four tissue inhibitors of MMPs. As shown in the Figure, the role of MMPs includes degradation of ECM structural proteins; for example, MMP-1, MMP-8, MMP-13, and MMP-14 cleave type I and III collagens, and MMP-9 and MMP-12 actively degrade elastin. This is probably necessary for vessel wall remodeling and invasion of artery walls by immune-inflammatory cells. Whereas MMP activity should reduce the amount of ECM and therefore limit atherosclerotic plaque growth, locally dysregulated MMP activity could weaken and cause mechanical failure of plaque caps, causing myocardial infarctions (MIs) or strokes, depending on the location. Consistent with this, MMP-1 and MMP-13 have been localized with epitopes of cleaved collagen in the vulnerable shoulder regions of atherosclerotic plaques. MMP-2 and 9 also cleave matrix proteins (e.g., basement membrane type IV collagen) and nonmatrix substrates (e.g., CD-44, cadherins), which, with MMP-14, promote migration, proliferation, and viability of vascular smooth muscle cells, processes expected to favor plaque-cap stability (see Figure). The same MMPs also influence endothelial loss, repair, and angiogenesis, which could have complex consequences for plaque-cap stability. Actions of MMPs on endothelial cells could contribute to plaque-surface erosion, which also precipitates MI. Given the large number of MMP genes and the potential complexity of their impact on plaque stability, it is not surprising that studies of broad-spectrum MMP inhibitors have so far failed to clarify their role. In experimental models, such inhibitors alter the kinetics (but not extent) of intima formation after balloon injury and do not appear to change atherosclerotic plaque growth. Clinical studies failed to show effects of MMP inhibitors on in-stent restenosis in the Brilliant registry or on unstable coronary events in a small pilot trial. Genetic manipulations hold a high potential for elucidating the importance of individual MMPs and therefore providing a rationale for therapy targeted to individual MMPs. Complementary approaches include knockout and transgenic experiments in mice and genetic epidemiological studies, such as that by Pearce et al in this issue of Circulation Research, which relates functional gene polymorphisms to the incidence of disease in humans.

Genetic manipulation of apolipoprotein E knockout mice has been widely used to study the function of MMPs, although important differences in dietary regime, background strain, and site of investigation hamper direct comparison among studies. For example, MMP-1 overexpression in macrophages led to smaller, less advanced plaques. These findings imply a protective effect on lesion development but do not rule out a role in rupture of advanced plaques. Deletion of MMP-3 accelerated plaque development in thoracic aortas and brachiocephalic arteries. However, whereas MMP-3 knockout reduced inflammation and aneurysm formation in the aorta, it increased inflammation and gave a more unstable plaque phenotype in the brachiocephalic artery. Both studies therefore concur that MMP-3 reduces plaque progression but imply opposite effects on plaque stability. In the only available study, deletion of MMP-7 had no effect on plaque growth or stability in the brachiocephalic artery. Deletion of MMP-9 reduced atherosclerosis and microaneurysm formation in the aorta but caused larger, less stable plaques in the brachiocephalic artery. Hence, MMP-9 was found to promote plaque growth and instability in the aorta but to prevent both in the brachiocephalic artery. Deletion of MMP-12 produced more consonant findings; it prevented internal elastic lamina degradation in the aorta and produced smaller more stable lesions in the brachiocephalic artery. In summary, the available evidence suggests that MMP-1 reduces lesion progression, whereas MMP-12 promotes bigger less stable lesions. MMP-3 inhibits lesion growth but plaques appear to be either more or less stable depending on the site. The effects of MMP-9 are ambivalent in the different models.

Genetic studies in humans require the identification of reasonably common and functional polymorphisms that can be evaluated in well-characterized patient groups. As with the genetic studies in mice, convergence of results between different studies is not always evident, but where it occurs, it
greatly increases confidence. The study by Pearce et al used 2 populations of cases and controls from the UK and Sweden. This exceptional design provided immediate corroboration in different genetic backgrounds. The authors first identified a number of polymorphisms by sequencing the MMP-1 coding sequence, intron–exon boundaries, and its gene promoter in 30 unrelated individuals. The authors found 7 promoter polymorphisms, namely: 1107 (GG/G, i.e., G insertion/deletion), 839 (G/A), 755 (G/T), 519 (A/G), 422 (T/A), 340 (T/C), and 320 (T/C), and 2 silent mutations in the coding region. Although no individual promoter polymorphism predicted disease, the authors used a statistical approach based on Akaike’s Information Criterion to deduce the simplest haplotypes that predicted MI. In the UK population, the analysis showed that compared with the A_519-T/340 haplotype, both the A_519-C/340 and G_519-T/340 haplotypes had a 30% less chance of MI, whereas the G_519-C/340 haplotype had an almost 100% more chance of MI. The results were essentially replicated in the Swedish population. The haplotypes associated with protection from MI were associated with lower promoter activity in THP-1 monocytic cells determined by transient transfection in vitro, by reduced binding of promoter fragments to THP-1 DNA and, where measurable, with levels of MMP-1 mRNA in carotid endarterectomy tissues. Taken together, the present results support the contention that high levels of MMP-1 promote plaque instability and precipitate MI.

Interestingly, a previous study from the same group showed, in relation to the -1607 GG/G polymorphism, that the 2G allele that is associated with lower promoter activity predicted symptomatic coronary heart disease in a different cohort of patients. The 2G allele also appears to favor carotid artery stenosis. One interpretation of this apparent paradox is that MMP-1 activity is associated with less matrix protein accumulation and hence smaller plaques, consistent with the observations in MMP-1 transgenic mice, but that these plaques are less stable.

Similar conclusions emerge from genetic studies of the MMP-3 gene. In particular, the 5A/6A promoter polymorphism has attracted a large number of independent studies, which facilitate some cross-correlation and hence generalization. Broadly summarizing, the 5A allele that drives greater transcription is associated with less advanced coronary and carotid lesions but with greater incidence of MI and strokes. Again these data can be rationalized by the hypothesis that MMP-3 decreases ECM accumulation, leading to smaller but less stable plaques. The mouse studies of MMP-3 knockout broadly reinforce this interpretation. Several studies have also addressed MMP-9 polymorphisms, with the -1562C>T being the most informative. The T allele that has greater promoter activity is associated with acceleration of atherosclerosis development. Whether these bigger, more complicated plaques give rise to more or less unstable events has yet to be clarified.

In summary, MMPs are a large family of proteases with established functions that might increase or decrease plaque growth and stability through a variety of mechanisms (Figure 1). Genetic epidemiological studies of polymorphisms in the MMP gene promoters are revealing exciting new relationships with atherosclerotic plaque progression and precipitation of unstable events such as MI and strokes. In combination with genetic manipulation in mice, present and future studies should have the power to dissect the roles of individual MMP genes and more precisely identify drug targets.

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