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MMP-8: a new target for atherosclerosis?

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Previously unseen expression of the matrix metalloproteinase-8 (MMP-8) enzyme in atherosclerotic plaques has been discovered by researchers at Millennium Pharmaceuticals (Cambridge, MA, USA) and Brigham and Women's Hospital (Boston, MA, USA). This finding provides a novel pathway that could potentially be exploited to treat cardiovascular disease [1]. One of a class of collagenases, MMP (also known as neutrophil collagenase) was thought to only be present in neutrophils. It is produced at sites of acute inflammation where it digests collagen during the process of tissue remodelling that is associated with wound healing. The expression of MMP-8 in atherosclerotic plaques was not studied previously because atheroma contain few, if any, neutrophils.

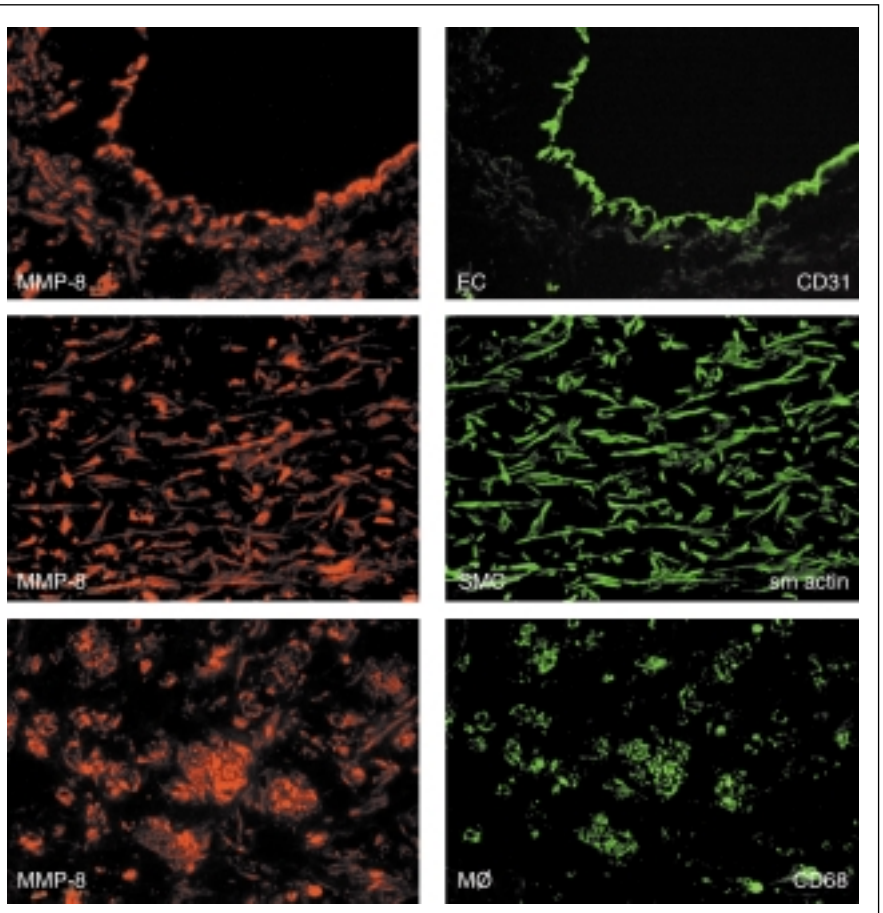

Figure 1. Matrix metalloproteinase-8 (MMP-8) colocalizes with human vascular endothelial cells (ECs), smooth muscle cells (SMCs) and mononuclear phagocytes (MØs) in human atherosclerotic lesions. Double immunofluorescence staining was used to colocalize MMP-8 (red) with ECs (using anti-CD31), SMCs (using anti-actin) or MØs (using anti-CD68) within atherosclerotic plaques. Figure reproduced, with permission, from Ref. [1].

Arterial thrombosis

Arterial thrombosis is recognized as the event responsible for the majority of acute ischemic syndromes, such as heart attack or stroke. Most thrombi occur at sites of rupture of thinned fibrous caps overlying lipid-rich atherosclerotic lesions with intimal and adventitial inflammation. Previous studies have established thinning and weakening of the fibrous cap as the mechanism that renders atheroma prone to rupture [2].

'From the mechanical perspective, preventing the rupture of the fibrous cap makes good sense in preventing the onset of a heart attack or stroke,' says Roger Breitbart, Senior Director of Cardiovascular Research at Millennium. Indeed, it appears that some caps are stable and others are more prone to rupture [3].

However, the mainstay of therapy for atherosclerosis has been the class of drugs known as statins, which partially inhibit 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, thus preventing cholesterol production. As a result, the liver produces additional low-density lipoprotein (LDL) receptors to bind LDL cholesterol in the blood, leaving less LDL to accumulate in the vessels within plaques. 'Apart from the low incidence of serious side effects, such as liver abnormalities and muscle problems, people still die from heart attacks while taking statins,' says Breitbart.

Fibrillar collagen type I is the major structural element and load-bearing molecule that confers tensile strength to

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the fibrous cap. Therefore, the activity of enzymes capable of digesting this matrix component are regarded as especially important in plaque stability. Interstitial collagen fibrils resist degradation by most proteinases and only interstitial collagenases I (MMP-1), II (MMP-8) and III (MMP-13) can initiate the breakdown of intact, triple-helical collagen. Previous studies have demonstrated the over-expression of MMP-1 and MMP-13 in human atheroma. However, a role for MMP-8 in atherogenesis had not previously been considered.

MMP-8 in atherogenesis

Breitbart and colleagues used transcriptional profiling to study which genes are expressed in cells that participate in the development of atherosclerosis. They studied the differences in gene expression in several types of human vascular cell – monocytes and macrophages, smooth muscle, and endothelial – and subjected them to inflammatory stimuli involved in atherosclerosis.

Three atheroma-associated cell types – endothelial, smooth muscle and mononuclear phagocytes – were found to express MMP-8 *in vitro* after stimulation with proinflammatory cytokines such as interleukin-1β, tumour necrosis factor-α (TNF-α) or CD40 (Fig. 1).

The expression of regulated genes identified in these DNA microarray experiments was compared in sections of healthy and diseased coronary and carotid arteries. In addition, the team also compared stable plaques with unstable plaques (as indicated by histological microscopic criteria and molecular markers). When gene expression was studied in sections of normal vessels, stable plaque and vulnerable plaque, MMP-8 appeared to be upregulated in vulnerable plaques (Fig. 2). 'The possibility that MMP-8 plays an important role in plaque vulnerability is a tantalizing hypothesis,' says Breitbart, adding that future experiments will investigate the potential mechanistic role of MMP-8 in this process.

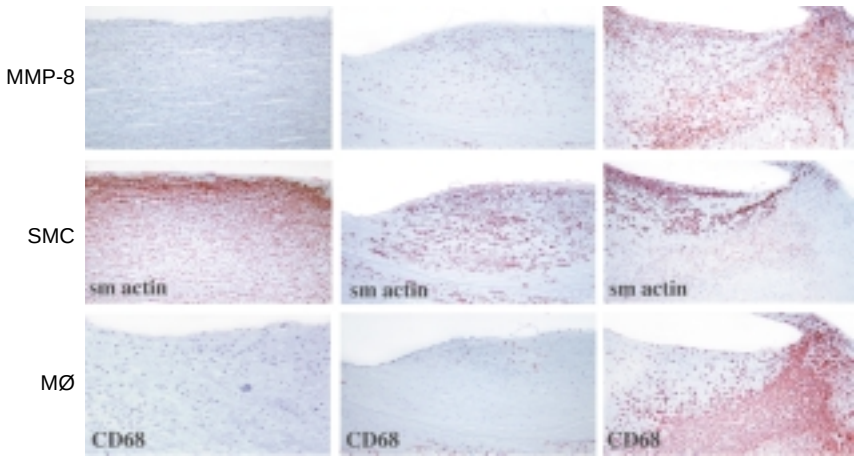

Figure 2. Enhanced expression of matrix metalloproteinase-8 (MMP-8) protein in human atherosclerotic lesions. Serial cryostat sections from non-atherosclerotic aortas (Normal; left) and atherosclerotic carotid atheroma, dichotomized by features associated with either stable (middle) or vulnerable (right) lesions, were analyzed for expression of MMP-8 (top), as well as smooth muscle α-actin (SMC; middle) or CD68 (MØs; bottom). Analysis of three non-diseased arteries, as well as three stable and vulnerable surgical specimens of atheroma obtained from different donors, showed similar results. Figure reproduced, with permission, from Ref. [1].

'If MMP-8 does play a role in plaque rupture, it offers the opportunity to block mechanisms that are not being addressed by existing medications,' says Breitbart. 'There are also ways in which you could consider using the presence of high levels of MMP-8 as a screening tool. If higher levels are found in the circulation it might indicate that the plaques are at risk of rupture. People found to be at risk could be given an MMP-8 inhibitor,' says Breitbart.

MMP-8 inhibitors

MMP-8 inhibitors already exist for research purposes, but none are currently marketed as drugs. The pursuit of MMP-8 as a drug target, therefore, would require further development of existing compounds and/or identification of novel inhibitors. Although several MMP inhibitors have been developed up to Phase III trials for several types of advanced cancer, they demonstrated no clinical efficacy in trials [4]. However, Breithart suggests this might be because MMPs appear to be important only in early cancer progression.

In an editorial [5], Prediman Shah from the Atherosclerosis Research Center at Cedars Sinai Medical Center (Los Angeles, CA, USA), commented: 'A direct causal connection between the matrix-degrading action of MMPs and plaque rupture has yet to be demonstrated. One major obstacle for demonstrating such a relation is the fact that spontaneous rupture with thrombosis has not been convincingly demonstrated in any of the many animal models of atherosclerosis, despite evidence of MMP expression in many of the human and experimental lesions.' However, he concluded that continued investigation of this crucial area in vascular biology is warranted.

References

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Gene therapy for Parkinson's disease

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Gene therapy using the gene encoding aromatic L-amino acid decarboxylase (AADC) has been shown to produce significant recovery from Parkinson's disease (PD). These results were announced by Krys Bankiewicz (University of California, San Francisco, CA, USA) at the recent *Society for Neuroscience 31st Annual Meeting* (10–13 November 2001, San Diego, CA, USA) [1].

L-Dopa (L-3,4-hydroxy phenylalanineone), one of the main drugs used to treat PD, requires AADC to convert it to dopamine; as the disease progresses, the cells that store AADC are lost causing patients to become resistant to L-dopa treatment. Using a primate model, Bankiewicz and colleagues have shown that transfer of the *AADC* gene into the striatum of the brain restores the conversion of L-dopa to dopamine to normal levels (Fig. 1) [1,2] and they are now developing the therapy for clinical trials.

PD affects 1–2 per 1000 of the population worldwide. However the incidence rises with age and increases to one person in 100 over the age of 65 and one in 50 over the age of 80. The disease occurs after degeneration of neurons in the midbrain that normally synthesize the neurotransmitter dopamine. Dopamine deficiency causes the main symptoms of the disease: slowing of emotional and voluntary movement, muscular rigidity, postural abnormality and tremor.

L-Dopa, the precursor of dopamine, can be given orally as therapy and was originally shown to alleviate disease symptoms in the 1960s [3]. L-Dopa therapy is initially successful in many patients, but the response declines as the disease progresses and is complicated by adverse side effects. Other drugs are also used; for example, anticholinergics (effective for tremor in some patients), catechol-*O*-methyltransferase (COMT) inhibitors (which slow down the breakdown of L-dopa), and selegiline (a selective inhibitor of monoamine oxidase type B, which metabolizes dopamine). However, all are associated with numerous side effects.

The gene therapy approach

'Gene transfer technology offers the possibility of achieving prolonged delivery of proteins into specific areas of the CNS, although at the moment, gene transfer does require brain surgery', says Bankiewicz. Several enzymes are involved in the synthesis of dopamine and a successful gene therapy strategy to restore endogenous dopamine production would involve the transduction of several genes. Bankiewicz's approach is to transfer only the gene for AADC – the enzyme that completes the last step in the pathway that converts L-dopa to dopamine. 'With disease progression, there is a severe loss of dopamine terminals in the striatum; the enzyme AADC is concentrated in these terminals and is, therefore, also reduced,' says Bankiewicz.

After successful *AADC* gene transfer, subsequent administration of exogenous L-dopa should then be converted to the functional neurotransmitter and could improve symptoms. 'Furthermore, since the cells that express the *AADC* gene following gene transfer are unaffected by the PD, levels of AADC should not be affected by the ongoing disease process,' he adds.

Effects of transgene expression

The preclinical studies have also examined the effects of *AADC* transfer in the unilateral 6-hydroxydopamine (6-OHDA) rat model of PD. In this model the dopamine pathway on one side of the brain is destroyed (in this study, a left nigrostriatal lesion). This causes the rats to turn away from the side of the lesion (from left to right) when injected with a dopamine-releasing drug. 'The 35 rats studied showed a good rotational response to apomorphine, but no response to L-dopa. After transduction with the *AADC* gene, animals showed a contralateral turning response to L-dopa that was significantly higher than that in control animals,' explains Bankiewicz. The improved response to L-dopa was observed 10–14 days after *AADC* transduction and persisted for at least eight weeks [4].

'This study demonstrates that AADC activity in the striatum was correlated with increased contralateral rotation, indicating that striatal neurons express the *AADC* transgene and are able to decarboxylate exogenous L-dopa to form dopamine,' says William Langston of The Parkinson's Institute (Sunnyvale, CA, USA).

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