COMMENTARY

Dilating the degradome: matrix metalloproteinase 2 (MMP-2) cuts to the heart of the matter

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With recent work revealing that MMPs (matrix metalloproteinases) cleave an increasingly large degradome of bioactive and signalling molecules, the dogma that MMPs are extracellular-matrix-remodelling proteases is under challenge. In this issue of the *Biochemical Journal*, Martínez et al. have reported that AM (adrenomedullin), a potent vasodilator predominantly expressed by blood vessel endothelial and smooth muscle cells, and microvasculature-rich tissues, is another new bioactive substrate for MMPs *in vivo*. Cleavage by MMP-2, but not MMP-9, generates a series of peptides; two of which retain receptor agonist and vasodilator activity, three are inactive and, excitingly, AM(11–22), a small product containing a canonical disulphide

loop, is a vasoconstrictor. In view of the robust vasodilatory and other cardiac protective activities of AM in inhibiting myocardial fibrosis this represents a potent new systemic role for MMP-2 in the cardiovascular system. Hence, the paper by Martínez et al. directly implicates MMP activity in the development of hypertension and paradoxically in stimulating myocardial fibrosis, therefore pointing to exciting new possibilities for utilizing MMP-2-specific inhibitors as a new mode to treat blood pressure and heart disease.

Key words: adrenomedullin, cardiovascular system, hypertension, matrix metalloproteinase-2 (MMP-2), myocardial fibrosis, vasoactive peptide.

Signalling circuits use proteolysis at key control points to activate prohormone or cytokine precursors, and to then inactivate these bioactive molecules to precisely regulate systemic, interstitial and extracellular environments. In the control of blood pressure the metalloproteinase angiotensin-converting enzyme and the aspartyl protease renin sequentially process pro-angiotensinogen to activate this potent hypertensive vasoactive peptide; big endothelin-1 is cleaved by MMP-2 (matrix metalloproteinase-2) to endothelin-1, which is also a potent vasoconstrictor; and natriuretic peptides are cleared by neutral endopeptidase. Together with adaptive changes in the extracellular-matrix compartments of the cardiovascular system, blood pressure is dynamically controlled. In this issue of *Biochemical Journal*, Martínez et al. [1] reveal a surprising direct intersection between blood pressure control and the MMPs, proteases that are considered to be the effectors of extracellular-matrix turnover. This report shows that MMP-2, but not its close relative MMP-9, controls blood pressure by the processing of the vasodilator peptide AM (adrenomedullin). At first active and then inactive products are produced, but also one cleavage fragment changes receptor specificity to function as a vasoconstrictor. With the detection of the cleaved products in human urine, it appears that AM is a biologically relevant substrate of MMP-2 *in vivo*. The work is significant in that it places MMP activity squarely in a central role in the control of blood pressure and so identifies a new drug target to treat hypertension.

Collagen was the first substrate to be identified for collagenase, the archetypal member of what would become known as the MMP family [2]. With subsequent discoveries that MMPs cleave other matrix components including laminin, fibronectin and proteoglycans *in vitro*, and the expansion of the family to 23 members [3,4], MMPs came to be thought of as critically important

proteinases in the turnover of extracellular matrix. Indeed, MMPs are proposed to be important contributors to the pathogenesis of cardiovascular disease by remodelling cardiovascular tissues in myocardial fibrosis, blood vessel wall thickening [5] and plaque rupture. Nonetheless, the dogma that the central role of MMPs is to cleave matrix proteins is becoming unstuck. It is usually stated that MMPs cleave all components of the extracellular matrix, when in fact probably less than 30 matrix proteins have actually been characterized as substrates *in vitro* with only two, collagen and aggrecan, directly shown to be cleaved *in vivo* [6]. In all but one case, MMP knockout mice show remarkably few differences in matrix turnover for enzymes deemed to be so important in these processes. Long-term clinical trials of MMP inhibitors to treat cancer were not only generally ineffective in preventing the spread of cancer through tissue and throughout the body, but also showed surprisingly little in the way of connective tissue side effects [3,7]. Furthermore, increasingly, new non-matrix substrates are being described that include signalling molecules, receptors and adhesion molecules, resulting in new biological roles for MMPs being recognized at an increasing pace. Hence, by the precise and limited processing of an expanding and diverse substrate repertoire, or degradome, that includes more and more bioactive molecules, such as chemokines, cytokines and receptors, MMPs profoundly control cell behaviour *in vivo* [4,8]. So, it is time to rethink the biological role of MMPs and to consider them to be potent proteases that control homeostasis of the extracellular environment at many levels, including regulation of cell function, growth and division; regulation of innate and acquired host defences; and control of the signals that orchestrate extracellular matrix synthesis. I consider these roles to surpass in importance the original function of MMPs as being mere effectors of extracellular-matrix turnover. In light

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of this, the study by Martínez et al. [1] is particularly timely for it points to new realms of interstitial and systemic homeostasis that fall under MMP control.

AM is synthesized as a 185-amino-acid precursor that is processed to an active 52-residue peptide with a six-member disulphide-constrained loop and an unusual amidated C-terminal tyrosine, both of which are essential for its activities in promoting vasodilation, natriuresis and diuresis (reviewed in [9]). Upon mechanical stretch and inflammatory cytokine stimuli, AM expression in vascular endothelial and smooth muscle cells exerts potent autocrine and paracrine vasodilatory and diuretic effects. Pleiotropic activities of AM also protect the myocardium by inhibition of extracellular-matrix production and thereby prevent cardiac tissue remodelling and fibrosis [10]. In addition, AM mediates the localized vasodilator component of inflammation that leads to tissue oedema.

Martínez et al. [1] report that MMP-2 specifically inactivates AM following a precise series of cleavages that generated six peptides. An initial two peptides retained vasodilator activity, but upon further processing this activity was lost. Until now, degradation by neutral endopeptidase was thought to be the major clearance mechanism of AM, as well as for the related atrial natriuretic peptide and kinins. The importance of the present study [1] is not only the new role of MMP-2 in the net inactivation of AM, but the interesting finding that one end product, AM(11–22), is a vasoconstrictor. Thus, rather than just a passive 'run down' of vasodilator activity by the inactivation of AM, a more potent control mechanism is evoked whereby MMP-2 generates a new vasoconstrictor. In homeostasis, the 'off' signal is as important as the 'on' signal, but this aspect of signalling is often overlooked and is not experimentally sought, because its importance is not as widely recognized as it should be. By the generation of antagonistic activities, either by use of different receptors and pathways to achieve biologically opposite effects, as is the case for AM, or by generating receptor antagonists, the conversion of an agonist into an antagonist is a robust and precise mechanism to achieve fine overall homeostatic control.

This is not the first time that MMP-2 has been found to play such an important role in signalling. The chemokine family of chemoattractant cytokines, which regulate the activation and trafficking of all leucocytic populations in innate and acquired immune responses, have recently been characterized as excellent substrates for MMPs, exhibiting higher kinetic rates of cleavage than many matrix molecules [11]. All MCPs (monocyte chemoattractant proteins) are cleaved and are inactivated by MMPs, and in all cases this generates receptor antagonists that dampen inflammation [11]. The localized induction of AM by inflammatory cytokines, such as IL-1 (interleukin-1) and TNF- α (tumour necrosis factor- α), results in the vasodilation that is characteristic and essential for inflammation. Thus it is likely that potent anti-inflammatory effects of MMP-2 will also be directed here to reduce vasodilation and oedema during termination of inflammatory responses. But what is equally intriguing in the paper [1] is that, whereas the two initial products of processing AM, AM(8–52) and AM(11–52), retain receptor binding and cAMP production, the vasoconstrictor peptide generated downstream by MMP-2 activity, AM(11–22), operates through a different receptor system, which is as yet unidentified. This too harkens back to chemokine processing where stromal-cell-derived factor-1 is cleaved and inactivated by MMP-2. In this case, CXCR4 (CXC chemokine receptor 4) binding is lost, and so the cleaved chemokine does not convert into an antagonist like the MCPs. Instead, the cleaved stromal-cell-derived factor binds an unidentified G-protein-coupled receptor that specifically and potently induces apoptosis in neurons leading to neurodegeneration in

HIV/AIDS dementia models *in vitro* and *in vivo* [12]. Thus the precise trimming of a bioactive signalling molecule of even a few residues by MMP-2 can reveal different receptor specificities in the processed ligand.

Although *in vitro* protease activities can be intriguing, it is another matter altogether as to whether these are biologically relevant substrates *in vivo*. Too often this assumption is reached too quickly. The fallacy of this premise was underscored in recent proteomic analyses of the biological activity of MT1 (membrane type-1)-MMP [8]. Fibronectin, a protein generally considered to be a very good substrate of MT1-MMP *in vitro*, was released from the plasma membrane without being cleaved in cell-based systems; therefore, just because a protease can cleave a protein does not mean it does. But how do you prove that a substrate is biologically relevant *in vivo*? The most elegant and direct method is to show the generation of the cleaved products *in vivo* [6,8]. Martínez et al. [1] utilized antibodies to detect probable cleavage fragments of AM in human urine, thereby showing that these products can be generated *in vivo*. Although urine has distinct advantages for such analyses, since many proteolytic events occur in the kidney and because MMP-2 is also present in urine, this is not absolute proof that the vaso-inactive products or vasoconstrictor fragment are generated in blood vessel walls where their activity is mediated. Although, the data are compelling, this requires additional confirmation, including the demonstration of these peptides in the rodent models of hypertension and their reduction upon administration of MMP-inhibitor drugs. Nonetheless, since AM regulates renal function, the generation of these peptides locally or systemically may also be important for regulating diuresis. This too requires further investigation.

Another intriguing aspect of the work by Martínez et al. [1] is that complement factor H, the AM-binding protein in serum, was discovered to specifically prevent the cleavage of AM. Most likely this is by steric blockade of protease access to the scissile bond in the bound complex or by allosteric rearrangement of the scissile bond region. The identification of this property for complement factor H clears up the mystery of its biological role and its mechanism of increasing the bioavailability of AM *in vivo*. Clearly, clinical assays for the quantification of AM levels in serum samples need to be modified to also measure the complement-factor-H-complexed fraction – doing so may reveal the clinical importance of altered AM levels in the control of blood pressure *in vivo* and so be useful for patient diagnosis and treatment monitoring.

An exciting therapeutic prospect stands out from the present study [1]. The loss of vasodilator activity through inactivation by MMP-2 and the concomitant generation of vasoconstriction effects with AM(11–22) indicate that MMP-2 inhibitors may be useful in the treatment of hypertension. Vasoepitidase inhibition of the renin–angiotensin system and neutral endopeptidase are potential new therapies for heart failure [13]. Therefore targeting MMP activity in the vasculature appears promising for maintaining local levels of AM with concomitant beneficial vasodilatory and renal effects. Moreover, by stabilizing the AM suppression of destructive extracellular matrix protein synthesis, MMP inhibitors may paradoxically protect against myocardial fibrosis. The effect of prolonging the half-life of AM would also be synergistic with prolonging the activity of the related and potent vasodilator, CGRP (calcitonin-gene-related peptide), which is degraded by MMP-2 [9]. This raises the intriguing possibility that other vasoactive peptides, such as atrial natriuretic peptide and amylin, may also be MMP substrates and so could also be stabilized by MMP inhibition. Thus retrospective analysis of MMP-inhibitor clinical trial data may reveal blood

pressure reduction in cancer patients who were also hypertensive.

In the light of recent evidence, we must now view MMPs as major controllers of homeostasis in the extracellular and systemic environments. By acting upstream in information pathways through the activation or decapitation of signal circuits, MMPs can profoundly control cell function, with the original role of MMPs as masters of connective tissue matrix turnover under question or at least representing just one minor aspect of the extracellular environment under the purview of MMP regulation. Hence, MMP involvement in blood pressure control [1] and neurodegeneration [12] are just two recent examples of MMP misactivity in perturbing signalling networks in the pathogenesis of diverse and important diseases. This raises exciting possibilities for revisiting MMPs as new drug targets with many more and diverse indications than previously considered based merely on controlling their potential tissue degradative activities in cancer and inflammatory diseases.

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