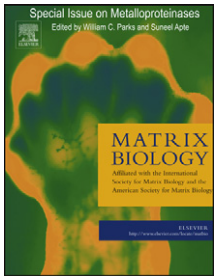
Review



Extracellular regulation of metalloproteinases

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Abstract

Matrix metalloproteinases (MMPs) and adamalysin-like metalloproteinase with thrombospondin motifs (ADAMTSs) belong to the metzincin superfamily of metalloproteinases and they play key roles in extracellular matrix catabolism, activation and inactivation of cytokines, chemokines, growth factors, and other proteinases at the cell surface and within the extracellular matrix. Their activities are tightly regulated in a number of ways, such as transcriptional regulation, proteolytic activation and interaction with tissue inhibitors of metalloproteinases (TIMPs). Here, we highlight recent studies that have illustrated novel mechanisms regulating the extracellular activity of these enzymes. These include allosteric activation of metalloproteinases by molecules that bind outside the active site, modulation of location and activity by interaction with cell surface and extracellular matrix molecules, and endocytic clearance from the extracellular milieu by low-density lipoprotein receptor-related protein 1 (LRP1).

Introduction

Matrix metalloproteinases (MMPs) and adamalysin-like metalloproteinases with thrombospondin motifs (ADAMTSs) degrade extracellular matrix (ECM) macromolecular components. Both MMPs and ADAMTSs are multi-domain proteinases, with the human genome containing 23 MMPs and 19 ADAMTSs. They are considered to play important roles in many physiological processes including embryonic development, morphogenesis, tissue remodelling, cell growth, migration and apoptosis [1,2] and pathological conditions such as inflammation, rheumatoid arthritis, osteoarthritis, cardiovascular diseases, nephritis, chronic wounds, pulmonary diseases, cancer and fibrosis [3]. The activity of these enzymes is tightly regulated by a number of mechanisms, and their activity is often not readily detected in steady state tissues. Expression is regulated transcriptionally by factors such as growth factors, hormones, inflammatory cytokines, UV irradiation, cell–cell and cell–matrix contacts, and the half-lives of their transcripts may be post-transcriptionally regulated by microRNAs [4]. Once translated, MMPs and ADAMTSs are produced as inactive zymogens (pro-MMPs). Many of the MMPs and ADAMTSs are secreted from the cell in this zymogen form, and are then activated extracellularly by other proteinases. The membrane-bound MMPs (MT-MMPs) and ADAMTSs are activated intracellularly by proprotein convertases such as furin. The activity of mature metalloproteinases is then regulated by endogenous inhibitors such as α_2-macroglobulin (α_2M) in blood plasma or body fluids and tissue inhibitors of metalloproteinases (TIMPs) in the tissue [5].

In addition to these well-recognized regulatory mechanisms, several secreted MMPs and ADAMTSs are known to bind to the ECM or to interact with specific cell surface molecules or receptors. More recently, some of the MMPs, ADAMTSs and TIMPs have been found to be very short-lived in the extracellular space, as they are rapidly endocytosed

by the cells that produce them. These new findings add further complexity to the regulation of metalloproteinase activity and ECM degradation. In this mini-review, we focus on the effect of allosteric activators on proMMP activation, and extracellular trafficking of MMPs and ADAMTSs.

Activation of proMMPs through allosteric sites

ProMMPs are kept in an inactive state by coordination of the catalytic zinc ion by the sulfhydryl group of the Cys residue in the conserved PRCGXPD sequence of the pro-peptide domain, called the 'cysteine switch' [6]. This interaction prevents H_2O from interacting with Zn^{2+} in the active site and thus blocks catalytic activity. Disruption of the cysteine switch Zn^{2+}-Cys interaction can activate proMMPs, and can be triggered by (i) step-wise cleavage of the prodomain by proteolytic enzymes (ii) oxidation of the cysteine switch by reactive oxidants or SH-reactive agents such as mercurial compounds and (iii) by structural perturbation by chaotropic agents and denaturants such as sodium dodecyl sulfate, low pH and heat treatment [7,8]. Hypochlorous acid generated by myeloperoxidase in macrophages and neutrophils has been shown to oxidatively activate and subsequently inactivate proMMP-1 and proMMP-7 [9,10]. S-nitrosylation also activates proMMP-9 [11].

In addition, a number of reports have shown that ECM components and cell surface molecules can promote proMMP activation. Binding of such components to allosteric sites on the proMMPs is thought to induce conformational changes that disrupt the cysteine switch. For example, insoluble elastin is able to bind to proMMP-2 and proMMP-9, probably through the three repeats of the fibronectin type II (FNII) domain that are attached to the catalytic domain. This interaction leads to auto-activation of proMMP-2, followed by autocatalytic inactivation of the mature enzyme [12]. However, elastin binding does not promote proMMP-9 activation, even when potential activator proteases are added [12]. ProMMP-9 can be allosterically activated by binding to gelatin, without removal of the pro-domain [13]. In both proMMP-2 and proMMP-9, the third domain of the three FNII repeats interacts with the pro-domain and the catalytic domain [14,15]. Gelatin, elastin and type IV collagen bind to these FNII domains, and may thus disturb the molecular interaction between the pro- and catalytic domains, facilitating the activation of proMMP-2 and proMMP-9.

ProMMP-7 can be activated on the cell surface by interaction with the C-terminal extracellular loop of the transmembrane tetraspanin CD151 [16] or with highly sulfated glycosaminoglycans (GAGs) [8]. Heparin, chondroitin-4,6-sulfate and dermatan sulfate augment intermolecular autolytic activation of proMMP-7, while heparan sulfate, less sulfated chondroitin sulfate and chondroitin-2,6-sulfate are ineffective.

Activation of proMMP-2 by MT3-MMP is enhanced by melanoma-specific chondroitin sulfate proteoglycan, leading to an increase in cell invasion in vitro [17]. The chondroitin sulfate chain of the proteoglycan binds to the catalytic domain of MT3-MMP and the hemopexin domain of proMMP-2. Activation can also be enhanced by the addition of isolated chondroitin-4-sulfate, but not by chondroitin-6-sulfate, hyaluronan or heparin. As with proMMP-7 activation, a specific sulfation pattern has been shown to be essential for MT3-MMP-mediated proMMP-2 activation.

A fraction of proMMP-9 secreted by the leukemic macrophage cell-line THP-1 is covalently linked to the core protein of a chondroitin sulfate proteoglycan. Activation of proMMP-9 in this complex is uniquely induced by Ca^{2+}, but not by mercurial compounds [18]. This involves sequential intramolecular release of the prodomain, cleavage of the proteoglycan core protein, truncation of a part of the hemopexin domain and release of active MMP-9.

The hemopexin domain of proMMP-9 can also bind to hemin and βhematin, which induces autoproteolytic processing of the pro-peptide and subsequent activation by MMP-3 [19].

Regulation by location

At the cell surface

Various mechanisms focus and modulate metalloproteinase activity in the extracellular environment (Fig. 1). For example, MMP-1 is considered to be a soluble collagenase, but it binds to the I domain of α2 integrins [20]. α2β1 integrin binds native collagen I with high affinity, clustering this integrin at contact points in migrating keratinocytes. MMP-1 binding to α2β1 integrin thus enables focal cleavage of collagen; weakening adhesion to the matrix and allowing keratinocyte migration [21]. MMP-1, collagen and α2β1 integrin thus coordinate together to drive and regulate keratinocyte migration during re-epithelialization. Similarly, proMMP9 has been shown to associate with α5 and β5 integrins and to co-localise with β5 at the leading edge of invading cells [22]. No specific effect on enzyme activity has been demonstrated in this case, but this is clearly an efficient localisation mechanism to focus enzyme function where it is required.

Emmprin (CD147, basigin) is a cell membrane MMP regulator originally identified as an MMP-inducer. It is a transmembrane glycoprotein with two Ig-like domains that interacts with a number of other membrane proteins, including caveolin, cyclophilin 60 and monocarboxylate transporters, as well as proMMP-1 and