Review

Metalloproteinases: role in breast carcinogenesis, invasion and metastasis

Michael J Duffy, Teresa M Maguire, Arnold Hill, Enda McDermott
and Niall O'Higgins

St Vincent's Hospital and University College Dublin, Dublin, Ireland

Abstract

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases. Their primary function is degradation of proteins in the extracellular matrix. Currently, at least 19 members of this family are known to exist. Based on substrate specificity and domain organization, the MMPs can be loosely divided into four main groups: the interstitial collagenases, gelatinases, stromelysins and membrane-type MMPs. Recent data from model systems suggest that MMPs are involved in breast cancer initiation, invasion and metastasis. Consistent with their role in breast cancer progression, high levels of at least two MMPs (MMP-2 and stromelysin-3) have been found to correlate with poor prognosis in patients with breast cancer. Because MMPs are apparently involved in breast cancer initiation and dissemination, inhibition of these proteinases may be of value both in preventing breast cancer and in blocking metastasis of established tumours

Keywords: breast cancer, carcinogenesis, invasion, matrix metalloproteinases, metastasis, tissue inhibitor of metalloproteinase

Introduction

The MMPs, which are also known as matrixins, are a family of structurally and functionally related endoproteinases that are involved in the degradation of the extracellular matrix (ECM). Physiologically, these enzymes play a role in normal tissue remodelling events such as embryonic development, angiogenesis, ovulation, mammary gland involution and wound healing. Abnormal expression appears to contribute to various pathological processes including rheumatoid arthritis and osteoarthritis, pulmonary emphysema, and tumour growth, invasion and metastasis (for review [1••]). Currently, at least 19 different MMPs are known to exist in mammalian systems.

The main characteristics of these proteinases have previously been described in detail [1••,2••], and are therefore only briefly mentioned here. All MMPs possess specific domains that are conserved between different members. Catalytic activity depends on the presence of zinc ions at the catalytic active site. Most MMPs are synthesized and secreted in a zymogen form. Activation is usually accompanied by loss of a 10-kDa amino-terminal domain. Most cleave at least one component of the ECM. Finally, proteolytic activity is inhibited by tissue inhibitors known as tissue inhibitors of metalloproteinase (TIMPs).

ADAM = α-disintegrin- and metalloproteinase-like; ECM = extracellular matrix; FGF = fibroblast growth factor; MMP = matrix metalloproteinase; MMTV = mouse mammary tumour virus; TACE = tumour necrosis factor-α-converting enzyme; TIMP = tissue inhibitor of metalloproteinase.

Subgroups of matrix metalloproteinases

Based on *in vitro* substrate specificity and domain structure, the MMPs have traditionally been divided into four main subgroups: the interstitial collagenases, gelatinases, stromelysins and membrane MMPs [1••,2••]. The collagenases comprise interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8) and collagenase 3 (MMP-13). These MMPs catalyze degradation of fibrillar forms of collagen (ie types I, II and III). MMP-1 shows a preference for the type III form, MMP-8 preferentially degrades type I collagen, and MMP-13 has highest affinity for type II collagen [3].

The gelatinases, which are also known as type IV collagenases, degrade gelatin (denatured collagen), and types IV, V, VII, IX and X collagen. Type IV collagen is particularly abundant in basement membranes, which are the membranes that separate organ parenchyma from the underlying stroma. Degradation of type IV collagen by gelatinases occurs within the triple helical regions. This subgroup has two distinct members, known as gelatinase A (MMP-2) and gelatinase B (MMP-9). Generally, these two gelatinases are thought to have similar substrate specificity with respect to ECM substrates, but may have different specificity toward growth factor receptors [4]. An example of the latter is the release of the soluble ectodomain of fibroblast growth factor (FGF) receptor-1 by MMP-2, but not by MMP-9.

The third subgroup of MMPs are the stromelysins (ie stromelysin-1 [MMP-3], stromelysin-2 [MMP-10], stromelysin-3 [MMP-11] and matrilysin [MMP-7]). The stromelysins have relatively broad substrate specificity, catalyzing degradation of many different substrates in the ECM [1••,2••]. The substrates include proteoglycans (core protein), noncollagenous proteins such as laminin, fibronectin and the nonhelical regions of collagen IV. Stromelysin-3, on the other hand, has not yet been found to degrade any matrix protein, but has been shown to hydrolyze the serine proteinase inhibitor α1-proteinase inhibitor [5]. It should be stated, however, that a carboxyl-terminal truncated form of mouse stromelysin-3 has been shown to exhibit weak stromelysin-like activities [6]. A further difference between stromelysin-3 and the other stromelysins is that stromelysin-3 is processed intracellularly by furin [7]. Thus, stromelysin-3 can be secreted as a potentially active protease. This intracellular activation distinguishes stromelysin-3 from most of the other MMPs, which are secreted as latent proteases and activated in the extracellular space. Because of its restricted substrate specificity and intracellular activation, it could be argued that stromelysin-3 represents the first member of a new MMP subgroup rather than being the fourth member of the stromelysin family.

The fourth subgroup consists of the membrane-type MMPs, which possess a transmembrane domain [8]. Five members of this group have been described, the best characterized species being membrane-type 1 MMP. This MMP has been shown to catalyze activation of progelatinase A [9], to degrade a variety of ECM substrates [9] and to function as a fibrinolytic enzyme in the absence of plasmin [10]. As with stromelysin-3, the membrane-type MMPs posses a consensus domain that is recognized by a furin-like enzyme.

The ADAMs (a disintegrin and metalloproteinase like) are a group of molecules that are related to the MMPs. The ADAMs share some or all of the following domains: a signal peptide, a propeptide, a MMP domain, a disintegrin domain, a cysteine-rich region, an epidermal growth factor-like sequence, a transmembrane region and a cytoplasmic tail (for review [11••]). Currently, 23 members of the ADAM family are known to exist, and at least three of these (ie ADAM-10, -12 and -17) have been shown to possess proteinase activity [11••]. Unlike the MMPs, little work has been done to address the role of ADAMs in cancer.

Inhibitors of matrix metalloproteinases

Four endogenous specific inhibitors of MMPs have been described: TIMP-1, -2, -3 and -4 [2••,12•]. The TIMPs inhibit protease activity by forming high-affinity 1:1 stoichiometric, noncovalent complexes with the active MMPs. In addition to binding to the active form, TIMP-1 can complex with pro-MMP-9, whereas TIMP-2 binds to the precursor form of MMP-2 [2••]. The complexes with the precursor forms involve the carboxyl-terminal domains of both the TIMPs and the MMPs. In contrast to the MMPs, at least one of the ADAMs (tumour necrosis factor-α-converting enzyme [TACE]) is not inhibited by TIMP-1, -2 or -4 [13]. TACE activity, however, is blocked by TIMP-3 [13].

Some TIMPs appear to act as multifunctional molecules. Thus, in addition to inhibition of MMP activity, TIMP-1 and TIMP-2 can stimulate cell proliferation, at least *in vitro* [2••,12•]. Furthermore, although both TIMP-1 and TIMP-2 have been found to inhibit apoptosis [14,15], TIMP-3 was shown to promote this process [16].

Role of matrix metalloproteinases in breast cancer

Tumour initiation and growth

It is generally believed that the key genes involved in breast carcinogensis are c-oncogenes such as c-*erbB-2*, c-*myc*, *ras*, some members of the *ets* family, and tumour suppressor genes such as *p53* and *Rb* [17•]. Recent evidence, however, suggests that certain MMPs may also play a role in breast cancer initiation and growth. Indeed some of the c-oncogenes may contribute to tumourigenesis by regulating the expression of MMPs. For example, transfection of MCF-10A breast cancer cells with either c-*erbB-2* or c-*ras* resulted in increased expression of MMP-2 [18], whereas transfection of MCF-7 cells with the *ets* gene, *PEA-3*, led to increased production of MMP-9 [19].

Evidence that implicates MMPs in breast cancer genesis and/or growth is as follows. Overexpression of stromelysin-1 in transgenic mice gave rise to preneoplastic and malignant mammary gland tumours [20,21]. Transfection of MCF-7 cells with stromelysin-3 constructs resulted in increased tumour take after subcutaneous injection into nude mice [22]. In the latter situation, overexpression of stromelysin-3 did not appear to either modify cell proliferation or confer an invasive phenotype on the breast cells. Inactivation of the stromelysin-3 gene led to decreased chemical-induced tumourigenesis in mice [23]. Transgenic mice expressing matrilysin under the control of mouse mammary tumour virus (MMTV-long terminal repeat promoter/enhancer) developed premalignant hyperplastic nodules [24]. Mating MMTV–matrilysin mice with MMTV–*neu* transgenic mice resulted in offspring that developed mammary tumours substantially earlier than MMTV–*neu* controls. Administration of batimastat (a synthetic inhibitor of MMPs) reduced the rate of tumour formation in mice injected with MDA-MB-435 breast cancer cells [25]. Finally, overexpression of TIMP-4 in the same cell line reduced tumour growth [26].

Possible mechanisms by which MMPs contribute to cancer initiation or to tumour cell growth include promotion of angiogenesis, activation of stimulating growth factors or their receptors, and inactivation of inhibiting growth factors.

Stimulation of angiogenesis
Angiogenesis is necessary for a tumour to grow to a size greater than approximately 2 mm in diameter. The process begins with local degradation of the basement membranes that surround capillaries, followed by invasion of the surrounding stroma by the underlying endothelial cells in the direction of the angiogenic signal. Endothelial cell migration is accomplished by cell growth at the leading edge of the migrating column. The endothelial cells then organize themselves into three-dimensional structures to form new capillary tubes (for review [27••]).

MMPs may promote angiogenesis by at least two different mechanisms: by degrading barriers and thereby allowing endothelial cell invasion; and by liberating factors that promote or maintain the angiogenic phenotype [28•]. An example of the latter is the degradation of the ECM protein laminin-5 by MMP-2, which results in enhanced mammary epithelail cell growth [28•]. Similarly, both MMP-1 and MMP-3 have been shown [28•] to breakdown endothelial-derived perlecan, releasing basic FGF, a potent endothelial mitogen.

It is important to point out that, although clear evidence exists that MMPs potentiate angiogenesis, these proteases also have the potential to inhibit this process. For example, a number of MMPs, such as MMP-3, -7, -9 and -12, can degrade plasminogen, generating the angiogenesis inhibitor angiostatin [28•]. Another potent inhibitor of angiogenesis is endostatin, which is a breakdown product of collagen XV111 [28•]. It is presently unknown whether MMPs play a role in generating endostatin.

Activation of growth factors and their receptors
It was mentioned above that MMP-1 and MMP-3 can release the endothelial cell mitogen basic FGF, which is bound in the ECM. MMP activity, however, may also release mitogens for epithelial cells. For example, a member of the ADAM family, known as TACE, has been shown to cause cell-shedding of transforming growth factor-α, tumour necrosis factor-α and other growth factors [29]. In a further study [30], MMP inhibitors were shown to reduce cell proliferation in direct proportion to their effect on transforming growth factor-α release. An MMP-like protease also appears to be responsible for the cleavage of the ectodomain of c-*erbB-2* [31]. The release of this amino-terminal sequence may lead to enhanced signalling by the residual membrane-associated oncoprotein [31]. The increased signalling may in turn lead to enhanced tumour cell proliferation. Also, as mentioned above, MMP-2, but not MMP-9, has been shown [4] to release the ectodomain of FGF receptor 1. Because the hydrolyzed ectodomain retains its ability to bind FGF, it has the potential to modulate the mitogenic and angiogenic activities of FGF. Finally, stromelysin-3 has been shown [23] to promote growth of MCF-7 cells by liberating ECM-associated growth factors.

Degradation of inhibitory growth factors
Although degradation of inhibiting growth factors by MMPs could theoretically lead to increased cell proliferation, there is currently no evidence for this mechanism.

Matrix metalloproteinases and metastasis
The evidence that links MMPs with invasion and metastasis is now extensive and has been widely reviewed [1••, 2••]. Consequently, the following discussion focuses only on the role of these proteinases in the spread of breast cancer.

Evidence that implicates MMPs in breast cancer dissemination is as follows. Batimistat reduced both lung colonization and spontaneous metastasis of a highly malignant rat mammary cancer [32]. In mouse mammary cancer cell lines, inhibition of stromelysin-1 by antisense oligodeoxynucleotides prevented invasion of an artificial basement membrane [33]. The ratio of active to latent form of MMP-2 increased with tumour progression in invasive breast cancers [34]. Transfection of TIMP-4 into the invasive human breast cancer cell line MDA-MB-435 reduced invasion in an *in vitro* model system [26]. Finally, overexpression of TIMP-2 in MDA-231 cells reduced osteolytic lesions after injection of these cells into nude mice [35].

Mechanisms by which matrix metalloproteinases promote invasion and metastasis

It is generally assumed that the primary mechanism by which MMPs promote cancer spread is by degradation of the ECM, which consists of two main components: basement membranes and interstitial connective tissue. Although collagen IV is the main component of basement membranes, other proteins such as laminin, proteoglycans, entactin and osteonectin are also present in this structure. To establish metastatic growth, cancer cells must pass through basement membranes at least three times. Breast cancer cells initially cross these membranes when an *in situ* carcinoma becomes an invasive lesion. Later, malignant cells transverse these structures during both entry into and exit from the blood stream.

The collagen IV component of basement membranes is thought to be degraded mostly by MMP-2 and MMP-9. These MMPs may therefore play a critical role in the conversion of *in situ* breast cancers to invasive lesions. Early work by Barsky *et al* [36] found that 'type IV collagenase immunoreactivity' was present in all of 25 invasive breast cancers, but not in any *in situ* malignancy.

In contrast to the acellular basement membrane, the interstitial connective tissue is composed of cells distributed in a meshwork of collagen fibres, glycoproteins, proteoglycans and hyaluronic acid. The main forms of collagen found here are types I, II and III. During cancer dissemination, the interstitial connective tissue is believed to be broken down mainly by the interstitial collagenases and some of the stromelysins.

A frequent site of breast cancer metastasis is to bone, where the presence of cancer cells upset the balance between bone resorption and bone formation, resulting in net bone loss. Thus, the main effect of breast cancer metastasis in bone is degradation, which appears to be primarily mediated by osteoclasts [37]. This osteoclast-induced bone resorption is also catalyzed by MMPs [37], although the specific MMPs that are involved have not yet been identified.

Matrix metalloproteinases as prognostic markers in breast cancer

The early data that implicated MMPs in metastasis [38•] were based on correlations between levels of specific MMPs and metastatic potential in model systems. Over 10 years ago, we originally proposed [39] that proteinases causally involved in experimental metastasis might be markers of metastatic potential or prognosis in human cancers.

Presently, high levels of two MMPs (ie MMP-2 and stromelysin-3) have been found [2••,40] to correlate with poor outcome in patients with breast cancer. Neither of these MMPs has thus far been shown to be prognostic in axillary node-negative breast cancer patients, however.

Paradoxically, high levels of both TIMP-1 and TIMP-2 have also been shown [2••,41,42] to predict adverse outcome in breast cancer patients. Similar results have also been found with plasminogen activator inhibitor-1, an inhibitor of urokinase-type plasminogen activator [43]. These findings suggest that certain endogenous proteinase inhibitors, rather than preventing metastasis, may potentiate the process.

Matrix metalloproteinases as targets for breast cancer prevention and antimetastatic therapies

The data from model systems, reviewed above, suggest that MMPs are involved in most phases of carcinogenesis from initiation to metastasis. Inhibition of these proteinases might thus lead both to prevention of cancer development and to inhibition of dissemination. Because of this potential, considerable research in recent years has focused on different approaches to block the actions of MMPs.

Two main types of MMP inhibitor exist: the TIMPs and low-molecular-weight synthetic inhibitors [2••,10]. Because of their protein nature and multiplicity of actions, it is unlikely that TIMPs will be widely used as anticancer molecules. Because of this, most research in recent years has focused on the synthetic inhibitors. Many of these are peptides and are similar to the cleavage site in collagen [44]. Inhibition is effected by a zinc-binding group that is adjacent to the P1′ position. Some of the zinc-binding groups that are currently being investigated in model systems include the hydroxyamates, carboxylates, amino carboxylates and sulphydryls [44,45]. Some of these inhibitors (eg the hydroxyamates) are presently undergoing clinical trials in patients with advanced cancers [45,46]. We are unaware of any studies so far in human breast cancer, however.

Although MMP inhibitors are currently being evaluated in patients with metastatic cancers, there are still many unanswered questions concerning the use of these compounds. Some of these are as follows.

Is it better to use a broad spectrum or specific matrix metalloproteinase inhibitor? In order to answer this question, it will be necessary to establish which are the MMPs whose involvement in the different phases of cancer progression is critical.

If the action of MMP inhibitors is blocking of MMP activity only, these compounds may not induce the type of tumour shrinkage that is seen with the traditionally used cytotoxic agents. Conventional approaches that are used to assess tumour regression may thus not be possible. A novel approach taken to address this issue has been to monitor the rate of rise in levels of serum tumour markers [46]. The use of these tests in phase 2 trials has shown a dose-dependent decrease in rate of rise after treatment with the MMP inhibitor Marimastat (British Biotech, Oxford, UK)

[45]. Furthermore, this decreased rate of marker rise appeared to correlate with extended patient survival [46].

Because MMPs have functional overlap with other proteases (eg plasmin), it is unclear whether blockage of the MMPs alone will prevent cancer initiation or progression. Preliminary data from model systems [47] suggest that arrest of invasion will require inhibition of plasmin as well as of the MMPs.

Do MMPs inhibitors promote apoptosis? The primary substrates of MMPs are generally thought to be the ECM components. As mentioned above, however, some MMPs also mediate cell shedding of membrane-bound growth factors. Recently, two different synthetic MMP inhibitors (BB-3103 and A-151011) were shown to induce apoptosis in Ewing's sarcoma cell lines [48], apparently by inhibition of a MMP-like enzyme that releases membrane-bound fas ligand. One of the endogenous inhibitors of MMPs, TIMP-3, has also been shown [15] to cause apoptosis. Both TIMP-1 and TIMP-2, on the other hand, have been shown [14,15] to suppress apoptosis.Clearly further work is necessary to clarify the role of MMP inhibitors in programmed cell death.

Conclusion

There is now strong evidence from model systems to suggest that MMPs are involved in both tumour initiation and progression. In these systems, administration of MMP inhibitors can prevent cancer cell growth as well as inhibit invasion and metastasis. Use of MMP inhibitors in humans has so far been limited to patients with advanced disease. Theoretically, it might be expected that the main anti-cancer benefit of these compounds would be in the adjuvant treatment setting (eg in combination with tamoxifen or chemotherapy for breast cancer). Finally, with the recent findings that MMPs are also involved in cancer initiation, MMP inhibitors could also be considered for evaluation as cancer chemopreventive molecules.

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Authors' affiliations: Department of Nuclear Medicine, St Vincent's Hospital, and Department of Surgery and Conway Institute of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland

Correspondence: Dr M J Duffy, Department of Nuclear Medicine, St Vincent's Hospital, Dublin 4, Ireland. Tel: +353 1 209 4378; fax: +353 1 269 6018; e-mail: Michael.J.Duffy@ucd.ie