Recent advances in understanding the roles of matrix metalloproteinases in tumour invasion and metastasis

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Short title: Matrix metalloproteinases and tumour invasion

Key words: extracellular matrix, matrix metalloproteinase, metastasis, microenvironment, protease, tumour invasion, tumour progression

Word count: 5363

Conflict of Interest. GAC declares no conflict of interest, GIM is a scientific advisor to Vertebrate Antibodies (www.vertebrateantibodies.com).

Abstract

This review aims to provide an overview of recent developments regarding the roles of matrix metalloproteinases in tumour invasion and metastasis. Much of the mortality burden belonging to cancer relates to its ability to invade adjacent tissue and form metastases at distant sites. This would not be possible without remodelling the extracellular matrix, a process which is enabled by the functions of matrix metalloproteinases. Recent studies provide a better understanding of the importance of the biophysical nature of the extracellular matrix, how this influences cancer cell motility, and how MMPs act to modify matrix stiffness. The regulation of matrix metalloproteinases and the role of immune cell generated matrix metalloproteinases has also become better understood. All of this provides a framework for the therapeutic targeting of matrix metalloproteinases and recent advances in the development of selective matrix metalloproteinases inhibitors are also reviewed.

Introduction

One of the hallmarks of a malignant tumour is its capacity to invade surrounding tissue, both locally, at the site of the primary lesion, as well as at distant sites to form metastatic foci. In carcinoma, epithelial cells assume the characteristics of mesenchymal cells and begin to traverse the extracellular environment. Supported by host endothelial cells, fibroblasts, and immune cells, tumour cells manipulate the microenvironment to optimise their potential for growth and motility. Once motile and within the vasculature, tumour cells must optimise the metastatic niche for colonisation.[1,2] Essential to this sequence are the matrix metalloproteinases (MMPs): a group of zinc-dependent endopeptidases involved in extracellular matrix (ECM) degradation and remodelling.[3–5]

In health, they are crucial during inflammation and the repair of tissues following injury, as well as organogenesis. However, critically, modification of the ECM (a structural framework comprising collagen, elastin, fibrillin, proteoglycans, glycosaminoglycans and other proteins [6]) is also required by cancer cells in order to invade tissues locally and at distant metastatic sites. Together, the MMPs are capable of degradation of most, if not all the protein components of the ECM and basement membrane. In this manner, the MMPs are important molecules in the complex systems that regulate tumour invasion and metastasis, as well as proliferation, differentiation and cell death.

Historically, their nomenclature was rather complicated, owing to gradual identification and elucidation of the various MMPs' structures, functions and cellular locations, and with several MMPs not expressed in humans. However, most broadly, the MMPs were usually categorised as collagenases, stromelysins, gelatinases,

membrane-type or other miscellaneous type based on substrate specificity (table 1, figure 1). Current convention identifies individual MMPs by number, rather than by substrate because it is clear that most MMPs act on multiple different substrates. Furthermore, MMP-4, MMP-5, MMP-6 and MMP-22 are not recognised as unique gene products, but actually are identical to existing MMPs. In total, 23 MMPs are recognised in humans. Their action is regulated by the interplay of the four naturally occurring tissue inhibitors of MMPs (TIMP-1, TIMP-2, TIMP-3 and TIMP-4).[7,8] In general, MMPs comprise a signal peptide, pro-peptide and a catalytic domain. All MMPs apart from MMP-23 and the matrilysins (MMP-7 and MMP-26) have a linkage hinge region between their catalytic domain and a haemopexin domain. The gelatinases (MMP-2 and MMP-9) have a fibronectin repeat insertion in their catalytic domain. Common to the entire family of proteins is a cysteine switch within the pro-peptide region, which interacts with the catalytic zinc ion and the zinc-binding region of the catalytic domain.[9] The MMPs are synthesised as zymogens, with regulated activation. The pro-peptide region, which confers latency to the MMPs, is removed in a stepwise manner by the action of tissue and plasma proteinases often in addition to the action of other active MMPs; MMPs can activate other MMPs or indeed they may autocatalyse, cleaving themselves once partially activated. A number of the MMPs, including MMP-14 and other membrane type MMPs (MT-MMPs), contain a consensus furin cleavage sequence within the pro-peptide and are thus activated by intracellular serine proteinases.[10] As well as a purely structural role in ECM breakdown, many of the MMPs also affect molecular pathways through the release of cytokines from the ECM as a result of its degradation. There is also some recent work suggesting nuclear functions for a number of MMPs.[11,12] Nuclear localised MMP-7 has been demonstrated in cells at the invasive edge of prostate cancer and

nuclear MMP-14 in hepatocellular carcinoma cells has been associated with larger tumours and poorer survival.[13,14] In colonic adenocarcinoma, nuclear localisation of a non-catalytic isoform of MMP-3 is associated with proliferation, migration and metastasis of adenocarcinoma cells.[15]

This review will outline the recent advances in the biology of MMPs with regard to their roles in tumour invasion and metastasis including their enzymatic role in the remodelling of the extracellular matrix, but also: their influence on cellular molecular signalling; interaction with the immune system; regulatory mechanisms and potential therapeutic manipulation.

Membrane-type MMPs

The MT-MMPs, a subgroup of six MMPs in humans, exert their action once localised to the cell surface. MMP-14, MMP-15, MMP-16 and MMP-24 are transmembrane proteins, with hydrophobic transmembrane domains and short cytoplasmic domains, whereas MMP-17 and MMP-25 are bound to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. The cytoplasmic domain is important in a number of non-proteinase functions of the transmembrane MT-MMPs, including localisation to invadopodia and interaction with hypoxia-inducible factors (HIFs), discussed in more detail later.[16] Phosphorylation of the tyrosine and threonine residues of the cytoplasmic tail mediates activation of intracellular signalling pathways, such as ERK1/2, Akt and Rac1, which are important in tumour cell proliferation and migration.[17,18] For this reason, MMP inhibitors which target only the proteinase function of MMPs are likely to lack clinical efficacy.

The MT-MMPs are fundamental in the process of ECM breakdown and also contribute to proteolytic protein processing. Key amongst the MT-MMPs, MMP-14 is widely expressed in cancer cells and cancer associated stromal cells. Accordingly, understanding its function has been of great interest.[19,20] Recent research, has identified in detail the role MMP-14 in cancer cell invasion and metastasis, as well as the role of the other MT-MMPs.

MMP-14

The function of MMP-14 and its role in tumour invasion and metastasis has been widely studied however, recent investigations have now provided more detail about the molecular processes that regulate the action of this crucial enzyme. Epithelial-mesenchymal transition (EMT), the process by which cancer cells begin showing mesenchymal characteristics, with expression of mesenchymal genes and reorganisation of the cytoskeleton to enable motility, is considered a major step in cancer cell metastasis. A key part of this process is the trafficking of proteins that interact with the ECM to the surface of tumour cells. Apart its well-established role as an enzyme involved in breakdown of the ECM, MMP-14, like many other MMPs, is known to interact with cell signalling mechanisms by both the proteolytic activation of extracellular molecules, such as transforming growth factor β (TGF-β) as well as the release of molecules sequestered in the ECM, such as vascular endothelial growth factor (VEGF). [21] Furthermore, it has been known for some time that MMP-14 activates MMP-2 and recently this process has been described in more detail.[22] The accepted model begins with dimerization of cell surface MMP-14 via the haemopexin and transmembrane regions. The catalytic region of one of the MMP-14 molecules binds the N-terminal domain of TIMP-2 and the exposed C-terminal domain binds proMMP-2. The second, non-TIMP-2 bound MMP-14 molecule is then

able to cleave the pro-peptide domain of proMMP-2, ultimately leading to its activation.[16] This process is significant in tumour invasion because MMP-14 cannot degrade type IV collagen within the basement membrane whereas MMP-2 does have this capability.[23]

In order to migrate through ECM, cells must assemble invadosomes and lamellae at their leading edge. These are membrane structures with protrusive actin elements, which permit actin-myosin forces to propel the cell. Invadopodia are part of the invadosome family and MMP-14 containing vesicles, formed in the Golgi apparatus, are directed along F-actin filaments to the surface of invadopodia, where MMP-14 becomes functional. Recent studies have provided detail about the regulation of this process. Localisation of MMP-14 to the cell surface is controlled, in part, by the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE proteins), a family of proteins involved in the fusion of adjacent plasma membranes.[24] The SNARE protein syntaxin 4 is involved in the formation of invadopodia and its activity is regulated by Munc18c.[25] Phospholipase D2 (PLD2), which generates a signalling lipid, phosphatidic acid, and binds to KIF5B, also enables surface localisation of MMP-14. Increased surface localisation of MMP-14 and association with invadopodia enables local invasion of tumour cells. Knockout of PLD2 inhibits lung metastasis in a mouse model of breast cancer and it is therefore a promising target for therapeutic modification.[26] In vitro, knockdown of N-WASP, an invadosome component that promotes trafficking of MMP-14 to invadopodia, abolishes invadopodia formation and lung metastasis in breast cancer cells.[27] Recent experiments using gels of varying matrix stiffness show that an increase in ECM stiffness increases the density of invadopodia on tumour cells and consequently, increases ECM degradation.[28] However, a balance must be

achieved between ECM degradation and invadopodia penetration because excessive ECM degradation actually destabilises invadopodia and impairs motility. Therefore, cancer cells must finely tune the spacing of invadopodia in order to optimise the creation of pores for movement.[28]

One of the non-ECM-degradative functions of MMP-14 is the modulation of Eph receptor tyrosine kinases, such as EphA2. EphA2 is expressed on the cell surface of normal epithelial cells and upon ligand binding, by the ephrin family of proteins, it exerts a downstream influence on maintaining cell morphology and suppressing cell growth.[29] The N-terminal portion of EphA2 is required for ligand induced activation. However, in human tumour xenografts in mice, MMP-14 appears to cleave the N-terminal portion of EphA2, ultimately increasing the growth, migration and metastasis of cancer cells.[30] Without ligand induced activation, EphA2 cannot suppress Ras and Akt as usual. Instead, ErbB-receptor activation of Akt phosphorylates EphA2 and leads to the recruitment of pro-migratory signalling molecules.[31]. This is compounded by the fact that MMP-14 actively promotes pro-oncogenic ErbB signalling.[30]

Another recently discovered non-collagenase function of MMP-14 is inhibition of the oxygen-dependent suppression of HIFs. The cytoplasmic tail of MMP-14 binds to factor-inhibiting HIF-1 (FIH-1). Munc18-1 interacting protein 3 (Mint3), which is normally involved in synaptic vesicle fusion with the plasma membrane, binds to furin and thus localises adjacent to the cytoplasmic tail of MMP-14. The N-terminal portion of Mint3 competitively inhibits the binding of FIH-1 with HIFα.[32] In this way, MMP-14 expression within cancer cells leads to elevated HIF activity, enabling increased metabolism of glucose and promoting angiogenesis. This novel finding regarding

MMP-14 demonstrates that MMP-14 has pro-oncogenic functions outside its well documented role in motility and invasion of cancer cells.[33]

There is recent evidence that MMP-14 may be, at least partly, under the regulation of p53. A p53 response element was found within at the -nt66 to -nt59 region of the MMP-14 promoter, where p53 competitively binds with the transcription factor Sp1, which is known to positively regulate MMP-14.[34–36] Deletion of the p53 response element prevents p53 mediated suppression of MMP-14. Moreover, it was shown that IL-6 down-regulates p53 protein levels, enhancing MMP-14 expression, underlining the role that inflammatory cytokines have in mediating oncogenesis.[37]

Other membrane-type MMPs

MMP-14 is widely expressed in cancer cells, cancer-associated fibroblasts, endothelial cells and immune cells, thus it has an established role in tumour cell invasion and metastasis in many tumour types.[19,20,38–41] However, less has been identified about the role of the other membrane-type MMPs on tumour progression, perhaps largely due to the fact that these MMPs are less widely expressed.[16,42] Recent studies have identified the role of these MMPs in tumour invasion.

In vivo, in colorectal cancer cells, inactivation of MMP-15 by lentiviral mutation renders the tumour cells epithelial-like, whereas those expressing wild-type MMP-15 retain their more invasive, mesenchymal-like phenotype.[43] Fragments of E-cadherin were immunoprecipitated from the medium of MMP-15-expressing human ovarian cancer cells. In contrast, inactivation of MMP-15 by either drug or mutation, suppresses cleavage of E-cadherin. In addition, MMP-15 was found to degrade ZO-1, a molecule involved in the formation of tight junctions.[43] Through cleavage of E-

cadherin and disruption of ZO-1, MMP-15 renders cancer cells less cohesive, increasing their invasive potential.

In melanoma cells, MMP-16 cleaves MMP-14, diminishing its collagenolytic activity and causing tumours to exhibit a more expansile, nodular pattern of growth, rather than invasive. Nests of melanoma cells become surrounded by bundles of collagen, but curiously this actually drives the malignant cells into adjacent lymphatics. In this instance, reduced invasion through the ECM, mediated by MMP-16, is actually associated with more aggressive disease.[44]

Regulation of MMPs

The action of MMPs is dependent on the complex interplay of various molecular systems. MMPs not only act as proteolytic enzymes, but also by responding to and generating the release of active signalling molecules at the cell surface from precursors. Constitutive expression of MMPs is low and only under certain physiological (such as embryogenesis or tissue repair) and pathological circumstances (such as neoplasia or arthritis) is MMP transcription induced. In the case of neoplasia, there are several signalling molecules that are particularly significant regulators of MMP activity.

TGF-β

In health, TGF-β suppresses cell proliferation by arresting the cell cycle at G1, but through mutation cancer cells may lose their susceptibility to regulation by TGF-β. Recent work has shown that in cancer cells, TGF-β may actually promote tumour progression due to its immunosuppressive and angiogenic functions, as well as its

ability to stimulate production of the dense, fibrotic stroma surrounding tumour cells, known as desmoplasia.[45] This matrix stiffening triggers the EMT in cancer cells.[46] MMP-2, MMP-9, MMP-13 and MMP-14 are all capable of activating TGF-β by solubilising ECM-bound TGF-β. MMPs are known to stimulate EMT in kidney, ovary, lung, pancreas and prostate cells by way of TGF-β activation.[47]

MMP-3, MMP-9, MMP-7 and MMP-15 have all been shown to induce EMT in various cell types through E-cadherin degradation and now it is clear that their activation is at least partially under the control of TGF-β.[48] MMP-14 catalysis proteolytically activates TGF-β and modulates its activity through the release of ECM-bound TGF-β-binding protein 1.[49] In this manner, MMP-14 expressing tumour cells can trigger EMT in nearby cells through the paracrine action of TGF-β. This action is distinct from MMP-14's role as a collagenase. A recent study showed that stromal expression of MMP-14 alone was capable of driving cell invasion, demonstrating the important influence of cancer associated stromal cells on tumour invasion.[50] Motile, mesenchymal-like tumour cells reverted back to an immotile epithelial phenotype when the stromal MMP-14 regulated activation of TGF-β was inhibited, despite endogenous expression of MMP within the tumour cells.[21]

Thrombospondin-2

Thrombospondin-2 (TSP-2) is a matricellular glycoprotein and is known to regulate cell proliferation, angiogenesis, cell adhesion and ECM remodelling.[51,52] In some cancers, expression of TSP-2, which exerts an anti-angiogenic function, is associated with improved prognosis.[53–55] However, in oral squamous, prostate, and non-small cell lung cancer (NSCLC), overexpression has been shown to confer a worse prognosis.[56–58] In the case of lung cancer, TSP-2 promotes cell migration

and invasion, doing so by integrin-αvβ3-mediated signal transduction of focal adhesion kinase (FAK) and protein kinase B (Akt).[56] FAK/Akt transduction causes NF-κB to bind to the promotor region of the MMP-13 gene, increasing transcription and subsequently cell motility.[56]

In prostate cancer, MMP-2 increases cell motility and invasion. Here, TSP-2 binds to both αvβ3 and CD36. This leads to phosphorylation of the mitogen activated protein kinase (MAPK) pathway molecules: p38, ERK and JNK. MAPK activation down-regulated the expression of the micro-RNA, miR-376c, which leads to increased expression of MMP-2 and increased cell motility both in vivo and in vitro.[58]

CD97

CD97 is a G-coupled protein receptor, a member of the epidermal growth factor-7 transmembrane proteins and is known to play a role in regulating cellular adhesion and cell-ECM interaction in a number of cancer types including: gastric, thyroid, oesophageal, pancreatic, brain and oral SCC. In hepatocellular carcinoma, CD97 cell-surface overexpression by tumour cells suppresses G-coupled protein receptor kinase 6 (GRK6), which increases expression of MMP-2 and MMP-9. This in turn promotes EMT and is associated with poor prognosis. In vivo, CD97 promotes tumour metastasis. CD97, which is dependent on interaction with CD55 in order to lead to downstream signalling, is usually internalised following activation by the binding of β-arrestin-1, preventing over-stimulation. However, in HCC, it was found that aberrant internalisation, due to disruption of GRK6-mediated arrestin binding, leads to overexpression of CD97 and increased secretion of MMP-2 and MMP-9.[59]

ST6Gal-I

The β-galactoside, α2-6-sialyltransferase 1 (ST6Gal-I), is overexpressed in many cancer types including breast, hepatocellular, colon and lung. In NSCLC, it was demonstrated recently that downregulation of ST6Gal-I leads to impairment of signalling by Notch1 and subsequently decreased protein levels of MMP-2, MMP-7, MMP-9 and VEGF. This reduces proliferation, migration and invasion of NSCLC cells in vitro. In vivo, in a mouse model, ST6Gal-I suppresses lung cancer growth.[60]

Biophysical properties of ECM and regulation of MMPs

The firmness of solid tumours when compared to normal healthy tissue is, in part, due to the stiffness of the ECM. Stiffness is defined as the extent to which a material resists deformation in response to an applied force, and is synonymous with rigidity.[61] The physical nature of the ECM itself is known to determine the growth and invasion of tumours. Increased matrix stiffness, determined primarily by type I collagen deposition and cross-linking, is a common feature of most types of carcinoma.[62] Stiffer ECM has been shown to upregulate MMP-14 activity, promoting tumour growth by supporting angiogenesis: both matrix invasion by new vascular growth and the branching of new blood vessels.[63] The angiogenic switch has been used to describe the activation of unregulated vascular proliferation in tumours and this has long been regarded as a fundamental hallmark of many neoplasms.[64] MMPs also play a role in regulating the biophysical properties of cancer cells themselves. Cancer cells interact physically with the ECM by way of integrins, which transmit forces to the actin cytoskeleton.[65] The complex array of cell-ECM proteins has been termed the adhesome.[66] Extracellular MMP proteolytic

activity has been shown to modulate integrin β1, with subsequent remodelling of the cytoskeleton, and an increase in cell spreading, motility, contractility and cortical stiffness. Signalling molecules, generated by the breakdown of the ECM by MMPs, stabilise membrane integrins and activate focal adhesion kinase, vital for cell adhesion, proliferation, survival, migration and invasion.[67,68]

Motile cells detect the stiffness of the ECM through their adhesions formed by actin-based protrusions. Actin-myosin interaction subsequently generates the force that enables motility. Mathematical modelling predicts that cells move optimally through ECM of intermediate stiffness and it has been shown that tumour cells actively remodel the microenvironment in order to increase its stiffness to a threshold that enables motility.[69] This remodelling of the ECM is enabled by MMPs, which leads to the aligning of collagen fibres, increasing cell-ECM adhesions, which in turn increases contractility in a parallel direction and polarises spheroid cells.[70,71] It has been shown that there is a “critical-stiffness” at which cell-cell adhesions are overcome and tumour cells undergo EMT.[71]

MMPs, cancer and the immune system

Tumours instigate an immune response and the role of the immune system in the natural history of tumours has been highlighted with the efficacy of immunomodulatory therapy in a number of cancers.[72–74] Recent evidence implicates natural killer (NK) cell, neutrophil and monocyte derived MMPs in contributing to tumour invasion and metastasis.

NK cells are cytotoxic lymphoid cells that have an anti-metastatic function separate to the MHC-mediated T-cell pathway. In colorectal cancer, NK cells assume an aberrant decidual-like phenotype, secreting MMP-2, MMP-9, TIMP-1 and TIMP-2 and proangiogenic factors. This stimulates angiogenesis and remodels the ECM to favour neovascular growth. Several studies have shown that angiogenesis and microvessel density are associated with worse prognosis in colon cancer.[75,76] Other immune cells, such as monocytes, perform a similar function. A proangiogenic subset of monocytes (CD16+) migrate to the site of tumours following chemokine gradients, namely CCL2, CCL3 and CCL5.[77,78] Once there, they secrete MMP-9 and this is associated with an increase in proangiogenic vascular growth factors, including VEGF-A, believed to be released following breakdown of the ECM. [79]

It is known that in the case of many types of solid tumours, there may be small numbers of circulating tumour cells (CTCs) in the blood, but most of these are inconsequential in terms of metastasis because they lack the ability to move through the extracellular microenvironment and remodel it in their favour.[80,81] In the case of any single tumour, the associated CTCs are not necessarily phenotypically identical, with some CTCs demonstrating the characteristics of having undergone EMT. In breast and prostate cancers, higher levels of expression of MMP-1 and MMP-2 respectively are associated with both more biologically aggressive primary tumours, as well as increased likelihood of successful metastasis by CTCs.[82,83]

Those cancer cells that do implant and begin to grow at a distant site must also evade immunosurveillance cells, such as NK cells. B7-H6 is a cell surface ligand that usually activates the activating-NK cell receptor, NKp30, and triggers NK cell-mediated cell death. However, metalloproteinases expressed by cancer cells are able to shed B7-H6 from the cell surface, causing it to become a soluble ligand,

which suppresses anti-tumour immunity, reducing the recognition of cancer cells by NK cells.[84] Inhibition of MMPs leads to increased surface levels of B7-H6 and greater NK-cell-mediated cell death. [85,86]

CD11b and CD15 expressing circulating tumour associated neutrophils (TANs) are also able to reduce the expression of NK cell activating ligands, namely CD69, on the tumour cell surface.[87] Indeed, TANs are now recognised as having multiple roles in enabling cancer cell metastasis.[88] Neutrophils produce MMP-8 and MMP-9, which collectively degrade collagen I, II III and IV, priming the ECM and basement membrane for invasion by cancer cells.[89,90] MMP-9 is also proangiogenic (due to its ability to release ECM-bound vascular endothelial growth factors [79]) and, as previously mentioned, the tumour vascular network not only fuels the metabolism of a growing tumour, but also provides the opportunity for entry into the vascular system and subsequent metastasis. Furthermore, in a breast cancer mouse model, TNFα, produced by cancer cells, causes chemokine receptor type 2 and C-X-C motif chemokine receptor 2 production by stromal cells, which attracts neutrophils to the site of the tumour. Neutrophils are then able to induce MMP-12 and MMP-13 production by the tumour cells, which, like MMP-8, also degrades type I, II and III collagen, in addition to elastin.[91] Later in the metastatic timeline, in a mouse model of colon cancer, MMP-2-expressing fibrocytes and MMP-9-expressing neutrophils were found to be essential to the establishment of liver metastases.[92]

Macrophages, primarily the pro-reparative M2 subtype, have been shown to increase the speed of migration and persistence of direction of movement of cancer cells through 3D assays. It was demonstrated that tumour associated macrophages release TGF-β1, which up-regulates MMP-14 expression, increasing cancer cell migration, both by increasing levels of integrin adhesions and by remodelling of the

ECM. In addition, TNFα and TGF-β1 secretion by macrophages up-regulates MMP-1 secretion by cancer cells. Increased MMP-1 secretion leads to increased cancer cell migration persistence through efficient breakdown of type I collagen, more so than MMP-14.[93] In the model described, MMP-14 determined cancer cell migration speed and MMP-1 determined directed cancer cell migration.

Anti-tumourigenic roles of MMPs

The failure of broad spectrum MMP inhibitors to provide clinical benefit in the treatment of cancers is perhaps the best indication that MMPs do not function solely as pro-oncogenic molecules. Understanding which MMPs are partially or primarily anti-tumourigenic will help inform future more specific MMP inhibitors. Perhaps the most well characterised anti-tumourigenic MMP is MMP-8, but those with putative anti-tumourigenic roles also include MMP-3, MMP-9, MMP-12, MMP-16 and MMP-26.[94]

In oral tongue squamous cell carcinoma (OTSCC), MMP-8 expression is tumour suppressive, reducing invasion and migration of OTSCC cells in a mouse tongue cancer model. MMP-8 reduces expression of the tumour promoting factors, MMP-1 and VEGF-C, by preventing TGF-β1 activation. Treatment with exogenous TGF-β1 overcomes this impediment.[95] Similarly, in breast cancer, MMP-8 is tumour suppressive, increasing adhesion of myoepithelial cells to the ECM and reducing invasion. This appears to be a key mechanism by which myoepithelial cells suppress tumour growth in breast, with loss of myoepithelial cells being a key feature of invasive breast cancer. It has recently been shown that over-expression of MMP-8 reduces TGF-β signalling in myoepithelial cell lines and reduced cell invasion in 2D

and 3D assays. In contrast, MMP-8 knock-down enhances cancer cell invasion. MMP-8 is expressed by normal breast myoepithelial cells and its expression is reduced in DCIS. It has been shown that expression of MMP-8 is even lower in the myoepithelial cells surrounding DCIS with concomitant invasive disease in the same breast, indicating that MMP-8 is key to the anti-invasive function of myoepithelial cells in breast cancer. [96] In a mouse model of breast cancer, MMP-8-null status accelerates tumour growth and increases the rate of lung metastasis. This is purported to be due to pleiotropic effects including promotion of angiogenesis, reduction of MMP-3 expression and reduced innate immune cell activity.[97]

In oesophageal squamous cell carcinoma cells, MMP-16 is downregulated in tumour cells versus healthy controls. What is more, downregulation of MMP-16 is correlated with higher rates of metastasis and poorer 5-year survival in a clinical cohort of patients with oesophageal squamous cell carcinoma. In contrast, MMP-14 and MMP-15 are both overexpressed in oesophageal cancer cells and their overexpression is correlated with tumour aggressiveness and increased tumour size. MMP-16 blocks G1/S transition in the cell cycle, arresting oesophageal cancer cells in the G1 phase by the upregulation of p21 and p27, thus preventing proliferation. Therefore MMP-16 that appears to be another MMP that may prevent tumour growth and metastasis.[98]

Recent advances in the development of MMP inhibitors

Given the central role of MMPs in tumour progression there has been considerable interest the development of anti-MMP therapies. There are even attempts to utilise the function of MMPs in tumours to activate nano-particles and deliver targeted

therapy to the site of the tumour.[99] Early attempts with broad spectrum MMP inhibitors were unsuccessful due in part to poorly designed trials, lack of knowledge of MMPs, and lack of drug specificity. High profile failures of early clinical trials of broad spectrum MMP inhibitors led to a considerable hiatus in MMPs being considered as therapeutic targets. However, a number of recent studies have provided a much greater understanding of the roles of MMPs in tumour invasion and metastasis and broader roles in cancer biology, which has led to a re-evaluation and renewed interest in MMPs as therapeutic targets.

Moreover, it is now clear that some MMPs may suppress tumourigenesis and their inhibition may promote tumour progression. That is why recently more narrow-spectrum MMP inhibitors have shown some promise.

Therapeutic targeting of MMP-2 and MMP-9

Therapeutic manipulation of MMPs targeting specific MMPs rather than broad spectrum MMP inhibitors should be expected to be more effective. The catalytic domains of the MMP proteins are highly conserved, but the haemopexin domains vary between the members of the group. The haemopexin domain of MMP-9 has been shown to interact with CD44 and integrin-α4β1on the surface of cells in order to activate EGFR-MAP kinase intracellular signalling and enhance invasion of cancer cells. The compound, N-(4-fluorophenyl)-4-(4-oxo-3, 4, 5, 6, 7, 8-hexahydroquinazolin-2-ylthio)butanamide, also known as, “3c,” was developed as a specific inhibitor of MMP-9 homodimerisation and it specifically targets the haemopexin domain of MMP-9. In doing so, it prevents MMP-9 interaction with cell surface molecules and thus blocks downstream activation of FAK and paxillin, molecules known to influence tumour growth, invasion and migration.[100]

In retinoblastoma (Rb) cell lines, specific inhibition of MMP-2 and MMP-9, using ARP100 and AG-L-66085 respectively, reduces secretion of TGF-β1, reducing cell migration in a highly metastatic subtype of Rb. In a less metastatic subtype of Rb, combined reduction of TGF-β1 and VEGF reduces angiogenesis and cell viability.[101]

Specific inhibition of MDA-9/syntenin, a highly conserved PDZ domain-containing scaffolding protein, by the drug, PDZ1i, was found to radio-sensitise glioblastoma cells by preventing EGFR activation of FAK signalling. This results in decreased secretion of MMP-2 and MMP-9. In vivo, this leads to smaller, less invasive tumours and the benefit was compounded in conjunction with radiotherapy, leading to significant survival benefit.[102]

Interestingly, in a recent study in a mouse model of pancreatic ductal adenocarcinoma (PDAC), systemic MMP-9 inhibition was not shown to be beneficial. MMP-9 is overexpressed in PDAC, but systemic genetic ablation of MMP-9 paradoxically leads to larger, more invasion tumours, with more abundant stroma. Systemic MMP-9 ablation prevents physiological shedding of stem cell factor (SCF) in the bone marrow. SCF, a cytokine which binds to CD117 and is involved in differentiation of haematopoietic cells, can stimulate production of IL-6.[103] In turn, IL-6 activates STAT3, increasing transcription of cMet, VEGFa, Car9, Hif1a, Vimentin and Icam-1; the net effect of which is enhanced tumour cell proliferation, survival, migration, invasion and angiogenesis.[104]

Therapeutic targeting of MMP-14

MMP-14 is a pivotal enzyme in the process of cancer cell invasion and metastasis. So for this reason, there is considerable interest in developing inhibitors of this MMP.

S100A4 is a calcium binding protein, correlated with more invasive cholangiocarcinoma, the action of which appears to be dependent on nuclear importation. A recent study found that in a mouse model of cholangiocarcinoma, inhibition of S100A4 with the drug, paclitaxel, a microtubule stabilising agent, is associated with a reduction in MMP-14 expression and MMP-9 secretion, due to reduction of RhoA and Cdc42 GTPase activity. This decreased lung metastasis, but did not affect primary tumour proliferation.[105]

In breast cancer cell lines, downregulation of MMP-14 decreases the invasiveness of cells and prevents radiation-induced enhancement of invasiveness. It is known that some triple-negative breast cancers may be cured by radiotherapy, whereas others recur following radiation, often with metastases.[106] By considering MMP-14 as a biomarker of poor response to radiotherapy, detection of MMP-14 in breast cancer cells, perhaps by using fluorescence microscopy, may inform treatment strategies.[107] Radiotherapy, in combination with an MMP-14 inhibitor, might confer improved survival on a subset of patients. Upstream targeting of MMP-14 activity might also prove beneficial as it has been shown in breast cancer cells that blockage of Pi3K-AKT dependent β-catenin accumulation prevents upregulation of cyclin D1, c-Myc, COX-2, MMP-7, MMP-14, and claudin-1, reducing invasion and migration.[108]

A promising development in the field of MMP inhibitors is monoclonal antibody MMP inhibitors. Some anti-MMP antibodies not only show specificity in the MMP that they target, but may also selectively target specific MMP functions. For example, a mouse monoclonal antibody that targets a surface epitope of MMP-14 is capable of inhibiting collagenolytic MMP-14 activity, whilst having little effect on the activation of proMMP-2.[109]

There are numerous challenges in creating potent and highly selective MMP inhibitory antibodies, not least the fact that inhibiting MMP by binding their active region, requires physical access to a region of the molecule which is often concave and physically inaccessible to human immunoglobulin. One method to overcome this has been to incorporate camelid antibody regions, which contain long complementarity-determining region-H3 regions encoding convex paratopes.[110] This design is based on the structure of TIMP2 the physiological inhibitor of MMP-14.[111] Recently, by screening a human Fab fragment library an inhibitory antibody has been isolated that incorporates a convex camelid-like paratope and is therefore able to access the convex pocket of proteinase.[110] A recombinant inhibitory human IgG Fab fragment has been developed which selectively inhibits murine MMP-14. It was shown to dramatically reduce tumour growth and metastasis in a mouse model of breast cancer.[112]

The naturally occurring inhibitor TIMP-2 has provided the starting point for the design of other selective MMP inhibitors. Using yeast surface display technology, fluorescently labelled catalytic domains of MMP-14 and MMP-9 can be used to identify mutant TIMP-2 that has greater specificity for either one MMP or the other.[113] The mutant TIMP-2 molecules inhibit the catalytic function of MMP-14 and MMP-2 in vitro, and reduce cell migration in a breast cancer cell line.[114] Using next generation sequencing, it should be possible to find many new antibodies with inhibitory function against specific MMPs.[115]

Conclusions

The roles of MMPs in cancer cell biology are diverse. The recent findings reviewed here provide more detail about the factors controlling their regulation, as well as their interaction with immune cells, stromal cells and the ECM (figure 2). There is overwhelming evidence that they are key effector molecules with which tumour cells remodel their microenvironment and undergo EMT. Lately, better understanding of the biophysics of this process implicates the MMPs in almost every stage of the process. The evidence that MMP inhibitors would make effective therapeutic targets is compelling and the focus on more specific inhibitors, rather than broad spectrum inhibitors is appropriate given the evidence that not all MMPs confer a pro-oncogenic effect in tumour progression. However, most research in this area has evaluated the benefit of MMP inhibitors on the progression of already well-established tumours. In reality, the clinical value of MMP inhibitors would seem most likely to be found in the case of early stage cancer, perhaps in a neoadjuvant setting, and in combination with radiotherapy or surgical intervention.

Statement of author contributions

Both authors conceived, drafted and revised the manuscript and figures.

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Table 1.The classification, nomenclature, cell type expression, chromosomal location and substrates of human MMPs

MMP group	MMP	Nomenclature	Cell type expression	Chromosomal location	Substrates
Collagenases	1	Interstitial collagenase	Fibroblasts, epithelial cells	11q22-q23	Collagens I, II, III, VI, VII, VIII, IX, X; gelatin; aggrecan; L-selectin; IL-1beta; proteoglycans; entactin; ovostatin; MMP-2
	8	Neutrophil collagenase	Neutrophils, macrophages, epithelial cells, fibrocytes	11q21-q22	Collagens type I, II, III, V, VII, VIII, X; gelatin; aggrecan; fibronectin
	13	Collagenase-3	Fibroblasts, myofibroblasts	11q22.3	Collagens type I, II, III, IV, IX, X, XIV; gelatin; plasminogen; aggrecan; perlecan; fibronectin; osteonectin; MMP-9
	18	Collagenase-4			
Gelatinases	2	Gelatinase-A 72 kD type IV gelatinase	Epithelial cells, endothelial cells, fibroblasts, fibrocytes, myofibroblasts	16q13	Gelatin type I, II, III; collagen type I, III, IV, V, VI, VII, X; fibronectin; elastin
	9	Gelatinase-B 92kD type IV gelatinase	Epithelial cells, fibrocytes, leukocytes	20q11.2-q13.1	Gelatin type I, V; collagen type I, III, IV, V, VII, X, XIV; entactin; aggrecan; elastin; fibronectin; osteonectin; plasminogen; MBP; IL-β1
Stromelysins	3	Stomelysin-1 Procollagenase	Fibroblasts, myofibroblasts	11q23	Collagen type III, IV, V, IX; gelatin; aggrecan; perlecan; decorin; laminin; elastin; casein; osteonectin; ovostatin; entactin; plasminogen; myelin basic protein IL-β1; MMP-2/TIMP-2
	10	Stromelysin-2	Macrophages	11q22.3-q23	Collagen type III, IV, V; gelatin; casein; aggrecan; elastin; MMP-1; MMP-8
	11	Stromelysin-3	Fibroblasts	22q11.2	Casein

Membrane-type MMPs	14	MT1-MMP	Fibroblasts, epithelial cells, macrophages	14q11-q12	Collagen type I, II, III; gelatin; MMP-2; casein; fibronectin; laminin; vitronectin; entactin; proteoglycans; MMP-2; MMP-13
	15	MT2-MMP	Epithelial cells	16q13-q21	Fibronectin; entactin; laminin; aggrecan; perlecan; MMP-2
	16	MT3-MMP	Epithelial cells, fibroblasts	8q21	Collagen type III; gelatin; casein; fibronectin; MMP-2
	17	MT4-MMP	Leukocytes	12q24.3	
	24	MT5-MMP	Basal bronchial cells, epithelial cells	20q11.2	Fibronectin
	25	MT6-MMP	Leukocytes	16p13.3	Progelatinase A
Others	7	Matrilysin 1 (PUMP-1)	Epithelial cells, macrophages, monocytes, fibrocytes	11q21-q22	Collagen type IV, X; gelatin; aggrecan; decorin; fibronectin; laminin; elastin; casein; transferrin; plasminogen; myelin basic protein; β4-integrin; MMP-1; MMP-2; MMP-9; MMP-9/TIMP-1
	12	Macrophage elastase	Macrophages, epithelial cells	11q22.2-q22.3	Collagen type IV; gelatin; elastin; casein; fibronectin; vitronectin; laminin; enactin; myelin basic protein; fibrinogen; fibrin; plasminogen
	19	Rheumatoid arthritis-associated	Fibroblasts, myofibroblasts	12q14	Collagen type I
	20	Enamelysin	Ameloblasts, odontoblasts	11q22.3	Amelogenin; aggrecan; cartilage oligomeric matrix protein
	21	Partially identified from human ovary cDNA	Embryonic cells, epithelial cells, fibroblasts	1p36	
	23a	Cysteine array matrix MMP	Embryonic cells, ovary, heart, lung	1p36.33	
	23b	Femalysin	Ovary, heart	1p36.3	
	26	Matrilysin 2	Endometrium	11p15	Collagen type IV; fibronectin; fibrinogen; casein; proMMP-9
	28	Epilysin	Keratinocytes, testis, lung, adipocytes, colon	17q21.1	Caesin

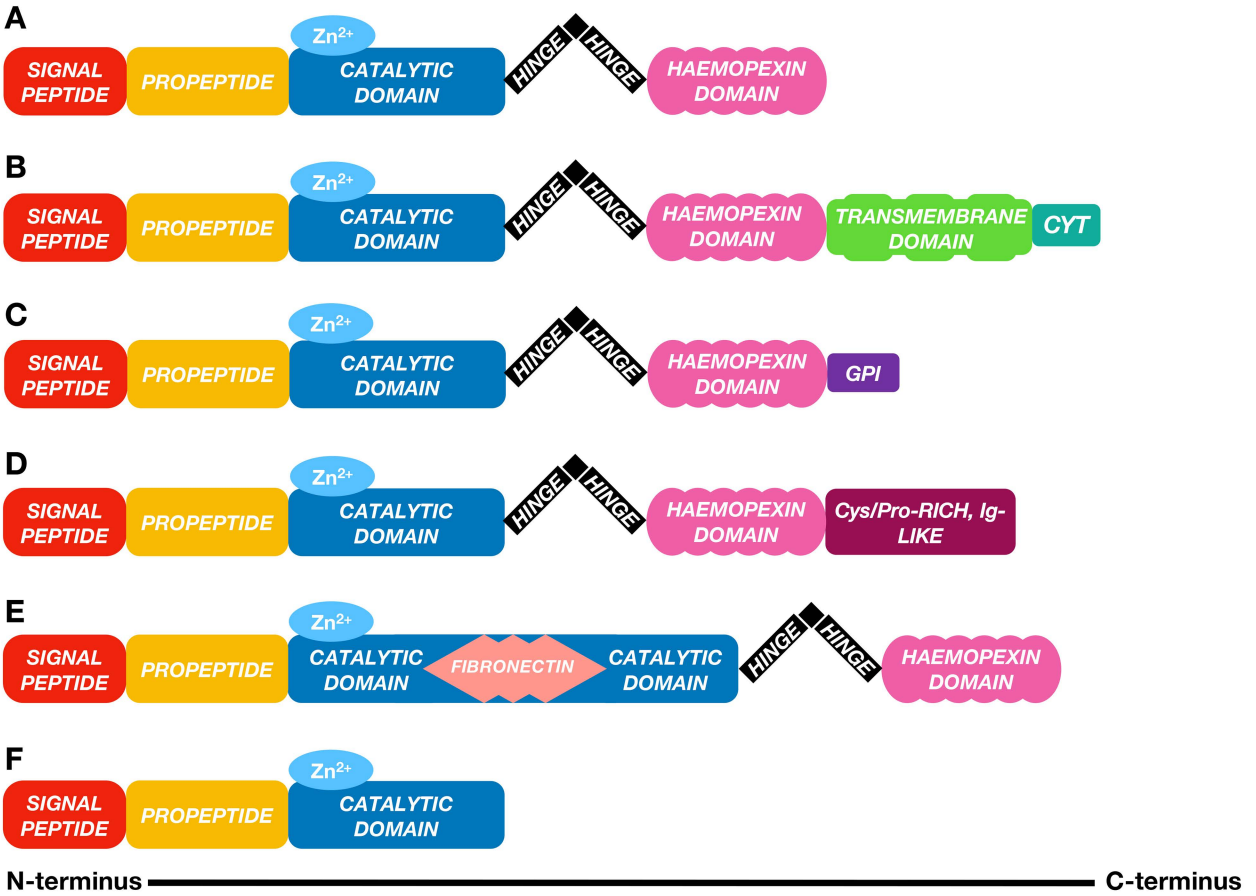
Figure legends

Figure 1

The domain structure of MMPs: A = simple haemopexin domain containing MMPs (MMP-1, MMP-3, MMP-8, MMP-10, MMP-12, MMP-13, MMP-19, MMP-20, MMP-22); B = transmembrane MMPs, with a C-terminal transmembrane insertion and cytoplasmic domain (CYT) (MMP-14, MMP-15, MMP-16, MMP-24); C = GPI anchored membrane MMPs (MMP-17, MMP-25); D = cysteine/proline rich, immunoglobulin-like domain MMP (MMP-23); E = gelatin binding MMPs, containing a fibronectin type 2-like insertion (MMP-2, MMP-9); F = minimal domain MMPs (MMP-7, MMP-26).

Figure 2.

An overview of the interactions between key MMPs, immune cells, tumour cells and stromal cells involved in the process of ECM degradation, invasion and metastasis.


A
SIGNAL PEPTIDE
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Zn2+
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HAEMOPEXIN DOMAIN
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SIGNAL PEPTIDE
PROPEPTIDE
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HAEMOPEXIN DOMAIN
TRANSMEMBRANE DOMAIN
CYT
C
SIGNAL PEPTIDE
PROPEPTIDE
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Cys/Pro-RICH, Ig-LIKE
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SIGNAL PEPTIDE
PROPEPTIDE
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FIBRONECTIN
CATALYTIC DOMAIN
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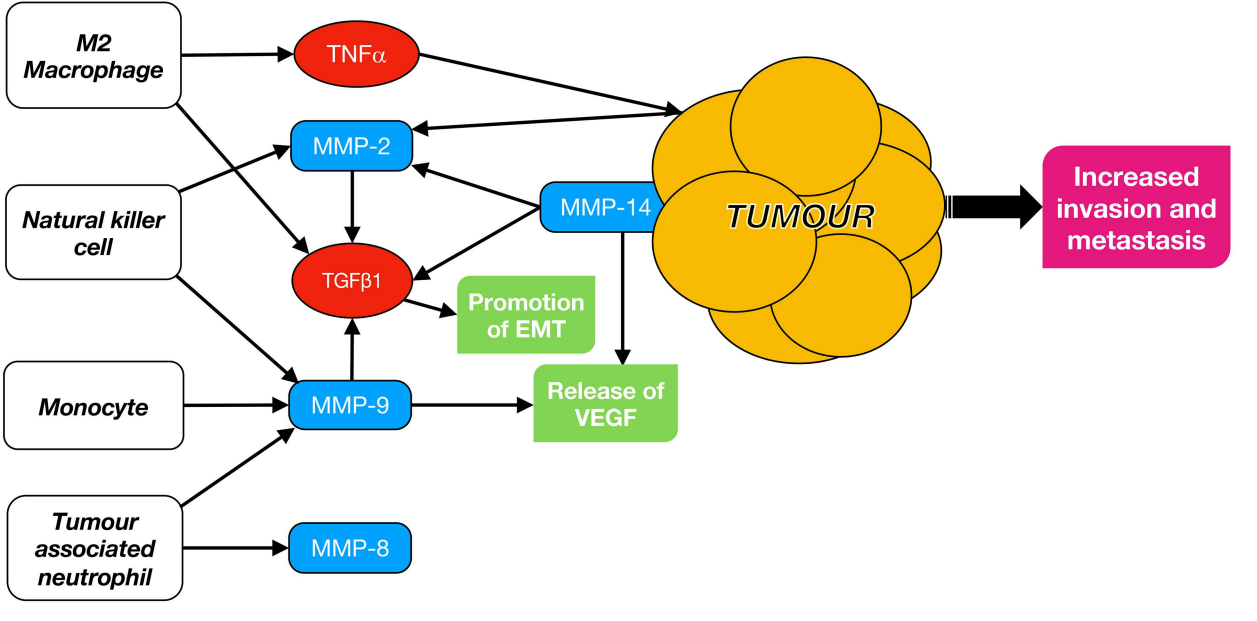
PROPEPTIDE
Zn2+
CATALYTIC DOMAIN
N-terminus
C-terminus


M2 Macrophage
TNFα
MMP-2
Natural killer cell
MMP-14
TUMOUR
Increased invasion and metastasis
TGFβ1
Promotion of EMT
Monocyte
MMP-9
Release of VEGF
Tumour associated neutrophil
MMP-8