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Matrix metalloproteinases: roles in cancer and metastasis

Barbara Fingleton

Dept of Cancer Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

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1. ABSTRACT

The matrix metalloproteinase (MMP) family of extracellular proteinases play roles in normal physiological processes as well as in multiple disease settings including cancer. The link between MMP activity and cancer was considered strong enough to warrant considerable investment in pharmacological inhibitors of MMPs as a potential therapeutic modality, however, multiple large-scale clinical trials have all failed to reach their primary endpoints. This has led us to re-evaluate our thinking with respect to MMPs in cancer and shown that, most importantly, we need to understand the range of functions of these enzymes before we can effectively modulate them. The MMPs contribute to every stage of tumor progression, not just the later stages as was originally assumed. Additionally, through processing of their various substrates, MMP activity can have both pro- and anti-tumorigenic functions, thus their broad inhibition is likely to have unwanted consequences in some settings. Interactions between MMPs and proteinases of other classes are another important aspect of tumor biology and understanding these interactions is also necessary for development of effective therapeutic strategies. The aim of this article is to summarize recent findings in these areas and put them in the context of our growing understanding of how MMPs function in cancer development and progression.

2. INTRODUCTION

The term cancer describes a set of insidious diseases in which mutant cells within the body use normal physiological processes in dysregulated ways to result in profound pathology. The matrix metalloproteinase (MMP) family of enzymes, although often associated with tumor progression and metastasis, represents a group of proteins that evolved to perform physiologically relevant functions. For example, the significant tissue remodeling that occurs as an embryo develops, the spatially and temporally controlled remodeling necessary for efficient wound repair, and the carefully controlled cleavage leading to activation or deactivation of potent signaling molecules in the immune system are all events dependent on MMP function. In this article, we will focus on the various contributions of MMPs to tumor progression which arise when such normal functions are hijacked by tumors as well as how MMP activity can have important anti-tumor effects. In addition, we will explore one of the mechanisms behind the increased levels of MMPs seen in many tumors. It is important to realize that MMPs are only one family within a large group of proteolytic enzymes and activities of the various different proteinase classes often affect each other. Hence we will examine some of the ways in which proteinases from different families can interact with the MMPs and how this may impact cancer and metastasis.

Table 1. *In vivo*–verified substrates of MMPs

MMP	Substrate	Reference
MMP-2	Macrophage chemoattractant protein-3	93
	Laminin	94
	Fibronectin	94
MMP-3	Entactin	95
MMP-7	Fas Ligand	96
	E-cadherin	97
	Pro alpha-defensins	98
	RANKL	99
	TNF-alpha	100
	Syndecan-1	101
	HB-EGF	102
MMP-8	LIX	48
MMP-9	alpha-1-proteinase inhibitor	72
	Collagen IV alpha3	31
	Kit ligand	30
	VEGF/VEGFr signaling	24
	Plasminogen	32,33
MMP-13	Type II collagen	11,12
	Aggrecan	12
MMP-14	Type I collagen	7
	PDGF-B/PDGFRbeta	36
	Pro-MMP-2	7
MMP-20	Amelogenin	103

Finally, we will briefly analyze the multiple failed clinical trials of pharmacological inhibitors of MMPs for cancer and what this means for future therapeutic strategies. As there have been many review articles covering the topics of MMPs and cancer [for example see (1-3)], here we are not providing a comprehensive survey of the field but rather focus on placing some of the most recent information in context. Additionally, a series of articles accompanying this one give detailed descriptions of MMP structure and function, MMP regulation and the roles of MMPs in multiple disease settings.

3. POSITIVE AND NEGATIVE CONTRIBUTIONS OF MMPs TO TUMOR PROGRESSION

3.1. MMPs and Invasion

The originally described tumor-associated role for members of the MMP family, specifically the type IV collagenases or gelatinases now usually known as MMPs-2 and -9, was in degradation of the basement membrane (4). Basement membrane is a specialized extracellular matrix (ECM) surrounding epithelial tissue compartments. Observation of this ECM-degrading activity led to the hypothesis that the primary function of MMPs in cancer was in tumor cell invasion. As the definition of malignancy is the ability to invade, this identified MMPs as prominent molecules in tumor biology. There is a vast array of literature describing enhanced invasive activity of multiple tumor cell lines or tumor tissue when MMPs are over-expressed [for review see references (5,6)]. Conversely, decreased invasive capacity of tumor cells was observed when the endogenous inhibitors of MMPs known as tissue inhibitors of metalloproteinases (TIMPs) were expressed. In many cases this apparent invasive function was also interpreted as enhanced metastatic capability, hence the particular association of MMPs with invasion and metastasis, which are late stages of tumor progression. Of course metastasis is much more than the ability to invade. It also involves survival in the circulation, arrest at secondary sites and, most importantly, growth at the secondary site. The assumption that a tumor cell with the ability to invade is automatically a metastasis-competent cell is therefore false and this suggests that some of the early literature linking MMPs with metastasis should be interpreted with caution.

The vast majority of literature describing invasive functions of MMPs is based on studies conducted *in vitro*. Based on the results of such *in vitro* analysis, the MMP family has been described as being able to degrade all components of the ECM. However, proteolytic activities seen *in vitro* are not always apparent in the more complex *in vivo* setting. Indeed results from *in vivo* analyses using transgenic, null or pharmacologically manipulated animals have thus far yielded a more limited ECM substrate spectrum (Table 1). Degradation of fibrillar collagens however is a specialized task for which several members of the MMP family (MMPs-1, 8, -13, -14) are competent. The most severe phenotype of any MMP-deficient animal produced to date occurs in the MMP-14 animal and is significantly related to the inability of this animal to adequately remodel collagen-containing matrices (7,8). In studies using various normal and tumor cell lines, the ability to produce activated cell-associated MMP-14 has been identified as the rate-limiting factor for whether invasion through collagen-containing ECM can occur (9). In fact the inability to degrade fibrillar collagen can prevent the outgrowth of a tumor cell mass as the collagen acts as a physical barrier to expansion (10). Mice deficient in MMP-13 also show collagen remodeling defects, particularly related to endochondral ossification (11,12). It may be anticipated that tumor studies in either the MMP-13 or-14 deficient animals will demonstrate striking tumor-associated phenotypes, however to date these have not been reported.

An important debate over the true contribution of MMPs to tumor cell invasion was stimulated by the publication of an article by Friedl and colleagues illustrating the phenomenon of "ameboid movement" when proteinase activity is inhibited pharmacologically (13). The data from the Friedl studies would suggest that both *in vitro* and *in vivo*, tumor cells change their mechanism of movement through ECM from predominantly proteinase-dependent invasion to proteinase-independent motility in which the tumor cells constrict themselves through gaps in the matrix in a manner reminiscent of leukocyte movement. While these studies apparently contradict the decades-worth of data illustrating a dependence on proteinases for tumor cell invasion, some explanation may be due to the composition of the ECM as well as the aggressive cell lines used. More relevant however is that fact that few other studies have inhibited all proteinase classes simultaneously and this may be necessary to observe the amoeboid movement seen. Given the spectacular failure of pharmacological inhibitors of MMPs in large-scale clinical trials in cancer patients, to be discussed later, the ameboid movement hypothesis is worthy of careful consideration as a possible contributory explanation.

3.2. MMPs and tumor growth

One of the earliest challenges to the idea of MMPs purely being mediators of invasion in cancer came

Table 2. Tumor studies in MMP-over-expressing transgenic animals

MMP	Tissue targeted (Promoter)	Mechanism of tumor induction	Result	Reference
MMP-1	Skin (haptoglobin)	No treatment DMBA [1]/TPA DMBA/chyrsarobin	Hyperplastic lesions Increased tumor incidence in MMP-1 skin	15 15 104
MMP-3	Mammary gland (WAP)		Increased tumor incidence in MMP-3 mammary glands	105
	Mammary gland (MMTV)	DMBA	Decreased tumor incidence in MMP-3 mammary glands	106
	Mammary gland (WAP)	None (although parity increased severity of phenotype)	Increased tumor incidence and enhanced progression in MMP-3 mammary glands	17
MMP-7	Mammary gland (MMTV)	No exogenous manipulation but animals multiparous	Hyperplastic lesions evident in MMP-7 mammary glands	16
	Mammary gland (MMTV)	Cross with MMTV-neu	Significant acceleration of tumor incidence and increased penetrance of tumor phenotype in mmp7/neu bigenic mice	16
MMP-14	Mammary gland (MMTV)	No exogenous manipulation but animals multiparous	Development of adenocarcinoma	18

[1] Abbreviations: DMBA, 7,12-dimethybenzanthracene; TPA, 12-O-tetradecanoylphorbal-13-acetate; WAP, whey acidic protein; MMTV, mouse mammary tumor virus

Table 3. Tumor phenotypes of specific MMP-null animals

MMP deleted	Tumor phenotype	Reference
MMP-2	Decreased number of metastatic foci in lungs in experimental metastasis assay; Decreased angiogenesis in dorsal air sac assay; Decreased growth rate of tumor cells implanted s.c [1] Decreased tumorigenesis in RIP-Tag insulinoma model	107 24
MMP-3	Increased tumor growth rate in DMBA/TPA and MNNG skin carcinogenesis models; Enhanced tumor progression in MNNG skin carcinogenesis model	47
MMP-7	Decreased incidence of adenomas in $Apc^{Min/+}$ model of intestinal tumorigenesis	108
MMP-8	Increased tumor incidence in males with DMBA/TPA skin carcinogenesis model ; Increased incidence and enhanced progression in males with MCA fibrosarcoma carcinogenesis model	48
MMP-9	Decreased number of metastatic foci in lungs in experimental metastasis assay; Decreased tumor incidence but enhanced progression in K14HPV16 skin tumorigenesis model Decreased tumorigenesis in RIP-Tag insulinoma model Decreased tumorigenesis in K14HPV16/E_2 cervical carcinoma model	109 110 24 92
MMP-11	Decreased tumorigenic potential of tumor cells when co-implanted with MMP-11-deficient fibroblasts; Decreased mammary tumor formation in DMBA model	111
MMP-12	Increased number of metastatic foci in lungs in experimental metastasis assay	112 [2]
MMP-19	Decreased tumor incidence in males with MCA fibrosarcoma carcinogenesis model	113

Abbreviations: [1] s.c., subcutaneous; RIP-Tag, rat insulin promoter driving T antigen; DMBA, 7,12-dimethybenzanthracene; TPA, 12-O-tetradecanoylphorbal-13-acetate; MNNG, 1-methyl-3-nitro-1-nitrosoguanidine; APCmin/+, murine intestinal neoplasia allele of adenomatous polyposis coli gene; MCA, methylcholanthrene; K14HPV16, keratin14 promoter driving human papillomavirus 16 early genes; E_2, 17beta-estradiol, [2] Cited as unpublished observations by Grisolano and Shapiro within review article

from experiments using *in vivo* imaging. In elegant experiments by Ann Chambers and colleagues, intravital video microscopy was used to observe tumor cells injected into the vascular system of chicken chorioallantoic membranes (CAMs). These investigators compared tumor cells over-expressing the MMP inhibitory protein TIMP-1 with non-inhibitor expressing tumor cells. Unexpectedly, the TIMP over-expressing cells, that is cells with reduced MMP activity, could extravasate and invade into the tissue parenchyma equally as well as the non-expressing cells. However the TIMP-expressing cells, once they arrived within the tissue, failed to proliferate. TIMP proteins, while potent MMP inhibitors, are multifunctional proteins and indeed growth and death regulatory activities have been ascribed to them (14). It is therefore quite possible that the lack of metastatic growth seen with TIMP-expressing tumor cells was a property quite distinct from TIMP effects on MMP function. Nevertheless, these results were provocative and among the first to suggest that MMPs play important roles in tumor development other than invasion.

The fact that MMPs could be involved in tumor-associated processes other than invasion was even more apparent in tumor-formation studies in transgenic animals in which particular MMPs were targeted by the use of tissue-specific promoters to be over-expressed in certain tissues (Table 2). In the absence of specific carcinogens, overexpression of some MMPs such as MMP-1 and MMP-7 was sufficient to cause hyperplastic growths within the targeted tissue (15,16). Even more striking was the demonstration that over-expression of certain MMPs was sufficient for the generation of fully malignant tumors (17,18). These studies were complemented with tumor generation experiments in animals genetically deficient in particular MMPs (Table 3). In the complex *in vivo* setting, changes in tumor growth can be related to other effects of MMPs such as angiogenesis. Several *in vitro* studies however have indicated that cleavage of particular substrates by MMPs can have direct effects on tumor growth. These substrates include pro-forms of growth factors such as transforming growth factor (TGF)-beta (19) and growth factor inhibitory proteins such as insulin-like growth factor binding proteins (IGFBPs), one of which has been demonstrated *in vivo* to have relevance to tumor growth (20).

3.3. MMPs and angiogenesis

Angiogenesis, that is the formation of new blood vessels from existing ones, is required for tumor progression beyond a certain size, typically 1-2mm^3(21). Blood vessels supply oxygen and nutrients to the growing tumor while removing waste products. They also provide a ready route for metastasis and, from a treatment perspective, are a conduit for the introduction of tumor-targeting therapies. For many years, the general perception has been that MMPs facilitate angiogenesis primarily through their ECM-degrading abilities as neo-vessels require some associated matrix remodeling in order to form appropriately. As we have come to appreciate the more diverse repertoire of MMP substrates, it has become apparent that MMPs contribute to angiogenesis in many ways. Importantly, we have also realized that ECM proteolysis is much more than forming holes in a quiescent structural material. Instead, regulated proteolytic processing can result in the release of bound signaling molecules stored in the matrix, can generate cryptic matrix fragments that have their own signaling ability and can alter ECM architecture, another powerful signal generator (22). Angiogenesis is one process that is highly dependent on the precise interactions between matrix molecules and MMPs.

One of the most important MMPs with respect to angiogenesis appears to be MMP-9. Animals deficient in MMP-9 showed a subtle phenotype that was observed in the growth and development of long bones and traced to defects in angiogenesis (23). The problem appeared to be generation of an unidentified signaling-competent angiogenic factor. Angiogenic factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are stored within the matrix and cannot interact with their receptors until freed from this milieu. The prevailing hypothesis has been that MMPs such as MMP-9 are required to proteolyze the matrix thus freeing embedded factors. In both the long bones of juvenile MMP-9-null mice and in a mouse model of pancreatic insulinoma (RIP-Tag) in which MMP9 was deleted (24), an angiogenic switch was prevented due to the lack of free angiogenic factors. In a complementary study, Hiratsuka and colleagues recently showed that a primary tumor can “prime” the lungs as a site of subsequent metastasis by the stimulation of MMP-9-expression on macrophages and lung endothelium in a manner dependent on activation of VEGFR1 (25). Recently, a careful study by Ireula-Arispe and colleagues illustrated how MMP-mediated processing of VEGF itself, rather than the ECM in which may be embedded, can serve to make the growth factor bioavailable (26). This is because defined proteolytic processing of full-length VEGF (specifically the VEGF-A isoform) can remove the domains responsible for its interaction with matrix proteins. A different role for MMP-9 in angiogenesis, apparently unrelated to VEGF availability, was demonstrated recently in a neuroblastoma model (27). Here, the lack of MMP-9 prevented the maturation of nascent vessels. In particular, vessels forming in tumors in MMP-9-deficient animals failed to recruit pericytes, cells of smooth muscle lineage that surround the endothelial cells of a blood vessel and that provide some of the structural integrity required for continued development and functioning of neovessels.

The cells that contribute to blood vessel formation include newly differentiated endothelial cells that appear to evolve from bone marrow derived progenitors. Two recent publications have illustrated that MMP-9 is required for the incorporation of such bone marrow-derived cells in tumor vasculature (28,29). Jodele et al (28) showed that EGFP-labeled transplanted bone marrow cells from MMP-9 sufficient animals comprise approximately 14% of the endothelial marker CD31-expressing tumor vasculature in xenograft neuroblastomas In contrast, bone marrow cells from MMP-9 deficient animals failed to produce any CD31-expressing endothelial cells in tumors examined. The cells of interest in the other study are a Gr+CD11b+ subgroup of immune cells, which represent an expanded myeloid suppressor population associated with the immunosuppression that commonly develops in tumor-bearing patients (29). The tumor-promoting function of this cell population can be completely abrogated when they are genetically ablated for MMP-9. This particular population also incorporates into tumor-associated endothelium and becomes CD31-positive, however such endothelial differentiation fails to occur in the absence of MMP-9. Both of these studies are reminiscent of an earlier study indicating that MMP-9 was important for mobilization of hematopoietic lineages from the stem cell niche, a process apparently dependent on MMP-9-mediated generation of soluble Kit ligand (30).

In contrast to the various mechanisms by which MMP-9 contributes to angiogenesis, MMP-9 is now also strongly associated with anti-angiogenic processes *in vivo.* The endogenous angiogenesis inhibitory peptides angiostatin, endostatin and tumstatin can all be generated by MMPs (21) with plasminogen-derived angiostatin and collagen IV alpha3-derived tumstatin particularly associated with MMP-9 activity. The activity of these potent inhibitors balances the activity of pro-angiogenic factors such as VEGF and bFGF. Illustration of the potency of tumstatin was given in a recent paper in which the generation of large angiogenic tumors in MMP-9-null mice could be reversed by intravenous infusion of physiological levels of tumstatin (31). Conversely, in mice with enhanced plasma levels of MMP-9 as a result of genetic deletion of the alpha 1 integrin (32), tumors that form are small and non-angiogenic but this anti-tumorigenic effect can be overcome by depletion of plasminogen, the MMP9 substrate from which angiostatin is generated (33). Other metalloproteinases, particularly MMPs-12 and -7 have also been identified as potent generators of the angiogenesis inhibitors angiostatin and endostatin. Obviously, interference with these potentially anti-tumorigenic roles of MMPs is an undesired consequence of broad-spectrum inhibition of MMPs in cancer.

MMP-2 is also a strongly angiogenesis-related enzyme. In particular, it associates with the tumor integrin alpha V beta 3 where it is thought to facilitate endothelial

cell invasion and migration (34). Critically, the invasive function of MMP-2 is dependent on its association with alpha V beta 3 and disruption of the binding between these molecules can significantly inhibit tumor angiogenesis (35). The activation of MMP-2 is predominantly achieved by the activity of another MMP, the membrane-associated MMP-14. In mice lacking MMP-14, angiogenesis is defective likely due to reduced levels of active MMP-2 (8). However, a novel role for MMP-14 was recently identified related to generation of an active PDGF-B/ PDGFRbeta signaling complex (36). PDGF-B signaling is critical for proper development of vascular architecture, specifically support of the endothelium by mural cells i.e. pericytes and vascular smooth muscle cells. Although the actual target of MMP14 proteolysis in this system is not completely clear, the catalytic activity of MMP-14 appears essential for competent PDGF-B signaling through PDGFRbeta. As PDGF-B signaling is considered highly relevant to tumor angiogenesis, this newly discovered dependence on MMP14 activity suggests a major role for this enzyme in tumor angiogenesis.

3.4. MMPs and apoptosis

The ability to evade programmed apoptotic mechanisms inherent in normal cells to prevent proliferation of mutants is considered a necessary hallmark of tumor development (37). Apoptotic programs can also be initiated by various chemotherapeutic agents and overcoming these gives rise to the significant problem of drug resistance. MMPs, particularly MMP-7, have been shown to contribute to both of these anti-apoptotic events. In normal or early tumor cells, the expression of MMP-7 can lead to the generation of a soluble form of the death protein Fas Ligand (FasL) (38,39). Soluble FasL is of lower death-promoting potency than the membrane anchored form (40). However, since it is a soluble protein, it is free to interact with its cognate receptor Fas, even on the same cells. Exposure of nascent tumor cells to this weak but constant apoptotic signal can act as a selective pressure for those that have upregulated anti-apoptotic programs and these can then expand and presumably acquire additional mutations thus further progressing as tumors. In later-stage tumors, there is generally sufficient resistance to the weak apoptotic signal resulting from soluble FasL to render it non-functional. Chemotherapeutic agents such as 5-flurouracil and doxorubicin function in part by causing increased expression of FasL (membrane-form) that, when it interacts with Fas, leads to a potent death signal. MMP-7-mediated shedding of FasL in this setting can render cells resistant to cytotoxic chemotherapeutics (41).

3.5. MMPs and immune modulation

The ability of MMPs to alter the behavior of immune molecules such as chemokines and cytokines through specific proteolytic cleavage events has only come to light in recent years. Typically, such molecules are present in low levels and so have not been easy to detect. Additionally, the profound contribution of these molecules to tumor progression has only begun to be appreciated. One of the most striking examples of how a chemokine can impact tumor development was the demonstration of how expression of CXCL12, also known as SDF1, in particular organs could explain the metastatic dissemination pattern of certain tumors shown to express CXCR4, the receptor for this chemokine (42). SDF-1 has been identified as an in vivo substrate of MMP-2 (43) as well as of other MMPs and its processing renders it unable to bind to its receptor CXCR4 (44). This attenuation of SDF-1 binding may be an important mechanism by which MMP activity can influence tumor cell behavior.

The association between tumors and inflammatory cells has long been recognized. Although the assumption has been that these cells are present within tumors as an attempt by the host to eradicate the tumor, there is now much evidence to suggest that instead these cells are suborned by the tumor to positively contribute to its development (45,46). MMPs produced within the tumor microenvironment appear to be largely responsible for the recruitment of inflammatory cells. In the case of both MMP-3 and MMP-8 this appears to be a protective function of these proteinases as their genetic ablation permits enhanced tumor development (47,48). In the case of MMP-8-deficient mice, initial inflammatory cell recruitment to the skin after administration of the chemical carcinogen methycholanthrene (MCA) was significantly attenuated. However, inflammatory cell presence dissipated in wildtype mice but was sustained in the MMP8-/- skin. These persistent inflammatory cells most likely supplied pro-tumorigenic molecules such as growth and angiogenic factors as well as reactive oxygen and nitrogen species that could exacerbate the tumorigenic potential of the carcinogen-exposed skin. A possible explanation for some of the altered inflammatory cell kinetics is the MMP-8-mediated processing of the chemokine LIX to result in a molecule with enhanced neutrophil chemotactic activity. Hence the wild-type mice could initially recruit neutrophils more effectively than could the MMP8-/- animals. The explanation for the sustained inflammatory cell presence in the absence of MMP-8 however remains elusive. Interestingly, an apparently protective effect of MMP-8 expression has also been reported in studies with human breast cancer cell lines (49). In studies using overexpression of MMP-8 or ribozyme-mediated knockdown, Montel and colleagues could demonstrate enhanced in vivo metastatic propensity when tumor cell expression of MMP-8 was low and complementary low metastatic potential when MMP-8 levels were increased. It is unclear however if these effects were mediated through inflammatory cells. In the MMP3-deficient mice, topical application of either of two carcinogens resulted in skin tumors that grew at a faster initial rate than did carcinogen-elicited tumors in wild-type mice (47). Animals on a 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) treatment protocol had a higher incidence of the more advanced spindle type tumors and of metastasis to the lungs when MMP3-null were compared to wildtype. As in the MMP8-/- mice, one of the processes that was different in the MMP3-/- mice that correlated with enhanced tumor development was a reduction in the tumor inflammatory cell infiltrate. Thus the presence of MMP3 appears to confer a degree of protection apparently by generating a chemokine responsible for an anti-tumorigenic neutrophil and macrophage influx to the area of carcinogenesis.

Table 4. MMP gene polymorphisms associated with cancer

MMP	Polymorphism	Association	Reference
MMP-1	Guanine insertion in promoter at -1607 (?1G vs 2G)	NSCLC – increased risk of lymphatic metastasis	114
MMP-1	Guanine insertion at -1607 (2G vs 1G)	Increased risk of lung cancer in never-smokers and males	115
MMP-1	Guanine insertion at -1607 (2G vs 1G)	Chondrosarcoma	116
MMP-1	Guanine insertion at -1607 (2G vs 1G)	Reduced survival in colorectal cancer patients	59
MMP-1	Guanine insertion at -1607 (2G vs 1G)	2 G allele with advanced disease in cervical cancer 1 G allele with lymph node metastasis Heterozygous patients (1G/2G) have longer survival	117
MMP-1	Guanine insertion at -1607 (2G vs 1G)	2G/2G genotype has increased risk of renal cell carcinoma in males	118
MMP-1	Guanine insertion at -1607 (2G vs 1G)	2G/2G genotype has worse prognosis in invasive colorectal cancer	119
MMP-1	Guanine insertion at -1607 (2G vs 1G)	2G with poorer prognosis in malignant melanoma	120
MMP-2	-1306 C→T in promoter -735C/T in promoter	Increased risk of occurrence and metastasis of esophageal squamous cell cancer with C alleles at both loci	121
MMP-2	-1306 C→T substitution	TT genotype had smaller breast tumors and lower ER In ER- tumors, TT has reduced survival but in ER+ tumors, TT has good survival	60
MMP-2	-1306 C→ T substiution	CC genotype has increased risk of gastric cardia adenocarcinoma, especially in smokers and younger subjects	122
MMP-3	Adenosine insertion in promoter at -1171(5A vs 6A)	NSCLC – increased susceptibility in smokers	114
MMP-3	G/A polymorphism in exon2 that causes Glu45Lys change	Risk of renal cell carcinoma (in combination with 2G genotype of MMP-1 promoter @-1607)	64
MMP-9	-1562 C→T substitution	T allele has better prognosis in breast cancer patients	60
MMP-9	-1562 C→ T substitution	T allele associated with tumor invasion/lymphatic invasion in gastric cancer	61

Immunosuppression is a significant problem associated with many cancers. A contribution of MMPs to this process was outlined in a paper describing MMP-mediated cleavage of IL-2Ralpha (50). The development and propagation of cytotoxic T cells is dependent on signaling through IL-2Ralpha, hence any downregulation of its expression on the surface of tumor-infiltrating CD8+ T cells can result in attenuation of a T cell-mediated anti-tumor response. MMPs, particularly MMP-9, expressed in cervical cancer tissues, were found to cause the cleavage of IL-2Ralpha from T cells and thus downregulated proliferation of specific anti-tumor T cells. Another mechanism by which activity of an MMP can modulate immune responses is through the cleavage of the complement receptor gC1qR (51). The hypothesis here is that active MMP14 can proteolyze soluble gC1qR released by tumor cells however, inactive or inhibited MMP14 may instead act as a cell surface receptor for gC1qR and subsequently the complement component 1q resulting in lysis of the tumor cell. Thus MMP inhibitors would have a beneficial effect by promoting complement-mediated destruction of tumor cells. For discussion of other interactions between MMPs and immune molecules, an excellent and comprehensive review article is available and recommended reading for those interested in this topic (52).

4. MMP PROMOTER POLYMORPHISMS AND CANCER RISK

Single nucleotide polymorphisms (SNPs) describe common variants of particular bases of DNA within the genome (53). Although these changes in base sequence frequently have no consequence and instead are useful purely as markers or identifiers, there are some changes that occur within the regulatory or coding regions of specific genes that alter that gene's expression or function. Within the MMP gene family, a series of such SNPs has been identified mostly within promoter regions that change the levels of the expressed MMP gene. There are approximately 40 studies reported in the literature where investigators identified specific cancer patient cohorts from whom genomic DNA was analyzed for the presence of a given SNP. Examples of the findings of such studies are presented in Table 4. In general, SNPs that result in higher expression of an MMP are found in the DNA of patients with more advanced cancer. These are not strong cause and effect relationships, the odds ratios found are usually between 1.5 and 2.5. However they indicate that MMP levels can contribute to disease progression. Such studies complement expression analyses where levels of gene expression at either the RNA or protein levels are statistically correlated with particular outcomes. Tables of these analyses are available in previously published review articles [for examples see references (54) and (2)].

One of the most striking cancer-associated MMP polymorphisms was identified within the promoter of MMP-1. At position -1607 upstream of the start site, a single guanine base is usual. In a significant percentage (30%) of normal samples, however, another guanine base is inserted adjacent to the first giving rise to a "2G" alternative genotype (55). The significance of this is that the insertion creates a transcriptional binding site ('5-GGA-3') for Ets transcription factors . The incidence of this 2G genotype was significantly higher, at 62.5%, in DNA samples from tumor cell lines. Transcription of MMP-1 from the 2G version of the promoter is elevated and results in higher levels of MMP-1 protein. Since higher levels of MMP-1 themselves have been shown to be of prognostic significance in some cancers (56,57), it is perhaps unsurprising that presence of a promoter polymorphism that leads to higher transcriptional activity of the MMP-1 gene also associates with poorer prognosis. However, the range of tumor types for which a MMP-1 promoter SNP has been associated with disease severity is greater than the limited number where levels of MMP-1 protein have a demonstrated link with disease. This is probably related to the relative ease with which genomic DNA samples can be collected and analyzed compared to the tumor tissue usually required for protein or transcript analysis. It should be understood, however, that not all cases with the 2G

genotype will have higher MMP-1 levels and, conversely, not all tumors with high MMP-1 levels will have the 2G genotype (58). There is a myriad of factors that contribute to regulation of MMP gene expression and to levels of functional protein all of which can influence the association between genotype and phenotype, nevertheless, these promoter polymorphisms appear to give some information with respect to cancer risk. It is also important to realize that few of the current studies have used the more stringent statistical tools such as multivariate analysis. One study that did indicated that the 2G genotype was an independent indicator of poor prognosis in colorectal cancer patients (59).

Other MMPs that have been shown to have promoter SNPs are MMP-2, MMP-3 and MMP-9. In the case of MMP-2, the C to T variant at position 1306 upstream of the transcriptional start site abolishes the binding site of the transcription factor SP-1 and hence results in reduced levels of MMP-2. While it may be anticipated and is evident from some studies that the "T" allele would be associated with a better scenario (Table 4), there is a definite interaction with other factors so that in the breast cancer cases where estrogen receptor is negative, a TT genotype actually correlates with poorer survival (60). A similar C to T variant in the MMP-9 promoter at a position 1562 bases upstream of the start site, increases transcription of the gene and correlates with increased invasiveness in gastric cancer (61) but actually is associated with a better prognosis in breast cancer (60). This last finding echoes another study in which higher MMP-9 expression levels were significantly associated with a better prognosis in node-negative breast cancer patients, possibly representing an anti-tumor role for this proteinase (62). The MMP-3 promoter can have either 5 or 6 adenines at a position -1608 bases from the transcriptional start site. The 5A variant is associated with higher levels of MMP-3 transcription apparently due to changes in binding and interactions between several transcription factors including the NF-kappaB p50/p50 homodimer (63). This polymorphism has been associated with disease severity particularly in heart disease (63) but also a diverse range of other pathologies in addition to cancer. Interestingly, a polymorphism within the coding region of MMP-3 has recently been described that changes a glutamate residue in the pro-domain to a lysine, however the functional significance is unclear (64).

5. INTERACTIONS OF MMPS WITH OTHER PROTEOLYTIC SYSTEMS

MMPs are only one of 5 major classes of proteinases that have been described. Others are members of the serine, cysteine, aspartic or threonine proteinase classes. Even within the MMPs, there are families other than the MMPs that can have significant associations with cancer. These are the ADAM (a disintegrin and metalloproteinase) and ADAM-TS (a disintegrin and metalloproteinase with thrombospondin) families. There is significant crosstalk among the various classes and families of proteinases making tumor-associated proteolysis a very complex subject. Ways in which proteases can interact include (i) activation cascades whereby active proteinases of one type process pro- or zymogen forms of proteinases of another class; (ii) proteolytic degradation of inhibitors of proteinases of other classes; (iii) generation of signaling pathways that serve to upregulate expression of proteinases or inhibitors of other classes; and (iv) functional redundancy with respect to substrates between protease classes. Examples of all of these interactions and possible implications for tumor progression are discussed below.

Interactions between MMPs and serine proteinases are perhaps the best-described and occur at all the levels listed above. Direct activation of zymogen forms of MMPs by the plasmin cascade have been well-documented and shown to promote tumor invasion and metastasis and to regulate angiogenesis (65-67). Conversely, the plasmin system can be downregulated by matrix metalloproteinase activity by for example cleavage of urokinase plasminogen activator (uPA) (68) or its receptor uPAR (69). Of note, the processing of uPAR by MMP-12 can inhibit outgrowth of capillary structures (69) thus inhibition of MMP-12 activity may allow angiogenesis, a further example of possible unwarranted effects of blocking MMPs in cancer patients. There is evidence that other serine proteinases, for example the tissue kallikreins (70) and mast cell chymases (71), can also activate MMPs. Endogenous inhibitors of serine proteinases (called serpins) have been identified as substrates for several MMPs. In fact the serpin alpha1-proteinase inhibitor (alpha1-PI) is an *in vivo*-verified substrate for MMP-9. MMP-9 is highly expressed by neutrophils as are multiple other proteinases such as the serine proteinase neutrophil elastase. Degradation of alpha-1-PI by MMP-9 allows the activity of neutrophil elastase and this can have pathological consequences as in the skin blistering disorder bullous pemphigoid (72). Alpha-1-PI is also a substrate for MMP-25 and its hydrolysis renders it inactive as an inhibitor of human neutrophil elastase, cathepsin G and proteinase 3 (73). This is thought to be of particular importance at sites of inflammation where rapid bursts of proteolysis by proteinases produced by infiltrating neutrophils are important for tissue remodeling. Of course, in the chronic inflammatory states that have been associated with many cancers (74), this excessive proteolytic activity may be contributory to the tumorigenic phenotype (45).

Protease-activated receptors are G-protein coupled receptors on cell membranes that instead of being activated by receptor binding, are activated by protease cleavage and subsequent re-organization of the extracellular domains (75). Typically they are activated by serine proteinases, especially thrombin and plasmin. As these are receptors whose activation unleashes signaling pathways with multiple downstream effectors, they are regarded as sensitive mechanisms for triggering protease-initiated biological events. Activation of PAR1 on tumor cells by thrombin has been associated with enhanced lung metastasis (76). One mechanism through which PAR signaling can potentially contribute to tumor invasion and metastasis is through the upregulation of MMP expression and/or activation as there is some evidence that increased

levels of active MMPs result from PAR stimulation (77-79). Hence, serine proteinases can indirectly contribute to tumor progression through PAR signaling and MMP secretion. Recently, an exciting new link between PARs and MMPs was demonstrated in breast cancer cells when MMP-1 from stromal fibroblasts was shown to directly activate PAR1 on cancer cells and promote their growth and invasion (80). This is a highly novel mechanism by which MMP-1 can contribute to tumor cell invasion and suggests that PAR receptors are not solely limited to serine proteinases but can also respond to metalloproteinases. This may have special relevance in scenarios where serine proteinases are limited, for example in the presence of an inhibitor, and thus could be one mechanism by which redundancy between the proteinase classes ensures continued tumor progression despite attempts to disable a proteolytic system.

The cysteine proteinases are usually regarded as intracellular but may become cell surface-associated or extracellular with malignancy (81). Cathepsin B has been shown to be involved in proteolytic cascades resulting in activation of MMPs (81,82) and down-regulation of cathepsin B by antisense in glioblastoma cells leads to reduced levels of active MMP9 and reduced angiogenesis in vivo (83). Cathepsin K is considered to be the major proteinase responsible for ostoclastic resorption of bone matrix seen in bone metastases (84) although MMPs can also contribute. In mice genetically deficient for cathepsin K however, expression of the MMPs that can also effect osteolysis is upregulated indicative of a compensatory mechanism that crosses proteinase classes (85).

6. PERSPECTIVE: PROSPECTS FOR THE PHARMACOLOGICAL INHIBITION OF MMPs FOR CANCER TREATMENT

The serious development of pharmacological inhibitors of MMPs (known as MMPIs) as potential anti-cancer agents began in the 1980s with results of the first clinical trials reported a decade later (86). As most readers are no doubt aware, the clinical use of these agents in oncology has been brought to a crashing halt with the repeated failure of various MMPIs in multiple large-scale phase III clinical trials. Extensive analyses of these trials and the development of the MMPIs have been published previously (5,54,87). In addition to the problems with the trials themselves – unclear endpoints for phase II, lack of markers of drug efficacy for dosing determination, patient populations with overly aggressive disease – we have also come to realize that the target, MMPs, is more complicated than initially thought. As should be evident from the preceding paragraphs, we now know of many instances where MMPs are apparently protective or anti-tumorigenic and consequently, their large-scale inhibition would likely have unintended consequences. Additionally, there is evidence for crosstalk among the different protease classes that can possibly lead to compensatory mechanisms if one class is inhibited. Finally, a significant problem with the inhibitor clinical trials was the frequency and severity of dose-limiting side-effects often musculoskeletal in nature. As yet, the cause(s) of these side-effects is unclear. This leads us to a re-evaluation of MMP inhibition as a therapeutic modality in cancer. When and how should MMPs be inhibited?

Given the issues with side-effects of unknown cause as well as the possible positive and negative contributions of various family members, one of the most critical requirements for any future clinical uses will be highly selective/specific inhibitors of each MMP. These can be mixed to give more broad-spectrum inhibition if warranted but could be used as single agents in certain settings. As the MMP family are quite similar structurally, and particularly with respect to the catalytic site, such specific inhibitors have proven extremely difficult to obtain. One possible strategy is to consider antibody-based inhibitors that can be produced with highly specific binding. Antibody-based therapeutics such as Avastin™, Herceptin™, Erbitux™ and Rituximab™ have been very successful in the cancer arena and there is now a wealth of experience in how they should be formulated and administered to patients.

Of course, even a highly specific inhibitor will be of no benefit if modulation of its target interferes with anti-angiogenic programs or immune cell-mediated tumor destruction. For this reason, it is critical that we fully understand the roles of the MMPs in each cancer setting. Inhibition of MMP-9 may be useful in preventing the priming of sites for metastatic formation but once metastasis has occurred and has engaged an angiogenic program, MMP-9 inhibition could promote angiogenesis and lead to larger and more aggressive metastatic lesions. This means that animal models in which inhibitors are tested should recapitulate as closely as possible the scenario in the patient populations being considered for treatment. Only then are the results of preclinical testing likely to predict the various consequences of the inhibitor. Animal models that do recapitulate human disease have proved successful in predicting which therapies are effective. For example, the *Apc*$^{Min/+}$ mouse model of intestinal neoplasia strongly resembles the human syndrome familial adenomatous polyposis coli where sufferers develop numerous polyps in the colon that can progress to malignancy (88). This is essentially a chemopreventative setting as the goal is to prevent the development of malignant disease. COX-2 inhibitors were remarkably effective at reducing the polyp burden in the Apc$^{Min/+}$ mice and proved similarly useful in FAP patients (89). Interestingly, MMP inhibitors, as well as genetic deletion of a single MMP, MMP-7, have been shown to be as effective as COX-2 inhibitors in the Apc$^{Min/+}$ mouse (90,91) and are worthy of consideration in a chemoprevention setting.

Since the different proteinase classes are so interconnected and likely to have substantial substrate overlap, combinations of MMP inhibitors with inhibitors of other classes may be valuable. For example, MMPIs can reduce the extent of osteolysis associated with metastasis to bone (84), however, since cathepsin K is the prime candidate for bone matrix destruction, MMPIs in combination with a cathepsin K inhibitor are likely to be much more effective.

Finally, it is worth considering the use of drugs already approved for clinical use for their MMP inhibition potential. An excellent example of this was recently shown from the lab of Doug Hanahan who used the bisphosphonate zoledronic acid in a mouse model of cervical carcinogenesis (92). The development of tumors in this model is MMP9-dependent as was demonstrated using MMP-9-deficient mice and the MMP-9 is produced by tumor-associated macrophages. Daily administration of zoledronic acid prevented progression of premalignant lesions and, in established tumors, induced regression. The mechanism of action involved the downregulation of MMP-9 expression by macrophages as well as inhibition of MMP-9 activity.

The goal of this review article has been to summarize recent advances in our understanding of how MMPs contribute to tumor progression and metastasis. As we have seen, the roles for MMPs are numerous and include all stages of tumor progression. Importantly, the results of MMP activity are not always pro-tumorigenic and there are certainly scenarios where broad inhibition of MMP activity may cause more harm than good. One reason why some patients fare better or worse than others may have to do with their genetic signature. The presence of SNPs within the promoter regions of several MMP genes has been documented and shown to alter the transcriptional regulation of these MMPs. Many of these SNPs have been identified as risk factors for the development or enhanced progression of certain cancers. Interactions of MMPs with proteinases of other classes can contribute to various tumor processes and illustrate that MMPs should not be considered in isolation. Finally, while further development of MMP inhibitors as cancer therapeutics has been hampered by the resounding failure of previous clinical trials, there are reasons for considering this therapeutic approach once more albeit with caution and only in well-understood settings.

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Send correspondence to: Barbara Fingleton, Ph.D. Department of Cancer Biology, Vanderbilt University Medical Center, 771 PRB, 2220 Pierce Ave, Nashville, TN 37232-6480 USA, Tel: 615-936-5877, Fax: 615-936-2911, E-mail: Barbara.fingleton@vanderbilt.edu

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