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Matrix metalloproteinases: protective roles in cancer

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- **Introduction**
- **MMP-3**
- **MMP-8**
- **MMP-9**
- **MMP-12**
- **MMP-19**
- **MMP-26**
- **Conclusions**

Abstract

The original notion that matrix metalloproteinases (MMPs) act as tumour and metastasis-promoting enzymes by clearing a path for tumour cells to invade and metastasize has been challenged in the last decade. It has become clear that MMPs are involved in numerous steps of tumour progression and metastasis, and hence are now considered to be multifaceted proteases. Moreover, more recent experimental evidence indicates that some members of the MMP family behave as tumour-suppressor enzymes and should therefore be regarded as anti-targets in cancer therapy. The complexity of the pro- and anti-tumorigenic and -metastatic functions might partly explain why broad-spectrum MMP inhibitors failed in phase III clinical trials. This review will provide a focussed overview of the published data on the tumour-suppressive behaviour of MMPs.

Keywords: cancer • matrix metalloproteinases • protective • anti-target

Introduction

Cancer is a leading cause of mortality worldwide, accounting for 13% of deaths (7.4 million) in 2004. Lung, stomach, liver, colon and breast cancer are responsible for the majority of cancer-associated deaths each year [1]. It has been reported that more than 30% of cancer incidence could be prevented by avoiding key risk factors such as tobacco and alcohol use, obesity, physical inactivity, low fruit and vegetable diet, sexually transmitted Human Papillomavirus (HPV) infection and occupational hazards [2]. On the other hand, cancer treatment is becoming more effective due to early detection and personalized cancer therapy. Metastasis, the development of secondary tumours at a distant site, remains the major cause of cancer mortality. Screening programs raise cancer awareness, resulting in earlier detection of precancerous and cancerous lesions and thus preventing metastasis. Much effort has been put into the development of targeted therapies to prevent tumour growth by interfering with the functions of specific molecules. Such anticancer approaches have focused upon, for example, the hormonal dependence of certain tumour types, targeting the oestrogen or progesterone receptor in breast cancer, or the need for rapidly growing tumours to promote angiogenesis by targeting pro-angiogenesis factors.

Among these candidate targets, the matrix metalloproteinases (MMPs) rose to prominence over two decades ago. In human beings, MMPs form a family of 23 endopeptidases which together degrade all protein components of tissue extracellular matrices and basement membranes. They can be subdivided into five groups depending on their domain structure and substrate specificity; collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs. They participate in various physiological and pathological processes such as embryonic development, wound healing, arthritis, atherosclerosis and tumour progression. In physiological tissue remodelling, MMPs are tightly regulated at the levels of transcription, activation and inhibition. In general, they are secreted as inactive zymogens and are converted into active enzymes by specific proteolytic cleavages on the cell surface or in the pericellular environment, providing spatial control of their function. In addition, they are inhibited by their endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs), which are represented by four family members in human beings, each with characteristic properties and expression patterns [3].

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Some TIMPs, in particular TIMP-3, can also inhibit MMP-related proteases of a disintegrin and metalloproteinase (ADAM) and a disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS) families that also have important roles in cell signalling and ECM organization [4]. This intertwined network of metalloproteases and inhibitors, along with proteolytic enzymes from other catalytic classes, has been termed the 'protease web'. It is responsible for maintenance of tissue homeostasis and its perturbation is undoubtedly linked with pathologies such as cancer, though the interconnectedness of the web can make it difficult to define unique functions for particular proteases [5].

Originally it was believed that MMPs were key players in tumour development and progression due to their ability to clear a path for cancer cells to invade matrix barriers and migrate through tissue stroma. This notion of MMPs as pro-tumorigenic and pro-metastatic enzymes that was prevalent in the 1980s to 1990s spawned the development of synthetic matrix metalloproteinase inhibitors (MPIs) as cancer therapeutics. Animal studies were encouraging, showing that broad-spectrum MPIs were in many instances effective in preventing metastasis and inhibiting invasion and angiogenesis. However, in the clinic, these agents proved largely disappointing. Several phase III clinical trials with broad-spectrum inhibitors failed due to lack of efficacy and severe musculoskeletal side effects. Moreover, small-cell lung cancer and pancreatic cancer patients treated with the more specific MPI Tanomastat showed a poorer survival than placebo-treated patients [6, 7]. There were however some positive indications: for instance, in a randomized trial of non-resectable gastric cancer patients a modest but not significant survival benefit was shown for treatment with the broad-spectrum inhibitor marimastat. Of interest, analysis of a subgroup of patients excluding individuals with more advanced or rapidly progressing disease revealed an impressive significant 2 year survival benefit of 13% [8].

So the key question is-why did the broad-spectrum MPIs fail? The consensus that has emerged over the past decade from analysis of the clinical trial data and from use of more sophisticated transgenic mouse models of cancer is that MPIs in general are less effective in advanced disease [7, 9]. Moreover, extensive subsequent research has made it clear that MMPs are multifunctional proteins and that their roles in cancer are much more complex than originally thought. In addition to ECM degradation, there is now considerable evidence for their involvement in the subtle regulation of cell growth, survival and differentiation, inflammation and angiogenesis through precise cleavage of various molecules, releasing matrix-sequestered growth factors or generating critical bioactive fragments [10]. These views have converged with a growing body of data that reveal that some MMPs consistently inhibit tumorigenesis and metastasis, whereas others can show either a pro- or anti-tumorigenic/metastatic action, depending on the tumour type, disease stage and the cellular source of the MMP [11, 12]. These latter findings emphasize the complex nature of MMPs and may further explain why broad-spectrum MMP inhibition failed as a therapeutic approach and may sometimes result in an unfavourable outcome [13]. Furthermore, it still needs to be determined whether the same MMP tumour-promoting or suppressive behaviours observed in chemically and genetically induced mouse tumour models are also manifest during the pathogenesis of human tumours.

With the above cautions in place, interest is now returning to the possibility of targeting MMPs in cancer therapy, and increasing effort is being put into the development of synthetic inhibitors or antibodies that are specific for a single MMP, which might incur less systemic toxicities. For instance, recent work demonstrates that selective inhibitors to MMP14 inhibit tumour growth, invasion, angiogenesis and metastasis in human xenograft tumour models while prolonging the survival of mice [14, 15]. It is important therefore to consider which MMPs are preferred targets and which are the anti-targets that need to be spared from blockade [16]. This is a huge field and this brief review will not attempt a comprehensive analysis of the various target and anti-target features of each member of the MMP family, but rather we have chosen to focus on recent findings suggesting a protective role for particular MMPs in cancer.

MMP-3

MMP-3 is a member of the stromelysin subfamily of MMPs, which comprises stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), and stromelysin-3 (MMP-11). Stromelysin-1 is overexpressed in a wide variety of tumour types, where it is almost exclusively found in the tumour stroma, *i.e.* fibroblasts, endothelial cells, immune cells [17, 18]. MMP-3 has a large repertoire of ECM and non-matrix substrates [19]. Hence, its wide distribution and large substrate specificity suggests that it could be a key player in tumour progression.

Conflicting data have been published on the role of MMP-3 in tumorigenesis. A protective role for MMP-3 was reported in a squamous cell carcinoma mouse model [20]. Both *Mmp3*-null mice and wild-type mice developed papillomas and carcinomas after treatment with the chemical carcinogen N-Methyl-N′-nitro-N-nitrosoguanidine or with a combination of 7,12-dimethylthracene (DMBA) and tetradecanoylphorbol acetate (TPA). No difference was seen in tumour onset or incidence. However, compared to wild-type mice *Mmp3*-null mice had faster initial tumour growth associated with increased cell proliferation, had more undifferentiated or highly metastatic tumours and more surface lung metastases. *Mmp3*-null mice showed an overall reduction in the number of tumour infiltrating macrophages and neutrophils, supporting a role for MMP-3 in the host defence response during tumorigenesis. As tumour expression of MMP-3 has been associated with invasive squamous cell carcinoma, it was suggested that although stromal-derived MMP-3 may account for its anti-tumorigenic functions, tumour-derived MMP-3 exerts pro-tumorigenic functions [21]. To investigate the tumour-derived effects of MMP-3, the DMBA-TPA chemical carcinogen protocol was applied to transgenic mice with keratinocyte-targeted *Mmp3* overexpression.

A reduced number of papillomas and carcinomas were found in *Mmp3* transgenic mice compared to wild-type littermates, without a difference in tumour onset. No changes were observed in cell proliferation, apoptosis or leucocyte infiltration; however, tumour vascular density was increased in *Mmp3* transgenic mice. In accordance, no tumours were found after orthotopic injection of *Mmp3* overexpressing SP-1 murine papilloma in immunocompromised mice whereas all mice inoculated with wild-type SP1 cells developed tumours. Furthermore, skin biopsies of *Mmp3*-SP1 injected mice revealed reduced levels of proliferation and enhanced differentiation, corroborating a role for MMP-3 in early events of tumour formation. In further support of a protective role for MMP-3, mouse mammary tumour virus (MMTV)-*Mmp3* transgenic mice, with *Mmp3* expression targeted to the mammary glands, which were subjected to the chemical carcinogen DMBA developed 33% less breast tumours than their non-transgenic littermates [22]. No difference in the extent of invasion or presence of metastases was found. In contrast to these observations, it has been demonstrated that MMP-3 can promote mammary carcinogenesis using phenotypically normal mammary Scp2 epithelial cells expressing MMP-3 in a tetracycline-regulated manner and a *Mmp3* transgenic breast cancer mouse model [23]. Orthotopic injection of *Mmp3* overexpressing Scp2 cells into severe combined immunodeficiency (SCID) mice resulted in the formation of normal duct-like and pseudo-glandular structures when MMP-3 expression was inhibited by tetracycline in the drinking water. However, in the absence of tetracycline MMP-3 expression induced the formation of small mesenchymal-like tumours within 6 weeks suggesting that MMP-3 triggers epithelial-to-mesenchymal transition. A WAP (whey acidic protein) *Mmp3* transgenic mouse model was used to investigate the long-term effects of MMP-3 expression on mammary tumorigenesis. *Mmp3* transgenic mice had a higher incidence of premalignant lesions and breast tumours, characterized by genomic changes, than their wild-type littermates. Crossing WAP-*Mmp3* mice with WAP-*Timp1* mice resulted in a reduced number of hyperplasia lesions suggesting that the proteolytic activity of *Mmp3* is required for the induction of neoplasia.

Because polymorphisms in *MMP* genes may result in changes in the expression of MMPs, single nucleotide polymorphisms (SNPs) in *MMP3* have been investigated in relation to the risk of developing cancer. A common haplotype across *MMP3* and the *MMP3*–6A allele (5A/6A SNP) have been found to associate with a decreased risk of lung cancer and head and neck squamous cell carcinoma respectively [24, 25]. Furthermore, the 6A allele has been linked with a reduced risk of lymphatic metastasis in lung cancer, breast cancer and oesophageal squamous cancer patients [26–29]. *In vitro* and *in vivo* work has demonstrated a 2–4-fold higher promoter activity and gene expression of the 5A allele variant compared to the 6A variant, which suggests overall MMP-3 has pro-tumorigenic and pro-metastatic effects, leading to reduced risk for individuals who have the 6A variant [30, 31].

Overall, conflicting data have been published on the role of MMP-3 in tumour development and progression. Discrepancy between studies might have arisen from differences in study design. For instance, the choice of mouse strain for *in vivo* work can have a substantial effect on the observed effects. This is clearly illustrated by the MMTV-*Mmp3* transgenic breast cancer mouse model where mammary tumours are observed on a CD1 background but not on a C57/bl6 genetic background [22, 23]. The use of exogenous carcinogens might also change the way MMP-3 is involved in cancer through the activation of diverse signalling pathways. Therefore, this area of research merits further investigation.

MMP-8 (Table 1)

MMP-8 was the first MMP recognized to be an anti-target for cancer therapy. MMP-8 belongs to the collagenase subfamily of MMPs and is also known as collagenase-2 or neutrophil collagenase. It is predominantly a product of neutrophils but is also expressed in fibroblasts, endothelial cells, keratinocytes, epithelial cells, chondrocytes, macrophages and plasma cells [32]. MMP-8 has been implicated in various inflammatory diseases, including osteoarthritis and periodontitis [33].

Experimental evidence for the protective role of MMP-8 in cancer arose from *Mmp8*-knockout mice subjected to a conventional chemical carcinogenesis protocol (DMBA, TPA) in a skin-tumour-formation study [34]. Male *Mmp8*-null mice developed a higher number of papillomas than female *Mmp8*-null mice or wild-type controls, and with a shorter latency period. The gender difference in tumour incidence was lost in ovariectomized female *Mmp8*-null mice and female *Mmp8*-null mice treated with the oestrogen antagonist tamoxifen, demonstrating a protective effect of female sex steroids. Host-derived MMP-8 from neutrophils in bone marrow transplants was sufficient to restore the protective effect in male *Mmp8*-null mice. An anti-metastatic role for MMP-8 was also revealed by *in vivo* studies [35, 36]. Two human cancer cell lines derived from the same parental cell line MDA-MB-435 showed a different metastatic potential in athymic mice (M-4A4, NM-2C5). Expression analysis further showed a 20-fold increase in MMP-8 expression in the non-metastatic cell line NM-2C5 compared to the metastatic cell line M-4A4. Overexpression of *MMP8* in M-4A4 cells and ribozyme knockdown in NM-2C5 cells reversed the metastatic phenotypes of both cell lines. Interestingly, orthotopic injection of mice with the non-metastatic cells treated with ribozymes resulted in a higher number of lymph node metastases than lung metastases. In collaboration with other research groups, we further explored the anti-metastatic potential of MMP-8 [33]. Injection of *Mmp8*-overexpressing B16F10 melanoma cells into the tail vein of C57BL/6 mice resulted in a 70% reduction in metastasis compared to the injection of control B16F10 cells. The anti-metastatic behaviour of MMP-8 was independent of effects on cell growth either *in vitro* or *in vivo*. Further, we found that MMP-8 expression in B16F10 melanoma cells or exogenous recombinant MMP-8 protein reduced cell invasion *in vitro* by 80%. More specifically, transendothelial migration was reduced by 50% in the presence of MMP-8. In parallel with the decrease in cell migration, MMP-8 was shown to enhance cell adhesion to collagen-1 and laminin-1. We were able to confirm previous findings

Table 1 Protective roles of MMP8 in cancer

MMP8	References	Study type	Cancer	Main findings
	[34]	*In vivo*	Skin cancer (chemical induced)	Pro-tumorigenic in male KO mice Protective effect restored by bone marrow transplantation from WT mice
	[35]	*In vitro*	Breast cancer (MDA-MB-435 cell line)	Elevated expression in non-metastatic-derived cell line Increased migration through Matrigel in absence of MMP8
	[36]	*In vivo*	Breast cancer (MDA-MB-435 cell line)	Pro-metastatic with ribozyme knockdown
	[37]	*In vitro*	Melanoma (Mel-STR cell line)	Inhibition of cell proliferation
		In vivo	Melanoma (Mel-STR cell line)	Inhibition of tumour growth
	[38]	*In vivo*	Tongue squamous carcinoma (chemical induced)	Pro-tumorigenic in female KO mice
		Human studies	Tongue squamous carcinoma	Prolonged OS
	[39]	Human studies	Breast cancer	Plasma levels positively associated with lymph node metastasis, negatively associated with distant metastasis
	[40]	Human studies	Breast cancer	SNP associated with reduced lymph node metastasis rs11225395 SNP confers better prognosis (DFS, OS)
	[41]	Human studies	Lung cancer	SNP associated with decreased risk of lung cancer
	[44]	*In vitro*	Melanoma (B16F10 cell line)	Inhibition of invasion and transendothelial migration Increased cell adhesion to collagen-1, laminin-1 No effect on cell proliferation
		In vivo	Melanoma (B16F10 cell line)	Anti-metastatic No effect on tumour growth
		Human studies	Breast cancer	Inversely associated with lymph node metastasis

DFS: disease-free survival; KO: knockout and OS: overall survival.

indicating that host-derived MMP-8 can also play an important role in protection against the formation of melanoma or Lewis lung metastases. Taqman RT-PCR analysis of breast cancer patients revealed that *MMP8* tumour expression inversely correlates with lymph node metastasis and confers good prognosis.

Another recent study has shown that *MMP8* is frequently mutated in malignant melanoma, the spectrum of mutations including ones that lead to loss of catalytic activity [37]. These authors also showed that expression of human MMP-8 in Mel-STR melanoma cells reduced both cell growth in soft agar *in vitro* and tumour formation *in vivo*. In tongue squamous cell carcinoma, tumour expression of MMP-8 was positively associated with improved survival, in particular in female patients [38]. Furthermore, female but not male *Mmp8*-null mice developed tongue squamous cell carcinomas more often than wild-type mice after treatment with the chemical carcinogen 4-Nitroquinoline-N-oxide. Oestrogen induced MMP-8 expression in HSC-3 tongue squamous cell carcinoma (SCC) *in vitro*, and MMP-8 cleaved the oestrogen receptors ER-A and ER-B. This is in contrast with the data obtained from the skin tumour mouse, where an increase in tumour formation was only seen in male mice or ovariectomized/tamoxifen-treated female mice. It will be important in future studies to unravel the interplay of MMP-8 and sex steroids, particularly in hormone-regulated cancers such as breast and prostate cancer.

We have evaluated plasma collagenase levels as diagnostic and prognostic markers of breast cancer [39]. Plasma MMP-8 levels were positively associated with lymph node involvement but showed a negative correlation with the risk of distant metastasis. We suggested that blood and tissue protein levels are in reverse association, with low levels in the blood when a protein is sequestered in the tissue and higher circulating levels upon secretion. As such, these findings suggest that MMP-8 in the tumour may have a protective effect against lymph node metastasis. We also previously investigated whether gene variation could affect the anti-metastatic role of MMP-8 and found four SNPs to be associated with lymph node metastasis [40]. Further analysis in a large case–control study with 7 years of follow-up, revealed that the minor T allele of the promoter region SNP rs11225395 was associated with a longer disease-free and overall survival in early stage cancer. Transient transfection of MDA-MB-231 breast carcinoma cells with reporter constructs in which reporter expression was driven by *MMP8* promoters containing either form of the SNP, showed that the minor T allele displayed higher expression. This was supported by the binding of nuclear proteins to oligonucleotide

probes containing the minor allele sequence. Furthermore, the minor G allele of the +17C/G *MMP8* promoter SNP has been associated with a decreased risk of lung cancer in a large case–control study of 500 patients and controls [41]. The polymorphism does not seem to be uniformly associated with an increased or decreased expression but differs in various cell types [42].

There is growing evidence to suggest that the protective effects of MMP-8 are propagated through the immune system. Recombinant MMP-8 has been shown to proteolytically activate the pro-inflammatory mouse lipopolysaccharide induced CXC chemokine (LIX) and its human orthologue interleukin-8, which are essential for a normal immune response by recruiting neutrophils to the site of infection or wound [43]. It has been shown that although initial recruitment is delayed in *Mmp8*-null mice, once established a sustained inflammation reaction with a greater influx of neutrophils is achieved, providing a microenvironment favourable for tumour development [34, 44]. The observation that MMP-8 increases cell adhesion to collagen-1 and laminin-1 in addition to its involvement in inflammation further supports an important role for MMP-8 in modulation of events associated with the initiation, progression and invasion of cancer [44]. However, MMP-8 substrates are largely unknown and require further investigation to gain greater understanding of how the protease exerts its protective effects.

MMP-9

MMP-9 or gelatinase-B and MMP-2 (gelatinase-A) form the gelatinase subfamily of MMPs. They are characterized by three repeats of a fibronectin type II motif in the catalytic domain and they share similar proteolytic activity against denatured collagens, gelatines and various extracellular matrix molecules [19]. MMP-9 expression has been found in a large variety of cell types, including epithelial cells, fibroblasts, endothelial cells and inflammatory cells [45]. Numerous studies have shown that MMP-9 expression is correlated with tumour development and progression and is an important regulator of angiogenesis by releasing VEGF and promoting vascular pericyte recruitment [46–48]. Immunohistochemical analysis of breast tumour tissue revealed a significant association between a strong expression of pro- and active MMP9 in breast tumour tissue and a shortened relapse-free survival, and one study reported this relation in particular in oestrogen positive tissue [49–51]. Further a relationship between MMP-9 overexpression and a prolonged overall and relapse-free survival in early breast cancer has been demonstrated, although this finding is debatable as only the expression of the inactive proform of MMP-9 was assessed [52]. To further elucidate the role of MMP-9 in skin carcinogenesis, the effect of MMP-9 deficiency was investigated in the K14-HPV16 skin cancer mouse model [53]. *Mmp9* knockout mice developed neoplastic lesions and squamous carcinomas at a later stage than *Mmp9* heterozygote or wild-type mice. Although a larger number of tumours developed in the presence of MMP-9, these were of a less aggressive phenotype suggesting that MMP-9 may protect against tumour progression rather than promote tumour development. Analysis of tumours from control mice revealed that MMP-9 was predominantly expressed in the tumour stroma by mast cells, neutrophils and macrophages. In accordance, transplantation of bone marrow from control mice restored the tumorigenic phenotype of lethally irradiated *Mmp9*-deficient mice. MMP-9 possibly inhibits tumour progression *via* the generation of the anti-angiogenic factors endostatin and tumstatin. Endostatin levels have been found to increase *in vivo* after intratumoral adenoviral delivery of *MMP9* [54]. Adenoviral delivery of *MMP9* after subcutaneous injection of MCF-7 cells in nude mice increased MMP-9 activity *in vivo*, decreased tumour growth, induced endostatin expression and reduced microvessel density. It has been reported that *Mmp9*-deficient mice have decreased circulating levels of tumstatin and an increased tumour growth of implanted Lewis lung cancer cells which could be inhibited by intravenous administration of tumstatin [55].

With regard to genetic variation, it has been reported that the –1562 C/T promoter polymorphism affects *MMP9* expression with a higher promoter activity for the T allele in macrophages but not in primary amnion epithelial cells, Wistar Institute Susan Hayflick (WISH) amnion-derived or THP-1 cells [56, 57]. Tumours from breast cancer patients carrying the CT or TT genotype are characterized by various features of good prognosis and confer a prolonged overall survival [58].

Collectively these observations argue that the balance between the pro- and anti-angiogenic actions of MMP-9 is critical in determining its overall impact on tumour growth and progression indicating that this area needs further investigation.

MMP-12 (Table 2)

Macrophage metalloelastase or MMP-12 was originally identified as an elastolytic MMP, but it has been shown to degrade a wide variety of substrates [59]. MMP-12 cannot be categorized in one of the MMP subfamilies but is part of a separate group of miscellaneous MMPs. MMP-12 is predominantly expressed by macrophages but can also be found in hypertrophic chondrocytes and osteoclasts [56, 58]. As summarized below numerous studies have demonstrated a protective role for MMP-12; however, pro-tumorigenic/pro-metastatic functions for MMP-12 have also been reported [59, 60]. This discrepancy may partly be caused by the variety of tumour types studied, or may be due to differences in the cellular source of MMP-12 [61]. In squamous cell carcinoma of the vulva, tumour-derived *MMP12* mRNA expression correlates with more aggressive histology whereas macrophage-derived *MMP12* mRNA has been shown to be more abundant in well-differentiated grade I than in grade III tumours. Irrespective of its cellular source, *MMP12* mRNA expression was not correlated with tumour vascularization, metastasis or survival.

Similar to MMP-9, MMP-12 can inhibit endothelial cell proliferation and angiogenesis by the production of angiostatin. For

Table 2 Protective roles of MMP12 in cancer

MMP12	References	Study type	Cancer	Main findings
	[62]	*In vivo*	Melanoma (B16 cell line)	Reduced tumour growth Anti-angiogenic
	[63]			Prolonged OS
	[64]	Human studies	Hepatocellular carcinoma	Anti-angiogenic
	[65]	*In vivo*	Colon cancer (CT26 cell line)	Inhibition of tumour growth Anti-angiogenic
	[66]	*In vivo*	Colon cancer (CT26 cell line)	Inhibition of tumour growth Anti-angiogenic Anti-metastatic Prolonged OS
	[67]	*In vivo*	Colon cancer (CT26 cell line)	Reduced tumour growth Anti-angiogenic
	[68]	Human studies	Gastric cancer	Increased in cancerous tissue Inversely associated with lymph node metastasis Better 2 year survival
	[69]	Human studies	Colorectal cancer	Increased in cancerous tissue Inversely associated with hepatic metastasis
	[70]	Human studies	Colorectal cancer	Inversely associated with metastasis Anti-angiogenic Prolonged OS
	[71]	*In vivo*	Lung cancer (Lewis lung cancer cell line)	Increase in tumour growth Pro-angiogenic in KO mice
	[72]	*In vivo*	Lung cancer	Increased metastasis growth
	[73]	*In vitro*	Breast cancer (MCF-7, MDA-MB-231 cell line)	Inhibition MVEC invasion, MVEC tube formation Anti-angiogenic (uPAR dependant)
		In vivo	Breast cancer (MCF-7, MDA-MB-231 cell line)	Anti-angiogenic Reduced tumour growth
	[75]	Human studies	Lung cancer	SNP associated with better OS

KO: knockout; OS: overall survival; MVEC: microvascular endothelial cells; SNP: single nucleotide polymorphism; uPAR: urokinase plasminogen activator receptor.

instance, subcutaneous injection of MMP-12 overexpressing B16 murine melanoma cells reduced primary tumour growth by 73%, and blood vessel formation by 76% which correlated with an increase in serum angiostatin [62]. Similarly, *MMP12* mRNA expression in hepatocellular carcinomas was associated with a reduced tumour vascularity, increased angiostatin expression and better overall survival [63, 64]. Further *in vivo* evidence for an anti-angiogenic and anti-tumorigenic role for MMP-12 was obtained in orthotopic colon cancer Balb/c mouse model studies [65, 66]. Subcutaneously injected MMP-12 overexpressing CT26 murine colon cancer cells formed smaller tumours with a longer latency, a lower microvessel density, reduced VEGF expression and increased angiostatin expression compared to control CT26 murine colon cancer cells. Mice bearing MMP-12-expressing cancer cells developed less metastases and had a longer overall survival. Consistent with these findings, liposomal delivery of *Mmp12* to tumours induced by subcutaneous injection of CT26 colon cancer cells inhibited tumour growth and vascularization [65, 67]. Furthermore, in separate investigations, increased *MMP12* expression in tumour specimens was associated with a lower rate of lymph node metastasis and a better 2 year survival in gastric cancer [68], with the absence of hepatic metastases [69] and less extensive invasion into the intestinal wall, lymphatics and blood vessels, which was linked with a better overall survival in colorectal cancer [70].

The expression of both human and mouse proteases have been investigated in an orthotopic model of lung cancer using the human/mouse Affymetrix protease microarray [71]. Host-derived *Mmp12* was found to be up-regulated in lung tumours compared to

normal lung tissue. Consistent with the mouse data, an increased expression of stromal *MMP12* was seen in human lung adenocarcinomas compared to normal lung tissue. To determine if host-derived MMP-12 has a functional role in lung tumour formation, an experimental metastasis assay was performed. *Mmp12*-knockout mice injected with Lewis lung cancer cells showed a 2-fold increase in tumours that reached >2 mm in diameter, an increase in angiogenesis and a decrease in angiostatin levels. Both a spontaneous and experimental lung metastasis assay of *Mmp12*-knockout mice revealed numerous lung metastases whereas no difference was observed in the size of the primary tumour [72]. However the number of micrometastases was equivalent in *Mmp12*-knockout and wild-type mice, suggesting that MMP-12 affects lung tumour growth rather than metastasis formation. Bone marrow transplantation from wild-type mice into *Mmp12*-null mice identified macrophages to be the source of MMP-12 in the experimental lung metastasis model. In accordance with these findings an increased microvessel density was found in tumours from *Mmp12*-knockout mice. Surprisingly although plasma angiostatin levels have been found to be decreased in *Mmp12*-null mice, serum angiostatin levels were shown not to be altered. Additionally, it has been suggested that MMP-12 may exert its anti-angiogenic function through urokinase plasminogen activator receptor (uPAR) cleavage rather than angiostatin production using MMP-12 overexpressing MCF-7 and MDA-MB-231 breast cancer cells and *nu/nu* (CD-1) BR mice [73]. MMP12 overexpression inhibited microvascular endothelial cell (MVEC) invasion through Matrigel and formation of capillary-like tubes. Interestingly, the anti-angiogenic activity of MMP-12 was not related to the generation of angiostatin as addition of exogenous plasminogen did not alter angiostatin production of MMP-12 overexpressing cells. On the other hand, immunohistochemical staining indicated that MMP-12 was involved in uPAR cleavage on MVECs, disrupting its ability to interact with integrins and eliminating uPAR-driven pericellular proteolysis that enables endothelial cells to move within tissues. *In vivo*, a reduced vascularization of Matrigel sponges was observed in C57/BL6 mice after subcutaneous injection of Matrigel suspension containing conditioned media from the overexpressing cells. Furthermore, orthotopic injection of MMP-12 overexpressing cells in *nu/nu* (CD-1) BR mice resulted in a reduced tumour volume.

An SNP analysis of eight SNPs in six genes (*MMP1*, *MMP2*, *MMP3*, *MMP7*, *MMP9*, *MMP12*) revealed that small cell lung cancer patients carrying the G allele of the –82A/G *MMP12* polymorphism, which is associated with a higher gene expression in reporter gene assays [74], had a significantly prolonged overall survival compared to patients with the common allele [75]. Based on the aforementioned findings, we can conclude that the role of MMP-12 in cancer is as yet not fully understood. Evidence indicates that its cellular source, whether macrophage- or tumour derived, dictates its function. Macrophage-derived MMP-12 has been shown to play an important pro-inflammatory role through cleavage, both activating and inactivating, of all but one of the human Glu-Leu-Arg^{+} (ELR^{+}) CXC chemokines, resulting in resolution of acute inflammation and a less favourable microenvironment for tumour development [76]. Numerous studies have pointed to a different substrate repertoire for tumour-derived MMP-12, and consequently to a different role in tumorigenesis. Tumour-derived MMP-12 is believed to inhibit angiogenesis by enhancing angiostatin production [62–64, 66, 67, 71], reducing VEGF expression [65, 66] and preventing uPAR-mediated endothelial cell migration [73], further highlighting its complex activity. Identification of the relevant bioactive molecules for each cellular source appears to be the key to understanding the function of MMP-12 in cancer.

MMP-19

Together with MMP-12, MMP-19 belongs to the subgroup of miscellaneous MMPs. MMP-19 comprises the basic structural domains of MMPs but also displays several distinctive structural features, including an unique insertion of glutamic acid residues within the linker region, an unusual latency motif in the propeptide domain, an additional cysteine residue in the catalytic region and a COOH-terminal extension lacking sequence similarity to equivalent regions in other MMPs [77–79]. Remarkably, MMP-19 can cleave basement membrane components, connective tissue and cartilage matrix but does not degrade triple-helical type I collagen [80, 81]. Vascular smooth muscle cells and endothelial cells of inflammatory lesions have been shown to express MMP-19 [82]. Furthermore, MMP-19 expression was shown to be up-regulated in benign breast epithelial cells, normal intestine tissue and hyperproliferative keratinocytes at the tumour surface of squamous cell carcinomas [83–87]. *Mmp19*-knockout and wild-type mice have been subjected to the transplantation chamber assay using malignant murine PDVA keratinocytes cultured on a collagen gel to examine the effects of MMP-19 on tumour invasion and angiogenesis [88]. Transplants from *Mmp19*-knockout mice showed a progressive infiltration of host-derived cells, increased endothelial cell migration and tumour invasion. Analysis of basic fibroblast growth factor (bFGF)-treated Matrigel implants confirmed an increase in vascularization in *Mmp19-null* mice. MMP-19 expression was found in host mesenchymal cells but not in capillary endothelial cells or inflammatory cells. *In vitro* it was shown that capillary-like formation of human MVECs was inhibited after addition of recombinant MMP-19 to the Matrigel. Peptide mass fingerprinting of the Matrigel matrix revealed nidogen-1 to be cleaved in the presence of MMP-19, disrupting its ability to crosslink collagen IV and laminin and stabilize microvessels [89].

From these data, it appears that MMP-19 is involved in vascularization of tumours. However how MMP-19 acts and whether its role is attributed to a single function or multiple distinct activities most likely needs to be clarified in relation to its cellular source being endothelial, mesenchymal or inflammatory.

MMP-26

MMP-26 or matrilysin-2 or endometase is the smallest MMP family member comprising only pro- and catalytic domains but lacking a

haemopexin-like domain. Together with MMP-7 (matrilysin-1) it forms the matrilysin subfamily of MMPs. MMP-26 has only been detected in human beings and other primates suggesting that it is the result of a recent evolutionary event [90]. The little data available on MMP-26 in cancer suggest a relation between MMP-26 expression and a favourable phenotype. For instance, higher protein levels of MMP-26 have been found in early stages of squamous cell cancer, prostate cancer and breast cancer as compared to its expression in more advanced invasive cancer [91–94]. Similarly, MMP-26 expression was reduced in the surroundings of the most dedifferentiated and invasive cancer islands of colon cancer [83]. MMP-26 down-regulation was also found in endometrial carcinoma compared to endometrial hyperplasia lesions or normal endometrial tissue [95, 96]. On the RNA level, however, we found a positive correlation between *MMP26* expression and Gleason score in prostate cancer patients whereas no expression was found in head and neck squamous cell carcinoma [97, 98]. This indicates that conflicting results may result from the use of different techniques focusing on either RNA or protein expression, and urges a thorough comparison of MMP expression levels and localization using different methods such as in situ hybridization and immunohistochemistry.

At present it is unclear how MMP-26 is involved in cancer, having only two known substrates. A complex interplay between the oestrogen receptor and MMP-26 has been unveiled. MMP-26 expression has been shown to be regulated by oestrogen in hormone-regulated tumours including breast and endometrial cancers as well as in the normal reproductive processes and menstrual cycle [99–101]. On the other hand, MMP-26 is capable of cleaving the ERβ1 isoform of the oestrogen receptor, disrupting its ligand-independent transactivation and pointing to an oestrogen-regulatory loop in hormone-regulated malignancies. Indeed, in breast cancer MMP-26 expression was inversely correlated with levels of intact ERβ1. Elevated levels of MMP-26 were found during the early stages of cancer and were associated with a longer overall survival. In later stages of tumour progression MMP-26 levels were shown to decrease [93]. Furthermore, hormone-regulated MMP-26 expression has been implicated in inflammation through cleavage of the serpin a1-antitrypsin thereby releasing the activity of inflammatory serine proteinases, in particular neutrophil elastase and thus promoting matrix destruction and tumour development [101]. Further study is clearly required to gain a better understanding of the role and regulation of MMP-26 in cancer, especially in oestrogen-dependent neoplasms.

Conclusions

As our knowledge of MMPs broadens, the complexity of their functions becomes more apparent. An increasing number of functional studies using *in vitro* and *in vivo* models reveal MMPs to have conflicting roles. Although some MMPs, particular the ones that have been the focus of this review, appear more consistently to antagonize malignant behaviour, others such as MMP-1 and MMP-14 appear instead predominantly to promote tumour progression. The precise mechanisms underlying their cancer promoting and/or inhibiting actions observed *in vitro* and *in vivo* are as yet not fully understood. Although no conclusive evidence has been found to date, it is surmised that monitoring of circulating (plasma, serum) levels of MMPs with a distinct tumour-promoting role may prove to be clinically useful for cancer diagnosis and/or prognosis. It is intriguing that TIMP levels in plasma and tumour tissue appear to have clinical utility as predictors of prognosis in certain cancers, in particular colorectal and breast cancer, with high levels of TIMP-1 equating with poorer outcome [102–105]. This association again supports the concept of MMPs as anti-targets, but more broadly these types of findings indicate that the circulating levels of particular MMPs or TIMPs could find clinical utility as diagnostic or predictive markers.

It has become clear that depending on the tumour type, cellular source of expression and disease stage, a specific MMP can promote or inhibit tumorigenesis and/or metastasis. The site of expression dictates the availability of particular substrates and hence the tissue of origin and cellular source of a specific MMP will impact on the biological outcome associated with its expression. We feel therefore that a more detailed understanding of the tissue- and disease stage-specific expression and function of individual MMPs is needed to inform the deployment in the clinic of specific MMP inhibitors. In addition, the dynamic nature of the tumour microenvironment during disease progression will affect the available substrate repertoire as well as the expression of various MMPs. Thus identifying the key substrates of MMPs that are essential for their anti-tumorigenic and anti-metastatic functions promises to be a fertile area for further investigation and this in turn may advance the development of new therapeutics mimicking such cleavage products. Another useful strategy for cancer therapy takes advantage of tumour proteases without needing to know whether they act to promote or inhibit tumorigenicity. In this situation specific proteases that are up-regulated in tumours can be used to proteolytically activate latent pro-drugs, thus increasing their cytotoxicity. Recently we were involved in the demonstration of the utility of this novel approach whereby an MT1-MMP-cleavable version of the vascular disrupting agent colchicine derivative was shown to be effective against MT1-MMP expressing tumours with markedly reduced systemic toxicity [106]. The novel vascular disrupting agent ICT2588 was specifically hydrolysed into its active metabolite by MT1-MMP in tumour tissue, liver homogenates and plasma of fibrosarcoma HT1080 xenografts and activation was inhibited by the MMP inhibitor ilomastat. We found that ICT2588 administration reduced tumour vasculature and induced haemorrhagic necrosis of the tumour with reduced toxicity, improved therapeutic index and greater efficacy than its active metabolite supporting the clinical development of ICT2588. This novel approach might be more successful in preventing tumour progression than the inhibition

of distinct MMPs as changes in expression of one MMP might also affect the expression of other MMPs or proteases resulting in a net pro- or anti-tumorigenic/ metastatic phenotype. It is now widely believed that all proteases form a complex protease web, where changes in expression in one protease perturb the web, resulting in a ripple effect with subsequent changes in more proteases [5, 107]. Hence, it is of utmost importance to take the degradome-the repertoire of all proteases and their substrates-into account when performing functional studies, as MMPs can activate other MMPs and cleave ECM proteins revealing new sites of interaction or abolishing a recognition sequence for other MMPs further disrupting the balance in the protease web [108]. Caution must be taken in designing a multi-targeted approach for inhibition of MMPs as such an approach is not without risk and may have more profound and possibly detrimental effects than anticipated.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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