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Matrix metalloproteinases: molecular aspects of their roles in tumour invasion and metastasis

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Abstract

The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, whose physiological functions include tissue remodelling and embryogenesis. The importance of this group of proteins in the processes of tumour invasion and metastasis is now widely acknowledged, and has led to the search for MMP inhibitors for use as anticancer treatments in a clinical setting. This review aims to bring the reader up-to-date with current research relating to MMPs, with particular emphasis on emerging mechanisms of regulation of these enzymes, and their interaction with cell adhesion molecules. The therapeutic inhibition of MMPs will also be discussed.

Keywords: Matrix metalloproteinase; Tumour; Pathology; Gene regulation

1. Introduction

The MMPs are a family of highly conserved metal atom-dependent endopeptidases, which, collectively, are capable of degradation of most, if not all components of the basement membrane and extracellular matrix. In particular, the MMPs include the only enzymes known to be capable of degrading fibrillar collagen. Fibrillar collagen refers to the polymeric structure adopted by collagens I, II, III, V and XI. Type I collagen is the most abundant collagen in humans, and comprises the principal collagen found in skin and bones.

There are currently at least 20 known human MMPs, with new members still being discovered (Table 1) [1,2]. Using many different methodologies, including gel zymography, immunohistochemistry and PCR-based techniques, the presence of individual members of the MMP family has been studied in most tumour types. Overexpression of MMPs is now known to be a characteristic of most malignant tumours, and, in the case of some carcinomas, the presence of specific MMPs has been shown to be of prognostic significance [3–12]. This review focuses on the role of MMPs in tumour invasion and metastasis, with emphasis on recent findings regarding the regulation of MMPs, and their interactions with cell adhesion molecules.

1.1. The role of MMPs in tumour invasion and metastasis

Tumour cell invasion and metastasis are now regarded as multi-step phenomena, involving proteolytic degradation of basement membranes and extracellular matrix (ECM), altered cell adhesion and physical movement of tumour cells. Angiogenesis, the formation of new blood vessels, is essential both for tumour growth and for successful tumour invasion and metastasis. Angiogenesis is complex and dynamic, and requires proliferation of endothelial cells from pre-existing blood vessels, breakdown of extracellular matrix (ECM) and migration of endothelial cells. Thus, growth and development of blood vessels within tumours requires the same factors that are crucial to tumour cell invasion and the MMPs play a central role in all of these processes. Individual MMPs may have different, possibly contradictory, roles in angiogenesis. Proteolysis of the ECM is a prerequisite for angiogenesis, and activated MMPs (specifically, MMP-2) are present in endothelial cells of blood vessels at sites of angiogenesis. However, several MMPs (MMP-2, MMP-

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Table 1
Classification and nomenclature of the human MMPs

MMP subfamily	MMP number	MMP name
Collagenases	1	Interstitial collagenase
	8	Neutrophil collagenase
	13	Collagenase-3
Gelatinases	2	72 kDa Type IV gelatinase
	9	92 kDa Type IV gelatinase
Stromelysins	3	Stromelysin-1
	10	Stromelysin-2
	11	Stromelysin-3
	18	Putative MMP, similar to stromelysins
Membrane-type MMPs	14	MT1-MMP
	15	MT2-MMP
	16	MT3-MMP
	17	MT4-MMP
	24	MT5-MMP
	25	MT6-MMP
Other MMPs	7	Matrilysin (PUMP-1)
	12	Macrophage elastase
	19	Rheumatoid arthritis-associated MMP
	20	Enamelysin
	21	Recently cloned MMP
	22	Recently cloned MMP
	23	Recently cloned MMP

7, MMP-9 and MMP-3) have recently been shown to be capable of proteolytic cleavage of plasminogen to form angiostatin, an endogenous angiogenesis inhibitor, which specifically inhibits proliferation of endothelial cells [13–15]. However, the role of MMPs in angiogenesis is a large topic, which will not be dealt with in detail in this review.

The MMPs are responsible for degradation of the constituents of basement membranes and the ECM. Through interactions with an array of cell adhesion molecules, MMPs are implicated in altered adhesion between the tumour cell and its environment, and recently have been shown to play a role in the movement of cells through the ECM.

In addition to their function in the breakdown of the ECM, MMPs also have growth regulatory effects on both primary and secondary tumours. *In vitro* studies have demonstrated degradation of insulin-like growth factor receptor binding proteins (IGFBP-3 and -5) by MMPs; this may contribute to the observed growth-regulatory functions of the MMPs [16].

There is also experimental evidence that MMPs are involved in the early stages of tumour growth and development. Goss and co-workers observed a 48% decrease in the number of adenomas in Min mice following administration of the synthetic MMP inhibitor Batimastat [17].

We have recently reviewed the literature on the presence of MMPs and their major physiological inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) in tumours [18]. In this review, we focus on the molecular mechanisms by which MMPs participate in tumour invasion and metastasis, with emphasis on emerging patterns of interaction with cell adhesion molecules, and examine recent research regarding the control of MMP expression. Lastly, we outline some studies of MMP inhibitors, which are being evaluated as anticancer agents.

2. Regulation of MMPs

The constitutive level of expression of MMP genes is normally low, the enzymes being induced under various physiological circumstances when ECM remodelling is required, for example during embryogenesis, wound repair and bone remodelling. Increased expression or activation of MMPs is observed in many disease states, in particular, arthritis and neoplasia. As befits a group of enzymes with such potentially devastating effects, MMP expression appears to be tightly regulated and to occur at a number of different levels. In addition, individual members of the MMP family are separately regulated and expression is highly tissue-specific. Thus, regulatory mechanisms operate in both a temporal and spatial dimension. Knowledge of the mechanisms by which MMP expression or activity is regulated is of interest because of the potential therapeutic applications of manipulating such processes, as well as enhancing our understanding of the mechanisms of tumour invasion and metastasis.

2.1. Transcriptional regulation of MMP genes

Some of the MMP genes (*MMP-1*, *MMP-3*, *MMP-7*, *MMP-9*, *MMP-10*, *MMP-12* and *MMP-13*) are known to be inducible and there is a high degree of conservation of regulatory elements within the promoter regions of these genes. The two most important types of binding sites found within the promoter regions of these genes are the Activating Protein-1 (AP-1) site and the Polyoma Enhancer Activator (PEA3) site.

2.1.1. AP transcription factors

A single AP-1 element is present at approximately −70 base pairs upstream in the promoter region of each inducible MMP gene. The AP-1 binding site has been the subject of much recent research and it is now known to be essential for basal transcription of *MMP-1*. Molecules that inhibit the expression of inducible MMP genes also appear to act via the AP-1 site.

The AP-1 site is necessary for the transcriptional response to a variety of signals, for example, inter-

leukin-1, tumour necrosis factor α, interferon-β and 12-O-tetradecanoylphorbol 13-acetate (TPA). Other agents that modulate the expression of MMPs, such as glucocorticoids, retinoids and transforming growth factor beta (TGF-β), also require the AP-1 site to exert their influence. The contribution of AP-1-independent mechanisms to MMP repression by these agents has not, however, been excluded. Other *cis*-acting elements (e.g. the PEA-3 site) are necessary for both basal transcription and *trans*-activation by phorbol esters, cytokines and growth factors [19–21].

AP-1 transcription factors are proteins, composed of Jun and Fos subunits. These subunits form heterodimeric leucine zipper proteins that bind to a consensus DNA sequence at the AP-1 site. In normal cells, Jun and Fos proteins are expressed transiently, following a mitogenic stimulus to the cell. The cellular concentration of these subunits is normally controlled by the stability of their mRNA (i.e. post-transcriptional regulation), as well as by the rate of gene transcription. Binding of Fos/Jun complexes to the AP-1 site is associated with transcriptional activation of MMP genes, but some members of the Fos and Jun families of proteins act as transcriptional repressors. Fos/Jun complexes that are weak activators of AP-1 interact with this site, thereby preventing interaction with more potent activators. For example, JunB has been shown to inhibit cJun-induced *MMP-1* expression. Thus, the relative abundance of various members of these protein families represents another level of control of MMP expression. Expression of JunB is associated with differentiated cells, which may contribute to the less aggressive biological behaviour observed in better-differentiated tumours.

A second activating protein (AP-2) has been described, and this site appears to play a significant role in MMP regulation [20].

Most of the promoters of inducible MMP genes also contain a PEA3 site, which binds members of the Ets family of oncoproteins. The combination of AP-1 and PEA3 binding sites has been referred to as an 'oncogene-responsive unit' and it appears to be a recurring motif, found in the promoter regions of other genes [22]. The AP-1 and PEA3 sites display functional cooperation, and there is evidence of synergism between these sites [21]. Thus, modulation of either of these sites may represent a therapeutic approach via downregulation of MMP synthesis.

There have been few studies on the regulation of genes encoding the TIMP proteins, but one such recent report concerns expression of *TIMP-3*. Aberrant methylation of the promoter region of the *TIMP-3* gene was identified in cells from a variety of solid tumours (kidney, brain, colon, breast and lung), but was not present in control cells. The abnormal methylation results in loss of expression of *TIMP-3*, which has previously been associated with tumour development [23].

2.1.2. *A polymorphism in the promoter of* MMP-1 *influences expression*

A single nucleotide polymorphism that influences transcription has recently been described in the *MMP-1* promoter. The presence of an additional guanine residue at −1607 bp in the promoter leads to the creation of an Ets binding site, 5′-GGA-3′, adjacent to an AP-1 site at −1602 bp. This genotype was observed in 30% of a control group ($n=100$). By contrast, in a polymerase-chain reaction (PCR)-based analysis of eight tumour cell lines (three breast cancer cell lines and five melanoma cell lines), the observed frequency of the GG genotype was 62.5% ($P=0.0001$). This polymorphism is associated with increased levels of transcription of *MMP-1* in both normal and malignant cells (human fibroblasts, melanoma cells and breast cancer cells). Normal cells transcribe *MMP-1* at a lower level than malignant cells, even under the influence of the GG allele, drawing attention to the fact that other factors are involved, for example, concentrations of growth factors and cytokines and cell-specific nuclear factors [24].

In another study of ovarian cancer by Kanamori and colleagues, it was reported that the GG genotype possesses greater transcriptional activity than the G genotype. This group examined blood samples from 163 ovarian cancer patients, genotyped them for the polymorphism and, using a semi-quantitative reverse transcriptase (RT)-PCR technique, also measured the level of mRNA expression of *MMP-1* in the corresponding tumours. Amongst patients with ovarian tumours, occurrence of the GG allele was significantly more frequent than amongst a control group without cancer ($P=0.028$). Levels of MMP-1 in patients with the GG alleles were found to be significantly higher than levels in patients with the G genotype ($P=0.0038$) [25].

2.1.3. *Some MMP genes are p53 target genes*

A p53 binding site has been identified in the promoter of the *MMP-2* gene, and interaction of wild-type p53 at this site induced *MMP-2* transactivation in *in vitro* and *in vivo* studies [26]. More recently, the *MMP-1* gene has been shown to be a p53 target gene. Wild-type p53 downregulates the promoter activity of *MMP-1* in a dose-dependent fashion. In contrast, several p53 mutants do not display this repression activity. The repression is mediated at least partly via the AP-1 site in the promoter [27].

2.1.4. *Transcriptional inhibition of MMP gene expression*

A variety of compounds are known to inhibit the synthesis of MMPs, including retinoids, thyroid hormones, glucocorticoids, progesterone and androgens. All these agents bind to members of the nuclear receptor subfamily. Nuclear receptors control gene expression via a number of different mechanisms.

Firstly, nuclear receptors act on the promoter regions of the MMP genes. To date, few hormone responsive elements (HREs) have been identified within the promoters, and inhibition of MMP expression appears to occur primarily via the AP-1 site. The nuclear receptors form complexes with the DNA through interactions with AP-1 proteins.

Glucocorticoids are known to repress transcription of the *MMP-1* gene by interaction with AP-1 proteins. Retinoids also suppress *MMP-1* transcription, the mechanism involving downregulation of *Fos* and *Jun* mRNA, sequestration of Fos and Jun proteins and the formation of complexes of retinoid acid receptors and AP-1 proteins.

Nuclear receptors and their ligands can also indirectly inhibit MMPs. Retinoids and glucocorticoids induce transcription of the tissue inhibitors of metalloproteinases (TIMPs). Nuclear receptors bind to coactivators, corepressors and components of the general transcriptional apparatus, but the significance of these interactions is unclear [19,20,28].

2.2. Post-transcriptional regulation of MMP activity

Although sophisticated transcriptional control mechanisms play an important role in modulating the rate of synthesis of MMPs, post-transcriptional mechanisms are central to the control of MMP activity, as all the soluble MMPs are secreted as inactive zymogens requiring activation by cleavage of the N-terminal propeptide domain (Fig. 1). The membrane-type MMPs (MT-MMPs) are distinguished by the possession of a transmembrane domain and differ in that they are activated intracellularly, prior to transport to the cell surface.

The major physiological inhibitors of the MMPs are a family of proteins known as the TIMPs, of which four members are currently known. These proteins are capable of specific inhibition of the active forms of MMPs, and inhibition by TIMPs represents an additional important level of control of proteolysis. The pathways by which MMPs interact with one another and with various activators and inhibitors are complex, and not yet fully elucidated.

The membrane-type MMPs, and stromelysin-3 (MMP-11), possess a decapeptide insert, which comprises a triad of basic residues, recognised by a family of endopeptidases known as pro-protein convertases. Furin, a member of this family, is concentrated in the Golgi apparatus, and activates a range of growth factors and membrane receptors. Furin has been shown to activate MT1-MMP *in vitro* [29]. Intracellular activation of MMP-11 (stromelysin-3) by furin has also been demonstrated [30]. Activation of MT1-MMP is important because this is one of the key molecules involved in the MMP activation cascade. MT1-MMP activates the zymogen of MMP-2 (proMMP-2) and pro-MMP-13. MMP-2 in turn activates MMP-9.

The interaction of the MMPs with the urokinase-type plasminogen activator (uPA)–plasmin system is complex, as proteolytic activation of MT1-MMP by plasmin has been described. Okumura and co-workers found that plasmin cleaved MT1-MMP in the multi-basic motif between its pro- and catalytic domains, which is

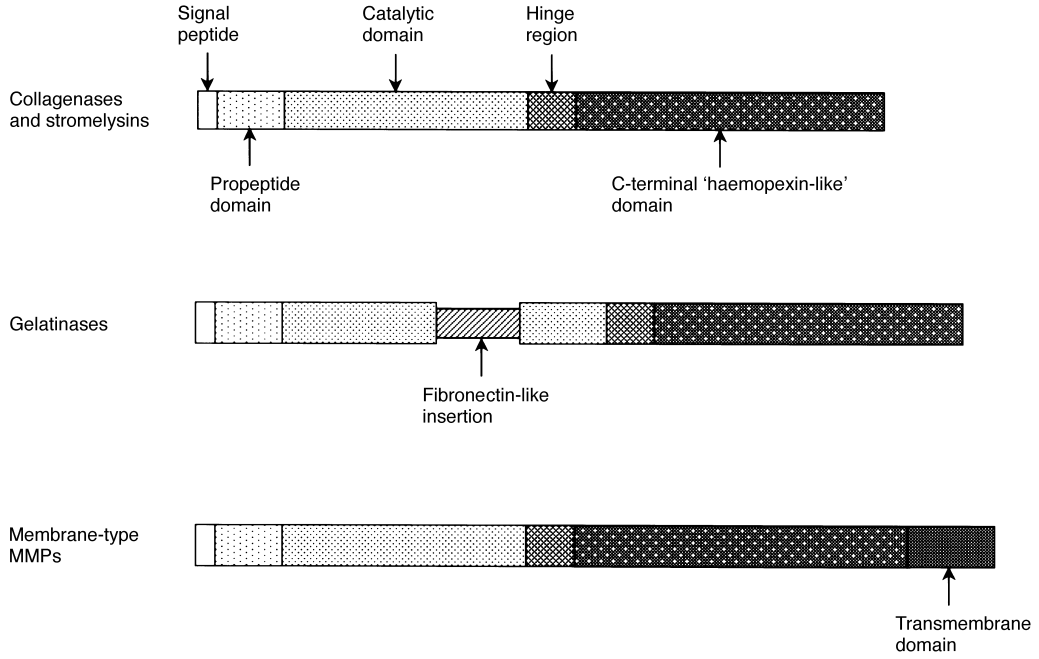

Fig. 1. Structure of the matrix metalloproteinases (MMPs). The MMPs display a domain structure: cleavage of the N-terminal propeptide domain yields the active form of the enzyme. The gelatinases are distinguished by the presence of a fibronectin-like region within their catalytic domain, whereas the membrane-type MMPs are characterised by a C-terminal transmembrane portion. The haemopexin-like repeat is absent from matrilysin (MMP-7).

the region associated with activation of pro-MMP-2. As plasmin is a soluble protein, and MT1-MMP is a transmembrane molecule, such interaction could occur at the cell surface [31].

2.2.1. Role of TIMPs in post-translational regulation of MMPs

The TIMPs are a family of low-molecular weight proteins, currently numbering four in total, which function as the major physiological inhibitors of the activated forms of the MMPs. However, the TIMPs do not simply act as inhibitors of MMPs; they also play important functions in their activation. For example, the activation of MMP-2 requires the formation of a ternary complex comprising MT1-MMP, proMMP-2 and TIMP-2. However, higher concentrations of TIMP-2 are reported to inhibit activation of proMMP-2. Once activated MMP-2 has been generated, it is capable of auto-proteolysis, with further production of the active form. The activation of soluble MMPs does not occur within the cytoplasm, and recent research offers an interesting hypothesis of how (and where) this activation occurs. This concept also offers an attractive mechanism by which the processes of cell movement and proteolysis can be interlinked. Specialised surface protrusions, called invadopodia and lamellipodia, are found on the surface of invasive cells, and make contact with the ECM. These structures serve as the 'motors' for cell movement through, and invasion of, the ECM. Chen and colleagues have localised MMP-2 and MT1-MMP to invadopodia, which are sites of ECM degradation. Lamellipodia are similar in structure and motility to invadopodia, but are accessible to TIMP-2. TIMP-2 co-localises with MMP-2 and MT1-MMP at lamellipodia but not at invadopodia. The co-localisation of MMP-2, MT1-MMP and TIMP-2 at lamellipodia may be a key mechanism for the regulation of MMP activity at the cell surface, which in turn governs expression of the cell invasiveness phenotype.

Tyrosine kinase inhibitors abrogate the degradative activity mediated by invadopodia, implying that an active process is occurring at the site of cell–matrix interaction [32].

2.3. The coagulation system and MMP activation

There are a number of points of intersection of the coagulation and fibrinolytic pathways with the MMP activation cascade. Several groups have observed that plasmin is an efficient activator of MMP-3 and MMP-13. Activated MMP-3 is, in turn, a potent activator of MMP-9, as well as MMP-1. However, even in the presence of potential activators, the molar ratio of MMP-9 to its inhibitor (TIMP) is the critical determinant of whether activation will occur. MMP-9 activation is observed to proceed at MMP-9/TIMP ratios of greater than 1:1.

A component of the fibrinolytic pathway, thrombin, has been identified as an activator of proMMP-2 in human umbilical vein endothelial cells (HUVEC); the mechanism is believed to involve MT1-MMP, a known activator of MMP-2 at the cell surface. However, recent work indicates that thrombin can activate MMP-2 in microvascular endothelial cells via an MT1-MMP-independent pathway [33–35].

3. Functions of MMPs: interactions with cell adhesion molecules

Both altered matrix degradation and cell adhesion are vital factors in promoting tumour invasion. Recent studies reveal mechanistic links between these two processes, and provide evidence that MMPs participate in both these processes, via multiple interactions with constituents of the cell adhesion apparatus. It is becoming apparent that complex, co-ordinated interactions between MMPs and cell adhesion molecules take place in order to facilitate the movement of cells through the ECM. There is increasing evidence of interactions between individual MMPs and cell adhesion molecules of different classes, as outlined below.

The mechanism (or mechanisms) by which MMPs facilitate the invasion of cancer cells remains to be completely elucidated. A report demonstrating cleavage of laminin-5 by MMP-2 sheds light on this fundamental question. Laminin-5 is a protein constituent of the basement membrane, which epithelial cells adhere to, and interact with, via transmembrane integrin molecules. Prior to this work, laminin had not been recognised as a substrate for MMP-2. Giannelli and coworkers demonstrated the presence of a cryptic site on the laminin-5 molecule, which was revealed following proteolytic cleavage of laminin-5, catalysed by MMP-2. Breast epithelial cells acquired motility following laminin-5 cleavage, which was abrogated by monoclonal antibodies directed against the cryptic site. This site is not involved in cell adhesion, but it does interact with epithelial cells and either induces motility or releases the epithelial cells from motility suppression. These investigators suggest that the cryptic site functions in a signalling capacity rather than by mechanical means to influence cell motility [36].

3.1. β-catenin/E-cadherin

β-catenin is a member of a group of intracellular attachment proteins; one of its functions is the attachment of cytoplasmic actin bundles to cadherin transmembrane adhesion molecules, at the site of cell–cell junctions. Tumour cells break away from their neighbours in order to invade nearby tissues and metastasise and therefore abnormalities of the β-catenin/E-cadherin

complex are likely candidates for involvement in this process.

In addition to its structural role, β-catenin, in combination with the DNA binding protein T Cell Factor (TCF-4), also acts as a transcriptional regulator of specific genes. Normally, the protein product of the tumour suppressor gene adenomatous polyposis coli (*APC*) binds to cytoplasmic β-catenin, leading to degradation of β-catenin. However, in most colorectal cancers, there is a loss of function mutation of *APC*, leading to an accumulation of β-catenin, which complexes with TCF, and activates transcription of TCF target genes. These genes include *cyclin D1* and *c-myc*, thereby connecting *APC* mutations to deregulation of the cell cycle. Recent evidence suggests that the *MMP-7* (matrilysin) gene is also a target of β-catenin/TCF. This finding is in agreement with the high proportion of colorectal cancers exhibiting both loss-of-function mutations of *APC* and overexpression of MMP-7. An alternative pathway in *APC*-positive tumours may involve dominant mutations in β-catenin, which render the molecule resistant to degradation. Additional details of this pathway come from the work of Crawford and colleagues, who have found that TCF actually represses the *MMP-7* promoter. In their study, promoter activity was upregulated, by as much as 12 times normal, by β-catenin [37–39].

An alternative mechanism whereby the β-catenin/e-cadherin complex may be implicated in tumour formation or progression is mutation of the *E-cadherin* gene. These mutations are frequently observed in lobular carcinomas of the breast and diffuse gastric carcinomas, but are infrequently observed in other types of human malignancies. Interestingly, these tumours display a characteristically diffuse growth pattern, with marked lack of cohesiveness between the tumour cells. The mutations in the E-cadherin gene occur at a very early, non-invasive stage of tumour development, suggesting that loss of cell–cell adhesion represents an early step in carcinogenesis, at least in these tumour types [40].

3.2. Integrins

The integrins are a family of transmembrane linker proteins, which function as cation-dependent cell adhesion molecules. Integrins are heterodimers, formed from α and β subunits; their primary function is in the formation of interactions between the cell and macromolecules in the ECM. Integrins provide anchorage for cells to the ECM and are involved in directed invasion and motility of cells.

Integrins possess recognition sites for specific matrix macromolecules on their extracellular portion. Proteolysis of the ECM alters the way in which integrins interact with it. For example, αvβ3 integrin does not adhere to native, intact collagen but it does adhere to collagen that has undergone proteolytic degradation, due to exposure of an RGD binding site. Expression of the αvβ3 integrin receptor confers an invasive advantage on melanoma cells [41]. More recently, Brooks and colleagues showed that melanoma cells expressing the αvβ3 integrin acquired the ability to bind MMP-2 in an active form, facilitating collagen degradation. The intact C-terminus of MMP-2 (the non-catalytic terminus) was required for binding to αvβ3 integrin. Thus, a cell surface receptor known to be involved in directed cellular motility can also bind an enzyme capable of breaking down the major constituent of basement membrane (collagen type IV) [42].

Integrins can affect the transcription of MMP genes; in an osteogenic cell line, overexpression of α2β1 is associated with an increase in *MMP-1* transcription. This could be a way for integrin to act as a signal transducer, influencing production of MMPs in response to information about the ECM [43].

The tetraspanin superfamily, (also known as the transmembrane-4 superfamily), consists of a group of cell-surface proteins that mediate a varied range of processes. They have been dubbed 'molecular facilitators' [44], due to their participation in such diverse functions as cell activation, differentiation, proliferation, adhesion and motility. These proteins are renowned for their ability to associate with integrins, lineage markers and other tetraspanins [44]. Tetraspanin–integrin complexes are implicated in integrin-mediated cell migration. Integrin (α3β1)–tetraspanin protein complexes are involved in tumour cell migration *in vitro* and these complexes have been shown to stimulate production of MMP-2. These complexes are also implicated in the rearrangement of the actin cytoskeleton. These observations provide a paradigm within which the co-ordination of proteolysis and cell migration may be considered [45,46].

Further evidence of the interaction between integrins and MMPs is provided by the work of Niu and colleagues who demonstrated induction of MMP-9 in colon cancer cells by αvβ6 integrin [47]. Increased expression of αvβ6 in colorectal cancer cells compared with normal colonic mucosa has been documented and is associated with enhanced cell growth. Agrez and colleagues report an increase in MMP-9 secretion relative to its inhibitor (TIMP-1) in colon cancer cells that overexpress αvβ6. The increase in secretion of MMP-9 parallels the level of cell surface αvβ6 expression, and is associated with increased proteolysis at the cell surface [48].

The cell surface receptor cell determinant (CD)44, which is known to promote the growth and metastasis of tumour cells, has been shown to associate with active MMP-9 at the cell surface of human melanoma cells. Disruption of MMP-9/CD44 aggregates diminishes the invasiveness of tumour cells *in vivo*. Recent studies of the interaction between CD44 and MMP-9 reveal that,

in normal keratinocytes, MMP-9 localisation to the cell surface is CD44-dependent. The localisation of MMP-9 to the cell surface is essential for its ability to promote tumour invasion and angiogenesis. These interactions between CD44 and MMP-9 provide a mechanistic link between cell adhesion and matrix degradation [49,50].

3.3. Cytokines

A role in the regulation of MMPs, either at the transcriptional level, or post-transcriptionally, has been assigned to a number of cytokines, in particular, the interleukins. Specifically, interleukin-1 (IL-1) is known to induce transcription of *MMP-1* and *MMP-3* in fibroblasts. This transcriptional activation is suppressed by IL-4, and this reduction occurs, at least in part, at the level of transcription [51].

IL-6 has been shown to be involved in the regulation of the expression of *MMP-2*, *MMP-9* and *TIMP-1* in non-Hodgkin's lymphoma (NHL) cells. The same group previously showed that increased MMP-9 and TIMP-1 levels confer a poor prognosis in NHL. Elevated expression of IL-6 correlated with elevated mRNA transcripts for *MMP-9*, *MMP-2* and *TIMP-1* [52].

IL-10 stimulates production of TIMP-1 and inhibits secretion of the gelatinases (MMP-9 and MMP-2) in immortalised human prostate cancer cell lines, thereby interfering with angiogenesis *in vitro* [53].

In contrast to the anti-angiogenic effects of IL-10, IL-8 is known to be 'pro-angiogenic'. Transfection of human melanoma cells with IL-8 resulted in upregulation of, and increased activity of, MMP-2 and was associated with increased invasiveness of the cells. Ultraviolet-B light and hypoxia are known inducers of IL-8; therefore, this study connects environmental factors with the aggressive phenotype in melanoma cells [54].

4. Therapeutic inhibition of MMPs

As the role of MMPs in tumour development and progression became apparent, many potential inhibitors of these enzymes (matrix metalloproteinase inhibitors, MMPIs) were assessed for anticancer properties. MMPIs can belong to a number of different chemical classes.

4.1. Synthetic low-molecular weight MMPIs

The TIMPs are unsuitable for therapeutic use due to their short half-lives. Therefore, a number of synthetic MMP inhibitors have been designed. Batimastat (British Biotech Inc., Oxford, UK), a potent broad-spectrum inhibitor of MMPs, is a synthetic hydroxyamate, whose structure imitates that of collagen. Batimastat functions by chelating the zinc ion present at the active site of each MMP enzyme. This was one of the first anti-MMP drugs to be clinically tested. While this agent inhibited tumour growth and metastasis in animal models, its major drawback was extreme insolubility in water. However, this feature does not affect its suitability for use as an intraperitoneal or intrapleural agent, for the relief of malignant ascites and peritoneal effusions. There is evidence for its' effectiveness in the latter setting. Recent animal experiments using intravital videomicroscopy have revealed that Batimastat inhibits angiogenesis *in vivo*, and this may be the basis of its' antitumour effects [55,56].

Marimastat (BB-2516) is a second-generation synthetic MMP inhibitor, and is the first orally administered synthetic MMPI to be clinically evaluated as an anticancer agent. In phase I and II trials, Marimastat showed dose-dependent biological effects (measured by evaluating serum tumour markers), in colorectal, ovarian and prostate cancer. The safety of combining Marimastat with cytotoxic chemotherapy has also been demonstrated. It is currently in comparative phase III clinical trials as a treatment for gastric and pancreatic adenocarcinoma and small cell lung carcinoma. Marimastat is also being assessed as adjuvant chemotherapy following resection of pancreatic adenocarcinoma. The dose-limiting side-effects of Batimastat and Marimastat are musculoskeletal, with inflammation of tendons and joints. These side-effects appear to be reversible on discontinuation of MMPI therapy [57,58]. The synthetic MMPIs Batimastat and Marimastat exhibit broad-spectrum inhibition of the MMPs, however, not all tumours express all MMPs. Additionally, it is not clear whether all the members of this large family of proteins are involved in tumour progression and/or metastasis. Therefore, the development of more specific inhibitors may be desirable, and may result in a diminution in side-effects. A recent report describes the development of specific gelatinase inhibitors, which inhibit *MMP-9* and *MMP-2*, and prevent the migration of tumour cells, tumour growth and invasion in animal models and prolong survival in xenograft-bearing animals [59].

4.2. Bryostatin compounds

The bryostatins are naturally occurring macrocyclic lactones, which inhibit MMPs by a mechanism that differs from the synthetic MMPIs. The mechanism of their inhibition is unclear, but appears to involve activation of protein kinase C (PKC), followed by its' downregulation. As *MMP-1*, *MMP-3*, *MMP-9* and *MMP-11* are all PKC-responsive genes, the bryostatins may act by suppressing transcriptional activation of these genes.

BAY 12-9566 (Bayer) is a bryostatin, a novel, non-peptide biphenyl MMP inhibitor with activity in a variety of tumour models, which is currently in clinical

development. This agent also appears to exert its anti-metastatic effects via an antiangiogenesis pathway, by inhibiting the ability of endothelial cells to invade through the matrix, without affecting cell proliferation [60].

Another bryostatin compound, AG3340 (agouron), inhibits growth and invasion of glioma cells in an *in vivo* model, and produces increased survival [61].

4.3. Modified tetracyclines

Tetracyclines, well established as antimicrobial agents, also possess MMP inhibitory properties and chemically modified tetracyclines (CMTs) have been synthesised, in which anti-MMP effects are retained, with elimination of antibacterial effects. One of these agents, CMT-3, shows promise in animal models of prostate carcinoma [62,63].

4.4. Therapeutic endpoints

As the MMPIs (matrix metalloproteinase inhibitors) are cytostatic rather than cytotoxic, traditional endpoints used in anticancer drug trials, such as reduction in tumour size, may not be appropriate in assessing their effectiveness. The concept of dormancy of metastases is well established, and in some circumstances, conversion of tumours from aggressiveness to a more 'quiescent' state may be an acceptable therapeutic outcome. In order to maintain dormancy of the tumour or metastases, such a drug would have to be administered on a long-term basis, and therefore, issues of toxicity would be of paramount importance. An example of a drug that may be capable of such modulation of tumour aggressiveness is a synthetic MMP inhibitor, (CT1746), which, in animal models of colorectal cancer, results in reduction in tumour growth, spread and metastasis and an increase in survival [64].

Related alternative endpoints have been utilised in assessing the efficacy of drugs, for example, the rate of change of serum levels of the tumour marker CEA (carcinoembryonic antigen) was used in early clinical trials of Marimastat. The validity of such an endpoint was, until recently, unproven. However, Watson and associates investigated the relationship between tumour growth and CEA levels in mice bearing gastric carcinomas. They found that serum CEA levels reflected tumour size and concluded that this supported the validity of using this outcome measure in early assessments of non-cytotoxic drugs [65].

4.5. Future development of MMPIs

The search for MMP inhibitors suitable for use as anticancer therapies continues, with many novel substances being proposed as candidates. An antibacterial agent called hypothemycin, which inhibits Ras-inducible genes, including *MMP-1*, *MMP-3* and *MMP-9*, has been described. This represents a new approach to MMP inhibition, through transcriptional regulation [66].

Antisense oligonucleotides to matrilysin (MMP-7) have been tested on two human colon cancer cell lines (CaR-1 and WiDr). The antisense oligonucleotide inhibited both the secretion of MMP-7 by cultured CaR-1 cells and their *in vitro* invasion through the basement membrane. The oligonucleotide liver metastasis of WiDr cells from the spleen of WiDr transplanted nude mice [67].

An MMP-9 ribozyme has also been evaluated in an experimental model — this approach involves inhibition of the synthesis of MMPs [68]. Other possible therapeutic approaches that exploit MMPs include gene-based treatments. A gene delivery system has been described which is activated by MMPs expressed preferentially by tumour cells [69].

5. Conclusions

Considerable information is now available about the role of MMPs and their inhibitors in tumour progression and metastasis; however, the challenge remains to apply this knowledge in a clinically useful fashion. To date, individual MMPs have been shown to be of prognostic significance in several types of tumours (often in small series). Precise information about which MMPs are critical to tumour invasion and/or metastasis in various types of tumours may enable the rational development of drugs aimed at specific MMPs, or their inhibitors. If, as some recent studies suggest, MMPs are involved in early stages of tumour development, it may be possible to use inhibitors as tumour prevention agents. Advancing our understanding of this important group of enzymes will enable us to fully exploit their potential clinical applications.

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