PERSPECTIVE

Matrix metalloproteinase biology applied to vitreoretinal disorders

C S Sethi, T A Bailey, P J Luthert, N H V Chong

Matrix metalloproteinases (MMPs) and their inhibitors are believed to have a significant role in a number of vitreoretinal diseases, from proliferative vitreoretinopathy to age related macular degeneration. The aim of this review is to summarise the current knowledge of their involvement in these diseases and to postulate potential therapeutic strategies.

MMPs are a tightly regulated family of zinc dependent endopeptidases that are capable of degrading all components of the extracellular matrix (ECM) and basement membranes.¹ The ECM is a complex structure that influences the behaviour of its resident cells, and those in the process of migration, by providing specific contextual information. Enzymes that modify the ECM thus have the potential to affect basic cell biology in many physiological and pathological processes.² Remodelling of the ECM must occur with spatial and temporal precision for normal development, morphogenesis, homeostasis, and tissue repair.³ As matrix degradation is a prerequisite for malignant invasion and metastasis, it is not surprising that MMP biology has attracted considerable interest, with clinical oncology trials under way in several organ systems.⁴⁻⁷ New proteolytic functions are also being defined for these enzymes. These include the degradation of non-matrix macromolecules such as myelin basic protein, inactivation of α_1 antitrypsin, release of sequestered growth factors from ECM, and cleavage of bioactive molecules from the cell surface.⁸

The tissue inhibitors of metalloproteinases, or TIMPs, are physiological MMP inhibitors that have now been shown to have additional biological activities independent of this primary function.⁹ The functional proteolytic activity of MMPs in a given biological situation is thus depend-

ent on the relative concentrations of regulatory TIMP molecules and *active* MMPs. Excessive MMP activity is associated with matrix degradation and a feature of destructive diseases such as rheumatoid arthritis, osteoarthritis,^{10 11} dermal photoageing,¹² periodontitis,¹³ and chronic ulceration.^{14 15} Aberrant regulation may also lead to excess matrix deposition, seen in chronic fibrotic disorders,¹⁶⁻¹⁹ and the formation of scar tissue following injury.²⁰

The interphotoreceptor matrix and Bruch's membrane are examples of ocular matrices that undergo slow physiological turnover. Any disruption in the exquisite homeostatic regulation of these structures may thus have a catastrophic effect on visual function. Understanding the control of MMP expression and activation and the cellular source and specific enzymes produced in a given situation, will contribute significantly to our understanding of many retinal disease processes and open new doors for therapeutic intervention.

Structure and biochemistry

The MMP family comprises at least 18 members in humans, sharing significant sequence homology and a common multidomain organisation.²¹ They have overlapping substrate specificities but patterns of expression are often distinct in different locations, suggesting precision in the control of matrix turnover. They are divided into three main groups with respect to the main activity of the purified enzymes in vitro (Table 1). The collagenases degrade fibrillar collagens type I, II, and III. The gelatinases cleave triple helical type IV collagen molecules at a single site and have high activity against gelatin. The stromelysins

Table 1 Members of the human MMP family*

	MMP designation	Molecular mass (latent)	Molecular mass (active)	Main substrates
Collagenases				
Interstitial collagenase	MMP-1	55	43	Collagen types I, II, III, VII, X (fibrillar)
Neutrophil collagenase	MMP-8	75	58	Collagen types I, II, III (fibrillar), proteoglycan core protein (PCP)
Collagenase 3	MMP-13	65	55	Collagen types I, II, III, IV, gelatin, PCP, fibronectin
Stromelysins				
Stromelysin-1	MMP-3	57	46	Collagen types II, IV, IX, X, XI, procollagen, PCP, fibronectin, laminin
Stromelysin-2	MMP-10	57	46	Similar to stromelysin-1
Stromelysin-3	MMP-11	51	44	α_1 proteinase inhibitor (serpin)
Gelatinases				
Gelatinase A	MMP-2	72	66	Gelatin, collagen types IV, V, VII, XI, fibronectin
Gelatinase B	MMP-9	92	86	Gelatin, collagen types IV, V, fibronectin, elastin
MT-MMPs				
MT1-MMP	MMP-14	64	54	pro-MMP-2, pro-MMP-13, collagens, PCP, fibronectin, tenascin
MT2-MMP	MMP-15	72	61	Similar to MT1-MMP
MT3-MMP	MMP-16	66	55	pro-MMP-2
MT4-MMP	MMP-17	unknown	54	pro-MMP-2
MT5-MMP	MMP-24	63	62	pro-MMP-2. Shed forms of MT5-MMP of 28 kDa and 44-46 kDa also exist and have some activity in vitro
Others				
Matrilysin	MMP-7	28	20	Collagen types IV, PCP, fibronectin, elastin, gelatin
Metalloelastase	MMP-12	54	45	Elastin

*Reviewed in references 1, 4, 22, 79.

degrade type IV collagen, other collagens with interrupted triple helices, and are also active against laminin, fibronectin, and proteoglycans.²²

Most members of the MMP family have well conserved structural domains²³:

- (1) the leader sequence targets the molecule for secretion and is then removed;
- (2) the amino terminal propeptide consists of 80–90 amino acids which are removed extracellularly to activate the latent enzyme;
- (3) the catalytic domain contains a zinc ion in the active site that is essential for proteolytic activity. There is a further “structural” zinc ion required to maintain a functionally stable conformation and at least one calcium ion;
- (4) the haemopexin-like domain has been shown to have a functional role in substrate binding and interaction with the TIMPs.²⁴

The membrane-type MMPs (MT-MMPs) comprise a subgroup that presently consists of five members. They differ in that they are processed to the active form intracellularly. A hydrophobic stretch of some 25 amino acids represents a transmembrane domain at the carboxy terminus and there is a recognition site for furin-like convertases at the end of the propeptide domain. They have been shown to degrade components of the ECM and activate classic MMPs *in vitro*.²⁵ The recently characterised MT5-MMP may contribute to the activation of pro-MMP-2 in tumour tissues in which it is overexpressed and, in contrast with other MT-MMPs, can also be shed from the cell surface as a soluble proteinase.²⁶ Abnormal function of such cell surface proteases associated with malignant tumour cells may contribute to their invasive potential.²⁵

Two recently cloned human MMPs, MMP-19 and enamelysin (MMP-20), cannot be classified into any of the above subgroups on the basis of structure or substrate specificity.²⁷ MMP-20 is expressed during tooth development and has been shown to degrade amelogenin.

REGULATION OF MMP ACTIVITY

The MMP axis must be highly regulated to avoid excessive tissue destruction, and this is achieved in three ways: transcriptional regulation,²⁸ proenzyme activation,²³ and the action of the TIMPs.²⁹ Most MMPs are not constitutively expressed *in vivo* but can be rapidly induced in response to exogenous signals such as growth factors, cytokines, phorbol esters, and cell-cell or cell-ECM interactions. Exceptions to the rule include MMP-8 and MMP-9, which are stored in the secretory granules of neutrophils and eosinophils, and MMP-7, stored in secretory epithelial cells of exocrine glands.³⁰ The 5'-flanking regulatory region of inducible MMPs contains an AP-1 *cis* regulatory element in the proximal promoter. Appropriate signals (such as platelet derived growth factor, basic fibroblast growth factor, epidermal growth factor) activate the AP-1 transcription factor complex, composed of c-fos and c-jun proto-oncogene proteins, which binds to the AP-1 *cis* element to activate transcription of the corresponding MMP gene.³¹ Other moieties, such as IL-4, IL-10, IL-13,

and transforming growth factor β (TGF- β) can downregulate some MMPs.

Latent MMPs are proteolytically activated in the extracellular space, with the exception of MMP-11 and MT-MMPs, which are activated before secretion by Golgi associated furin-like proteases. Plasmin is an important physiological activator of pro-MMPs and can initiate a cascade of MMP activation at the cell surface.³² For example, upon activation of MMP-9 and MMP-3, the latter can activate more MMP-9, and also MMP-1, amplifying the response. Localising this activation machinery on the cell surface, via integrin receptors and MT-MMPs, ensures that the proteolytic response is maximal in the immediate pericellular environment.³³ Another important, though often overlooked, activation mechanism is via reactive oxygen species released from inflammatory cells.¹³

Activated MMPs can then be specifically inhibited in tissues by forming a 1:1 non-covalent complex with TIMPs,³⁴ though inactivation can also occur non-specifically in plasma by binding to proteins such as α_2 macroglobulin. The formation of such complexes determines the rate at which physiological factors can activate MMPs. Four TIMPs have been identified in humans³⁵ (Table 2). They have 30–40% sequence homology at the amino acid level and contain two domains held in rigid conformation by six disulphide bonds. One domain is largely responsible for MMP inhibition, while the other can bind to pro-MMPs and may control their autocatalytic activation. They show little difference in their specificity for MMPs, with each TIMP largely capable of inhibiting most MMPs, though TIMP-1 is an ineffective inhibitor of MT-MMPs. TIMP gene expression is also regulated,³⁶ which allows coordinate or reciprocal patterns of MMP/TIMP regulation, depending on the nature of the stimulus and the target cell.³⁷ Paradoxically, TIMP-2 is also involved in the activation of pro-MMP-2, to which it binds at the cell surface, along with MT1-MMP, facilitating proteolytic activation of pro-MMP-2 by adjacent MT1-MMP.

TIMPs are expressed by a variety of cells and are present in most tissue and body fluids. TIMP-1, TIMP-2, and TIMP-4 are present in a soluble form, but TIMP-3 is insoluble and bound to the ECM.³⁸ There are several reports demonstrating the ability of TIMP-1 and TIMP-2 to promote growth and alter cell morphology in a variety of cultured cell lines.^{39–40} Furthermore, TIMP-3 has been found to be anti-angiogenic⁴¹ and differs from other TIMPs in inhibiting tumour necrosis factor- α converting enzyme (TACE), suggesting a role in the modulation of inflammation. Clearly, these molecules have important regulatory activities independent of MMP inhibition that require further characterisation.

INTERACTION BETWEEN MMPs, CYTOKINES, AND GROWTH FACTORS

There are many important areas of interaction between the MMP axis and the cytokine network.^{42–45} TGF- β inhibits stromelysin and collagenase expression via an upstream element in their promoter sequences referred to as the TGF- β inhibitory element.⁴⁶ Interestingly, it also upregu-

Table 2 Properties of tissue inhibitors of metalloproteinases (TIMPs)*

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Chromosome location (human)	Xp11.23–11.4	17q2.3–2.5	22q12.1–13.2	unknown
Protein (kDa)	28	21	24	22
mRNA (kB)†	0.9	3.5 (1.0)	4.5 (5.0, 2.4, 2.6, 2.8)	1.2 (1.4)
Expression	inducible	mainly constitutive	inducible	unknown
Main form of molecule	secreted	secreted	ECM associated	secreted

*Reviewed in references 9 and 86.

†Numbers in parentheses denote possible splice variants.

lates TIMP-1 expression.⁴⁷ The combined effect is to prevent the destruction of newly synthesised matrix⁴⁸ and helps explain why elevated levels of TGF- β are associated with fibrosis within the eye⁴⁹ and elsewhere in the body. MMP mediated degradation of matrix proteins such as decorin can release active TGF- β and insulin-like growth factor (IGF) from sequestered stores in connective tissue and complexes with binding proteins.^{50 51}

Cytokines and their receptors can also act as substrates for MMPs. Tumour necrosis factor α (TNF- α) is a pro-inflammatory cytokine that can be secreted following cleavage of the membrane bound pro-form by MMPs.⁵² Such “shedase” or “convertase” action by MMPs may play a part in perpetuating or damping down inflammatory reactions, depending on whether the molecule released is an active protein or a soluble form of receptor. MMP-2, for example, releases an active soluble ectodomain of FGF receptor-1 that may modulate the mitogenic and angiogenic activities of FGF.⁵³ It is likely that repolysin and adamalysin metalloenzymes contribute significantly to this convertase activity at the cell membrane.^{54 55} They have a catalytic site similar to classic MMPs, but a different domain structure, and will not be discussed further as part of this review.

The key issue raised by these findings is that MMP inhibition is likely to do more than just inhibit matrix breakdown *in vivo*. The functional consequence of such action requires careful characterisation and may produce results that cannot be predicted from the observation of simple systems *in vitro*.

Role in physiological and pathological processes

MMPs are determinants of basic cell behaviour in a number of physiological processes.^{56 57} Proteolytic remodelling of the ECM has an “instructional” effect on cellular phenotype that can occur directly via receptor ligation and triggering of appropriate intracellular transduction cascades, or indirectly, by modulating the cellular response to growth factors. For example, ligation of cell surface integrins by matrix components can influence the cellular response to mitogenic signals, suggesting that cell-matrix interactions are important regulators of the cell cycle. Moreover, ECM remodelling has the potential to alter cell phenotype by the release of matrix bound growth factors. Binding proteins for insulin-like growth factor (IGFBP) and latent transforming growth factor β (LTBP) can be sequestered in the ECM and released by proteases to influence the behaviour of resident cells. MMP cleavage of functional IGF from complexes with IGFBP has implications for cell proliferation and tissue growth.⁵⁸ Matrix fragments generated through proteolytic remodelling (for example, endostatin and restin) can themselves have a profound influence on cell phenotype, discussed below, with reference to angiogenesis.

Cell migration underlies many biological processes, including inflammation, wound healing, and tumour metastasis. Proteolytic mechanisms do not merely clear a path for migrating cells in these situations, but facilitate crucial cell-cell and cell-matrix interactions, in some cases by revealing cryptic sites previously inaccessible to cell surface receptors. For example, MMP-2 cleaves the α_2 subunit of laminin-5 to expose integrin binding sites that trigger cell motility.⁵⁶

The ability of cells to degrade matrix components is thus an essential homeostatic function, but it also forms the basis of a number of disease related processes. These will be discussed more fully below, as they explain the pivotal role of MMPs in ocular pathology and suggest rational approaches for devising new therapies.

INFLAMMATION AND IMMUNITY

Dynamic modulation of cell-cell and cell-matrix interactions must occur between T lymphocytes and endothelial cells to mount a normal inflammatory response and allow effector cells access to injured tissue. However, inappropriate or unregulated MMP expression can have deleterious effects. Experimental autoimmune encephalomyelitis (EAE) is characterised by multifocal perivascular inflammatory infiltrates in the central nervous system (CNS) and is a disease model of multiple sclerosis (MS), the prototype inflammatory demyelinating disorder of the CNS.⁵⁹ MMP-2 is induced and activated in autoreactive T cells in EAE by α_4 integrin binding to vascular cell adhesion molecule-1 on endothelial cells.^{56 57} Activated MMPs elaborated by migrating lymphocytes may then process the ECM to an “active” form (analogous to endothelial activation), facilitating further recruitment. MMP dependent cleavage of the transmembrane domain of TNF- α contributes to the release of this proinflammatory cytokine and enhances neuroinflammation. Moreover, MMPs modulate blood-brain barrier leakage⁶⁰ and have been shown to degrade myelin basic protein *in vitro*, widely considered to be an important autoantigen in MS. It has thus been postulated that MMPs may generate immunogenic fragments of host proteins as “remnant antigens” to drive autoimmune inflammatory processes. Suppression of EAE induction and reversal of clinical changes on treatment with a broad spectrum MMP inhibitor suggest that MMPs are contributing directly to tissue damage in this disease model.⁵⁹ Downregulation of trans-basement membrane migration of T lymphocytes by inhibition of their MMP-9 activity is likely to be a major mechanism by which interferon beta prevents clinical progression in MS patients.⁶¹

Leucocytes, particularly macrophages and polymorphonuclear leucocytes (PMNs), are a major source of MMP production.⁶² Our colleagues in oral medicine and dentistry have more ready access to biological samples and have established a direct causative role for elevated leucocyte derived collagenase in the pathology of periodontal disease.¹³ The inflammatory process of periodontitis is initiated by subgingival microflora, but the destruction of tooth supporting tissue derives from the host response to these organisms. MMP-8 (neutrophil collagenase) is elevated slightly in simple gingivitis, but present mostly in its latent form, in both tissues and gingival crevicular fluid (GCF). During periodontitis, the levels rise markedly and are activated by host and microbial derived proteases and reactive oxygen species from triggered PMNs. The levels in GCF fall with successful conventional treatment, so active MMP-8 is not only a target for therapy, but also a marker of disease severity, which has led to the development of an immunochromatographic dipstick test for MMP-8 that can be performed in the office.¹³ Subantimicrobial dose doxycycline (SDD) is the first drug acting as an MMP inhibitor to obtain US Food and Drug Administration approval, for treatment in adult periodontitis.⁶³ Marketed under the trade name Periostat (CollaGenex Pharmaceuticals Inc, Newtown, PA, USA), it reduces tissue destruction by direct MMP inhibition and also by preventing the influx of inflammatory cells across basement membranes, as discussed above in suppression of EAE.

Clearly, these observations suggest that MMPs can be active agents, and not merely passive markers, of tissue damage. But are MMPs always involved in the initiation of tissue damage or can their presence indicate an attempt at physiological repair? Numerous reports have suggested a role for MMP-3 in the pathology of rheumatoid arthritis, with levels three times higher than controls, and correlation with disease activity. However, in systemic lupus ery-

thematosis, the prototype autoimmune disease, serial measurements of MMP-3 do not correlate with fluctuation in disease activity scores, suggesting that this MMP may not be directly involved in tissue damage, but may be participating in a later phase of inflammation, perhaps involved with tissue repair.⁶⁴ So, MMP inhibitors may be beneficial in the early phases of some diseases, when the MMP activity is predominantly destructive, but later in the disease course the participation of MMPs in repair may actually be a desirable effect.

MMPs are capable of binding to connective tissue matrix, so local production in disease does not necessarily translate into a proportional increase in plasma levels. Comparisons between clinical studies are often difficult, owing to analytical variations. Enzyme linked immunosorbent assay (ELISA) measurements can be higher with diluted samples than undiluted plasma, because of reduced inhibitory effects of specific or non-specific inhibitors or matrix components, so accurate comparisons can only be made between samples at equivalent dilution.⁶⁵ Plasma levels are more reliable than serum levels as they exclude MMPs and TIMPs released from platelet activation and *in vitro* neutrophil degranulation.

MMPs are expressed in T cells and macrophages but there has been little evidence for their role in specific immune responses. "Knockout" mice, deficient in specific genes, are providing insights into potential roles for MMPs in these complex phenomena. In a DNFB induced model of contact hypersensitivity (CHS),⁸ the antigen is processed and taken up by Langerhans cells in the suprabasal epidermis, and transported via dermal lymphatics to T cell rich areas of regional lymph nodes. Naive cells presented with the antigen proliferate to form a specific T cell population that on subsequent challenge will migrate to the challenge site and release pro-inflammatory cytokines to attract other inflammatory cells. By applying DNFB to the dorsal surface of the ear, the ear thickness can subsequently be used as an index of inflammation. MMP-3 knockouts exhibit a markedly impaired CHS response to topical DNFB, though non-specific inflammatory responses to cutaneous irritants remain normal. MMP-9 knockouts exhibit a prolonged inflammatory reaction, accompanied by a lack of IL-10 production at the challenge site.

Mast cells have also been shown to produce MMP-9,⁶⁶ and inhalation of corticosteroids reduces submucosal expression of MMP-9 in asthmatics. As interest intensifies in the potential immunomodulatory roles of MMPs, we are likely to see advances in our understanding of allergic disorders of the anterior segment, as well as presumed immuno-inflammatory disorders of the posterior segment.

WOUND HEALING

Classic, cutaneous wound healing is characterised by haemostasis, re-epithelialisation, granulation tissue formation and remodelling of the ECM. Inappropriate or failed wound healing represents a major healthcare burden, in which excess proteinase activity has been implicated. Tissue injury produces a fibrin-rich provisional matrix. Cell migration through this scaffold utilises fibrinolytic (plasminogen activator) and proteolytic (MMP) systems.⁶⁷ Subsequent tissue repair can be divided into three phases: the acute inflammatory response, cellular proliferation, and remodelling of ECM.

Healing by primary intention involves surgical approximation of wound edges, using sutures to maintain tensile strength during repair. MMPs are unlikely to have much part to play in this scenario. Healing by secondary intention, however, requires epidermal keratinocyte migration over a denuded surface, which begins within hours of

injury.⁸ Migration off basement membrane and onto dermal matrix brings the $\alpha_2\beta_1$ integrin receptors on these cells into contact with type I collagen and triggers transcription of MMP-1 (collagenase-1), MMP-9,⁶⁸ and MMP-10. The epithelium further away from the healing edge of the wound expresses MMP-3, perhaps to remodel newly formed basement membrane. MMP-1 denatures fibrillar collagen type I and exposes cryptic sites in the ECM that facilitate migration.

As new basement membrane is synthesised, MMP-1 expression is suppressed and epithelial interactions with the basement membrane are stabilised. This occurs through hemidesmosomes forming within keratinocytes, linking them to anchoring fibrils of the subtending dermis, induced by remodelled ECM components.⁸ It is clear, therefore, that appropriate wound healing is accompanied by a temporally regulated increase in MMP activity, but that there are stop signals to reduce MMP production once reparative cells have completed their tasks. Chronic, non-healing ulcers demonstrate increased levels of MMPs, which may partly be due to the lack of appropriate stop signals normally provided by the presence of appropriate repair matrix. The elevated MMP level is thus a primary indicator that the wound bed is failing to heal, and the excess proteinase activity can then cause further tissue damage.⁶⁹

Recent studies have also demonstrated that, while TIMP-1 and TIMP-3 are found in proliferating keratinocytes in normally healing wounds, they cannot be detected in chronic ulcers.⁷⁰ This may contribute to excessive net proteolytic activity and failure to heal the wound bed. What we do not yet know is whether MMP inhibitors can be of use in chronic, failed wound healing, and whether the systemic use of such agents in other applications will interfere with normal re-epithelialisation in wounds that heal by secondary intention. Ophthalmic surgical intervention will also elicit a modified form of wound healing, which may have direct visual sequelae if there is abnormal matrix deposition, or scar contraction involving critical visual structures such as the retina. This is discussed below with reference to proliferative vitreoretinopathy, and other authors have implicated MMPs and TIMPs in subconjunctival healing following trabeculectomy.⁷¹

ANGIOGENESIS

MMPs and TIMPs have complex regulatory activities in angiogenesis, which involves the degradation of basement membrane, endothelial cell migration, capillary tube formation and endothelial cell proliferation.⁷² The importance of MT-MMPs in this process is becoming apparent, and is hardly surprising, as it enables endothelial cells to restrict proteolytic activity to the pericellular milieu, retaining surrounding matrix for structural support.³³ Whatever the "primary" stimulus in physiological or pathological angiogenesis (for example, hypoxia, growth factors) the MMP-TIMP axis is utilised as an effector pathway to direct the cellular processes involved. It is therefore to be expected that neovascularisation is associated with changes in resting levels of MMPs and TIMPs. In pathological neovascularisation, therapy may be directed against the primary stimulus or the effector pathway. If the exact nature of the primary stimulus is not known, then the effector pathway becomes an attractive target for intervention.

Several groups have documented the anti-angiogenic effects of MMP inhibitors,⁷³ which have therapeutic potential for angioproliferative disorders such as proliferative diabetic retinopathy. The anti-angiogenic effect of TIMPs may be through downregulation of MMPs required for endothelial cell migration and invasion, or direct suppres-

sion of these cells: TIMP-2 has been shown to inhibit basic fibroblastic growth factor driven endothelial cell proliferation⁷⁴ and TIMP-3 inhibits endothelial cell motility. The role of MMPs as processors of angiogenic modulators is also attracting considerable interest.⁷⁵ Macrophage metalloelastase (MMP-12) can process angiostatin, a 38 kDa internal fragment of plasminogen, to its active anti-angiogenic form.⁷³ It is a potent inhibitor of endothelial cell proliferation and induces apoptosis. A proteolytic fragment of MMP-2, termed "PEX", is angiostatic by inhibiting the association of MMP-2 with an integrin receptor on vascular endothelial cells.⁷⁶ The accumulation of PEX in developing retinal vessels suggests the presence of a regulatory mechanism that might be exploited to halt abnormal neovascularisation. Moreover, bioactive fragments released during ECM remodelling, such as endostatin and restin (carboxy terminal fragments of collagen XVIII and XV, respectively), strongly suppress neovascularisation by antiproliferative, antimigratory, and apoptotic mechanisms.⁵⁷

The role of vascular endothelial growth factor (VEGF) in angiogenesis is well established, but it may also induce MMP production.⁷⁷ Interestingly, VEGF immunostaining as a marker of neoangiogenesis has been found to be indicative of aggressiveness in serous ovarian tumours, and correlates closely to the presence of MMP-2.⁷⁸ Malignant tumours are angiogenesis dependent diseases, as primary tumour growth, invasion, and metastasis all require neovascularisation.

TUMOUR INVASION AND METASTASIS

Remodelling of the surrounding matrix permits local tumour cell growth, following which MMPs can facilitate tumour cell invasion and metastasis by degrading ECM barriers,⁷⁹ modulating cell-cell and cell-matrix adhesions and promoting angiogenesis. Metastatic disease is responsible for the majority of cancer related deaths, so understanding the mechanisms behind tumour invasion and metastasis is key to devising new therapies aimed at limiting tumour spread. However, considerable care must be taken in interpreting the two main sources of data on putative roles for MMPs in malignant disease—namely, animal models and studies on human pathological specimens.

There are several potential pitfalls in developing antimetastatic treatments on the basis of animal models.⁸⁰ Firstly, cell proliferation tends to occur more rapidly in primary and metastatic tumours in animals and there is usually less local invasion, resulting in pseudocapsule formation. Secondly, the MMPs are produced by experimental cancer cells rather than the stromal cells. In human pathology, the carcinoma cells produce a factor, EMMPRIN (Extracellular Matrix MetalloProteinase Inducer) which induces stromal fibroblasts to produce MMPs that are then sequestered by the cancer cells. MMP-7 (matrilysin) is the main exception, being produced by carcinoma cells rather than stromal cells.⁸¹ Thirdly, experimental cell lines have been selected by *in vitro* growth characteristics and tend to have more frequent and more bizarre chromosomal abnormalities than human cancers arising spontaneously *in vivo*. Finally, there is more central necrosis in experimental tumours, suggesting that angiogenesis is a less prominent feature, or at least, cannot "keep up" with tumour growth.

Most studies on human tissue merely reveal increased total MMP expression in cancer versus normal tissue, rather than active MMPs themselves, and do not comment on the potential importance of enzyme-inhibitor complexes. Nevertheless, MMP activity has been linked to the invasive potential of systemic tumours in a number of

studies, prompting immunohistochemical investigations to establish whether these enzymes may be predictive of tumour stage and survival time.⁸² MMP-11 (stromelysin-3) was recently identified as an independent prognostic factor for reduced disease free survival in breast carcinoma⁸³ and a similar association has been described for MMP-2 immunoreactive protein in melanoma cells of primary skin melanoma.⁸⁴ It is difficult to prescribe an exact role to these MMPs without knowing the nature of the preferred substrates *in vivo*, but MMP-11 does cleave and inactivate serpins such as α_1 antitrypsin, and may thus potentiate the action of other proteinases such as urokinase-type plasminogen activator.⁸⁵ Such observations have supported the use of synthetic MMP inhibitors in numerous clinical trials for cancer currently in progress. However, such pathological findings are by no means universal, perhaps due to technical limitations in our ability to detect active MMPs reliably in tissue extracts. The role of TIMPs in tumour progression is complex and paradoxical, reflecting the bimodal function of TIMPs as inhibitors of MMPs, but also facilitators of cell surface activation cascades.⁸⁶ Several mechanisms could explain a tumour suppressor effect. First is their anti-angiogenic activity, though this has yet to be established for tumours *in vivo*. Secondly, by preventing the degradation of growth factor binding proteins by MMPs in the ECM, TIMPs may limit the bioavailability of growth factors such as TGF- β and IGF-II to the tumour cells. Thirdly, by limiting proteolysis of the tumour ECM, they may promote encapsulation. New data support a further, indirect level of control, through maintenance of the integrity of the ECM, which is known to influence basic cell behaviour, including growth, differentiation, and apoptosis. Exogenous TIMP-2 inhibits the growth of human melanoma cells in the presence of intact fibrillar collagen, but this effect is lost if MMPs derived from these cells degrade fibrillar collagen, in the absence of excess TIMP-2. The anti-proliferative effect involves upregulation of the cyclin dependent kinase inhibitor p27, which occurs when cells encounter intact ECM and blocks cell cycle progression.⁸⁷ Transfection of the TIMP-1 gene into melanoma cell lines has resulted in reduced invasive and metastatic potential,⁸⁸ and overexpression of TIMP-1 in transgenic mice can inhibit the growth of primary tumours.⁸⁹

However, evidence also exists to support a tumour *promoting* activity of TIMPs. In colon cancer and non-Hodgkin's lymphoma, TIMP-1 expression is a positive index of malignant progression.⁸⁷ In bladder and breast cancer, TIMP-2 is an unfavourable prognostic indicator. Are there any mechanisms to account for such observations? TIMP-2 can act as an adaptor molecule between proMMP-2 and MT1-MMP at the cell surface, enhancing the generation of active MMP-2 which might then facilitate invasion of the basement membrane. The TIMPs can also stimulate the growth of a large variety of normal and malignant cells *in vitro* and TIMP-1 has also been shown to suppress apoptosis of malignant Burkitt cells.⁸⁷ We will understand more if a putative "TIMP receptor" can be found at the cell surface, with characterisation of the signalling pathways involved in these functions.

Whether TIMPs suppress or promote tumours may depend on many factors, such as local concentration, pericellular distribution, and association with proMMPs. Perhaps at low concentrations, TIMPs promote tumour cell growth and stimulate proMMP-2 activation by MT1-MMP, while at higher doses they predominantly suppress invasion. It may be that the MMP/TIMP ratio will prove more informative than either measurement alone, an elevated ratio having been correlated with aggressive

phenotype in pancreatic carcinoma.⁹⁰ At present, there are too many unanswered questions for the therapeutic use of TIMPs in cancer and it will take a lot more work to unravel the paracrine regulation of MMPs in tumour cell biology.

MMPs and TIMPs in vitreoretinal diseases

The posterior segment provides a unique environment for viewing the evolution of wound healing, angiogenesis, carcinogenesis, and degenerative processes. However, as biological sampling is not straightforward, with specimens often obtained at the end stage of disease processes, there are many unanswered questions regarding the cellular source of MMPs and TIMPs in vitreoretinal diseases and their role in physiological and pathological processes.

Human tissue and cell culture studies suggest that MMPs and TIMPs may have a role in normal retinal homeostasis. Plantner and colleagues have demonstrated the presence of MMP-1, MMP-2, MMP-3, MMP-9 and TIMP-1, TIMP-2, and TIMP-3 in human interphotoreceptor matrix (IPM) and vitreous, using western blot analysis.⁹¹ Subsequent observations suggest that MMP-2 and TIMP-1 produced by the retinal pigment epithelium (RPE) are secreted from the apical surface bordering the IPM.⁹² MT1-MMP has been demonstrated in retina, vitreous, and choroid, where it may play a part in activating pro-MMP-2.⁹³ TIMP-3 has been identified as an ECM associated component of Bruch's membrane and is discussed more fully below.

These investigations have led to studies of human pathological material to investigate whether MMPs and TIMPs may have pathogenic significance in disorders of the posterior segment. For this to be established, the candidate enzyme should be present in diseased tissue, at a level proportional to disease severity. Its biological function should have a plausible role in the aetiology of the disease, and inhibiting this function should modify or abrogate the disease process. With this in mind, we will now discuss those disorders where a significant body of evidence exists to suggest a pathogenic role for these molecules, even if *all* the aforementioned criteria cannot yet be satisfied, on the basis of present knowledge.

PROLIFERATIVE VITREORETINOPATHY

Proliferative vitreoretinopathy (PVR) occurs in 5–10% of all retinal detachments and is the commonest cause of anatomical failure in retinal detachment surgery.⁹⁴ PVR may be viewed as a maladapted wound healing phenomenon in which cellular proliferation occurs on both surfaces of the detached neuroretina, the posterior vitreous face and within the vitreous base, with the formation of contractile periretinal membranes.⁹⁴ Such contraction is likely to be an important mechanism in preventing break closure in detached retina with PVR, and Scott and co-workers have shown that MMP activity is a determinant of cell mediated collagen contraction *in vitro*.⁹⁵ Many growth factors and cytokines have been proposed as initiators of the cell proliferation, migration, matrix elaboration, and contraction that characterise PVR, making specific molecular inhibition an unreliable therapeutic proposition. As each of these processes is MMP dependent, this family of enzymes may represent a final common pathway in the evolution of PVR that may be more amenable to manipulation. We have discussed above that a temporally coordinated and spatially controlled increase in specific MMP activity is a feature of normal wound healing processes, and that failure to regulate this activity can be detrimental to healing.

MMPs and TIMPs have been demonstrated in subretinal fluid and vitreous,^{96–98} with pro-MMP-2 found constitutively in most studies. The cellular source of

MMPs and TIMPs in the vitreous is not known. Vitreal hyalocytes may be capable of producing these enzymes, but we can still detect MMPs and TIMPs in the vitreous cavity fluid of vitrectomised eyes (unpublished observation). It is noteworthy that the major cellular constituents of PVR membranes, such as RPE cells, glial cells, fibroblasts and inflammatory leucocytes have all been shown to produce MMPs and TIMPs *in vitro*, the profile varying with cytokine or growth factor stimulation.^{99–101} Limb and co-workers also report detection of active and latent forms of MMP-1 and MMP-2 by immunohistochemistry, in epiretinal and subretinal membranes of PVR.¹⁰² Vitreous levels are likely to represent proteins produced by retinal and periretinal tissues, as well as leucocytes derived from breakdown of the blood-retinal barrier and cells that have entered the vitreous cavity by transretinal migration or mechanical dispersal. Vitreous levels of these proteins may thus represent the potential for adverse pathological sequelae, in the same way that leaching of MMPs from tissues to the blood stream in patients with biologically aggressive cancer provide plasma markers that might be useful for predicting metastases.⁶⁴

Interpretation of vitreous levels of MMPs requires data on active rather than total protein levels, and information on the level of inhibitory TIMP proteins present, factors that have not been addressed satisfactorily in studies to date. Moreover, given that we expect a proportion of these proteins to be derived from breakdown of the blood-retinal barrier, it is important to investigate whether elevated levels of a particular enzyme correlate with a marker of blood-retinal barrier breakdown, such as total protein concentration. In viral meningitis, TIMP-1 is upregulated in the CSF, but correlates with total protein concentration, and therefore cannot be excluded as a passive circulatory derivative from blood-brain barrier breakdown.¹⁰³ Reduced oxygen tensions may be another factor contributing to elevated TIMP-1 in PVR (and proliferative diabetic retinopathy), as the TIMP-1 promoter contains a hypoxia response element.¹⁰⁴ The ophthalmic literature contains reference to elevated TIMP-1 as pathogenic in PVR, with no reference to blood-retinal barrier integrity or oxygen tensions.⁹⁷ However, total MMP activity, measured in a peptide substrate cleavage assay, appears to correlate with total TIMP-1 levels in uncomplicated retinal detachment, but this correlation is lost and the total MMP activity is significantly higher in retinal detachment complicated by PVR.¹⁰⁵ This would suggest that an MMP/TIMP imbalance may be associated with abnormal wound healing processes in PVR.

Vitreous levels of MMPs might not be a direct representation of what is occurring at the tissue level, but if we consider the posterior segment as the “wound healing environment” in PVR, they might conceivably be indicators of adverse outcome, as in non-healing wounds elsewhere in the body, discussed above. It is also conceivable that active MMPs in the vitreous might facilitate remodelling events at the inner and outer limiting membranes of detached retina, leading to preretinal and subretinal proliferation, respectively. In a prospective study of PVR, Kon and co-workers demonstrated a significant association between vitreous levels of MMP-9 and the development of postoperative PVR.¹⁰⁶ Work in our laboratory has also consistently demonstrated MMP-9 in retinectomy specimens from advanced cases of PVR (manuscript in preparation).¹⁰⁷ This provides a mechanism for RPE cell dissociation from their basement membrane and migration through the retina.

Whether elevated MMP-9 is truly “pathogenic” in PVR, or merely a reflection of the underlying cellular activity, can only be answered by demonstrating inhibition of PVR

induction in a physiological model with a strategy aimed at eliminating active MMP-9. There is some evidence to suggest that broad spectrum MMP inhibitors may prevent induction of experimental PVR¹⁰⁸ and, as discussed above, such agents prevent induction of EAE. The mechanisms may not be dissimilar. The lesions in EAE contain inflammatory cells, activated glial cells, and macrophages which are also components of PVR membranes. Agents that prevent extravasation of inflammatory cells, transretinal migration of RPE cells and contraction of mesenchymal cells are thus rational choices for adjunctive therapy in PVR. Broad spectrum inhibitors might even have advantages over more selective therapy, as they will also protect the blood-retinal barrier by reducing release of pro-inflammatory TNF- α by cleavage at the cell surface. We propose that such therapy would best be instituted at an incipient or preclinical stage, to suppress the activation cascades that might evolve into PVR. This requires selecting patients at high risk of PVR¹⁰⁶ and treating them at primary vitrectomy for retinal detachment, with a locally delivered MMP inhibitor. If a suitable long acting compound, or depot preparation, can be found, it is conceivable that, following a single application, induction of PVR could be suppressed until normal retinal homeostasis has been established. Current approaches to the pharmacological prevention of PVR have focused on non-specific cellular proliferation inhibitors, such as 5-fluorouracil.¹⁰⁹ It is possible that local MMP inhibition, introduced early enough, might form part of a targeted approach for PVR prevention, and perhaps to reduce recurrence following conventional PVR surgery.

RETINAL NEOVASCULARISATION

Retinal neovascularisation is an important cause of blindness, contributing to sight threatening complications associated with haemorrhage or retinal detachment in proliferative diabetic retinopathy (PDR), retinopathy of prematurity (ROP) and retinal vascular occlusions. Neovascularisation involves remodelling and proteolysis of the capillary basement membrane by migrating endothelial cells, which constitutively express mRNA for MMP-2, MMP-9, TIMP-1, and TIMP-2 in culture,¹¹⁰ enabling tight physiological control over this process. A well established model of retinal neovascularisation involves exposure of newborn mice to hyperoxia.¹¹¹ This closely resembles ROP and has some features of PDR, such as capillary dropout and disc neovascularisation. Das and co-workers have demonstrated increases in active forms of retinal MMP-2 and MMP-9 by zymography, during the active phase of angiogenesis in this model.¹¹¹ MMP-2 interacts with $\alpha_v\beta_3$ integrin on endothelial cells to localise proteolytic activity, and it has been postulated that ECM fragments produced by local remodelling bind to further $\alpha_v\beta_3$ integrins to promote cell survival and matrix invasion.⁵⁶ Such interactions might be disrupted by MMP inhibitors, and these authors document a reduction of neovascular nuclear counts by 72% in this model, with intraperitoneal administration of a synthetic MMP inhibitor. This suggests a pathogenic role for active gelatinases in the progression of neovascularisation. Further studies planned with topical applications at higher doses may have relevance for adjuvant treatment in patients undergoing vitreoretinal surgery for complications of established neovascularisation.

Proliferative diabetic retinopathy

Early histopathological features of diabetic retinopathy include basement membrane thickening, loss of pericytes, and microaneurysm formation. PDR supervenes when an initiating hypoxic stimulus drives expression of angiogenic

proteins such as VEGF, and both hypoxia and VEGF can stimulate retinal capillary endothelial cells in culture to produce active MMP-2.¹¹² Analysis of diabetic neovascular membranes and vitreous has produced interesting and somewhat contrasting results. In vitreous, proMMP-2 levels are similar between diabetic and non-diabetic subjects, whereas MMP-9 levels are significantly higher in diabetics, and correlate with disease progression, though most of the MMP-9 is in the latent form.¹¹³ The source of this proMMP-9 is unknown, but in the vitreous, at least, it seems to be more a marker of disease progression than actively involved in pathology. It may be produced in situ, reflect production by ischaemic retinal cells, or leak into the vitreous from new vessels. As in PVR, diabetic vitreous has significantly higher levels of TIMP-1 than found in non-proliferative vitreoretinal pathologies.⁹⁷ This may contribute to maintaining vitreous MMPs in their latent form, limiting active proteolysis to sites of neovascularisation. At the tissue level, however, human diabetic neovascular membranes contain active forms of MMP-2 and MMP-9,¹¹⁴ demonstrated by zymography of extracted proteins. Sawicki and colleagues have reported that platelet derived MMP-2 is released during activation and mediates platelet aggregation and adhesion to the endothelium.¹¹⁵ This may be a generic process that proves to be a component of many vasculopathies, including PDR.

Clearly, MMP activity is critical for the cell-cell and cell-matrix interactions involved in capillary tube morphogenesis, so it represents a "final common pathway" in the process of retinal neovascularisation, from whatever cause. Inhibition at this level is thus potentially more attractive than targeting individual systems such as VEGF, as even if this could be effectively achieved, there may be some "escape" by other inducers of neovascularisation employing this final common pathway. Given the chronicity of diabetes, MMP inhibitors would have to be used long term to prevent or halt progression of PDR. This would require agents with long half lives, good bioavailability, and few side effects. To our knowledge, there are no clinical trials currently in progress for the use of MMP inhibitors to prevent PDR, but this could change if appropriate drugs were available and a case could be made for long term MMP inhibition reducing other complications of diabetes.

One consequence of hyperglycaemia is the irreversible, non-enzymatic glycosylation and oxidation of proteins to form advanced glycation end products (AGEs). These glucose derived cross links reduce collagen solubility and turnover and, it has been suggested, might affect MMP activation directly by protein modification, or indirectly, by their propensity to generate reactive oxygen intermediates, which can activate some proMMPs.¹¹⁶ The best characterised binding site for AGEs is a member of the immunoglobulin gene superfamily, referred to as Receptor for AGE, or "RAGE". Macrophages and other cells possess RAGEs that can ligate AGEs and stimulate the production of MMPs, adhesion molecules, and pro-inflammatory cytokines, such as TNF, IL-1, and IL-6, which can induce transcription of further matrix degrading enzymes.

Conventional glycaemic control is clearly important, and there is some experimental evidence that MMP inhibition may contribute to preventing hyperglycaemia. Subcutaneous administration of KB-R7785, a new MMP inhibitor, reduces plasma glucose levels in a rodent model of insulin resistance. This agent also inhibits a lipopolysaccharide induced increase in TNF- α and may thus exert its anti-diabetic effect by ameliorating TNF- α mediated insulin resistance.¹¹⁷ The Diabetes Control and Complications Trial (DCCT) has shown that prolonged elevated serum glucose levels contribute to retinopathy and nephropathy.

However, only 4% of subjects in the intensely treated cohort could maintain normal values for serum glucose and glycosylated haemoglobin. So, the majority of diabetics are exposed to elevated glucose concentrations during the course of their disease and developing strategies that can reduce long term complications independent of glycaemic status would considerably reduce morbidity.

Models of type I and type II diabetes in the rat demonstrate increased collagenase and gelatinase activity in skin and gingival extracts.¹¹⁶ Chemically modified tetracyclines (CMTs) are drugs that have lost their antimicrobial properties, but act as MMP inhibitors by influencing intracellular modulatory pathways through effects on a variety of cytokines, inducible nitric oxide synthase, cyclooxygenase, and AGE formation. CMT treatment in the rat diabetic models not only reduces elevated MMP levels, but also significantly reduces proteinuria, lens opacities, and prolongs survival.

Abnormal matrix turnover in mesangial cells has been postulated in the pathogenesis of diabetic nephropathy.¹¹⁸ Interestingly, plasma levels of MMP-9 increase before the development of microalbuminuria in non-insulin dependent diabetes and are significantly reduced after appropriate treatment with angiotensin converting enzyme inhibitors.¹¹⁹ Matrix components may also modulate the MMP activity of retinal microvascular cells in response to glucose,¹¹⁰ which may be important in the development of diabetic retinopathy. More detailed examination of experimental material will be required to establish the effect of CMT treatment on renal tissue and proliferative retinal disease, but these preclinical results are promising.

UVEAL MELANOMA

Uveal melanoma is a common primary intraocular neoplasm in adults with a 5 year survival rate of approximately 70%. Metastasis is by haematogenous spread and there are several well established clinical markers of prognosis, including tumour size and location, cell type, patient age, and sex.¹²⁰ MMPs and plasminogen activators have been shown to play a part in progression of cutaneous melanoma, which has a similar embryological origin.¹²¹ Evidence has recently emerged that MMP-2 may be a prognostic marker in uveal melanoma. An immunohistochemical study of 29 uveal melanomas by Vaisanen and colleagues demonstrated that positive immunostaining for MMP-2 in tumour cells correlated significantly with poorer 5 year relapse free and overall survival rates and a higher incidence of visceral metastases.¹²² Levels of MMP-2, TIMP-1, and TIMP-2 in the vitreous, where available for analysis, were not elevated compared with controls, and had no prognostic significance. Positive immunostaining for MMP-2 was demonstrated in the RPE, photoreceptors, and fibroblasts of the ciliary body and choroid of enucleated eyes. The most significant difference in 5 year survival rates was between patients with MMP-2 positive non-spindle cell uveal melanoma (38%) and MMP-2 negative spindle cell tumours (100%).

However, these authors used a monoclonal antibody recognising a *latent* form of MMP-2. To determine whether MMP-2 is merely a marker of adverse outcome or actively involved in disease progression will require assessment of activation mechanisms and demonstration in tissue of active MMP-2, TIMP-2, and MT1-MMP. The cellular source of MMP-2 is uncertain and could be clarified by *in situ* hybridisation, which has demonstrated MMP-2 mRNA mainly in the stromal component of other tumours.¹²³ Sequential activation and production of MMP-2 has been demonstrated during breast cancer progression¹²⁴ and several lines of experimental evidence suggest that increased MMP-2 levels might have a causal

role in an invasive phenotype.⁷⁹ Exogenously added *active* gelatinases (MMP-2 and MMP-9) facilitate the *in vitro* invasion of a mammary tumour cell line. Transfection of the C127 breast cancer cell line with MMP-2 cDNA produces cells that metastasise to the lung when injected into the tail veins of nude mice. Importantly, there is a dose-response relation between the amount of active MMP-2 produced by the transfected cells *in vitro* and the number of lung nodules generated *in vivo*, and the administration of a synthetic gelatinase inhibitor prevents lung colonisation.

These observations suggest that gelatinase inhibition offers a strategy for preventing basement membrane destruction that accompanies cancer invasion. Several MMP inhibitors are presently in clinical trials for treatment of invasive malignant tumours, such as gastric, pancreatic, and ovarian carcinomas.³⁰ As the results of such studies have implications for the management of ocular neoplastic invasion and metastasis, we will discuss the rationale behind this approach.

MMP inhibitors and the treatment of cancer

A "cure" for cancer through eradication of malignant cells has so far evaded us. The most effective alternative is to prolong survival through long term control of the disease. As an adjuvant treatment for malignancy, MMP inhibitors represent a fundamentally different approach to conventional cytoreductive therapy and may have a complementary role in treatment. Cytotoxic drugs target cells with a high proliferative index, but as these are also genetically unstable, adaptation can lead to treatment failure. However, tumours are composed not only of genetically altered neoplastic cells, but also genetically normal inflammatory, stromal, and endothelial cells, which are collectively required for manifestation of the malignant phenotype.

MMP inhibitors encourage the development of stromal fibrosis which inhibits tumour growth and invasion by promoting encapsulation. Necrosis of tumour tissue may be related to a subsequent increase in interstitial pressure or a direct anti-angiogenic effect of MMP inhibition.^{125 126} There is mounting evidence that MMP activity may play a part in the survival and growth of malignant cells. Tumours produced by injecting nude mice with ocular melanoma cells overexpressing TIMP-3 grow significantly more slowly than controls transfected with vector alone.¹²⁷ Inhibition induced apoptosis¹²³ may be contributing to a reduction of tumour burden, as well as a direct effect of MMP inhibition on tumour growth.

There are many excellent reviews on synthetic MMP inhibitors, to which the reader is referred for further information.^{85 128} The first generation of inhibitors were developed through structure based design to fit stereospecifically into the active site of MMPs. Broad spectrum peptide based MMP inhibitors such as batimastat suffer from poor oral bioavailability and have been used intraperitoneally for treatment of malignant ascites in ovarian cancer. Marimastat is water soluble and is "biologically active" given orally at 50 mg twice daily. After 4 weeks of treatment, endoscopic examination of advanced gastric tumours demonstrated an increase in fibrotic stromal tissue.⁸⁵ Randomised comparative trials are being conducted with marimastat in patients with pancreatic, lung, gastric, ovarian, and breast cancer. However, prolonged treatment is associated with arthralgia, directing research towards more selective inhibitors. More selective targeting of MMPs may be preferable in cancer treatment, as MMP-3, MMP-7, MMP-9, and MMP-12 can generate angiostatin from plasminogen, and may thus suppress tumour growth by limiting angiogenesis. Trials are thus

under way with gelatinase “selective” compounds such as AG3340 and BAY 12-9566.

Combination treatments with conventional cytotoxic chemotherapies are also under evaluation in cancer patients and animal models, with some indication that an additive effect can be achieved. We have already referred to CMTs acting as broad spectrum MMP inhibitors, independent of their antimicrobial effects. They can also induce apoptosis of malignant cells and have more favourable pharmacokinetics and tissue absorption than conventional MMP inhibitors, with well recognised safety profiles for long term management. They may prove useful in the adjuvant treatment of malignancy and have been effective in reducing metastases in a rat model of prostatic carcinoma.¹²⁹ The real test for MMP inhibitors will be whether they can increase overall survival in the adjuvant treatment of micro-metastatic disease, once clinical trials move beyond the treatment of advanced pathology.

HEREDITARY AND DEGENERATIVE DISEASES

TIMP-3 is a protein of approximately 24 kDa, whose main physiological function is probably to control ECM remodelling via regulation of MMPs. It is distinct from other TIMPs in being an insoluble component of the ECM⁹ and is demonstrated in Bruch's membrane by immunohistochemistry¹³⁰, with in situ hybridisation locating production to the RPE and choroid.¹³¹ TIMP-3 has anti-angiogenic activity, demonstrated by reduced chemotaxis of vascular endothelial cells towards growth factors such as vascular endothelial growth factor in the presence of TIMP-3.¹³² Ophthalmology has led the search for genetically determined causes of abnormal matrix turnover by extensive study of the TIMP-3 gene in Sorsby's fundus dystrophy, discussed below. Polymorphisms in the other TIMP genes are now being discovered in individuals with connective tissue remodelling dysfunction leading to aneurysmal dilatation.¹³³

Sorsby's fundus dystrophy

Sorsby's fundus dystrophy (SFD) is an autosomal dominant inherited macular dystrophy first described by Sorsby in 1949.¹³⁴ Central visual loss occurs secondary to geographic atrophy and choroidal neovascularisation in the fifth decade of life, and this is accompanied by a thickening of Bruch's membrane.¹³⁵ The disease links to chromosome 22q13ter, and is associated with mutations in exon 5 of the TIMP-3 gene.¹³⁶

One common mutation of TIMP-3 has been identified in SFD within the UK, although four others have also been described elsewhere in the world.¹³⁶⁻¹³⁹ As the normal function of TIMP-3 is to control ECM remodelling and inhibit angiogenesis, the link between mutation and the SFD phenotype is clear, though we have yet to characterise exactly how mutant TIMP-3 is compromised in these functions. The mutations reported so far all result in the incorporation of an additional cysteine residue, which allows the possibility of inappropriate disulphide bridge formation that could affect structure and stability of the mature protein. Indeed, when the mutant form of TIMP-3 was introduced into an expression system, a 48 kDa species was produced, suggestive of a dimer. This might be more difficult to degrade and could accumulate within Bruch's membrane.¹³⁹ The mutant form retained some ability to inhibit MMPs and localise to the ECM¹⁴⁰ suggesting that SFD may result from a subtle alteration in activity or turnover, rather than loss of function.

Anande-Apte and co-workers have suggested that, being a dominant disease, half the TIMP-3 in SFD will be the mutant form, so the phenotype may arise from haploinsufficiency or through an interference effect of the mutant

protein.¹²⁷ If SFD were merely a consequence of half the functional protein being produced, we might expect net proteolytic activity to increase, resulting in a thinned Bruch's membrane. Histology of SFD eyes, however, has revealed irregular thickening of Bruch's membrane, strongly immunopositive for TIMP-3, except where there is associated RPE degeneration, suggesting a role for the RPE in TIMP-3 turnover.¹⁴¹ The sub-RPE deposits may be interfering with nutritional support of the RPE and photoreceptors, leading to their degeneration. Other organs are unaffected, emphasising the exquisitely sensitive control of matrix turnover in the posterior segment of the eye. The abnormal material may represent mutant TIMP-3 that is resistant to turnover, or binding of TIMP-3 to abnormal matrix accumulations. Presently, antibodies do not distinguish between normal and mutant TIMP-3, so such questions remain unanswered. Work has intensified on transgenic models and in vitro systems to understand the function and turnover of mutant TIMP-3. Understanding SFD may provide insights to the pathogenesis of age related macular degeneration, which it resembles in several respects.

Age related macular degeneration

Age related macular degeneration (AMD) is the leading cause of blindness in the developed world, and its prevalence is increasing.¹⁴² The disease may be broadly classified into exudative or non-exudative types according to the presence or absence of choroidal neovascularisation. Exudative AMD accounts for only about 10% of cases, but has a worse visual prognosis.¹⁴³

Despite differences in age of onset, the clinical manifestations of exudative AMD and Sorsby's fundus dystrophy show several similarities, including choroidal neovascularisation and thickening of the Bruch's membrane. The observation of these similarities has therefore led to the investigation of the TIMP-3 locus in AMD. However, analysis of this gene in AMD and other macular dystrophies failed to identify mutations in diseases other than SFD,¹⁴⁴ as did linkage analysis of AMD families in the USA.¹⁴⁵ Bruch's membrane thickening is a characteristic of AMD, and accumulation of TIMP-3 in this layer has been shown by immunohistochemistry.^{130 139} Western blotting and quantitative reverse zymography have been used to demonstrate an age related increase in normal donor eyes.¹⁴⁶ Moreover, single and continuous drusen deposits in AMD are highly reactive with TIMP-3 antibodies, as are sub-RPE deposits found in a dominant form of retinitis pigmentosa, also lacking any TIMP-3 mutation. Here, they have been demonstrated to occlude lumina of retinal vessels, contributing to hypoxic neuroretinal degeneration.¹⁴¹ In these conditions, TIMP-3 may be accumulating by binding to abnormal constituents of Bruch's membrane.

While the TIMP-3 gene is normal in AMD, there remains the possibility that defective gene regulation may be responsible for the ocular phenotype, and understanding such regulatory mechanisms may be just as important as identifying candidate genes in AMD. In X linked progressive retinal atrophy, a canine model of X linked retinitis pigmentosa (XLRP), TIMP-1 has been excluded as a candidate gene, despite being in close proximity to one of the two XLRP loci, and overexpression of TIMP-1 months before histologically evident retinal degeneration.¹⁴⁷ If subtle changes in matrix turnover could be identified—for example, by serial measurements of Bruch's membrane thickness using a modality such as high resolution optical coherence tomography,¹⁴⁸ we might, in the future, be able to identify patients at risk of AMD before the pathological sequelae are too advanced.

A rational approach to therapy would entail first identifying the matrix components that are responsible for the histopathological changes in Bruch's membrane and then establishing which MMP(s) might mediate effective turnover of these components. Quantitative analyses of the effect of ageing on the MMP content of human retina, Bruch's membrane, or choroid are difficult to interpret, owing to variation in technique and postmortem fixation time, which might influence enzyme degradation or diffusion out of the relevant compartment. The source of MMPs and TIMPs in Bruch's membrane is likely to be the RPE. Authors have described increases in MMP-2 in RPE associated interphotoreceptor matrix in AMD,¹⁴⁹ and increases in MMP-2 and MMP-9 with age in isolated human Bruch's-choroid complex.¹⁵⁰ In both cases, the enzymes were almost entirely inactive forms, and attributing a direct pathogenic role for these findings in AMD is thus oversimplistic. Accumulation of *inactive* MMPs is more likely to be a consequence of Bruch's membrane remodelling than causal. Increased deposition of collagen and other ECM components may provide a signal for increased MMP synthesis. Appropriate remodelling fails, however, as the enzymes are not activated. This may be due to the increased concentration of TIMP-3 present, or to decreased porosity of Bruch's membrane from increased collagen cross linking, denying access to legitimate MMP activators. This would also limit access of MMPs to appropriate substrates and hence cause further ECM accumulation. Subsequent zymogen turnover might be slower, contributing to the observed increase with age.

An intriguing question is whether *activation* of these enzymes precedes or contributes to neovascularisation. The answer might lie in studying large numbers of Bruch's membrane isolates from donor eyes with a spectrum of pathology covering "dry", "wet", early, and advanced forms of AMD. Treatment of AMD by modulating the MMP axis is thus fraught with difficulty. It may be that, in the dry form of the disease, MMP activation or supplementation would be beneficial. Presumably, tissue specific upregulation of MMP production by the RPE could only be achieved by subretinal gene therapy, or by direct introduction of the relevant enzyme(s) or activator(s), both involving extensive surgery.¹⁵¹ Perhaps there are less invasive alternatives for enhancing proteolytic activity in Bruch's membrane, such as laser stimulation, which we know can promote clearance of drusen and improve visual acuity and contrast sensitivity.¹⁵² Even if these issues are addressed, there are many unanswered questions. Would "proteolytic housekeeping" of Bruch's membrane be a one off treatment, or would more frequent "spring cleaning proteolysis" be required to maintain a healthy ECM? Once the disease has progressed to an exudative stage, MMP inhibition might be the appropriate strategy to suppress neovascularisation. That we can even postulate such strategies does offer some hope for patients, and their ophthalmologists, though there are major, unresolved issues regarding when to offer treatment in the natural history of the disease and the relative merits and efficacy of local versus systemic treatment.

CHOROIDAL NEOVASCULARISATION

Subfoveal choroidal neovascularisation is an important cause of visual loss in patients with age related macular degeneration, ocular histoplasmosis syndrome, and other disorders characterised by defects in Bruch's membrane.^{153 154} Surgical excision of neovascular membranes is under evaluation as a potential treatment and has provided tissue specimens to study pathogenesis. Steen *et al* recently reported the results of analysis for MMP and TIMP mRNA by in situ hybridisation in surgically excised

membranes.¹⁵⁵ Localisation of MMP-2 and MMP-9 expression to areas of new vessel formation and Bruch's-like membrane, suggests a possible role for these gelatinases in the growth of neovascular complexes. MMP-7 has been implicated in tumour progression¹⁵⁶ and has also recently been demonstrated in Bruch's membrane-like material around RPE cells in CNV membranes.¹⁵⁷ Inhibition of MMP activity might not be an effective treatment for established CNV complexes, but could conceivably prevent their development and growth, or reduce recurrence after laser photocoagulation or surgical excision.

A phase II clinical trial is currently being undertaken in the United States to assess an oral MMP inhibitor (AG3340) for treatment of subfoveal CNV secondary to AMD.¹⁵⁸ This agent is a potent, non-peptidic MMP inhibitor with selectivity for a subclass of MMPs involved in angiogenesis, including the gelatinases MMP-2 and MMP-9, and MT1-MMP. AG3340 is also currently undergoing advanced oncology clinical trials¹⁵⁹ and can inhibit cellular proliferation and induce apoptosis in tumours. It has been shown to decrease angiogenesis by approximately 50% in a variety of tumour models, on twice daily oral dosing, and by 77% after once daily intraperitoneal dosing in a newborn mouse model of retinal neovascularisation.¹⁶⁰ Plasma concentrations associated with preclinical efficacy in tumour models are readily achieved with twice daily oral dosing in human subjects.¹⁶¹ Sparing of MMP-1 may reduce adverse effects such as arthralgia, as MMP-1 is found in normal joints, but the potential systemic toxicity of AG3340 calls for caution when its use is proposed for a non-life threatening ophthalmic condition. The oncology trials will, however, provide useful data on the safety and tolerance of long term therapy, as prevention of CNV recurrence may require chronic treatment. An interesting evolution of such work would involve high dose, tissue specific application of a related derivative following surgical excision of CNVs, perhaps followed by maintenance on a lower oral dose. For this to be efficacious in AMD, however, the major issues of pigment epithelial loss and photoreceptor degeneration will need to be addressed.

Future directions

MMP research is an exciting and rapidly evolving field, covering a variety of disease related processes in a number of medical and surgical disciplines. Improvements in antibody specificity will enable us to reliably differentiate between active and latent forms in tissues, which might assist diagnosis and contribute to prognostic information, as well as suggest targets for therapy. New techniques such as cDNA microarray analysis will enable us to profile, and ultimately quantify, multiple gene expression in small specimens.¹⁶² Such an approach is likely to be instructive in the complex vitreoretinal disorders discussed above, where multiple MMPs may be participating in pathology, and where net proteolytic activity is dependent on a balance between MMP and TIMP expression. Novel disease related genes have already been discovered in anterior segment disorders, using this methodology.¹⁶³

The posterior segment of the eye presents potential problems regarding the biological activity of agents in relevant compartments following systemic treatment. Local drug or vector delivery is, however, possible with a degree of precision that may not be possible in other organs. Where conventional treatment has been surgical, such as in PVR, adjuvant strategies to manipulate the MMP axis can be assessed, once issues of safety and specificity have been resolved. Treatment of "medical" retinal disorders may follow, once we understand more completely the control of expression and activation of MMPs and TIMPs.

Chemical inhibitors currently being evaluated in trials are relatively non-specific, but as MMP structure-function relations continue to be teased apart, more specific inhibitors will emerge for individual MMPs or subclasses, based on α ray crystallography of active enzyme sites.¹²⁸ Protease activatable gene delivery vehicles to target cells overexpressing MMPs in disease are currently in development.¹⁶⁴ The important role of MMPs in normal physiological processes suggests that inhibition should ideally be targeted to diseased tissues, which remains a challenge. Perhaps targeting "inducible" regions of MMP genes would selectively protect "constitutive" expression and thus not interfere significantly with physiological processes.

Molecular strategies for manipulating the MMP axis will also be developed, as every level of regulation of MMP expression and activity is a potential target for therapeutic intervention. Inhibition might be achieved by targeting messenger RNA production with antisense oligonucleotides or ribozymes¹⁶⁵ and improvements in transfection technology would enable TIMP overexpression in selected cells. It is also possible that selective chemical inhibitors for distinct signalling pathways, such as mitogen activated protein kinase (MAPK) and protein kinase C (PKC), may soon be available for clinical trials. This would provide a novel strategy for reducing MMP expression by inhibiting MMP promoter activation.¹²⁴ Retinoids and tetracyclines are among compounds being explored for this purpose.¹⁶⁶

Such directed research will facilitate studies on the specific role of individual MMPs in biological processes involving matrix turnover. We will see potential therapies tested in new models of disease, and advances in transgenic technology¹⁶⁷ will provide opportunities to perform controlled experiments in complex biological systems. Finally, while we continue to tackle established vitreoretinal pathologies in the new millennium, matrix biology will be contributing significantly to our fundamental understanding of ocular developmental and homeostatic processes.

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C S SETHI

Department of Pathology, Institute of Ophthalmology, and Moorfields Eye Hospital, London

T A BAILEY
P J LUTHERT

Department of Pathology, Institute of Ophthalmology, London

N H V CHONG

Department of Pathology, Institute of Ophthalmology, and Moorfields Eye Hospital, London

Correspondence to: Mr C S Sethi, Vitreoretinal Surgery, Moorfields Eye Hospital, City Road, London EC1V 2PD

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