Metalloproteinase inhibitors: biological actions and therapeutic opportunities

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Summary

Tissue inhibitors of metalloproteinases (TIMPs) are the major cellular inhibitors of the matrix metalloproteinase (MMP) sub-family, exhibiting varying efficacy against different members, as well as different tissue expression patterns and modes of regulation. Other proteins have modest inhibitory activity against some of the MMPs, including domains of netrins, the procollagen C-terminal proteinase enhancer (PCPE), the reversion-inducing cysteine-rich protein with Kazal motifs (RECK), and tissue factor pathway inhibitor (TFPI-2), but their physiological significance is not at all clear. α2-Macroglobulin, thrombospondin-1 and thrombospondin-2 can bind to some MMPs and act as agents for their removal from the extracellular environment. In contrast, few effective inhibitors of other members of the metzincin family, the astacins or the disintegrin metalloproteinases, ADAMs have been identified.

Many of these MMP inhibitors, including the TIMPs, possess other biological activities which may not be related to their inhibitory capacities. These need to be thoroughly characterized in order to allow informed development of MMP inhibitors as potential therapeutic agents. Over activity of MMPs has been implicated in many diseases, including those of the cardiovascular system, arthritis and cancer. The development of synthetic small molecule inhibitors has been actively pursued for some time, but the concept of the use of the natural inhibitors, such as the TIMPs, in gene based therapies is being assessed in animal models and should provide useful insights into the cell biology of degradative diseases.

Key words: MMP, TIMP, RECK, Therapy

Introduction

Metalloproteinases (MPs) play key roles in the responses of cells to their microenvironment. By effecting proteolytic degradation or activation of cell surface and extracellular matrix (ECM) proteins they can modulate both cell-cell and cell-ECM interactions, which influence cell differentiation, migration, proliferation and survival. Both secreted and membrane-bound forms of metalloproteinases have been implicated in pericellular proteolysis, including the matrix metalloproteinases (MMPs), the adamalysin-like proteinases with both metalloproteinase and disintegrin-like domains (ADAMs and their counterparts that have a thrombospondin-1-like domain, ADAM-TSs) and the astacins (Werb, 1997). Cells use various strategies to regulate extracellular proteinases: transcriptional regulation, trafficking of membrane-bound forms (secretion and endocytosis), activation of latent proenzyme forms, extracellular binding proteins and the action of endogenous inhibitors. Here we will discuss the role of metalloproteinase inhibitors, from the well-known tissue inhibitors of metalloproteinases (TIMPs) and α2-macroglobulin through to newer and less well-understood putative inhibitors (Fig. 1). We look at the available evidence that their other roles in cell biology do not all relate to their metalloproteinase inhibitory activity. Finally, we discuss the potential for use of such natural metalloproteinase inhibitors as therapeutic agents.

Tissue inhibitors of metalloproteinases (TIMPs): basic structure and activity

Four mammalian TIMPs have been cloned, purified and characterised. These secreted proteins are thought to regulate MMP activity during tisssue remodelling. One TIMP gene has been identified in *Drosophila*, and its ablation generates a phenotype similar to that of integrin mutants, which indicates that it has a role in ECM function (Godenschwege et al., 2000). All four mammalian TIMPs have many basic similarities, but they exhibit distinctive structural features, biochemical properties and expression patterns (Table 1). This suggests that each TIMP has specific roles in vivo. As in *Drosophila*, the mammalian TIMP genes are embedded intragenically in intron 5 of synapsin genes (Edwards, 2000).

The TIMPs have molecular weights of ~21 kDa and are variably glycosylated (Table 1). They have six disulphide bonds and comprise a three-loop N-terminal domain and an interacting three-loop C-subdomain. Most of the biological functions of these proteins discovered thus far are attributable to sequences within the N-terminal domain, although the C-subdomains mediate interactions with the catalytic domains of some MMPs and with the hemopexin domains of MMP-2 and MMP-9 (Brew et al., 2000). The TIMPs are secreted proteins, but may be found at the cell surface in association with membrane-bound proteins; for example, TIMP-2, TIMP-3 and

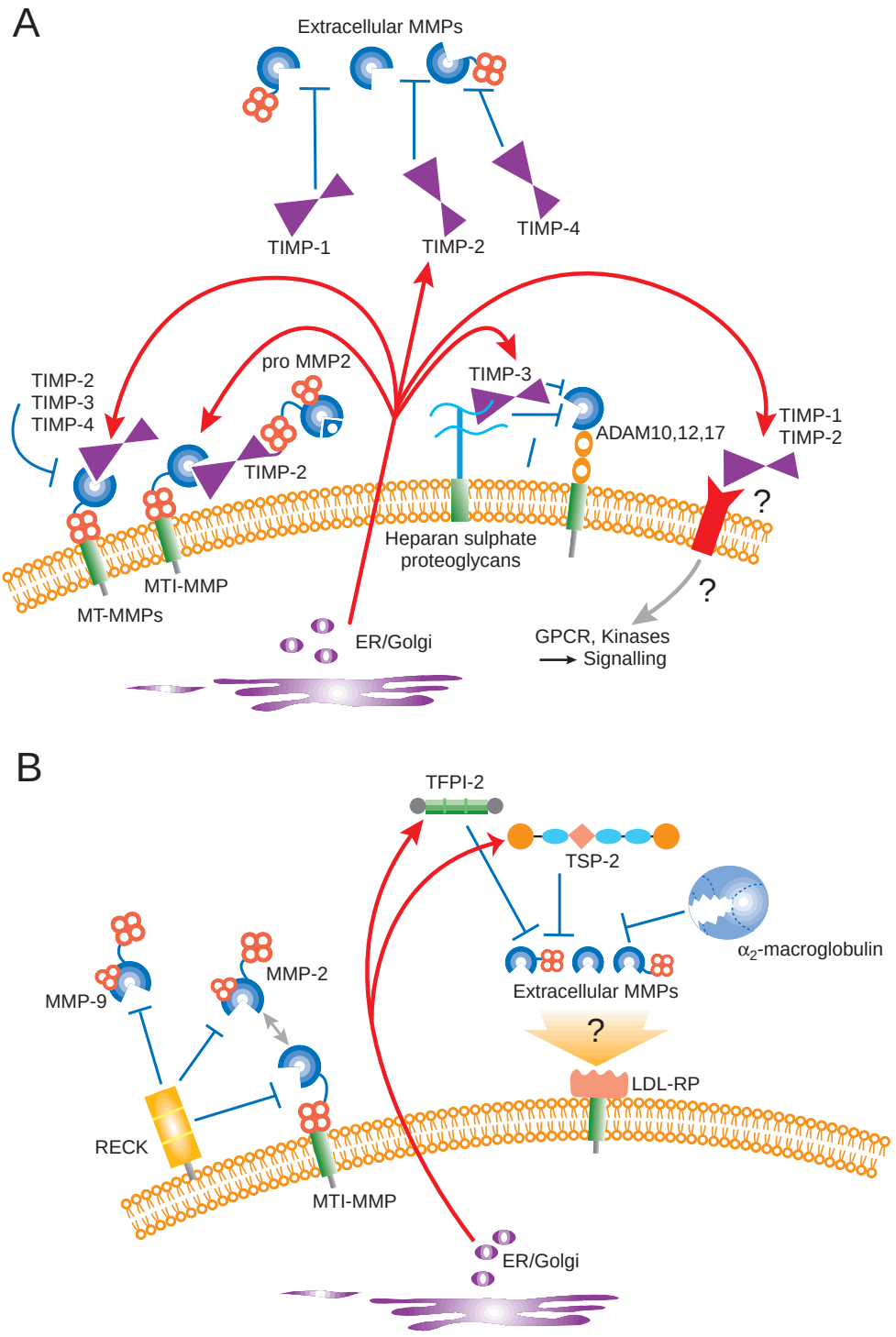

Fig. 1. Metalloproteinase inhibitors in the pericellular environment. (A) Tissue inhibitors of metalloproteinases (TIMPs). TIMPs-1-4 are largely matrix metalloproteinase (MMP) inhibitors modulating the activity of soluble, matrix bound and cell associated MMPs. TIMP-3 is an extracellular matrix protein, probably bound to heparan sulphate proteoglycans and is a potential inhibitor of the function of some membrane-associated ADAM s (a disintegrin and a metalloproteinase), as well as the matrix-associated ADAM-TS (ADAM-thrombospondins, not shown). TIMP-2 acts in conjunction with MT1-MMP as a receptor for the pro-form of MMP-2 at the cell surface, allowing an efficient activation and focussing of the active form of this soluble proteinase. In some cell types, TIMP-1 and TIMP-2 may have receptors directly linked to intracellular signalling pathways regulating cell behaviour. (B) Other inhibitors. RECK (reversion inducing cysteine rich protein with Kazal motifs) is a GPI-anchored glycoprotein that binds and inhibits a number of MMPs. The pan proteinase inhibitor α2-macroglobulin, although very large, has some access to the pericellular space in vascularised tissues and may be involved in MMP endocytosis through the low density lipoprotein receptor-related protein (LDL-RP). The roles of the LDL-RP in MMP2 removal via a thrombospondin-2 (TSP-2) complex and in direct MMP9 removal have been described. The tissue factor pathway inhibitor (TFPI-2) has also been described as an MMP binding agent

TIMP-4 could bind MMP-14, a membrane-type (MT) MMP. Uniquely, TIMP-3 is sequestered to the ECM by binding to heparan-sulphate-containing proteoglycans and possibly chondroitin-sulphate-containing proteoglycans (Yu et al., 2000). All four TIMPs inhibit active forms of all MMPs studied to date, their binding constants being in the low picomolar range, although TIMP-1 is a poor inhibitor of MMP-19 and a number of the MT-MMPs (Table 1). TIMPs have no significant activity against the astacins (J. Bond, personal communication), but some activity of TIMP-3 (and to some extent TIMP-1) against the ADAMs has been shown. TIMP-3 inhibits ADAM 12 and ADAM 17 and the aggrecan-degrading enzymes ADAM-TS4 and ADAM-TS5, and TIMP-1 inhibits ADAM 10. Here, dissociation constants are in the sub-nanomolar range (Amour et al., 2000; Amour et al., 1998; Kashiwagi et al., 2001).

Table 1. Common and unique features of TIMPs

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Protein kDa	28	21	24/27	22
N-glycosylation sites	2	0	1	0
Protein localization	Soluble	Soluble/cell surface	ECM	Soluble/cell surface
Pro-MMP association	pro-MMP-9	pro-MMP-2	pro-MMP-2/-9	pro-MMP-2
MMPs poorly inhibited	MT1-MMP MT2-MMP MT3-MMP MT5-MMP MMP-19	None	None	None
ADAM inhibition	ADAM 10	None	ADAM 12 ADAM 17 ADAM 19 (ADAM 10) ADAMTS-4, TS-5	None
Cell proliferation	↑ Erythroid precursors ↑ Tumour cells	↑ Erythroid precursors ↑ Tumour cells ↑ Fibroblasts ↑ Smooth muscle cells ↓ Endothelial cells	↑ Smooth muscle cells and cancer cells*	↑ Mammary tumour cells ↓ Wilm's tumour cells
Apoptosis	↓ Burkitt's lymphoma cells	↑ Colorectal cancer cells ↓ Melanoma	↑ Smooth muscle cells ↑ Tumour cells ↑ Retinal pigmented epithelial cells	↑ Cardiac fibroblasts
Tumour angiogenesis	↑ Mammary ↓ Liver	↓ Melanoma ↓ Mammary	↓ Melanoma	
Angiogenesis in 3D collagen/ fibrin gels	No effect	Inhibits	Inhibits	Inhibits
Tumourigenesis effects	Inhibits	Inhibits	Inhibits	Inhibits
Metastasis effects	Stimulates			Stimulates

Distinguishing characteristics of human TIMPs with respect to MP inhibitory activity and phenotypic modulation of cells both in vitro and in vivo are illustrated. We have also documented detailed aspects of TIMPs from studies in cancer models.

*Refers to the ability of TIMP-3 overexpression to instigate S-phase entry in vitro rather than a direct effect on promotion of cell proliferation per se.

Phenotypic effects of TIMPs

TIMPs have effects on cell growth and survival that cannot always be clearly reconciled with their ability to abrogate MMP activity. TIMP-1 was first identified as an erythroid potentiating activity (Gasson et al., 1985) and subsequently as an agent that stimulates growth of some cell lines (Bertaux et al., 1991; Hayakawa et al., 1992). TIMP-2 also has erythroid potentiating activity (Stetler-Stevenson et al., 1992) and stimulates the growth of lymphoma cells (Hayakawa et al., 1994) and fibroblasts (Corcoran and Stetler-Stevenson, 1995); in addition, it might be comitogenic with insulin (Nemeth et al., 1996). Point mutations of the TIMP-1 MMP-inhibitory domain uncouple MMP inhibition and erythroid precursor growth stimulation (Chesler et al., 1995). Moreover, recently Sobue et al., showed that both TIMP-1 and TIMP-2 stimulate the bone-resorbing activity of osteoclasts through tyrosine kinase and MAP kinase pathways, and that synthetic MMP inhibitors do not have this effect. (Sobue et al., 2001). TIMP-1 binds to the cell surface of MCF-7 breast carcinoma cells (Ritter et al., 1999) and is translocated to the nucleus, although no receptor or distinct function of nuclear-localised TIMP-1 has yet been identified. Accumulation of TIMP-1 in the nuclei of gingival fibroblasts had previously been shown to be maximal in S phase of the cell cycle (Li et al., 1995).

TIMP-2 is thought to act through specific, saturable high-affinity receptors (K_d ~0.15 nM) and links to G protein and cAMP signalling pathways (Corcoran and Stetler-Stevenson, 1995). Since reduced and alkylated TIMP-2 is mitogenic (Hayakawa et al., 1994) and an inactive mutant that has an additional N-terminal alanine residue promotes fibroblast growth (Wingfield et al., 1999), these activities are probably distinct from its ability to inhibit MMPs. Importantly, some TIMPs are associated with the tumour progression (Grignon et al., 1996; Jiang et al., 2001; Kossakowska et al., 1991; Stetler-Stevenson et al., 1997), although, paradoxically, others suppress tumour formation (Ahonen et al., 1998; Baker et al., 1999; Bian et al., 1996; Edwards et al., 1996) (Table 1). TIMPs also have divergent effects on programmed cell death. In Burkitt's lymphoma cell lines, high TIMP-1 expression correlates with the increased expression of activation and survival markers, and TIMP-1 confers resistance to Fas-ligand-dependent and -independent apoptosis (Guedez et al., 1998). Conversely, TIMP-2 can promote apoptosis in an in vivo colorectal cancer model (Brand et al., 2000) but protects B16 melanoma cells from apoptosis (Valente et al., 1998). High levels of TIMP-3 promote apoptosis in many cell types in vitro and in vivo (Ahonen et al., 1998; Baker et al., 1998; Bond et al., 2000; Smith et al., 1997; Yang and Hawkes, 1992), and this effect is associated with death receptor modulation (Bond et al., 2002; Smith et al., 1997). It is not clear whether TIMP-3-

induced apoptosis has any physiological parallel. In normal development, high-level TIMP-3 expression occurs in uterine decidual cells during embryo implantation and has been linked with the survival of these differentiated cells (Alexander et al., 1996). TIMP-4 can also instigate apoptosis in transformed cardiac fibroblasts but inhibits apoptosis in human breast cancer cells in vitro and mammary tumours in vivo; it is thus a tumour promoter when overexpressed. The elucidation of the mechanisms involved in controlling these distinctly opposing phenotypic effects of TIMPs is of paramount importance if we are to understand TIMP biology and assess the potential therapeutic roles of these proteins (see below).

TIMP-knockout phenotypes

Knockout mouse studies have addressed the biological roles of TIMPs (reviewed by Coussens et al., 2001). To date, TIMP-$1^{-/-}$, TIMP-$2^{-/-}$ and TIMP-$3^{-/-}$ mice have been described. All of these are viable and fertile, although the fertility and reproductive lifespan of female TIMP-1-null mice are reduced (Nothnick, 2001). Mice lacking TIMP-3 show spontaneous lung airspace enlargement by 2 weeks after birth, which progresses until the animals become moribund shortly after 1 year (Leco et al., 2001). This emphysema-like phenotype correlates with disturbance of the MMP/TIMP balance, which leads to increased collagen turnover in the alveolar interstitium. TIMP-$3^{-/-}$ mice also show accelerated mammary epithelial apoptosis during post-lactation involution of the gland (Fata et al., 2001). In TIMP-$2^{-/-}$ mice the only phenotype that has been remarked upon is impairment of pro-MMP-2 activation (Caterina et al., 2000; Wang et al., 2000), which reflects the unique role that TIMP-2 has in cell surface activation of pro-MMP-2 by MT1-MMP (Bigg et al., 2001; Toth et al., 2000). In the mouse, MMP-2 is dispensable for normal development, although there is reduced growth and vascularization of transplanted tumours in MMP-2-null host mice (Itoh et al., 1998). However, mis-sense mutations in the human *MMP*-2 gene give rise to multicentric osteolysis and arthritis (Martignetti et al., 2001), and so it is possible that disruption of the *TIMP-2* gene may likewise have more extensive effects in human. A curious aspect of the TIMP-$1^{-/-}$ mice is their increased resistance to infection by *Pseudomonas aeruginosa*, which has been linked with increased inflammatory and complement-dependent immune responses (Coussens et al., 2001). Upregulated production of TIMPs could be a factor in various fibrotic diseases but neither TIMP-$1^{-/-}$ mice nor TIMP-$2^{-/-}$ mice show differences in hepatic fibrosis following *Schistisoma* parasite infection (Vaillant et al., 2001). TIMP-1-null mice also show no change in renal fibrosis induced by unilateral ureteral obstruction (Kim et al., 2001). However, the issue of compensation by altered MMP/TIMP expression needs to be addressed comprehensively in these systems.

TIMP-like molecules

A number of proteins contain sequences with some similarity to the N-terminal sequence of the TIMPs, including the netrins, secreted frizzled-related proteins and type I collagen C-proteinase enhancer protein (PCPE) (reviewed by Banyai and Patthy, 1999), and might act as MMP inhibitors. Tissue factor pathway inhibitor 2 (TFPI-2), a serine proteinase inhibitor, also has an internal region that has some sequence similarity to the TIMPs and inhibits MMP-1, MMP-2, MMP-9 and MMP-13. TFPI-2 appears to bind and co-precipitate with the MMPs, including proMMP-2, and hence it may act by a sequestration mechanism (Herman et al., 2001). The importance of these interactions in cell biology has not yet been studied. None is likely to act by a mechanism directly analogous to that of a TIMP, because a critical feature of the latter is the liganding of the Zn^{2+} ion at the active site by the free amino group of the N-terminal cysteine residue (Gomis-Ruth et al., 1997). The NC1 domain of type IV collagen weakly inhibits MMP-2 and MMP-3, but this is unlikely to be physiological.

Thrombospondin-2 is another MMP inhibitor. It can regulate MMP-2 by forming a complex that facilitates scavenger-receptor-mediated endocytosis. Similarly, thrombospondin-1 has been shown to inhibit proMMP-2 and proMMP-9 activation and modulate MMP-2 production (Egeblad and Werb, 2002).

α2-macroglobulin

The plasma proteinase inhibitor α2-macroglobulin is a 772 kDa protein comprising four nearly identical, disulphide-bonded domains. It is synthesised mainly in the liver by hepatocytes, but production by some other cell types (e.g. macrophages) has also been described. α2-macroglobulin can almost universally inhibit endoproteinases, including the MMPs and the ADAMs, but does not inhibit astacins. Inhibition is effected by a novel mechanism involving the presentation of a cleavable 'bait' region that, once proteolytically cleaved, causes a conformational change that entraps the proteinase, which becomes covalently anchored by transacylation. α2-macroglobulin has proved to be a useful tool for the identification of proteolytic activity in less well-characterised proteinases: one can simply look for the formation of a covalent complex (Nagase, 1997). Although some TIMPs are also present in plasma, α2-macroglobulin can be regarded as the major plasma inhibitor of metalloproteinases. However, its role and importance in the regulation of the pericellular function of metalloproteinases is still a matter of debate. α2-Macroglobulin-serine-proteinase complexes have long been known to be associated with the α2-macroglobulin receptor, the low density lipoprotein receptor-related protein (LDL-RP) prior to internalisation and degradation (Moestrup et al., 1993), and it is likely that MMPs could be similarly removed. However, Barmina et al., have shown that MMP-13 associates with the LDL-RP through a specific receptor on osteoblastic cells, leading to internalisation (Barmina et al., 1999). More recently, Hahn-Dantona et al., showed that MMP-9 and its TIMP-1 complex can be internalised by binding to LDL-RP (Hahn-Dantona et al., 2001). α2-macroglobulin also has a variety of effects that do not directly relate to its ability to inhibit proteinases. These include binding to TGFβ and cytokines (Armstrong and Quigley, 1999).

Reversion inducing cysteine-rich protein with Kazal motifs: RECK

The *RECK* gene is expressed by v-Ki-Ras-transformed NIH 3T3 cells during the induction of a flat morphology. It encodes

a 110 kDa glycoprotein that contains serine-proteinase-inhibitor-like domains and associates with the cell membrane through a GPI anchor (Takahashi et al., 1998). RECK is widely expressed in human tissues, but its levels appear to be low in many tumour-derived cell lines. Overexpression of RECK in such lines resulted in a downregulation of MMP-9 at an unidentified post-transcriptional level. Concomitantly, the invasive potential of the cells decreased. RECK also inhibited the cellular activation of proMMP-2 beyond the MT1-MMP-generated activation intermediate, which is thought to be an autoproteolytic step (Butler et al., 1998). Soluble recombinant RECK is a moderately weak inhibitor of MMP-2, MMP-9 and a soluble form of MT1-MMP, but its activity against serine proteinases has not been studied as yet. Its importance is emphasised by the study of ablation of the gene in mice (Oh et al., 2001). RECK$^{-/-}$ embryos die at E10.5 and show severe disruption of mesenchymal tissues and organogenesis. Notably, vascular endothelial cells in these mice do not form tight tubules, which suggests that they have a defect in vessel maturation. Since MMP-2 and MT1-MMP are expressed at this stage, their dysregulation could be responsible for the phenotype. Alternatively, RECK might have other functions that have not been elucidated. Indeed, RECK$^{-/-}$MMP-2$^{-/-}$ double-knockout mice survive to E11.5, are larger than RECK$^{-/-}$ mice and exhibit improved vascular development. In a tumour model in which Oh et al., injected HT 1080 cells overexpressing RECK subcutaneously into nude mice, tumour angiogenesis was compromised (Oh et al., 2001). The sprouting stage of angiogenesis appeared to be impaired but lumenal growth continued, yielding a few vessels with wide lumens and extensive tumour cell death. Note that ablation of any of the TIMP genes has very little effect on embryonic development. Since RECK is cell associated, it could function in a much more specific and directed way. However, at this stage, it is prudent to suggest that RECK has other biological properties that remain to be elucidated.

Development of TIMPs as therapeutic molecules

Although it is now clear that TIMPs are multifunctional proteins that possess both mainstream (but differing) MP-inhibitory activities and divergent other functions, their attributes may be exploited in the search for novel therapies. The current trend of seeking to readdress the MP:TIMP balance to block or reverse disease progression involves either inhibition of MMP activity by small molecule drugs or increasing the local concentration of TIMPs by recombinant protein administration or gene transfer. The majority of clinical trials using synthetic MP inhibitors (MPI) have proven disappointing, principally owing to lack of efficacy and untoward side effects. However, note that in animal models MP inhibitors are most effective in preventing growth and vascularisation of pre-malignant tumours and are relatively ineffective against advanced disease (Bergers et al., 1999). Hence, their poor performance is, in retrospect, unsurprising given the design of the trials that have been undertaken to date (Brown, 2000; Coussens et al., 2002), many of which involved the use of MMP inhibitors as single agent therapies for patients with advanced disease. Recently however, survival benefits are being detected in some trials, including a phase II study of marimastat in advanced pancreatic cancer (Evans et al., 2001) and a phase III gastric cancer study (Bramhall et al., 2002). This suggests that, when we know more about the precise roles of MPs in tumour progression, these compounds may be of clinical value. Despite setbacks the basic principle of therapeutic MP inhibition remains intact and is applicable to diverse diseases, including cardiovascular disease and cancer. The rational design and evaluation of more selective synthetic inhibitors may be one route to efficacious therapies. However, recent evidence in pre-clinical studies suggests that gene-based therapy using TIMPs may have broad clinical potential.

MMP blockade and cardiovascular disease

Deregulation of MMPs has been implicated in diverse cardiovascular diseases, acute and chronic. These include atherosclerosis (George, 1998), myocardial infarction and heart failure (Creemers et al., 2001), development and rupture of aneurysms (Pyo et al., 2000), restenosis following balloon angioplasty (Bendeck et al., 1994; Zempo et al., 1994) and the failure of vein grafts following coronary artery bypass graft failure (George et al., 1997). Given the predicted involvement of MMPs in this range of cardiovascular events, many of which lack suitable surgical or pharmacological treatments, intense research has focused on blockade of MP activity. However, certain applications are more realistic than others. Chronic, progressive conditions such as atherosclerosis would require long-term administration of MP inhibitors to prevent disease progression or ideally reverse it. A short-term study using TIMP-1 in a pre-clinical model of atherosclerosis demonstrated this proof of concept (Rouis et al., 1999). Following systemic adenovirus administration, ectopic TIMP-1 reduced the severity of atherosclerosis relative to controls. The limitation, however, was the transient nature of adenovirus-mediated gene transfer, and thus long-term gene therapy studies are required. Paradoxically, atherosclerotic apoE$^{-/-}$ mice exhibit a reduction in atherosclerotic lesion size when crossed with TIMP-1$^{-/-}$ mice compared with controls (although the incidence of aneurysm increased). We must therefore define fully the underlying complexity of the MP:TIMP system in the development of atherosclerosis (Silence et al., 2002).

Many MPI studies have been performed in acute cardiovascular complaints, particularly restenosis and vein graft failure. These applications offer the opportunity to prevent disease progression in a time frame that is applicable to the current technologies available to gene therapists, by high-level transient TIMP overexpression locally within the vasculature. Post-balloon angioplasty procedures (although dwindling in frequency owing to use of stents, which act as scaffolds to hold arteries open) were an obvious target for TIMP gene therapy. MPs were envisaged to play an integral role in the pathological development of restenotic lesions, which are mediated by the proliferation and migration of vascular smooth muscle cells (Bendeck et al., 1994; Southgate et al., 1996; Zempo et al., 1994). However, small molecule MMP inhibitors fail to prevent restenosis, even though they efficiently block early smooth muscle cell migration (Bendeck et al., 1996). Recently, the broad spectrum MP inhibitor RO113-2908 failed to prevent angioplasty- or stent-induced intermal hyperplasia over 4 weeks in atherosclerotic primates (Cherr et al., 2002), although MP inhibitors do reduce

constrictive remodeling in a porcine angioplasty model (de Smet et al., 2000). Similarly, TIMP overexpression (Cheng et al., 1998; Dollery et al., 1999; Forough et al., 1996) prevented smooth muscle cell migration but long-term benefits were not fully established. Vein graft failure, in common with restenosis, involves smooth muscle cell migration and proliferation as a central mechanism, and increased MP synthesis and activation have been observed in appropriate models (George et al., 1997; Southgate et al., 1999). Local overexpression of TIMPs (TIMP-1, TIMP-2 or TIMP-3) in a human vein graft model prevented MMP-induced neointima formation (George et al., 1998a; George et al., 1998b; George et al., 2000). In vivo, overexpression of TIMP-3, but not other TIMPs, significantly inhibited disease progression through its ability to promote apoptosis (George et al., 2000). This is an important demonstration of how unique attributes of individual TIMPs can be used to therapeutic advantage.

Cancer

Cancer is another major clinical condition in which the use of MMP inhibitors is under investigation. Descriptive and intervention studies have defined the potential importance for MMPs in all major facets of cancer progression, including tumour growth, invasion and extravasation into distant sites, and the angiogenic effect required to nourish new tumour formation (Coussens et al., 2002; Egeblad and Werb, 2002). Many studies have attempted to correlate MMP and TIMP expression with tumour type and grade. Although there is distinct heterogeneity in the findings for different cancers (reviewed by Egeblad and Werb, 2002), the majority of studies underscore the potential feasibility for MP inhibition as a route to cancer therapy. Important early studies used recombinant TIMPs or basic gene transfer systems (plasmids or retroviruses) and demonstrated that inhibition of MPs by TIMP blocks both tumour growth and local invasion through extracellular matrices. However, as discussed previously, the use of MP inhibitors in clinical trials has proven largely disappointing, which highlights several key points. First, more selective inhibitors that block the functions of specific MPs involved in key steps in tumour progression are required. This is important because some MPs might act to limit tumour growth and metastasis through local production of angiogenesis-suppressing molecules such as angiostatin and endostatin or other factors that have negative effects on tumour growth (Coussens et al., 2002). Second, it is likely that MP inhibition alone is insufficient for treatment of late-stage disease and there is a need to consider the use of MPIs in patients who have early disease or after surgical debulking and in concert with other treatments. Third, localised MMP inhibition may be a pre-requisite to achieving efficacy. Although the results of long-term studies using selective MP inhibitors are eagerly awaited, TIMPs offer the opportunity to provide localised therapy, particularly using gene transfer technology. Since TIMPs are secreted they have a profound bystander effect, causing phenotypic modulation of cells that are not transduced directly by the gene delivery vector. This advantage over intracellular or cell-surface-associated transgenes may compensate for the low percentage of cells-transducted by certain vector systems. There are now many studies demonstrating pre-clinical efficacy for overexpression of TIMPs to prevent the growth and/or spread of cancers as well as protecting normal, non-cancerous tissue, from metastases that would normally form in the targeted tissue (Brand et al., 2000).

There are, however, safety issues relating to the biological effects of TIMPs and, in particular, their growth promoting activity. This paradigm is highlighted by two recent studies. First, Celiker et al. delivered a DNA plasmid encoding TIMP-4 via intra muscular injection into nude mice with tumours

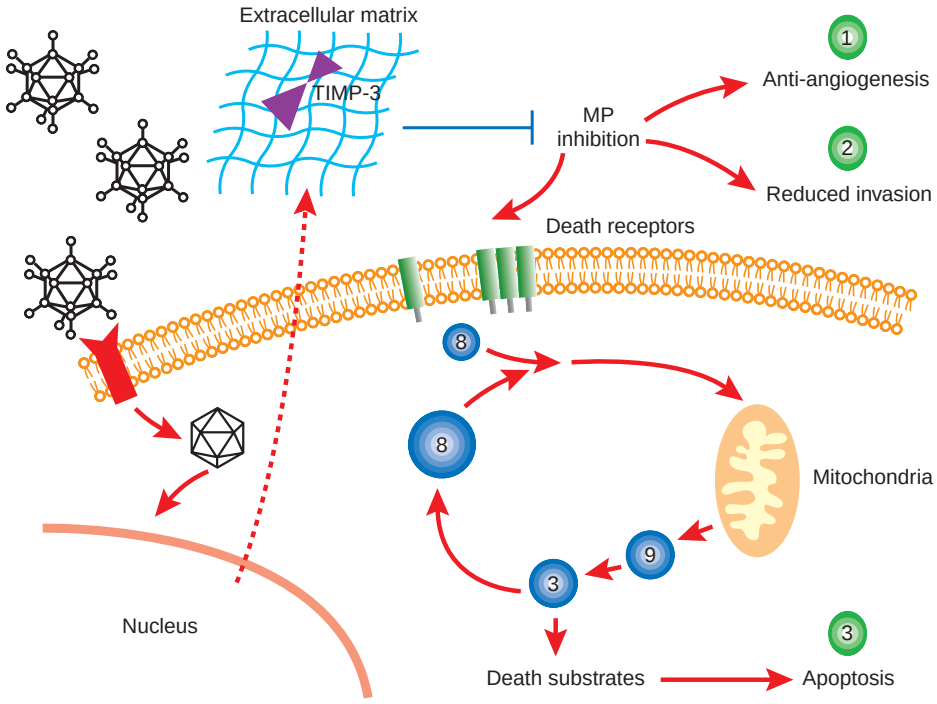

Fig. 2. Mechanistic aspects of TIMP-3 gene therapy. Highly efficient replication-deficient adenoviral vectors engineered to express TIMP-3 infect cells through receptor-mediated endocytosis, traffic to the nucleus and use the host DNA machinery to transcribe and secrete TIMP-3. Recombinant TIMP-3 binds the ECM where it initiates its desired phenotypic effects on cells (indicated by 1-3 in green). TIMP-3 overexpression is associated with anti-angiogenic activity (1), reduction in cell migration and invasion (2) and initiation of apoptosis (3), mediated through modulation of MP activity. The induction of apoptosis occurs though modulation in death receptor/death ligand activity at the cell surface resulting in activation of caspases (in blue). Apoptosis is mediated through a type-2-dependent pathway involving caspase-8, -9 and -3 as well as mitochondrial components. Ultimately, caspase-3-mediated cleavage of death substrates leads to apoptosis.

derived from G401 Wilm's tumor cells. They observed a significant reduction in tumour growth through the elevation of circulating TIMP-4 and uptake of the transgene into tumour cells. These effects were observed at TIMP-4 levels below the level required for MMP inhibition (Celiker et al., 2001). However, when the same transgene, route of delivery and nude mouse model were used to investigate breast cancer growth, TIMP-4 stimulated tumorigenesis through an MMP-independent anti-apoptotic activity (Jiang et al., 2001). Clearly different cancers were under investigation but the anti-apoptotic/pro-survival effects of TIMPs have also been defined for TIMP-1 in B-cell lymphoma (Guedez et al., 1998) and Hodgkin/Reed-Sternberg cells (Oelmann et al., 2002).

The biological characteristics of TIMP-3 have also been exploited in cancer gene therapy (Fig. 2). As previous studies have shown, TIMP-3 inhibits local invasion of cancer cells, promotes apoptosis, inhibits angiogenesis (Anand-Apte et al., 1997) and binds locally to the ECM (Leco et al., 1994), which suggests that overexpression of TIMP-3 is a rational multi-phenotypic approach for localised destruction of cancerous tissue. Indeed, a recent study has demonstrated this potential (Ahonen et al., 2002). TIMP-3 overexpression in melanoma-derived subcutaneous tumours in nude mice reduced gelatinolytic MMP activity, reduced blood vessel density, promoted apoptosis and significantly reduced tumour growth. Importantly, in side-by-side comparative studies, TIMP-3 overexpression was significantly better than overexpression of p53 (Ahonen et al., 2002). Such studies use the attributes of individual TIMP molecules to therapeutic benefit and may, in the longer term, show promise for cancer gene therapy in the clinic.

Concluding remarks

Together these findings illustrate that, of the known natural MMP inhibitors, TIMPs possess proteinase inhibitory properties that may be exploited for therapeutic benefit in diverse pathologies. However, caution will need to be exercised until the other biological activities of the TIMPs are fully understood, because they could have unwanted side effects. Although some clinical studies using broad spectrum MP inhibitors have drawn disappointing conclusions, further research on more selective MP inhibitors and TIMPs in appropriate target disease is warranted to define the therapeutic potential of these molecules, as well as to elucidate their varied mechanisms of action. The potential for RECK, or parts of the RECK molecule, to be used as therapeutic agents is currently not known, but will be clearly the subject of future work.

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