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Expression and biological significance of tissue inhibitors of metalloproteinases

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Abstract. Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent proteases that are involved in degrading extracellular matrix (ECM). Tissue inhibitors of metalloproteinases (TIMPs) are naturally occurring proteins that inhibit MMP activity by binding noncovalently with the active forms of MMPs at molar equivalence. Of the four TIMPs characterized so far, TIMP-1 and TIMP-2 participate in the inhibition of tumor growth, invasion, and metastasis. They also promote growth, inhibit angiogenesis, and modulate cell morphology. TIMP-3 is unique among the TIMPs in being a component of the ECM itself and in suppressing tumor cell growth. TIMP-4, the most recently discovered TIMP, has its gene allocated to human chromosome 3p25 and is perhaps the most tissue-specific of the TIMPs being expressed largely in the heart. Synthetic low-molecular-weight MMP inhibitors with a hydroxamate structure that mimics collagen have reduced the tumor burden and altered the growth of primary tumors in animal models. Understanding the biological significance of TIMPs and their involvement in tumorigenicity will be valuable for the development of effective novel therapeutic strategies for controlling tumor growth and metastasis. This chapter provides a brief review of the four TIMPs characterized thus far, focuses on their roles in tumorigenesis and angiogenesis and concludes with a brief look at the use of synthetic MMP inhibitors in cancer treatment.

Keywords. Tissue inhibitors of metalloproteinases; extracellular matrix; cancer.

1. Introduction

The major classes of extracellular matrix (ECM)-degrading enzymes are the serine proteases, which include plasminogen activators (urokinase- and tissue-type plasminogen activators; uPA and tPA), the cysteine proteases (cathepsin B and S), the aspartic proteinases (cathepsin D), and the MMPs. Many normal and pathological processes that require degradation and remodeling of the ECM involve MMPs. In fact, most ECM degradation is thought to be mediated by MMPs produced by a variety of cells [1]. The MMP family includes at least 16 secreted and transmembrane enzymes. All MMPs have a catalytic domain with an HEXGH motif that ligates Zn^{2+} and is essential for catalytic function and all require Ca^{2+} for their activity. Collectively, MMPs are capable of degrading all fibrillar and nonfibrillar collagens, fibronectin, laminin, elastin, and basement-membrane glycoproteins [2]. These enzymes are secreted in latent proenzyme form and require activation before they can degrade ECM components. Activation involves proteolytic cleavage of a propeptide domain at the N-terminus of the MMP

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molecule and is accomplished by other proteinase cascades involving other MMPs as well as other enzyme classes[3]. The expression of MMPs is modulated by growth factors and cytokines[3].

TIMPs are proteins that regulate the activity of secreted MMPs and modulate the proteolysis of the ECM[4]. Four distinct gene products have been isolated thus far that specifically inhibit the MMPs. In general, TIMPs have two distinct structural and functional domains. The N-terminal domain efficiently inhibits all the MMPs by interacting with the enzyme's catalytic domain. Structural features shared by the TIMPs include the presence of 12 cysteine residues in conserved regions of the molecule forming six disulfide bonds, conserved sequence VIRAK in the NH_2 terminal domain, and a 29-amino-acid leader sequence that presumably is cleaved to produce the mature protein[4].

2. TIMP-1

The gene encoding TIMP-1 has been localized in Xp11.23 in humans[5]. The promoter lacks the TATA sequence and contains multiple response elements in the 5' region of the gene, including a TPA-responsive element (TPE) and the AP-1, SP-1, and Ets sites[6]. The Ets binding site occurs as a single copy that lies between the AP-1 and SP-1 sites and has been shown to act synergistically with the AP-1 protein in activating TIMP-1 transcription[6]. Human TIMP-1 is encoded as a 0·9-kb mRNA. Stimulators of TIMP-1 expression include phorbol esters, Interleukin (IL)-1β, IL-6, epidermal growth factor (EGF), basic fibroblast growth factor (FGF), oncostatin and leukemia inhibitory factor[7–10]; suppressors include concanavalin A[9] and dexamethasone[11]. Site-directed mutagenesis of the 28·5 kDa TIMP-1 glycoprotein has verified the importance of the N-terminal amino acids, His7 and Gln9 for inhibition[12]. TIMP-1 seems to have several functions in addition to inhibiting MMP activity, including stimulation of steroidogenesis[13] and erythroid potentiating activity[14]. Recent studies of TIMP-1 structure and function have revealed separate domains for the MMP-inhibition and erythroid potentiating activities[15]. Moreover, transfection of rat mammary carcinoma cells with TIMP-1 led to flattened cell morphology and a decrease in MMP-2 activity of those cells[16].

3. TIMP-2

Human TIMP-2 is encoded by a gene that has been localized in human chromosome17q25[17]. The TIMP-2 promoter has a TATA-like element, several SP-1 binding sites, and an AP-1 site. The constitutive expression of TIMP-2 is believed to be due to the distance of the AP-1 site from the TATA box[18]. TIMP-2 is detected as two transcripts of 1·0 and 3·5 kb. TIMP-2 shares ~40% sequence identity with TIMP-1 but is not glycosylated[19]. It has a Mr of 21 kDa and its message levels are unaffected by transforming growth factor (TGF)-β, IL-1, or tumor necrosis factor (TNF)-α[20]. Overexpression of the TIMP-2 gene and the corresponding suppression of MMP-2 activity in melanoma cells has been associated with flattened cellular morphology and changes in cellular adhesion to different substrates[21].

4. TIMP-3

The TIMP-3 gene has been localized in human chromosome 22q13.1[22]. Point mutations in the gene encoding TIMP-3 have been found in patients with Sorsby's fundus dystrophy

(SFD)[23]. The human TIMP-3 gene has no TATA sequence but has several AP-1 binding sequences. The human TIMP-3 gene consists of five exons and is at least 30 kb in size; 5·0 kb transcript and two minor transcripts of 2·4 and 2·6 kb have been identified[24,25]. TIMP-3 shares identity with another gene product and was found to be identical to mig-5, a mitogen inducible gene[26]. Like TIMP-1 mRNAs, TIMP-3 mRNAs can be induced by EGF, platelet-derived growth factor (PDGF), IL-1β, and TGF-β[27–29]. TIMP-3 is found exclusively in the extracellular matrix but not in the conditioned medium of many cultured cells[30]. A secreted, 188 amino-acid, N-glycosylated protein TIMP-3 has significant structural similarity to TIMP-1 and TIMP-2 and has 30% sequence homology with TIMP-1 and 38% with TIMP-2[30]. Expression of TIMP-3 is related to the cell cycle and is highest during the G1 phase[30]. TIMP-3 effectively inhibit MMP-1, MMP-2, MMP-3, and MMP-9[24].

5. TIMP-4

First cloned from a human heart cDNA library[31], TIMP-4 has been localized in human chromosome 3p25[32]. Like other TIMPs, the TIMP-4 protein is encoded by five exons that span 6 kb of genomic DNA. The exon-intron boundaries of TIMP-4 are at locations very similar to those of other TIMP genes[32]. A TIMP-4 transcript of 1·4 kb has been reported and the mature protein is expected to have a molecular mass of 22 kDa. Recombinant TIMP-4 was shown to migrate as a 24 kDa-protein by reverse zymography under nonreduced conditions[31,33]. Comparison of the predicted amino-acid sequence of human TIMP-4 with that of other known TIMPs showed 37% homology with TIMP-1 and 51% homology with TIMP-2 and TIMP-3[31]. Expression of TIMP-4 transcripts in adult human tissues was abundant in the heart but low in other tissues[31]. TIMP-4 specifically binds to progelatinase A and COOH-terminal hemopexin-like domain of the gelatinase A in a manner similar to that of TIMP-2[34].

6. Role of TIMPs in tumorigenesis

ECM homeostasis during physiological processes depends on the maintenance of a balance between the deposition and the removal of connective-tissue matrix components. Changes in the balance between the matrix-degrading proteinases and their inhibitors can have profound effects on ECM composition. TIMPs expression is influenced by many cytokines, growth factors, hormones, and retinoids. TIMPs modulate the proteolysis of the ECM influencing the activation of the prometalloproteinases, notably, during tissue remodeling and inflammatory processes. Several TIMPs have been associated with cancer. Specifically, imbalance between MMP and TIMP activities resulting in excessive degradation of the ECM has been implicated in tumor invasion and metastasis. Although, some tumors have increased rather than decreased TIMP levels[35,36], many malignant tumors have been associated with increased MMP and decreased TIMP expression[37,38]. Moreover, MMPs and TIMPs can be located both in and around a tumor and can be expressed variably by the tumor and by the adjacent stromal cells. Overexpression of TIMP-1 directly inhibited the tumorigenic as well as the metastatic potential of B16F10 cells in mice suggesting that TIMP-1 plays a role in controlling malignancy[39]. In a recent study, the incidence of brain metastases in mice that overexpressed TIMP-1 was 75% less than that in their wild-type littermates[40]. Experiments with recombinant or genetically manipulated levels of TIMP-1 have helped to reveal the functional role of MMPs in

metastasis. For example, the administration of recombinant TIMP-1 reduced colonization of intravenously injected B16F10 melanoma cells in the lungs of mice.[41] Conversely, antisense reduction of TIMP-1 levels in murine fibroblasts resulted in the formation of metastatic tumors and colonization of the lungs in nude mice[42]. Down-regulation of TIMP-1 expression has been associated with increased invasiveness in embryonic stem cells carrying a mutated, nonfunctional TIMP-1 gene[43]. Human recombinant TIMP-1 was also shown to inhibit metastasis of c-H-ras transformed rat embryo cells[44] and transfection of the TIMP-1 gene in human gastric[45,46] and astrocytoma cell lines[47] reduced the invasive potential of those cells. The effect of TIMP-1 on the initiation and growth of liver tumors was recently documented in transgenic mice expressing either sense or antisense TIMP-1 constructs. In that study, TIMP-1 overexpression inhibited SV40 T-antigen-induced tumor initiation, growth, and angiogenesis whereas TIMP-1 reduction resulted in a more rapid tumor initiation and progression[48]. Notwithstanding the differences among experimental protocols and sources of the TIMP-1 (tumor versus host), these results provide strong indirect evidence of the role that MMPs play in tumorigenicity perhaps through inhibition of MMP activity by TIMPs.

Overexpression of TIMP-2, like that of TIMP-1, suppresses tumor growth, invasion, and metastasis. In experimental animal studies, transfection[49] or retroviral introduction[50] of TIMP-2 into transformed rat embryo fibroblasts reduced primary tumor growth. On the other hand, a human melanoma cell line that overexpresses TIMP-2 did not show any reduction in the rate of spontaneous metastasis, although growth of localized tumor was inhibited[51]. Purified TIMP-2 inhibited the invasion of HT-1080 human sarcoma cells in a Matrigel chemoinvasion assay[52]. TIMP-2 also inhibited the invasion of c-Ha-ras transformed rat embryo cells through a smooth muscle cell layer[53].

As for TIMP-3, introduction of a TIMP-3 expression plasmid in mouse JB6 cell, variants lacking TIMP-3 did not suppress tumorigenicity[54]. In another study, TIMP-3 was expressed in preneoplastic but not in neoplastic mouse epidermal cells[55]. TIMP-3 overexpression induced DNA synthesis and promoted rat vascular smooth muscle cell death by apoptosis[56]. Overexpression of TIMP-3 in human colon carcinoma cell line DLD-1 was associated with suppression of tumor cell growth *in vivo* and with the induction of cell death in suspended-cell cultures[57]. TIMP-3 overexpression in human breast carcinoma and malignant melanoma cell lines had no effect on growth of tumor cells *in vitro* but reduced tumor growth *in vivo*[58]. Adenovirus-mediated gene delivery of TIMP-3 inhibited the invasion of the metastatic melanoma cell lines SK-Mel-5 and A2058 even more potently than TIMP-1 or TIMP-2 and produced apoptosis in both cell types[59]. Finally, TIMP-3 mutations seen in dominantly inherited adult-onset blindness from SFD were found to contribute to disease progression through the accumulation of mutant protein rather than through the loss of functional TIMP-3[60].

In a recent study, human breast cancer cells MDA-MB-435 transfected with TIMP-4 cDNA exhibited decreased (4-10 fold) tumor growth and lung metastasis as compared to controls when injected orthotopically into nude mice[61].

7. Inhibition of angiogenesis

TIMPs seem to be involved in angiogenic processes in several ways e.g., by blocking endothelial cell migration, by preventing MMP-mediated endothelial cell detachment, and by inhibiting the degradation of the ECM. TIMP-1 inhibited tumor induced angiogenesis in experimental systems and blocked the endothelial cell response to known angiogenic

factors in an *in vivo* angiogenesis assay[62]. Findings that TIMP-2 inhibited endothelial tube formation in an *in vitro* Matrigel assay[63], and that TIMP-1 blocked endothelial cell invasion of an amniotic membrane[64], support the concept that TIMPs can affect the angiogenic processes in several ways. In an *in vivo* angiogenesis assay, human TIMP-2 cDNA transfected B16F10 murine melanoma cells showed reduced levels of blood vessel formation[65]. TIMP-3 effectively inhibits chemotaxis of vascular endothelial cells toward vascular endothelial growth factor (VEGF) and bFGF and inhibits bFGF-induced angiogenesis in the chorioallantoic membrane (CAM) assay *in vivo*[66].

Interpreting the effects of TIMPs on the growth of primary tumors and metastatic lesions is further complicated by the observations that TIMP-1 and TIMP-2 also promote growth in a variety of cell types[14,67]. Several recent examples have been reported in which TIMPs either enhanced, or had no effect on, tumor growth or metastasis[54,56] contrary to what would be expected from their antimetalloproteinase activity. Although all TIMPs inhibit MMP activity, subtle differences are evident in their interactions with the zymogen forms of MMPs. For example, TIMP-1 forms a complex with proenzyme form of the 92 kDa gelatinase (pro-MMP-9)[68]; this binary pro-MMP-9/TIMP-1 complex can inhibit active MMP and form the more active and stable ternary complex pro-MMP-9/TIMP-1/MMP. In another example, TIMP-2 can bind to the proform of the 72 kDa gelatinase (pro-MMP-2) which prevents autoactivation of MMP-2. The pro-MMP-2/TIMP-2 complex is activated by cell surface membrane-type-1 matrix metalloproteinase (MT1-MMP). The catalytic domain of MT1-MMP can activate pro-MMP-2 in the absence of TIMP-2; however, TIMP-2 inhibits this activation by binding to the catalytic domain of MT1-MMP[69]. In a recent study, pro-MMP-2 activation in cancer tissues is caused by both up-regulation of MT1-MMP and down-regulation of TIMP-2[70].

8. Synthetic MMP inhibitors

TIMPs prevent cancer progression in different experimental model systems. These findings raised the possibility of using an agent that affects expression or activity of MMPs as an anti-cancer therapy. Considerable research in recent years has focused on the development of synthetic low molecular weight MMP inhibitors. Synthetic peptides mimic collagen structure and contain a chemical group that binds the Zn^{2+} in the active site of the MMPs. Two of the MMP inhibitors i.e., batimastat (BB-94) and marimastat (BB-2516) are currently undergoing evaluation for treatment of cancer. Batimastat which is poorly soluble in water, decreased tumor growth in athymic nude mice with a human colon carcinoma xenograft[71]. It also prolonged survival and suppressed the formation of malignant ascites in athymic mice given a human ovarian carcinoma xenograft[19]. In a model of rat mammary carcinoma, batimastat was shown to reduce both the spread of metastatic cells and the growth of established metastasis[72]. Batimastat administration resulted in a significant decrease in the number of tumors in Min mice, which spontaneously develop pre-malignant small and large intestinal tumors[73]. In contrast to batimstat, marimastat has relatively good oral bioavailability. It has inhibited lung colonization in mice injected with B16Bl6 melanoma and has reduced tumor growth in rats inoculated with HOSP1 mammary carcinoma cells[19]. Single and repeated oral doses of marimastat in healthy male subjects appear to be well tolerated[74]. Recently, marimastat had been tested in patients with advanced lung cancer[75]. Administration of an orally-active synthetic MMP inhibitor CT1746 resulted in inhibition of solid tumor growth, spread and metastasis in nude mouse model with orthotopic implantation of human colon

tumor cell line Co-3[76]. Synthetic MMP inhibitors also have been shown to block angiogenesis and endothelial tube formation in collagen gels[77]. The mechanism by which these compounds inhibit tumor growth may reflect both indirect effects on angiogenesis as well as more direct effects on the growth of the tumor cells themselves.

9. Summary

Both MMPs and TIMPs clearly play key roles in tumor growth, metastasis, and angiogenesis. Experiments with recombinant or genetically manipulated levels of TIMPs have verified the functional role of MMPs in metastasis. Studies with synthetic MMP inhibitors have shown similar results. Both MMPs and TIMPs seem to regulate the sustained growth of tumors. Means of controlling of MMP activity have generated considerable interest as a possible way of inhibiting tumor progression. TIMPs also have other functions apart from inhibiting MMP-induced matrix degradation. The molecular and cellular mechanisms responsible for suppression of tumor invasion by TIMP-3 and TIMP-4 should be explored and compared with those of TIMP-1 and TIMP-2. Several pharmaceutical agents designed to block MMP synthesis or activity such as the hydroxymates, have been tested in human clinical trials. These and other MMP inhibitors may be well suited for use in combination with existing chemotherapeutic agents. The use of TIMPs or synthetic inhibitors as means of controlling excessive MMP activity also has potential therapeutic value in several non-neoplastic diseases e.g. intimal hyperplasia. For cancer therapy, the key is elucidation of the mechanisms by which TIMPs and MMPs create an environment that favors the initiation and continued growth of primary and metastatic tumors.

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