

REVIEW

Complex roles of tissue inhibitors of metalloproteinases in cancer

Yangfu Jiang¹, Itzhak D Goldberg¹ and Y Eric Shi^{*1}

¹Department of Radiation Oncology, Long Island Jewish Medical Center, The Long Island Campus, Albert Einstein College of Medicine, New Hyde Park, New York, NY 11040, USA

Matrix metalloproteinases (MMPs) is tightly associated with extracellular matrix (ECM) turnover, which plays a very active role in tumor invasion and metastasis. Tissue inhibitors of metalloproteinases (TIMPs) plays a critical role in the homeostasis of ECM by regulating the activity of MMPs. TIMPs are well-known for their ability to inhibit MMP activity thereby inhibiting tumor growth and metastasis. However, many evidences suggest that TIMPs are multifunctional proteins, which regulate cell proliferation, apoptosis, proMMP-2 activation, and angiogenesis. These effects may be through MMP-dependent or MMP-independent pathways. Recent data indicate that TIMPs have many paradoxical roles in tumorigenesis. In particular, both inhibitory effect and stimulatory effect on tumorigenesis have been demonstrated in many animal models in which TIMPs were overexpressed in cancer cells or in mice. Elevated TIMP levels are reported in association with cancer progression and identified as poor prognostic indicators in several human tumor types. Herein, we review the complex roles of TIMPs in cancer growth and metastasis.

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Introduction

Matrix metalloproteinases (MMPs) play a critical role in extracellular matrix (ECM) remodeling, which is involved in many physiological or pathological conditions including tumor growth and metastasis (Nagase and Woessner, 1999). Tissue inhibitors of metalloproteinases (TIMPs) can block the activity of MMPs. The inhibitory activity of TIMPs might be important in inhibiting tumorigenesis and subsequent malignant progression. However, the effects of TIMPs on tumorigenesis are multifunctional and paradoxical. First, although the inhibitory effect of TIMPs on tumor growth and metastasis was achieved by overexpression of the TIMP gene into tumor cells (Albini *et al.*, 1991; Baker *et al.*, 1999), TIMPs also have growth

stimulatory and anti-apoptotic effect (Hayakawa *et al.*, 1992; Guedez *et al.*, 1998; Li *et al.*, 1999). Whereas MMPs are important in the late stage of tumor progression leading to metastasis, the anti-apoptotic effects of some TIMPs may favor the tumor growth during the tumor onset and early primary tumor growth (Jiang *et al.*, 2001; Guedez *et al.*, 2001). Second, the effect of TIMPs on tumor angiogenesis is controversial. TIMPs have antiangiogenic activity either by inhibiting the activity of MMPs or by a direct inhibitory effect on endothelial cell proliferation (Murphy *et al.*, 1993; Fernandez *et al.*, 1999). However, some MMPs also play a critical role in generations of angiostatin and endostatin, potent angiogenic inhibitors (Dong *et al.*, 1997; Wen *et al.*, 1999). Therefore, TIMPs that inhibit angiogenic inhibitor-converting MMPs may prevent angiostatin and endostatin production and thus play a positive role in tumor angiogenesis. This review summarizes the current understanding of the complex roles of TIMPs in cancer growth and progression.

Old paradigm: inhibition of tumorigenesis and tumor metastasis by TIMPs

The down-regulation of MMPs may occur at the levels of transcriptional regulation of the genes; activation of secreted proenzymes; and interaction with four homologous TIMPs, TIMP-1 (Docherty *et al.*, 1985), TIMP-2 (Stetler-Stevenson *et al.*, 1990), TIMP-3 (Hammani *et al.*, 1996), and TIMP-4 (Greene *et al.*, 1996). Thus, the net MMP activity is the result of the balance between activated enzyme levels and TIMP levels. The overproduction and unrestrained activity of MMPs have been linked to malignant conversion of tumor cells (Bernhard *et al.*, 1994; Koshiba *et al.*, 1998; Sehgal *et al.*, 1998; Coussens *et al.*, 1999; Sternlicht *et al.*, 1999; Coussens *et al.*, 2000). Decreased production of TIMP could also result in greater effective enzyme activity and invasive potential (Denhardt *et al.*, 1992; Khokha *et al.*, 1989). These results suggest that the inhibitory activity of TIMPs might be important in inhibiting tumor malignant progression leading to invasion and metastasis. In fact, tumor invasion and metastasis can be inhibited by up-regulation of TIMP expression in tumor cells (Albini *et al.*, 1991; Baker *et al.*, 1999; Valente *et al.*, 1998; Matsuzawa *et al.*, 1996; Wang *et al.*, 1997). Intraperitoneal injection of rTIMP-1 and

*Correspondence: YE Shi; E-mail: shi@lij.edu
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rTIMP-2 has been shown to inhibit lung colonization of B16 melanoma cells (Schultz *et al.*, 1988; Alvarez *et al.*, 1990). Overexpression of TIMP-1 inhibits tumor growth and metastasis of melanoma (Khokha, 1994), suppresses metastatic ability of human gastric cancer cells (Watanabe *et al.*, 1996) and oral squamous cell carcinoma (Nii *et al.*, 2000). Adenoviral transfer of TIMP-3 into Hela, fibrosarcoma cell line HT1080, and melanoma cell inhibits the invasive ability of those cells and induces apoptosis (Ahonen *et al.*, 1998; Brand *et al.*, 2000). Overexpression of TIMP-4 in a human breast cancer cell line MDA-MB-435 cells by cDNA transfection inhibits the invasion, metastasis, and tumor growth (Wang *et al.*, 1997).

In addition to inhibiting tumor cell invasion and metastasis, overexpression of TIMPs also inhibits primary tumor growth (Wang *et al.*, 1997; Khokha, 1994; Brand *et al.*, 2000). Both invasiveness and tumorigenic potential of murine 3T3 cells are conferred when TIMP production is impaired by an antisense approach (Denhardt *et al.*, 1992; Khokha *et al.*, 1989). *In vivo* tumorigenic and metastatic potentials of human breast cancer cells can also be inhibited by overexpression of a single TIMP-4 gene (Wang *et al.*, 1997). There are several mechanisms that may attribute to the anti-tumor effect of TIMPs. First, there is evidence supporting a role for MMPs in early stages of tumor onset and primary tumor growth. Specifically, MMP-3 and MMP-9 have been reported to be involved in mammary carcinogenesis (Sternlicht *et al.*, 1999), skin carcinogenesis (Coussens *et al.*, 2000), and carcinogenesis of pancreatic islets (Bergers *et al.*, 2000). MMP-3 promotes spontaneous premalignant changes and malignant conversion in mammary glands (Sternlicht *et al.*, 1999). MMP-9, which is upregulated during early neoplastic progression in skin, stimulates tumor growth of oncogene-expressing keratinocytes (Bergers *et al.*, 2000). Second, MMPs also play a critical role in tumor-induced angiogenesis (Bergers *et al.*, 2000), and therefore TIMP-induced tumor inhibition may be mediated by inhibition of angiogenesis. Although these data indicate a tumor-suppressing effect of TIMP, this old paradigm is being challenged by many new experimental evidences, which indicate that TIMP may also function in favor of tumor growth.

Paradox 1: regulation of cell growth and apoptosis by TIMPs

In contrast to their anti-MMP activity, TIMPs also promote cell growth. The stimulatory effect on cell growth was initially recognized when TIMP-1 and TIMP-2 were identified having erythroid-potentiating activities (Docherty *et al.*, 1985; Stetler-Stevenson *et al.*, 1992). It is now clear that TIMP-1 and TIMP-2 are also mitogenic for non-erythroid cells, including normal keratinocytes (Bertaux *et al.*, 1991), fibroblasts, lung adenocarcinoma cells, and melanoma cells (Hayakawa *et al.*, 1992, 1994). Regulation of apoptosis by TIMPs has been reported. While TIMP-3 induces apoptosis

(Baker *et al.*, 1999; Ahonen *et al.*, 1998). TIMP-1, TIMP-2, and TIMP-4 have anti-apoptotic effect (Guedez *et al.*, 1998; Li *et al.*, 1999; Jiang *et al.*, 2001). TIMP-1 inhibits apoptosis of B cells and human breast epithelial cells *in vitro* (Guedez *et al.*, 1998; Li *et al.*, 1999) and rescues mammary epithelial cell apoptosis in transgenic mice (Alexander *et al.*, 1996). TIMP-2 overexpression protects B16F10 melanoma cells from apoptosis (Valente *et al.*, 1998). TIMP-4 also protects human breast cancer cells from apoptosis and systemic delivery of TIMP-4 gene promotes the growth of human breast cancer xenografts (Jiang *et al.*, 2001).

TIMPs may regulate cell survival by at least two pathways. One pathway correlates with their anti-MMP activity, and the other is MMP-independent. Cell-matrix interactions have been shown to influence cell viability, and withdrawal of anchorage-dependent cells from their association with the ECM results in apoptotic cell death. The turnover of ECM by specific ECM-degrading MMPs has been shown to modulate cell survival (Lukashev and Werb, 1998). While stromelysin-3 might favor epithelial cell survival in both physiological and pathological conditions (Boulay *et al.*, 2001), most members of the MMP family are known to exert pro-apoptotic function (Lukashev and Werb, 1998; Noel *et al.*, 1997). Overexpression of stromelysin-1 can induce apoptosis in mammary epithelial cells *in vitro* and in transgenic mice (Witty *et al.*, 1995). Therefore, TIMPs, except TIMP-3, may rescue cells from apoptosis by inhibiting MMPs. TIMP-3 is of the unique pro-apoptotic function among TIMPs. It was reported that the N-terminal domain of TIMP-3 alone is sufficient to induce-apoptosis and a TIMP-3 mutant that lacks anti-MMP activity failed to induce apoptosis in rat vascular smooth muscle cells and Hela cells (Bond *et al.*, 2000). These data suggest that inhibition of proteinases is required for TIMP-3-induced apoptosis.

TIMPs may also regulate apoptosis in an MMP-independent pathway. Reduced-alkylated TIMP-1, completely devoid of MMP inhibitory activity, suppresses apoptosis in B cells (Guedez *et al.*, 1998). In addition, the synthetic inhibitor BB-94 fails to protect cells from apoptosis, even though the levels of BB-94 used should completely inhibit a wide spectrum of MMPs (Guedez *et al.*, 1998). TIMP effects on cell survival may be mediated by yet undefined signaling pathways. TIMP-1 and TIMP-2 have been shown to stimulate tyrosine kinase and mitogen-activated protein kinase activity in a human osteosarcoma cell line MG-63 and in human breast cancer cell lines (Yamashita *et al.*, 1996; Luparello *et al.*, 1999). TIMP-1, which can be upregulated by Bcl-2, seems to function at the downstream of Bcl-2 (Li *et al.*, 1999). TIMP-1 also upregulates Bcl-X_L expression in B cells (Guedez *et al.*, 1998). TIMP-4 upregulates Bcl-2 and Bcl-X_L expression in a breast cancer cell line (Jiang *et al.*, 2001). Neutralization of secreted TIMP-1 by monoclonal antibody against TIMP-1 can reverse the suppression of apoptosis (Guedez *et al.*, 1998). Recombinant TIMP-1 complexed with proMMP-9

doesn't bind to cell surface and fails to promote cell growth (Hayakawa *et al.*, 1992; Luparello *et al.*, 1999). A nuclear translocation of GFP-TIMP-1 construct in MCF-7 cells was reported, indicating that TIMP-1 may also function in nucleus (Ritter *et al.*, 1999). These data suggest that TIMPs might regulate apoptosis by targeting yet unidentified TIMP-binding proteins on cell membrane and initiating signaling pathways. Consistent with this hypothesis, it was demonstrated that exogenous recombinant TIMP-4 (rhTIMP4) not only binds to the cell surface, but is also internalized into the cytoplasm in a specific manner since pro-MMP-2 is not internalized under the same conditions. By using immunohistochemical and immunofluorescence staining, TIMP-4 protein was detected exclusively on the cell membrane when G401 Wilms' tumor cells were incubated with rhTIMP4 at 4°C, while TIMP-4 was detected predominantly intracellularly when the cells were incubated with rhTIMP4 at 37°C (Celiker *et al.*, 2001). In addition, TIMP-2 also binds to the surface of A549 human lung adenocarcinoma cells in a specific fashion that is not competed by the synthetic MMP inhibitor BB-94 and is independent of MT1-MMP (Hoegy *et al.*, 2001). Many studies are underway to isolate and characterize the putative TIMP receptor.

Paradox 2: TIMPs regulate pro-MMP activation and tumor angiogenesis

All members of MMP family are secreted as inactive zymogen and require extracellular activation. TIMP-2 plays a dual paradoxical function in regulating MMP-2 activity. While TIMP-2 is a potent inhibitor for MMP-2, TIMP-2 is also an adaptor molecule that is required for proMMP-2 activation at cell surface. TIMP-2 can bind to proMMP-2 via C-terminal interaction, and to MT1-MMP by its N-terminal domain. This dual binding brings proMMP-2 close to cell surface, where it can be activated by neighboring TIMP-2-free MT1-MMP molecules (Shofuda *et al.*, 1998; Butler *et al.*, 1998). It has been reported that the entire propeptide domain of MT1-MMP is required for the TIMP-2 binding and subsequent proMMP-2 activation (Cao *et al.*, 1998). However, other study showed that TIMP-2 binds to active MT1-MMP but not to latent MT1-MMP (Hernandez-Barrantes *et al.*, 2000). Fully functional TIMP-2 is essential for efficient activation of proMMP-2 both *in vitro* and *in vivo* (Caterina *et al.*, 2000). However, the active 65-kDa MMP-2 can be inhibited by plasma membrane-bound TIMP-2 (Itoh *et al.*, 1998). These results suggest that the pericellular activity of MMP-2 is tightly regulated by membrane-bound TIMP-2 and surrounding extracellular matrix components. TIMP-4 and TIMP-3 can also bind to proMMP-2 with high affinity (Butler *et al.*, 1999; Bigg *et al.*, 1997), but do not promote MT1-MMP mediated activation of proMMP-2 (Hernandez-Barrantes *et al.*, 2001).

The effects of TIMP on angiogenesis are multifunctional and paradoxical. TIMPs have antiangiogenic activity either by their ability to inhibit the activity of

MMPs (Fernandez *et al.*, 1999), or by a direct effect on endothelial cell proliferation. MMPs promote endothelial cell migration and trigger angiogenic switch. MMP-9 participates in switching angiogenesis by releasing VEGF from extracellular matrix (Bergers *et al.*, 2000). MMP-2 activity was suggested to be necessary for the switch to angiogenic phenotype in an animal model (Fang *et al.*, 2000). TIMPs may also inhibit endothelial cell proliferation directly. TIMP-2 inhibits the growth of basic FGF-stimulated endothelial cells (Murphy *et al.*, 1993). TIMP-3 inhibits endothelial cell motility, and the proliferation of stimulated endothelial cells (Anand-Apte *et al.*, 1997). The tube formation of endothelial cells cultured in Matrigel is inhibited by TIMP-4, indicating an anti-angiogenic effect of TIMP-4 (unpublished data). Therefore, it seems to be logical that the suppression of MMPs activity by TIMP may inhibit tumor angiogenesis.

However, some MMPs also play a critical role in generation of potent angiogenic inhibitors. MMP-2, MMP-7, MMP-9, and MMP-12 can convert plasminogen into angiostatin (Patterson and Sang, 1997; Cornelius *et al.*, 1998; O'Reilly *et al.*, 1994, 1999; Pozzi *et al.*, 2000), a well-known antiangiogenic factor. The generation of another potent antiangiogenic factor, endostatin, may also require MMPs and elastase (Wen *et al.*, 1999). Therefore, TIMPs that inhibit these angiogenic inhibitor-converting MMPs may prevent angiostatin and endostatin production and thus playing a positive role in tumor angiogenesis. In addition, it has been reported that overexpression of TIMP-1 enhances VEGF expression in mammary carcinoma (Yoshiji *et al.*, 1998), and promotes VEGF-induced neovascularization in the retina (Yamada *et al.*, 2001). Therefore, *in vivo* systemic assessment of the net effects between TIMPs-mediated anti-angiogenic effect and their pro-angiogenic activities, such as inhibiting the angiogenic inhibitor-converting MMPs, need to be investigated more thoroughly and explicitly.

Paradox 3: stimulation of tumorigenesis by TIMP gene delivery in some tumor models

Although the inhibitory effects of TIMP on tumor growth and metastasis were achieved in many model systems by overexpression of TIMP gene in tumor cells, most MMPs and TIMPs are not expressed in genetically altered cancer cells but synthesized and secreted by adjacent stromal fibroblasts (Jiang *et al.*, 2001; Rudolph-Owen and Matrisian, 1998; Masson *et al.*, 1998; Hewitt and Dano, 1996; Hoyhtya *et al.*, 1994; Coussens and Werb, 1996). An imbalance between MMPs and TIMPs in favor of enzymatic inhibition might be important in inhibiting tumor progression, and therefore lead one to expect that an increase in the amount of TIMPs relative to MMPs could function to block tumor cell invasion and metastasis. However, recent evidence indicated that tumorigenesis was stimulated by TIMP gene delivery in some model system (Jiang *et al.*, 2001; Guedez *et al.*, 2001; Yoshiji

et al., 1998). So far, the net effect of systemic administration of TIMP on tumorigenesis has not been extensively determined yet. Unexpectedly, systemic delivery of TIMP-4 by intramuscular administration of naked TIMP-4 DNA significantly stimulated mammary tumorigenesis *in vivo* (Jiang *et al.*, 2001). TIMP-4 upregulates Bcl-2 and Bcl-X_L protein and protects breast cancer cells from apoptosis both *in vitro* and in nude mice (Jiang *et al.*, 2001). The divergent effects of TIMP-1 on tumor growth were also reported (Guedez *et al.*, 2001). TIMP-1 transfected Burkitt's lymphoma cells show an initial fast growth phase in nude mice, due to the TIMP-1 mediated stimulation of cell proliferation and the anti-apoptotic effect. However, the tumor growth is inhibited in the later stage when tumors reach certain size and develop necrosis, due to the inhibition of tumor angiogenesis by TIMP-1. Two breast carcinoma cell lines overexpressing TIMP-1 show stimulated tumor growth resulted from enhanced VEGF expression (Yoshiji *et al.*, 1998). In addition, this TIMP-1-induced stimulatory effect is also reported in intestinal tumor model in TIMP-1 transgenic mice (Goss *et al.*, 1998). Given the paradoxical roles of TIMPs on tumor growth and progression, in contrast to previously established anti-tumor and anti-metastatic effects, TIMP expression may favor the tumor onset or early primary tumor growth by their anti-apoptotic effects.

Paradox 4: association of poor prognosis with increased TIMP expression

The increased TIMP expression was demonstrated in a variety of different tumors, such as breast cancer (Jones *et al.*, 1999; Yoshiji *et al.*, 1996), colorectal cancer (Powe *et al.*, 1997; Zeng *et al.*, 1995), gastric cancer (Joo *et al.*, 2000), and lung cancer (Michael *et al.*, 1999). If TIMP's main biological activity is an inhibition of MMPs, elevated TIMPs levels would be expected to inhibit invasion and metastasis, and thereby improving prognosis. However, increased TIMPs expression is often associated with apparently paradoxical negative prognosis in many solid tumors. Increased expression of TIMP-1 is associated with poor prognosis in colorectal cancer (Zeng *et al.*, 1995; Rowe *et al.*, 1997; Hewitt *et al.*, 2000), breast cancer (Yoshiji *et al.*, 1996; McCarthy *et al.*, 1999), gastric carcinoma (Joo *et al.*, 2000), lymphoma (Kossakowska *et al.*, 1991), prostate (Still *et al.*, 2000), and lung cancer (Fong *et al.*, 1996). High preoperative plasma TIMP-1 levels are associated with short survival of patients with colorectal cancer (Holten-Andersen *et al.*, 2000), lung cancer (Ylisirnio *et al.*, 2000), and gastric cancer (Yoshikawa *et al.*, 2000). Elevated TIMP-1 mRNA in colorectal cancer stroma correlates with lymph node and distant metastases (Zeng *et al.*, 1995). The plasma levels of TIMP-1 in prostate cancer patients with metastasis are significantly higher than that in controls, suggesting that TIMP-1 is a marker of malignant progression of prostate cancer (Lein *et al.*, 1999). High levels of TIMP-2 correlate with

adverse prognosis in cervical carcinoma (Davidson *et al.*, 1999) and breast cancer (Visscher *et al.*, 1994). It was demonstrated that the clinical outcome of breast cancer is more closely related to the presence of TIMP-2 in peri-tumoral stroma than to the corresponding MMPs (Visscher *et al.*, 1994; Ree *et al.*, 1997). High levels of messenger RNAs for TIMP-1 and TIMP-2 in primary breast carcinomas are associated with development of distant metastases (Ree *et al.*, 1997). By using immunohistochemical analysis of clinical breast specimens, a higher level of TIMP-4 expression in breast carcinoma cells than in normal breast epithelial cells is demonstrated (Jiang *et al.*, 2001).

There are two scenarios to understand why the elevated TIMP expression is associated with malignant cancer cells. One scenario is that the increased expression of TIMPs may be related to the increased expression of MMPs during tumor progression. Therefore, this elevated level of TIMP in the invasive carcinomas may represent one of the subsequent acute host responses to the remodeling stimuli and try to balance the local tissue degradation. Alternatively, the high level of TIMP expression in cancer may favor the proposed MMP-independent growth regulatory and apoptotic regulatory functions (Hayakawa *et al.*, 1992; Guedez *et al.*, 1998; Li *et al.*, 1999; Jiang *et al.*, 2001; Guedez *et al.*, 2001). In this regard, the stimulation of mammary tumorigenesis by systemic administration of TIMP-4 provides some rationale for unexpected results of these clinical studies.

New paradigm: net function of TIMP on tumorigenesis: stimulation or inhibition?

The experimental data on TIMPs in tumorigenesis indicate a new paradigm for TIMPs in cancer. TIMPs block the activity of MMPs, which is important in inhibiting tumorigenesis and subsequent malignant progression. On the other hand, TIMP may also promote tumorigenesis. These contradictory facts promote one to investigate and redefine the net role of TIMPs on tumorigenesis. As summarized in Table 1 and Figure 1, in contrast to its well-established anti-tumor effect, TIMP may also function in favor of tumor growth either in an MMP-independent or MMP-dependent manner, including (a) growth promotion and anti-apoptotic effect (Hayakawa *et al.*, 1992;

Table 1 Summary of cancer-related biological functions of TIMPs

Tumor invasion	Erythroid potentiating		ProMMP-2		
	activity	Cell growth	Apoptosis	activation	Angiogenesis
TIMP-1 ↓	↑	↑	↓	-	↓ or ↑
TIMP-2 ↓	↑	↑	↓	↑	↓
TIMP-3 ↓	N.D.	N.D.	↑	-	↓
TIMP-4 ↓	N.D.	↑ or ↓	↓	↓*	↓

N.D., Not determined; -, No function; ↑, Stimulation; ↓, Inhibition. ↓*, by competing with TIMP-2, TIMP-4 inhibits TIMP-2 mediated proMMP-2 activation

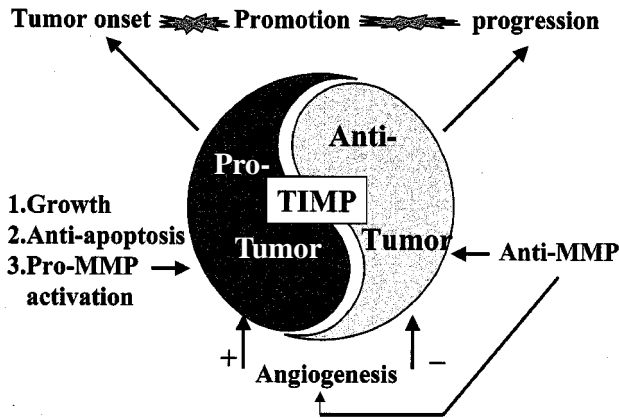


Figure 1 Schematic illustration of the functions of TIMP in tumorigenesis. While the anti-MMP activity of TIMPs is important in inhibiting tumor growth and particularly tumor malignant progression. TIMPs also have a growth stimulatory, anti-apoptotic effect, and participate in the pro-MMP2 activation, which favor the tumor growth. In addition, TIMPs can have antiangiogenic activity either by inhibiting the activity of MMPs or by a direct effect on endothelial cell proliferation; on the other hand, TIMPs may prevent angiostatin and endostatin production by inhibiting angiogenic inhibitor-converting MMPs and thus stimulating tumor angiogenesis. While the anti-MMP function of TIMP may play a major inhibitory role during the late stage of tumor progression, the growth promotion and anti-apoptotic effect of TIMP may play a stimulatory role during the tumor formation and early stage of tumor growth

Guedez *et al.*, 1998; Li *et al.*, 1999; Jiang *et al.*, 2001); (b) inhibition of angiostatin and endostatin-converting MMPs or upregulation of VEGF (Yoshiji *et al.*, 1998), and therefore stimulation of angiogenesis; and (c) involvement of activation of pro-MMP-2 (Shofuda *et al.*, 1998; Butler *et al.*, 1998). The net effect of TIMP on tumorigenesis may depend on bioavailability of local amount of TIMPs in tumor microenvironment, the time when the TIMP is presented to the tumor cells, and the presence of putative TIMP receptor on tumor cells.

The effect of TIMPs on tumorigenesis can be reviewed from several *scenarios*. The first scenario correlates with the bioavailability of TIMP protein in tumor microenvironment. TIMPs have both anti-MMP activity, which favors the tumor-suppressing effect, and growth stimulatory or apoptosis regulatory activity, which exerts pro-tumor effect. The balance between anti-MMP and anti-apoptotic effect on tumor growth may depend on the amounts of bio-available TIMP protein in tumor microenvironment. In this regard, higher levels of TIMPs may have tumor suppressing effect, due to its dominant anti-MMP activity, while lower levels of TIMP may favor tumor growth, due to its anti-apoptotic activity. In addition, lower levels of TIMP-2 may also favor proMMP-2 activation by MT1-MMP thereby favoring tumor progression (Shofuda *et al.*, 1998; Butler *et al.*, 1998). Many factors may affect the activities of TIMPs in tumor. For example, it has been demonstrated that tumor growth was inhibited when breast cancer cells were transfected with TIMP-4 (Wang *et al.*, 1997), whereas intramus-

cular TIMP-4 gene delivery stimulated tumorigenesis (Jiang *et al.*, 2001). When taking a gene transfection approach, it usually ends up with selecting the highly TIMP-expressed clones. The overexpression of TIMPs in every cancer cell would generate abundant inhibitory proteins in the tumor-stromal interface where the pro-tumor MMP activity is blocked. In contrast, in the intramuscular gene therapy approach, TIMP protein has to cross a vast amount of extracellular matrix proteins and circulation before reaching the target tumor cells, in which the anti-MMP function of circulating TIMP may be neutralized in part by circulating MMPs. Therefore, the amount of TIMP bio-available to the tumor cells may be much lower than that from locally expressed TIMP in transfected cells and the balance was shifted in favor of its anti-apoptotic activity when it reached the tumor.

The second scenario correlates with the timing of TIMP function. When tumor cells are inoculated into animal or spread to a secondary organ, many tumor cells will suffer from apoptosis, due to the host reaction. Therefore, the growth stimulatory effect or anti-apoptosis effect may be more important during the tumor onset and early tumor growth. Once the tumor established either in a secondary organ or in an animal challenged with tumor cells, the anti-apoptosis activity of TIMP is supposed to have little influence on tumor progression at this stage. On the contrary, sustained angiogenesis is far more important for tumor growth at later stage. Therefore, the antiangiogenesis activity of TIMP may become a dominant negative factor affecting tumor growth at this stage. Consistent with the timely differential dual functions, it has been demonstrated that TIMP-1 overexpression was of biphasic functions on lymphoma tumorigenesis. TIMP-1 has a significant tumor-stimulating effect during the tumor onset, but suppresses the tumor growth during the late state of tumor progression (Guedez *et al.*, 2001). Our research also indicated that the systemic stimulatory effect of TIMP-4 on mammary tumor is much more profound at the relatively earlier stage (Jiang *et al.*, 2001). TIMP-1 or TIMP-4-mediated stimulation at the early phase of tumorigenesis may be due to more survived inoculated cells because of its anti-apoptotic effect. These data support the timely divergent functions of TIMP on tumorigenesis. Finally, the selective presence of TIMP-binding proteins in cancer cells may be another factor which may direct the activity of TIMPs toward tumor promotion. So far, there is no evidence for a TIMP receptor, however, TIMPs can bind to some membrane bound molecules, such as MT-MMPs and ADAMs, that may act as TIMP 'receptors'.

Clinical application and future directions

There have been high hopes for small MMP inhibitors (MMPi) as a breakthrough in cancer treatment, thinking MMPs were ideal targets because of their causal involvement in metastasis, angiogenesis, and

therefore tumor growth. Many synthetic MMP inhibitors, such as Batimastat (BB-94), Marimastat, and Prinomastat, have been developed and evaluated. Although the anti-tumor effects of some MMPIs were confirmed in animal models (Lein *et al.*, 2000; Wylie *et al.*, 1999; Prontera *et al.*, 1999; Low *et al.*, 1996; Wojtowicz-Praga *et al.*, 1997; Shalinsky *et al.*, 1999), many MMPIs have met bad fate in clinical trials. Marimastat is the first orally bioavailable MMPI entered clinical trials in the field of oncology. Marimastat has now failed in trials for glioblastoma, pancreatic, gastric, breast, and ovarian cancers (Fletcher, 2000). Tanomastat failed in phase III trials on small-cell lung cancer. Phase II trial of MM1270 has been discontinued. Phase III trials of Prinomastat in prostate cancer and non-small-cell lung cancer was also halted because of a lack of efficacy (Fletcher, 2000). The promise of inhibiting MMP as a cure for cancer has clearly not been realized and analysis now believe that there is a large question mark over the usefulness of MMPI to treat cancer.

There are several potential rationales for the unsuccessful clinical trials of MMPI against cancer. First of all, some MMPIs can cause serious side effects and therefore can be easily reached to dose limitation for effective efficacy. For example, Marimastat can cause severe joint pain, which has to be minimized by reducing the dose, but at the expense of efficacy (Steward and Thomas, 2000). Secondly, MMPI, like TIMP, can also function in favor of tumor growth by up-regulation of angiogenic factors (Kruger *et al.*, 2001) or by inhibiting the angiostatin or endostatin-converting MMPs (Patterson and Sang, 1997; Pozzi *et al.*, 2000) and therefore playing a positive role in angiogenesis. In consistence with this scenario, an induction of liver metastasis was reported on intraperitoneally growing esophagus and ovarian carcinoma, intravenously inoculated T-cell lymphoma cells, and human breast carcinoma, when the mice were treated daily with Batimastat (Kruger *et al.*, 2001). Thirdly, most of the MMPIs have been tested as a single agent without conventional chemotherapy or radiotherapy. Since many MMPIs act as cytostatic drugs, which only slow tumor growth but not shrink tumors, MMPI alone may not be effective to control tumor growth and progression, particularly when most of the MMPIs entered the clinical trials targeting the tumors in an advanced stage. In addition to single agent therapy, several MMPIs have entered trials of combination therapy. The objective of combining chemotherapy with MMPI is to enhance tumor cytotoxicity as well as to reduce the size and number of metastatic lesions. Marimastat can be safely co-administered with conventional chemotherapy drugs and radiotherapy and phase III studies using these approaches are currently ongoing. Several MMPIs have entered phase III combination therapy trials. Whether or not MMPIs alone or in a combination therapy have a value for the treatment of early stage cancers or cancers with a low tumor burden will be judged according to the results of ongoing clinical trials.

Although there are many data which indicate an increase in the amount of TIMPs or MMPIs could function to block cancer progression, as outlined above, there is also experimental evidence suggesting that TIMPs may function in a manner that promotes rather than suppresses tumor growth. Whether the tumor stimulatory functions can prevail in the tumor environment and surpass the tumor-suppressing functions will direct the net functions of TIMP toward tumor promotion or tumor suppression. There are several possible factors which may affect the net function of TIMPs, including local concentration of TIMPs, cellular and pericellular distribution, the forms of presence in tumor and in circulation, the presence of a possible putative TIMP receptor on tumor cells, and the time when TIMP is available during the tumor onset and progression. Given these paradoxical facts, systemic administration of TIMP clinically may inhibit the invasion of tumor cells and the growth of established tumor, on condition that the tumor-suppressing effects are dominant. Otherwise, it may not inhibit tumor growth, even enhance tumor cells survival and therefore promote tumorigenesis in some patients. Specifically, significant proportions of patients with epithelial tumors have micro-metastases or minimal residual disease. These disseminated cells and residual cells can die quickly, grow progressively or become dormant. Dormancy may last for a long time. The persistence of cancer dormancy is due to a balance between proliferation and death. If the death of dormant cells is inhibited, disseminated or residual cancer cells may escape from dormancy and then metastases may establish or a tumor may recur (Uhr *et al.*, 1997; Karrison *et al.*, 1999). In this regard, administration of TIMP clinically may promote disseminated cells to establish overt metastases or to lead to residual minimal disease recurrence. This scenario is consistent with the clinical observation that high levels of TIMP-1 and TIMP-2 expression is associated with development of distant metastases in breast cancer patient (Ree *et al.*, 1997).

The potential cancer treatment by targeting MMP with TIMP warrants further investigation. Future direction should focus on a new conceptual basis for design of strategies that use TIMP as a probe for MMP function in cancer gene therapy. Such directions include the study of structure-bases for TIMP-mediated growth and apoptosis regulation, identification of putative TIMP receptor and study the signaling pathways of TIMP-mediated growth and apoptosis regulation, and more systemic approaches to evaluate the net effects of each TIMP at the different stages of tumor growth and progression.

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