Commentary

A Case of Tumor Betrayal

Biphasic Effects of TIMP-1 on Burkitt's Lymphoma

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Among a number of different proteinases that are capable of digesting extracellular matrix (ECM) components, the matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, play a major role.¹ Collectively, MMPs have been shown to cleave most ECM components either independently or in a collaborative manner. Because excessive digestion of ECM is a hallmark of many pathological conditions including tumor growth and metastasis, it is critical that MMP activity be precisely regulated both spatially and temporally. In addition to the transcriptional and translational control of MMP expression by growth hormones/factors, cytokines, cell-ECM or cell-cell contacts, and oncogene expression, MMP activity is regulated by their endogenous inhibitors, the tissue inhibitors of matrix metalloproteinases (TIMPs). TIMPs not only directly control the activity of active MMPs, but also have substantial influence on the activation process of MMP zymogens.²⁻⁵

Given the ability of TIMPs to inhibit the proteolytic activity of MMPs, it is not surprising to find that TIMPs are capable of blocking tumor metastasis, either by inhibiting tumor invasion of basement membrane or by restraining tumor angiogenesis.⁶ However, recent studies have suggested that the inhibitory effects of TIMPs on tumor progression are not only due to their ability to inhibit MMP activity, but also because of their ability to directly modulate the cell growth and apoptosis of tumor cells, as well as host endothelial cells (ECs).

In an intriguing study published in this issue of *The American Journal of Pathology*, divergent effects of TIMP-1 on Burkitt's lymphoma growth in nude mice are reported by Guedeux and co-workers.⁹⁶ When Epstein-Barr virus-negative Burkitt's lymphoma cells were forced to overexpress TIMP-1 by retroviral transfection, a biphasic tumor growth pattern was observed. After injection into nude mice, these cells showed an initial fast proliferation phase due to the stimulation of tumor cell proliferation and the protection from apoptosis elicited by TIMP-1. However, once these tumors reached a certain size (0.4 mm²), the initial fast growth phase was replaced by a slowdown of

tumor progression accompanied by tumor necrosis and regression. The authors provide convincing evidence to show that this late stage inhibition was caused by the suppression of tumor-induced host angiogenesis. These novel *in vivo* results highlight the importance of MMPs and their endogenous inhibitors during tumor progression and angiogenesis.

TIMPs and Tumor Growth

TIMPs and Cell Proliferation

Since first being discovered as having the ability to stimulate the proliferation of erythroid progenitor cells,⁷ in addition to inhibiting MMP activity,⁸ TIMP-1's growth promoting effects have been extended to a variety of cells, including mammary epithelial cells,⁹ keratinocytes,¹⁰ lymphoid, myeloid, endothelial, fibroblasts, hepatoma, breast carcinoma, and chondrocyte cells.¹¹ Like TIMP-1, TIMP-2 was shown to stimulate cell proliferation in human osteosarcoma cells and cultured rabbit corneal epithelial cells, potentially via a tyrosine kinase-dependent signaling pathway,^{12,13} and to promote fibroblast or fibrosarcoma cell growth by stimulating cAMP production.¹⁴ Unlike TIMP-1,¹¹ TIMP-2 also exhibits inhibitory effects on the proliferation of ECs,¹⁵ renal carcinoma cells,¹⁶ and several other carcinoma cells.¹⁷ Interestingly, TIMP-1 and TIMP-2 exert different effects on EC processes in that TIMP-2 inhibits fibroblast growth factor-driven EC proliferation and TIMP-1 does not.¹⁵ In fact, the latter has been shown to exert a stimulatory effect on EC growth *in vitro*.¹¹ Both inhibitors can, however, suppress capillary EC migration and tube formation in response to angiogenic stimuli.¹⁸

Supported in part by grants from The National Cancer Institute, National Institutes of Health (RO1 CA83106), and The American Cancer Society (RPG-97-013-04-CSM).

Accepted for publication February 2, 2001.

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These regulatory effects of TIMPs on cell proliferation may not necessarily be a function of their MMP inhibitory activity.^{15,19} For example, modulation of cell proliferation by TIMPs has been attributed to their interactions with unknown cell surface receptors,^{10,14} as well as to their potential regulation of DNA synthesis in the nucleus.^{20,21} Moreover, the divergent and sometimes even contradictory effects of TIMPs on cell growth *in vitro* are cell type-specific. Although these provocative findings could be taken to presage the divergent effects of tumor growth and angiogenesis that is demonstrated by Guedez and co-workers,⁹⁶ their study is the first to document these two divergent *in vivo* effects within the same tumor system. It will be important to learn whether this biphasic effect is observed in other tumor systems and whether all TIMPs are capable of exerting this same effect.

TIMPs and Tumor Cell Apoptosis

In addition to their growth-promoting ability, TIMPs can also affect programmed cell death, or apoptosis, in both normal cells and transformed tumor cells. TIMP-1 expression levels have been shown to correlate with cell apoptosis in a series of B lymphoma cells.^{22,23} Overexpression of TIMP-1 can rescue mammary epithelial cells from apoptosis induced by uncontrolled ECM degradation.²⁴ Similarly, TIMP-2 expression has inhibitory effects on apoptosis of melanoma cells.²⁵ In contrast, however, it has been reported that TIMP-3, a matrix-associated inhibitor, induces cell apoptosis in melanoma cells,²⁶ colon carcinoma cells,²⁷ and vascular smooth muscle cells.²⁸ The cell-death domain of TIMP-3 has been localized to the amino terminus and its apoptotic effect appears to correlate with the inhibition of metalloproteinase activity.²⁹

According to Guedez and colleagues,⁹⁶ the initial growth-stimulatory effect of TIMP-1 on Burkitt's lymphoma *in vivo* is likely achieved through its inhibition of apoptosis. In fact, the same group has recently reported that TIMP-1 expression in Burkitt's lymphoma cell lines inhibits induction of apoptosis by Fas-dependent and -independent pathways, and up-regulates BCL-X_L expression.²² Based simply on the anti-apoptotic effect of TIMP-1, one would expect that TIMP-1-positive tumors would grow much more aggressively than their TIMP-1-negative counterparts. However in this study, after reaching a certain size (0.4 mm²), the growth rate of TIMP-1-positive tumors decreased significantly. These observations led the authors to invoke the anti-angiogenic activity of TIMP-1 as a potential mechanism for the tumor suppression observed in their model. This anti-angiogenic mechanism is strongly supported by the immunostaining and microvessel counts provided in this study. In addition, their finding that tumor regression after the initial period of increased tumor growth was marked by the presence of only microscopic foci of residual proliferating tumor cells observed only on biopsy of the tumor site is reminiscent of the tumor dormancy elicited by a number of angiogenesis inhibitors^{30–32} and begs the question as to how long this dormant period might last. Based on recent work demonstrating that MMPs may be required for the acqui-

sition of the angiogenic phenotype during tumor progression,^{33,34} we would hypothesize that any shift in the proteolytic balance in favor of MMP activity would activate the switch to the angiogenic phenotype, thereby bringing an end to the period of tumor dormancy.

TIMPs and Tumor Metastasis

The inhibitory function of TIMPs during tumor metastasis has been well demonstrated in a variety of tumors. Overexpression of TIMP-1 or TIMP-2 inhibits the invasive behavior of highly invasive and metastatic B16F10 murine melanoma cells,²⁵ fibrosarcoma cells,³⁵ the osteolytic bone metastases of breast carcinoma,³⁶ and the extravasation of pulmonary metastasis of a bladder carcinoma.³⁷ The newly identified TIMP-4 has also been shown to inhibit breast carcinoma cell invasion *in vitro* and tumor metastasis *in vivo*.³⁸ Furthermore, targeted disruption of TIMP-1 expression increases the invasive behavior of primitive mesenchymal cells derived from embryonic stem cells.³⁹ Adenovirus-mediated expression of TIMP-3 resulted in a similar, and even more potent, inhibition of cell invasion than that caused by TIMP-1 and TIMP-2, in melanoma, fibrosarcoma, and ovarian carcinoma cells.^{26,40} In addition, TIMP-3 can also induce apoptosis by preventing cell adhesion to underlying matrix.²⁶

TIMPs and Tumor Angiogenesis

Tumor angiogenesis is required for sustained tumor growth because the new capillaries are conduits for oxygen and nutrients. Without angiogenesis, tumor growth is restricted to the tissue diffusion distance of ~0.2 mm.⁴¹ MMPs and TIMPs have been shown to be involved in various stages of the angiogenic process, from EC migration and proliferation, to deposition and remodeling of basement membrane of newly formed blood vessels.¹⁸ Furthermore, modulation of MMP or TIMP expression has a profound influence on angiogenesis at various stages in different tumors.^{33,34,42–44}

TIMPs and EC Proliferation and Migration

It is only in recent years that the notion that certain TIMPs might be capable of exerting a pleiotropic effect on tumor growth, metastasis, and angiogenesis has come to be appreciated. Since the first report that a TIMP could inhibit angiogenesis *in vitro* and tumor angiogenesis *in vivo*,^{45,46} a series of studies using a variety of endogenous MMP inhibitors have supported this suppression of neovascularization.^{15,47,48} At the time, the potential mechanisms of action focused on the ability of TIMPs to suppress mitogen-driven angiogenic events such as capillary EC proliferation, migration, and capillary tube and sprout formation. However, it was also reported that select TIMPs might also exert a stimulatory effect on EC functions required for successful angiogenesis. For example, TIMP-1 unlike TIMP-2, did not inhibit capillary EC proliferation¹⁵ but rather was reported to stimulate it.¹¹

The inhibitory effects of TIMPs on EC migration have been shown both *in vitro* and *in vivo*.^{25,45,47,49} Given the critical role of EC migration during angiogenesis,⁵⁰ this inhibition may account, at least in part, for the reduced tumor angiogenesis observed in TIMP-1-positive lymphoma tumor implants *in vivo*.

MMPs and TIMPs: Regulators of Angiogenic Signals

The process of tumor angiogenesis is governed by a complex signaling network.⁵⁰ The establishment of the neovasculature is modulated by soluble growth factors, hormones, and cytokines,⁵¹ as well as by insoluble ECM that underlies the participating vessels.^{52,53} Metalloproteinases, together with TIMPs, have been shown to play pivotal roles during the angiogenic process, from processing latent growth factors and shedding receptors, to maintaining and remodeling EC basement membrane.¹⁸ These roles are discussed briefly here.

Bioavailability of Soluble Factors

Many growth factors involved in angiogenesis reside in the ECM, associated with either their endogenous inhibitors or extracellular proteoglycans.⁵⁴ Therefore, to actively participate in angiogenesis, these sequestered growth factors must be released from their ECM compartments. A recent study has demonstrated that MMP-9 can liberate vascular endothelial growth factor from its ECM storage in a mouse pancreatic-islet carcinoma model.³⁴ MMP-9 has also been shown to process latent transforming growth factor- β or interleukin-8, which in turn, promote tumor invasion and angiogenesis, or activate neutrophils, respectively.^{55,56} In addition to governing the releasing and processing of active growth factors, MMPs and TIMPs are also involved in modulating cell surface receptors for these signaling molecules. For example, proteolytic cleavage mediated by MMP-2 has been shown to release a soluble active ectodomain of fibroblast growth factor receptor 1.⁵⁷ In another case, the processing of cell-surface tumor necrosis factor- α and its receptors can be inhibited by broad-spectrum MMP inhibitors, as well as by TIMP-2 and TIMP-3.^{27,58–60} This inhibition, in turn, augments tumor necrosis factor- α signaling and induces tumor cell apoptosis *in vitro* and suppresses tumor growth *in vivo*.^{27,61}

MMPs also process extracellular molecules into fragments with potent anti-angiogenic activities. For example, angiostatin, an internal fragment of plasminogen, is a potent inhibitor of angiogenesis, which specifically inhibits EC proliferation and tumor growth.^{31,62} The production of angiostatin has been linked to various MMPs,⁶³ including tumor-expressed MMP-2,⁶⁴ MMP-12 derived from tumor-associated macrophage,⁶⁵ MMP-3,⁶⁶ MMP-7, and MMP-9.^{67,68} Another angiogenesis inhibitor, endostatin, a 20-kd C-terminal fragment of collagen type XVIII, specifically inhibits endothelial cell proliferation and potently inhibits angiogenesis and tumor growth.³² Several lines of evidence have demonstrated that the release of en-

dostatin from its precursor is mediated by a metal-dependent early step, followed elastase cleavage.⁶⁹ The production of endostatin may also be mediated by cathepsin L secreted from endothelial-origin tumor cells.⁷⁰ Recently, more matrix-derived peptide fragments have been shown to have anti-angiogenesis activity.^{71,72} Although the identity of proteases responsible for their release is still unclear, MMPs are expected to play an essential role in these anti-angiogenic-processing events as well.

Taken together then, MMPs not only proteolytically release matrix-bound pro-angiogenic growth factors, but can also directly process matrix molecules into anti-angiogenic fragments. The balance of these two opposing activities, pro-angiogenic and anti-angiogenic, respectively, is regulated by the activity of TIMPs, which in turn, can independently modulate the angiogenic process.

Modulation of Insoluble Factors Signaling

Tumor cell and EC behavior is also regulated by the underlying ECM and signals transduced by integrins after engagement of ligands.^{52,73} Both ECM integrity and subsequent signaling events are profoundly influenced by the local balance between MMPs and TIMPs. Changes in ECM structure and integrity can also feed-back to modulate the proteolytic activity elicited by ECs.^{74,75} Excessive proteolytic cleavage of ECM by MMPs results in cell apoptosis, which can be rescued by overexpressing counteracting TIMPs.^{24,25} MMPs also influence cell behavior by exposing cryptic binding sites via proteolytic cleavage of ECM molecules.⁷⁶ Similarly, the angiogenic potential of vascular EC is also regulated by the ECM, which in turn, is constantly remodeled by the synchronized actions of MMPs and TIMPs.^{74,77}

TIMPs in Cancer Therapy and Diagnosis/Prognosis

For many of the reasons discussed above, MMP inhibitors, both synthetic and endogenous, have become attractive therapeutic candidates in recent years.^{78–80} The limited clinical success of broad-spectrum MMP inhibitors has been suggested to be due, at least in part, to their lack of specificity and associated side effects.⁸¹ Some of these side effects could be limited by designing more specific MMP inhibitors and a significant effort is being made to do so.⁸² However, targeting MMP specificity may represent only one of a number of potential approaches to resolving these problems. As shown in the study by Guedez and colleagues,⁹⁶ TIMP-1 can initially promote tumor growth in the early stage by inhibiting apoptosis, whereas at a later stage, the secreted TIMP-1 acts on host ECs to block tumor angiogenesis and to ultimately suppress tumor growth. Given such divergent and even contradictory functions of MMPs and TIMPs during tumor progression and angiogenesis, it becomes very important that the schedule of administration of MMP inhibitors as cancer therapeutics be carefully determined based on the stage of tumor progression in order to

achieve optimal therapeutic efficacy. Therefore, the clinical monitoring of tumor progression becomes a critical adjunct to inhibitor therapy.

The detection of MMPs and TIMPs in body fluids may provide a useful way to accomplish such monitoring. Elevated MMP levels in biological fluids, including serum, plasma, and urine from animals bearing experimental tumors or from cancer patients have been reported in several studies.^{83–88} In addition, elevated plasma levels of TIMP-1 and TIMP-2 have been detected in patients of late stage breast, colorectal, lung, and gastric cancer.^{89–95}

Some of these options represent highly sensitive, specific, and noninvasive monitoring systems. For example, it has recently been reported that intact and biologically active MMPs can be detected in the urine of cancer patients and are independent predictors of disease status.⁸⁸ These urine samples were obtained from patients with a variety of cancers, including prostate, renal, bladder, and breast carcinomas. Urinary MMP-2 and MMP-9 have been shown to be independent predictors of cancer. In addition to these two major gelatinase species, several MMP activities with molecular sizes greater than 100 kd were observed and were shown to be predictive of metastatic diseases. Combined with other conventional diagnostic tools, the detection of urinary MMP activity may provide the relevant information regarding tumor burden, tumor staging, and tumor angiogenic status that will be necessary to optimize both current and future cancer therapies.

Conclusion

The authors note in closing that their study highlights the need for a further understanding of the role of TIMPs in tumor progression. For example, it now becomes very important to identify the molecular players regulating the switch in TIMP-1's activities in early and later stage tumor progression. At the very least, this information might lead to the discovery of new drug candidates for cancer therapy and to a new therapeutic and diagnostic paradigm for the monitoring and treatment of neoplastic disease.

Acknowledgment

We thank Dr. Jay Harper for helpful discussions.

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