Mini-Review

An Alternate Perspective on the Roles of TIMPs and MMPs in Pathology

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Tissue inhibitors of metalloproteinases (TIMPs) are pleiotropic extracellular proteins. TIMPs are recognized as endogenous regulators of matrix metalloproteinases (MMPs), a large family of extracellular enzymes with proteolytic activities that participate in cellular homeostasis, adaptation, and tissue remodeling. In addition to their roles as endogenous potent MMP inhibitors, accumulating evidence indicates important physiological roles for TIMPs that are independent of their ability to block MMP activities. For instance, MMP-independent actions of TIMP-1 in the central nervous system have been implicated in synaptic plasticity, neuroprotection, oncogenesis, and oligodendrocyte differentiation. Expression of TIMP-1 is dramatically increased in response to a variety of injurious and inflammatory insults. In the context of disease pathogenesis, MMP and TIMP expression are interpreted with respect to the proteolytic consequences of increased MMP/TIMP ratios. Here, we provide an alternative perspective on the homeostatic balance of TIMP and MMP proteins, whereby consideration is given to the possible role of MMPs as cognate inhibitors of the signaling functions of TIMPs. Thus, MMPs may regulate the receptor-mediated actions of TIMPs, inasmuch as TIMPs are themselves inhibitors of MMP-mediated proteolytic activities. This broader view reflects our emerging understanding that TIMP signaling and MMP inhibition represent two important functions of TIMPs that have the potential to affect tissue pathology. (*Am J Pathol* 2012, 180:12–16; DOI: 10.1016/j.ajpath.2011.09.008)

Tissue inhibitors of metalloproteinases (TIMPs) are a family of small extracellular proteins known to inhibit the enzymatic activity of matrix metalloproteinases (MMPs), a large and diverse family of extracellular, zinc-dependent endopeptidases implicated in the pathophysiology of many diseases. The four known mammalian TIMP genes

share largely redundant properties of broadly blocking MMP-mediated proteolysis by noncovalently binding to the MMP active site in a 1:1 stoichiometric ratio.¹ Tissue homeostasis is thus achieved by a tight balance of MMP proteolysis to TIMP expression. When this balance is upset, dysregulated MMP activities result. Elevated MMP/TIMP expression ratios have been associated with many diseases of the central nervous system (CNS), including Alzheimer's disease, ischemic brain injury, CNS infections, Parkinson's disease, and multiple sclerosis (MS).^{2–5} Elevated MMP/TIMP protein level ratios are often viewed and interpreted with respect to the net increase in proteolytic activity of MMPs and disease pathology. Although this interpretation is certainly valid and supports many aspects of disease pathology, such a perspective does not take into account the potential implications of TIMP signaling functions as relevant for mechanisms of tissue injury or repair.

The impetus for developing an alternative view of TIMPs and MMPs stems from two observations. First, the identification of functional receptors that mediate downstream signaling by TIMPs provides an additional perspective to consider when examining MMPs and TIMPs in relation to pathology. Second, findings indicate that TIMPs and MMPs can be coincidentally expressed in pathology, and sometimes from the same cells. For example, application of the proinflammatory cytokine interleukin-1 β to primary glial cultures results in robust induction of both MMP-3 and its potent inhibitor TIMP-1.⁶ Similarly, during immune-mediated models of CNS demyelination, including experimental autoimmune encephalomyelitis and virus infections of the CNS, activated T lymphocytes simultaneously express both MMPs and

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TIMPs.^{7,8} Why do cells produce both an enzyme and its inhibitor at the same time? One could reasonably argue that these expression patterns represent cellular heterogeneity within the same population: some cells express MMPs and others express TIMPs. Considered strictly from a proteolytic perspective, increased production of both MMPs and TIMPs within the same cellular population would ostensibly nullify the activities of MMPs. If, however, we consider the MMP-independent actions of TIMPs, these examples of coincident production and release of MMPs and TIMPs could instead reflect counter-regulation of MMPs in TIMP signaling.

Our objective in this mini-review is to expand on the traditional interpretation of TIMPs, not only as MMP inhibitors but also as molecules with MMP-independent activities. Indeed, in an excellent recent review of the biological actions of TIMPs, Stetler-Stevenson⁹ provides an overview of TIMP functions unrelated to inhibiting MMPs. We will not attempt to recapitulate that information here, but rather will elaborate on TIMPs as pleiotropic molecules using two examples from neuropathology: the role of TIMPs in CNS myelin pathology and also the role during angiogenesis. These examples are used to illustrate this alternate view of TIMP signaling and how it may be applied in interpreting pathology of other tissues and in different disease states.

TIMPs and Myelin Pathology

TIMP-1 is a well-characterized 28-kDa secreted protein best known for its function as an endogenous regulator of MMP activities, although additional biological activities of TIMP-1 have been identified.¹⁰ The TIMP-1 protein is encoded from six exons that can be physically and functionally defined as an N-terminal domain [amino acids 1 to 126; TIMP-1(N)] that is sufficient to retain MMP inhibitory function¹¹ and as a C-terminal domain [amino acids 127 to 184; TIMP-1(C)] that mediates protein-protein interactions.¹² The C-terminal domains of TIMPs are highly variable, but have been shown to participate in binding to MMPs¹³ and, in the case of TIMP-2, to facilitate the activation of pro-MMPs.^{14,15} The C-terminal domain of TIMP-1 has also been found to mediate intracellular signaling via receptors. To date, two TIMP-1 receptors have been identified: a CD63- β 1 integrin complex in MCF10A (a breast cancer cell line)¹² and, more recently, α v β 3 integrin in an MG-63 (an osteosarcoma cell line).¹⁶ In both of these instances, TIMP-1 interactions with the respective receptor complex mediate the antiapoptotic activity of TIMP-1^{17–19} and also growth factor-like function.^{20,21} These findings provide strong evidence for direct cellular actions of TIMP-1 that are independent of its ability to regulate MMP activities.⁹ Moreover, the identification of receptors for all TIMP family proteins now suggest that signaling is a biological function of all known TIMPs, thus providing a new model for understanding how TIMPs may function in CNS diseases.

In inflammatory neurological diseases, such as MS, evaluation of MMPs and TIMPs has provided insight into mechanisms of immune-mediated pathology. For in-

stance, measuring MMPs in blood serum, CSF, or brain tissues of MS patients identified increased levels of MMP-2, MMP-7, MMP-9, and MMP-14, compared with healthy age-matched control groups.^{4,22–26} These changes in MMP expression during MS are thought to reflect increased production by lymphocytes, with MMP-mediated proteolysis facilitating the extravasation of these autoreactive and proinflammatory immune cells into the CNS parenchyma.^{27,28} Indeed, expression of MMP-9 in MS cases has been positively correlated with periods of worsening disease, or clinical relapse, and with development of new gadolinium-enhancing myelin lesions in the CNS.²⁵ Accordingly, the profiling of MMP-2 and MMP-9 as potential biomarkers has been proposed for MS disease course, subtype, and disease activity.^{4,28,29}

TIMPs have ubiquitous expression, which varies with the type of tissue and can be altered with pathology. Whereas many peripheral tissues have constitutive homeostatic expression of TIMP-1, levels of TIMP-1 in the healthy adult CNS are basally low to undetectable. In animal models of immune-mediated CNS demyelination, expression of TIMP-2 and TIMP-3 have been reported to increase,³⁰ although TIMP-1 consistently exhibits robust expression in response to CNS myelin injury, in some cases up to 165-fold above basal levels.^{7,31–33} Acute disseminating encephalomyelitis (ADEM) in humans is an acute demyelinating disease of the CNS that spontaneously resolves. In ADEM, TIMP-1 levels have been shown to be markedly higher than in controls.³⁴ In MS, however, TIMP-1 expression is not elevated, compared with healthy age-matched controls.^{4,23,35} Also, TIMP-1 levels are decreased in the cerebrospinal fluid of relapsing-remitting MS patients, compared with levels in other inflammatory diseases and also in noninflammatory neurological diseases.^{28,36} These findings support a positive correlation between TIMP-1 expression and preservation of CNS myelination.

In animal models of CNS diseases, TIMP-1 plays important roles in neuroprotection, neural plasticity, and tissue repair.^{10,37–39} We recently identified a novel MMP-independent function for TIMP-1 in CNS myelination.^{40,41} Specifically, we determined that TIMP-1 can directly stimulate the differentiation of oligodendrocytes from their precursor cells.⁴¹ This finding suggests that elevated TIMP-1 expression in the CNS can represent an endogenous signal for remyelination. Recent work by Pagenstecher and colleagues⁴² demonstrated that overexpression of TIMP-1 in the CNS dramatically attenuated clinical experimental autoimmune encephalomyelitis and CNS demyelination. In another study, overexpression of TIMP-1 was shown to lessen clinical disease progression and to preserve CNS myelination in a transgenic model of spontaneous demyelination.⁴³ In contrast, we had previously reported that global TIMP-1 knockout mice exhibited minimal remyelination after experimental autoimmune encephalomyelitis.⁴⁰ These findings led us to speculate whether a paucity of TIMP-1 in the brain during chronic diseases, such as MS, could contribute to CNS pathology.^{40,41} Furthermore, these experimental findings point to a parallel with clinical data providing a compel-

ling correlation between increased TIMP-1 and improved potential for CNS remyelination.^{40,42}

TIMP Signaling and Angiogenesis

TIMP-3 is an antagonist of vascular epithelial growth factor receptor 2 (VEGF-R2),⁴⁴ and has been shown to inhibit angiogenesis.^{45–47} Advances in our understanding of the angiogenic regulatory potential of TIMP-2 and TIMP-3 are credited to study of their roles in cancer.⁹ In the CNS, expression of TIMP-3 is notably increased by cerebral ischemia.⁴⁸ Rosenberg and colleagues⁴⁸ showed that TIMP-3 contributes to delayed neuronal death after middle cerebral artery occlusion, an animal model of ischemic stroke. In the context of the present mini-review and our revised perspective on the dynamic balance between MMPs and TIMPs, we note that Lo and colleagues⁴⁹ showed that delayed pharmacological inhibition of MMPs after middle cerebral artery occlusion exacerbates cortical neuronal loss. The negative effect of blocking MMPs after ischemia was associated with impaired angiogenesis, related to reduced VEGF signaling.⁴⁹ In that model, the limited VEGF signaling was a consequence of limited bioavailability of VEGF.^{49,50} From the perspective of a TIMP-centric perspective, these previous studies support our hypothesis that MMPs are endogenous inhibitors of TIMP signaling. For instance, when MMP inhibitors are administered after middle cerebral artery occlusion, the blockade of MMPs in that setting could provide a shift in the balance that favors function of TIMP-3 as a signaling molecule. Increased bioavailability of TIMP-3 could act as an endogenous antagonist for VEGF-R2, resulting in impaired angiogenesis, as is reported in this ischemic brain injury model.⁴⁹ Given the well-described function of TIMP-2 as a physiological antagonist of intracellular VEGF signaling,^{9,51} pathological impairment of angiogenesis resulting from MMP inhibition after ischemia could also result from increased TIMP-2-related activity. Further study will be required to test whether these ancillary actions of TIMPs are truly implicated in ischemic pathology.

Additional support for the importance of the signaling functions of TIMP-3 in pathology can be gleaned from the genetics of Sorsby's fundus dystrophy (SFD), an autosomal dominant form of macular degeneration that has been linked to a point mutation in TIMP-3.⁵² Individuals with SFD develop increased choroidal neovascularization that ultimately impairs vision. Known mutations in TIMP-3 linked to SFD are all point mutations that add a cysteine residue to the C-terminus of this protein, the component of TIMP-3 that mediates binding to the VEGF-R2 receptor. Notably, SFD mutations in TIMP-3 result in disrupted TIMP-3-mediated signaling, but not MMP inhibition,⁵³ and this loss of TIMP-3 signaling results in enhanced VEGF-mediated signaling and angiogenesis.⁵³ SFD thus represents the first instance of a human disease directly linked to the loss of TIMP signaling. Moreover, SFD provides an example of how the role of TIMP-3 as a signaling molecule provides insight into and a new understanding of the pathophysiology of disease.

Pathological roles for TIMP-2 and TIMP-3 may also have relevance for the neuroinflammatory condition of MS and chronic demyelination. Because both TIMP-2 and TIMP-3 antagonize VEGF signaling,^{44,46,51} it is relevant that increased angiogenesis and VEGF expression have been reported in MS and in experimental autoimmune encephalomyelitis.^{54,55} Notably, virus-mediated overexpression of TIMP-2 or TIMP-3 has been reported to protect the CNS from inflammatory demyelinating injury.⁵⁶ When considered from the alternate perspective offered here, the excessive production of MMPs during neuroinflammation and chronic CNS demyelination could be viewed as overwhelming the beneficial potential of TIMP-related signaling. For instance, excess production of MMPs could indirectly promote VEGF-mediated angiogenesis by restricting TIMP-2 and/or TIMP-3 from regulating VEGF signaling in response to neuroinflammation. Further study is needed to explore and validate the potential neuroprotective and antiangiogenic functions of TIMPs, and to identify the mechanism or mechanisms underlying their beneficial roles in CNS remyelination and MS. Studies exploring the signaling potential of TIMPs will be necessary to identify the specific contribution of these TIMP signaling functions in the etiology of these and other types of pathologies.

An Alternate Perspective

The prevailing perspective on MMP/TIMP ratios in pathology is that increased MMP production without counterbalancing TIMP expression represents a net increase in proteolytic activity. From the alternate perspective described here, however, MMP/TIMP ratios may also reflect a counter-regulation of TIMP-mediated signaling of target

Figure 1. MMP regulation of TIMP-mediated signaling. Under physiological conditions, there is a homeostatic balance between the production of MMPs and expression of TIMPs. The regulation of MMP proteolysis by TIMPs is mediated through their N-terminus, whereas the C-terminus of TIMPs interacts with cell surface receptors to initiate intracellular signaling cascades. Under conditions of pathology, increased MMP expression results in a net increase in MMP-mediated proteolysis, which also results in the depletion of TIMP proteins available to provide receptor-mediated signaling. This loss of TIMP-mediated signaling may have important implications for disease pathology.

cells through depletion of free (ie, unbound) TIMP protein (Figure 1). This alternative viewpoint suggests that MMPs function as inhibitors of TIMPs.

How might this alternative perspective on TIMPs translate into improved treatment of diseases? It may well reveal how the beneficial actions of TIMPs contribute to the clinical benefit of MMP inhibitors. For example, pharmacological inhibition of MMPs has been shown to mitigate demyelination in experimental autoimmune encephalomyelitis.²⁷ During inflammatory demyelination, the inhibition of MMPs is anti-inflammatory and limits the extravasation of inflammatory immune cells into the CNS. Could part of this benefit also be attributed to elevated TIMP signaling, resulting from MMP inhibition in the CNS? Further study is needed to address this question. Our alternate perspective may also lend support for strategies to replace or augment TIMP signaling in situations in which TIMPs have been shown to prevent pathology or promote tissue repair. For instance, TIMP-1 has been shown to be neuroprotective against stroke, and after intravenous infusion of human recombinant TIMP-1 in ischemic mice measurable levels of the human protein were identified in the CNS.⁵⁷ Therapies that augment TIMPs to treat disease, such as a peptide delivery approach, offer the potential to deliver rTIMP-1 to the ischemic or chronically demyelinated brain.

In summary, the recognition that TIMPs have MMP-independent ancillary functions calls for reinterpretation of MMP and TIMP expression in the context of disease. We propose that this alternate perspective could provide important insights toward understanding disease pathology and/or tissue repair.

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