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Tissue Inhibitors of Metalloproteinases In Cell Signaling:

Metalloproteinase-independent Biological Activities

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Abstract

Over the last twenty years the tissue inhibitors of metalloproteinases (TIMPs) have been implicated in direct regulation of cell growth and apoptosis. However, the mechanisms of these effects have been controversial. Recent work by several laboratories has identified specific signaling pathways and cell surface binding partners for members of the TIMP family. TIMP-2 binding to the integrin α3β1 is the first description of a cell surface receptor for a TIMP family member. TIMP-2 has been shown to induce gene expression, promote G_1 cell cycle arrest, and inhibit cell migration. TIMP-1 binding to CD63 inhibits cell growth and apoptosis. These new findings suggest that TIMPs are multifunctional and can act either directly through cell surface receptors or indirectly through modulation of protease activity to direct cell fate. The emerging concept is that TIMPs function in a contextual fashion so that the mechanism of action depends on the tissue microenvironment.

Introduction

As their name implies tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of metalloproteinase activities and as such were initially thought to function principally to modulate matrix metalloproteinase activity and suppress extracellular matrix turnover. In 1989, Khokha *et al*. demonstrated that suppression of the endogenous tissue inhibitor of metalloproteinases (TIMP, now known as TIMP-1) with antisense RNA conferred an oncogenic phenotype in Swiss 3T3 cells (1). This was the first suggestion that TIMPs may present an intrinsic barrier to tumor progression. Subsequently, many laboratories observed that most human malignancies were associated with increased matrix metalloproteinase (MMP) activity and that TIMPs could modulate the invasive activity and metastatic capacity of tumor cells (2-5). These findings stimulated an effort for the development of synthetic MMP inhibitors as novel anti-cancer therapeutics (6). Although successful in a number of murine tumor models, these agents failed in human clinical trials (7). Retrospectively, it became apparent that the assumption that MMP activities were exclusively pro-invasive and necessarily facilitated tumor progression was naïve. It is now widely recognized that MMPs, like many

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Gloss: The extracellular matrix influences cell behavior through interaction with various cell surface receptors. Cells also influence the structure and the composition of the extracellular matrix. Remodeling of the extracellular matrix is mediated by protease activity, which in turn is modulated by endogenous protease inhibitors. The tissue inhibitors of matrix metalloproteinases (TIMPs) were first identified as inhibitors of the matrix metalloproteinase (MMP) family, a class of proteases closely identified with cell growth and invasion during the process of tumor progression and metastasis. Over the last twenty years TIMPs have been implicated in direct regulation of cell growth and apoptosis. However, these biological activities of the TIMP family have been controversial. Recent work by several laboratories has identified specific signaling pathways and cell surface binding partners for members of the TIMP family. These new findings suggest that TIMP are pluripotential regulators of the extracellular matrix and can act either directly through cell surface receptors or indirectly through modulation of protease activity to direct cell fate.

other extracellular matrix (ECM) components, play a dual role in the process of tumor progression with both pro- and anti-tumorigenic activities (8).

This dual role extends to the TIMPs. Although originally characterized by their ability to inhibit MMP activity, TIMPs have additional biological activities that are just beginning to be recognized and characterized. Over the past twenty years TIMPs have been shown to regulate a number of cellular processes including cell growth, migration, and apoptosis. Despite mounting evidence suggesting direct cell signaling capacity for the TIMPs, however, the requirement for MMP-inhibitory activity in mediating these cellular activities of the TIMPs remains controversial. Although TIMP-mediated inhibition of MMP activity is an important determinant of cell function, the concept of MMP-independent TIMP regulation of cell fate is now supported by the identification of specific cell binding partners--and specific signaling events--for TIMP family members. This is particularly relevant to the cell signaling mechanisms mediated by TIMP-1 and TIMP-2. The recognition of these MMP-independent TIMP activities and understanding of the mechanisms involved have important implications for development of new therapies for cancer and other chronic diseases.

The TIMP Gene Family

TIMPs have been identified in species ranging from *Caenorhabditis elegans*, to *Drosophila*, zebra fish, and humans, suggesting that they are ancient eukaryotic genes (9-11). Recent studies have shown developmental defects in TIMP-deficient organisms, in both non-mammalian and mammalian systems, revealing the importance of these proteins during embryonic development, as well as the possible functional redundancy of some TIMPs in mammalian development (12-16). However, the study of TIMP function remains most closely associated with their role in tumor development and cancer progression.

The mammalian TIMP family has four members, which share substantial sequence homology and structural identity at the protein level. TIMPs have basically two structural domains: an N-terminal domain consisting of six conserved cysteine residues forming three disulfide loops, which possesses MMP-inhibitory activity, and a C-terminal domain that also contains six conserved cysteine residues and forms three disulfide loops (17). The overall structure of the N-terminal inhibitory domain is that of the oligosaccharide/oligonucleotide binding fold (OB-fold) that is found in DNA or oligosaccharide binding proteins. This OB-fold is dominated by β-sheet structures separated by small flexible loop regions (17).

By definition, all members of the TIMP family inhibit MMP activity. This is accomplished through co-ordination of the Zn^{+2} of the MMP active site by the amino and carbonyl groups of the TIMP N-terminal cysteine residue. However, selective inhibition of some members of the MMP has been observed. For example, although TIMP-1 is the prototypic inhibitor for most MMP family members, it is a poor inhibitor of the membrane-type MMPs (MT-MMP) and MMP-19 (9,18). TIMP-3 inhibits members of the A Disintegrin And Metalloproteinase (ADAM) family of proteases, although the mechanism for this inhibition appears to be distinct from that of MMP inhibition (11,19). TIMP-2 is unique in that, in addition to inhibiting MMP activity, it selectively interacts with MT1-MMP to facilitate the cell-surface activation of pro-MMP-2 (9,18,20). Thus, TIMP-2 functions to both inhibit MMP activity and promote cell surface activation of pro-MMP-2 by MT-1-MMP.

TIMP-3 specifically interacts with sulfated glycosaminoglycans and as a result is sequestered in the extracellular matrix (21), whereas the other TIMP family members remain soluble and diffusible. Although the biological relevance of the matrix association of TIMP-3 has not been determined, it suggests that TIMP-3′s pericellular distribution and availability for interactions with cell surface proteins may be more restricted than that of other members of the TIMP family. Many in vitro studies of TIMP-3 cellular functions have been conducted by addition

of soluble, exogenous recombinant TIMP-3 and have not addressed the role of matrix binding, which is a unique feature of this inhibitor. By comparison relatively few studies have focused on TIMP-4, so less is known about this member of the TIMP family.

TIMPs can be regulated at the transcriptional level by various cytokines and growth factors, resulting in tissue-specific, constitutive, or inducible expression (22). TIMP-1 is expressed in organs of reproduction and TIMP-3 expression is found in the heart, kidney, and thymus. TIMP-4 shows the most selective pattern of tissue localization, being expressed predominately in heart and brain, as well as to a lesser degree in muscle and ovary (18,23). Analysis of the *TIMP* promoters reveals that the *TIMP-2* promoter contains several Sp1 sequences characteristic of housekeeping genes, a finding consistent with the constitutive pattern of TIMP-2 protein and mRNA expression observed in adult murine tissues (22,24,25).

Three members of the *TIMP* gene family are nested within the synapsin gene family (26-28). The *synapsin 1* gene nests *TIMP-1*, *synapsin 2* nests *TIMP-4*, and *synapsin 3* nests *TIMP-3*. *TIMP-2* is the only member of the *TIMP* gene family that is not nested within a member of the *synapsin* family. Synapsins are a multigene family of phosphoproteins that are neuron specific and are the most abundant protein of the synaptic vesicle (29). They are proposed to tether synaptic vesicles and regulate neurotransmitter release. The *synapsin-TIMP* gene nesting relationship began phylogenetically as far back as *Drosophila*, suggesting a strong linkage between these two gene families, although the implication of this association is not known (28).

TIMP-1 differs from the other members of the family in having a short exon 1 that is transcribed but not translated. The function of exon 1 appears related to the control of the specificity of tissue expression and may contain tissue-specific repressor elements (22,30). *TIMP-2* also has a distinct gene structure in that a nested gene, known as DDC8 (for differential display clone 8) (31), is present within the very large (~60 kb) first intron of the *TIMP-2* gene (32). Furthermore, tissue extracts from the brain of the *TIMP-2*-deficient mouse contain TIMP-2 mRNA encoding exons 2-5 downstream of DDC8, suggesting alternative splicing between these two genes (31,33). This would have important implications for delineating TIMP-2 functions associated with exons 2-5 in these mice.

MMP-Dependent TIMP Regulation of Cell Signaling

TIMP regulation of cell fate is a highly complex process whose interpretation is further complicated by the indirect effects of TIMP-mediated inhibition of MMP activity. Moreover, there are numerous conflicting reports of TIMP effects on the same cellular process. The complexities of understanding TIMP regulation of cell fate are highlighted here by focusing briefly on TIMP-1.

Recent studies have begun to identify signaling pathways involved in the MMP-dependent growth-promoting effects of TIMP-1. For instance, TIMP-1 specifically enhances the formation and growth of micrometastases in the liver (34). This effect is mediated through both the induction of hepatocyte growth factor (HGF) and the suppression of shedding its cognate receptor, cMet. It has been proposed, but not directly demonstrated, that the latter effect depends on inhibition of ADAM-10-mediated shedding of cMet. Thus high TIMP-1 expression in the liver creates a microenvironment that is conducive to tumor dissemination, and explains the observation that synthetic metalloproteinase inhibitors enhance formation of liver metastasis through inhibition of cMet shedding.

This research contrasts with reports that TIMP-1 inhibits MMP-mediated activation of HGF in the extracellular matrix and consequently reduces hepatocyte proliferation in a model of murine liver regeneration (35), and that TIMP-1 inhibition of MMP-mediated degradation of

insulin-like growth factor (IGF) binding protein-3 (IGFBP-3) reduces bioavailability of IGF-II, resulting in reduced growth of simian virus 40 (SV40) large T antigen-induced hepatocellular carcinoma (36).

These three studies emphasize the complexities of TIMP-associated cell signaling and the importance of recognizing that the observed effects of TIMPs on cell fate are highly dependent on the cellular context and details of the specific model system utilized.

TIMP Functions Independent of MMP Inhibition

TIMP Promotion of Cell Growth

Sequencing of a TIMP-1 cDNA clone revealed its identity with a cloned protein demonstrating erythroid-potentiating activity (designated EPA) (37). EPA is a T lymphoblastic factor present in serum that supports the growth of erythroid precursors in vitro by a mechanism involving direct cell surface binding (38). TIMP-2 also has erythroid-potentiating activity (39). The maximal erythroid-potentiating activity observed in vitro was at TIMP-1 or -2 concentrations of 80-100 pM, much lower than its plasma concentration of 10-20 nM (40,41). This suggests that TIMPs are unlikely to be critical regulatory factors for this activity.

Several laboratories have reported that TIMP-1 and TIMP-2 promote cell division, as measured by DNA content or ^{3}H-thymidine uptake, in various epithelial (keratinocytes), mesenchymal (fibroblasts), and tumor cell lines (42-47). TIMP-2 produced by the metanephric mesechyme rescued ureteric bud epithelia from apoptosis and stimulated ^{3}H-thymidine incorporation (25). These activities were not observed with use of synthetic MMP inhibitors. Collectively, these reports repeatedly demonstrated that this growth stimulation was observed with TIMP concentrations in the range of 10-50 nM, although some reports suggested higher concentrations were required. Moreover, these studies indicated that growth stimulation by TIMPs was independent of their inhibition of MMP activity as shown through the use of reductive alkylation or sequence mutations, both of which disrupted TIMP MMP inhibitory activity but not growth stimulatory activity (45,48). These studies also demonstrated the requirement for free TIMPs in stimulating cell division, in that the growth-promoting activity was abolished by complex formation with either pro- or active MMPs (45). Binding studies suggested the presence of high affinity receptors, with K_D values reported in the low nanomolar range (< 10 nM) by a number of laboratories (42,49). These studies suggest that the effects of TIMPs on cell growth may be mediated by their direct binding to the cell surface, i.e. through a cell receptor mechanism.

Several distinct signaling pathways have been implicated in TIMP growth-promoting activity, including the mitogen activated protein kinase (MAPK) and adenosine 3′,5′-monophosphate (cAMP)-protein kinase A (PKA) pathways (43,50). The growth promoting activities of TIMP-1 and TIMP-2 may require activation of Ras, albeit by distinct pathways suggesting independent receptor mechanisms (51). Collectively these studies demonstrate that the functions of TIMPs are contextual; for instance, growth-promoting activity was only observed in the presence of free TIMPs, independent of MMP-binding or MMP inhibition.

TIMP-3 accelerates the morphological changes associated with cell transformation and stimulates the proliferation of growth-retarded, nontransformed cells under low serum conditions (52), suggesting that TIMP-3 may promote the development of the transformed phenotype in cultured cells. It is not clear if this is an MMP-independent effect of TIMP-3 mediated by a cell autonomous mechanism.

TIMP Anti-apoptotic Activity

TIMP-1 expression inversely correlates with the susceptibility to induction of programmed cell death (apoptosis) in various human Burkitt's lymphoma cell lines (53). These studies demonstrated that treatment with recombinant TIMP-1 (1.4-140 nM) or forced expression of TIMP-1 in a TIMP-negative cell line reduces susceptibility to induction of apoptosis (intrinsic and extrinsic pathways), suppresses caspase-3 activity, and sustains DNA synthesis up to five days under serum-free culture conditions. TIMP-1 neutralizing antibodies block these effects, suggesting an autocrine mechanism. This anti-apoptotic effect is not observed with TIMP-2 or the synthetic MMP inhibitor BB-94, but is retained following reduction and alkylation of TIMP-1, demonstrating that the mechanism is independent of MMP inhibition. Furthermore, TIMP-1 suppression of apoptosis correlates with increased abundance of Bcl-X_L and IκBα (inhibitor of nuclear factor κB α) protein, but not Bcl-2 or NF-κB (nuclear factor κB), suggesting that these effects are mediated by a specific anti-apoptotic signaling pathway. Subsequently, it was observed that TIMP-1 enhances expression of survival and differentiation cytokines, such as interleukin-10 (IL-10), that also contribute to the anti-apoptotic effect (54). These findings are consistent with an apparent growth stimulatory activity of TIMP-1, in that the survival factor activity prevents cell loss and allows the continued unstimulated cell growth to dominate because of the exponential nature of cell proliferation.

In vivo, forced expression of TIMP-1 in Burkitt's lymphoma xenografts results in a biphasic effect on tumor growth, with initial enhancement and subsequent suppression of tumor growth (55). Tumor regression is associated with a failure of these tumors to develop an angiogenic response and TIMP-1 inhibition of endothelial cell migration (55).

TIMP-1 also inhibits apoptosis and anoikis (apoptosis induced by loss of cell adhesion) in human breast epithelial cells in vitro (56). Here, Bcl-2 overexpression increased the abundance of TIMP-1 protein in breast epithelial cell lines (MCF10A and MCF7), whereas it has no effect on MMP or TIMP-2 expression. Furthermore, TIMP-1 overexpression suppresses anoikis in MCF10A cells. These findings show that the anti-apoptotic activity of TIMP-1 does not depend on its ability to stabilize cell-matrix interactions, and is independent of MMP inhibition. In this model system TIMP-1 activates the focal adhesion kinase (FAK)-phosphoinositol-3 kinase (PI3K) pathway to protect cells from intrinsic and extrinsic cell death (56-58). These studies indicate that TIMP-1 mediates activation of specific signal transduction pathways that protect cells from apoptosis in a fashion that is independent of its metalloproteinase inhibitory activity.

The anti-apoptotic activity of TIMP-1 is not restricted to tumor cells. Expression of TIMP-1, but not that of TIMP-2, prevents cytokine-mediated apoptosis and cytokine-mediated glucose-stimulated insulin secretion in rat pancreatic islets (59). These effects are mediated by TIMP-1 inhibition of cytokine activation of NF-κB, suggesting a similar mechanism to those reported for malignant cells. TIMP-1 produced by embryonic stem cells implanted for the treatment of myocardial infarction inhibits apoptosis in cardiomyocytes (60). These data suggest that the effects of TIMP-1 on programmed cell death may be mediated by cell-specific pathways, and that these pathways may not differ greatly between normal and malignant cell compartments.

Unlike the relatively well-established anti-apoptotic effects of TIMP-1 discussed, the effect of TIMP-2 on programmed cell death has not been well characterized, with only two conflicting reports in the literature and no clearly defined signaling pathway (61,62). TIMP-3 promotes apoptosis in several in vitro systems, including vascular smooth muscle cells (63) and various tumor cell lines (64,65). Recent findings in TIMP-3-null mice suggest that TIMP-3 can either promote or inhibit apoptosis depending on the model system examined (15,66-68). In either case, there is little or no data to support the concept that TIMP-3 initiates cell signaling directly to mediate effects on apoptosis. Instead TIMP-3 functions indirectly by modulating metalloproteinase activity (of MMPs or ADAMs or both) to alter cell fate.

The fourth member of the TIMP family, TIMP-4, has been reported to stimulate tumorigenesis of human breast cancer cells in nude mice (69). Consistent with this stimulation of tumorigenesis, TIMP-4 increases the abundance of Bcl-2 and Bcl-X_L protein. TIMP-4 also inhibits apoptosis in human breast cancer cells in vitro and mammary tumors in vivo. A synthetic MMP inhibitor BB-94 did not have such an antiapoptotic effect, suggesting that TIMP-4, like TIMP-1, can promote tumor growth by inhibiting apoptosis. Thus the signaling pathways and receptor mechanism for the anti-apoptotic effect of these two TIMPs may be similar.

TIMP Growth-Inhibitory Activity

In addition to the numerous reports describing TIMP-1 and TIMP-2 growth-stimulating activity, there are reports documenting growth-inhibitory activity for these TIMP family members. Again, this suggests that the specific effects of the TIMPs on cell fate depend on the cell context and specific model system under study. For example, in TIMP-1-deficient mice there is an increase in mammary epithelial proliferation resulting in increased ductal branching and expansion of the ductal structures, which is reversed by introduction of exogenous TIMP-1 in slow release pellets (70). Also, transgenic mice expressing auto-activating MMP in the mammary epithelial show enhanced apoptosis and depletion of entactin levels from the basement membrane but these phenotypic changes can be rescued by cross breeding with TIMP-1 transgenic mice (69). Although these effects were initially attributed to inhibition of MMP activity, specifically that of MMP-3, recent findings suggest that TIMP-1 may arrest mammary cell proliferation directly. A recent report shows that TIMP-1 induces cell cycle arrest in G_1 in association with down regulation of cyclin D_1, up regulation of the cyclin-dependent kinase inhibitor $p27^{Kip1}$, and hypophosphorylation of the retinoblastoma (Rb) protein (71). These data suggest that TIMP-1 mediates a cell signaling pathway that results in altered gene expression and inhibition of cell growth. Although arrested in G_1 phase of the cell cycle, the TIMP-1 over-expressing cells remain viable and do not show detectable levels of apoptosis, consistent with the anti-apoptotic effect of TIMP-1 described above.

A novel anti-angiogenic agent from bovine cartilage, the cartilage-derived inhibitor (CDI) of angiogenesis, was isolated and characterized in 1990 (72). CDI co-purifies with MMP-inhibitor activity, and inhibits angiogenesis in vivo and in vitro. The N-terminal amino acid sequence of CDI shows close identity with TIMP-2, suggesting that CDI is TIMP-related (72). Analysis of the angio-inhibitory effects of TIMP-1 and TIMP-2 indicated that TIMP-2, but not TIMP-1 or the synthetic MMP inhibitor, BB-94, inhibits the fibroblast growth factor 2 (FGF-2)-stimulated proliferation of human endothelial cells (73). This inhibitory effect is not observed with a pro-MMP-2-TIMP-2 complex, suggesting that only free TIMP-2 inhibits endothelial cell growth in response to FGF-2 stimulation. These studies suggest that TIMP-2 selectively inhibits the growth of endothelial cells through an MMP-independent mechanism; however, the signaling mechanism was not identified.

As described previously, TIMP-3 modulates apoptosis through its regulation of metalloproteinase activity. A direct inhibition of cell growth has not been definitively demonstrated, although a recent report suggests TIMP-3 may act additively with other cellular components to inhibit endothelial cell growth (vide infra) (74,75). Electroporation-mediated intramuscular injection of TIMP-4 expression plasmid results in a sustained increase in plasma TIMP-4 concentration, which inhibits the growth of G401 Wilm's tumor xenografts and is associated with cellular internalization of the exogenous TIMP-4; however, a signaling mechanism or cellular receptor mediating these effects has not been described (76).

Uncoupling TIMP-mediated Growth Inhibition from MMP Inhibition

Appending a single amino acid alanine to the amino-terminus of TIMP-2 to produce Ala +TIMP-2 yields a TIMP-2 mutant devoid of MMP-inhibitory activity (77). Ala+TIMP-2 does not inhibit MMP-2 or MT1-MMP activity or mediate MT1-MMP activation of pro-MMP-2 (77,78).

Both TIMP-2 and Ala+TIMP-2 bind to the surface of human A549 lung cancer cells with very high affinity (K_D= 147 pM) and this binding is not competed by the synthetic MMP inhibitor BB94 or TIMP-1 (78). Furthermore, the binding of Ala+TIMP-2 shows only partial competition by MT1-MMP blocking antibodies, and co-localization studies demonstrate that TIMP-2 and Ala+TIMP-2 binding is, at least in part, independent of MT1-MMP. These findings have been confirmed by research showing two distinct cell surface binding sites for TIMP-2 (79), that cell-surface binding of TIMP-2 is independent of the level of MT1-MMP expression (80), and that not all cell surface-bound TIMP-2 can be competed by synthetic MMP inhibitors (79).

Studies using Ala+TIMP-2 showed that TIMP-2 mediated growth suppression in vitro is independent of MMP inhibition (78) and demonstrated that TIMP-2 or Ala+TIMP-2 growth suppression is mediated by reduced tyrosine kinase growth factor receptor phosphorylation and activation. These changes correlate with an increase in the protein tyrosine phosphatase activity of Shp-1 (78).

More recent research has definitively demonstrated uncoupling of the MMP-inhibitory and anti-angiogenic activities of TIMP-2 (81). The ability of TIMP-2 to inhibit endothelial cell proliferation localizes to the C-terminal domain of TIMP-2, specifically to the C-terminal disulfide loop, referred to as loop 6 (81). This region is encoded by exon 5 of the TIMP-2 gene, and, as noted above, TIMP-2-deficient mice may produce alternative splice variants of the DDC8 gene that could express proteins containing a TIMP-2 loop 6 structure, which might retain anti-angiogenic activity (31).

Establishing a New Paradigm: Identification of TIMP Receptors

TIMP-2 binding to the endothelial cell surface and its ability to inhibit endothelial cell proliferation are independent of MMP inhibition, as demonstrated by Ala+TIMP-2 (82). The binding of TIMP-2 to the human microvascular endothelial cell surface is saturable and reversible with K_D= 900 pM. Competition binding studies demonstrated that TIMP-2 binding to the surface of human microvascular endothelial cells can be competed by anti-β1 and anti-α3 integrin blocking antibodies. The interaction of TIMP-2 with α3β1 cell surface integrin was confirmed by co-immunoprecipitation of α3β1 integrin using anti-TIMP-2 antibodies, and loss of TIMP-2 growth suppressive activity in β1-null fibroblasts. This was the first demonstration of TIMP interaction with a specific cell surface protein, identifying this integrin as a TIMP-2 receptor.

Subsequent studies demonstrated that TIMP-2 or Ala+TIMP-2 binding through α3β1 integrin results in G1 growth arrest and enhanced de novo expression of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ (83). Further studies revealed that TIMP-2 and Ala+TIMP-2 enhance the expression of the reversion-enhancing-cysteine-rich protein with Kazal motifs, also known as RECK (84). RECK is a membrane-associated inhibitor of MMPs (MMP-2, MMP-9, MT1-MMP) as well as ADAM-10 (85), and enhanced RECK expression is associated with inhibition of endothelial cell migration (84). TIMP-2 induction of RECK expression is mediated by inhibition of the activity of the Src tyrosine kinase, resulting in an altered pattern of phosphorylation of the adaptor protein paxillin at residues 31 and 118 (86). This altered phosphorylation results in inactivation of the small guanosine triphosphatase (GTPase) Rac1

and a reciprocal activation of the small GTPase Rap1, resulting in loss of a migratory phenotype. Collectively, these findings suggest that TIMP-2 inhibits angiogenesis by inducing endothelial cell differentiation to a quiescent state with G_1 cell cycle arrest, enhanced expression of RECK, and inhibition of cell migration, through a specific cell signaling paradigm (Fig. 1).

A recent report suggests that TIMP-2 binding to MT1-MMP can stimulate MCF7 breast cancer cell growth through activation of the MAPK pathway (87). This study showed that TIMP-2 and Ala+TIMP-2 bind to MT1-MMP, and that both elicit MAPK activation. Because Ala +TIMP-2 does not bind to the catalytic site of MT1-MMP, the authors proposed that the observed growth effect is mediated by TIMP-2 binding to other sites on MT1-MMP, possibly the hemopexin or hinge domain. Additional studies are needed to assess the contribution of this reported mechanism to tumor growth in vivo.

Identification of α3β1 integrin as the first TIMP signaling receptor stimulated the search for additional binding partners for other TIMP proteins. As previously noted, the high-affinity cell surface binding of TIMP-1 to myeloid leukemia cells and keratinocytes suggested the presence of cellular binding partners (38,42,88). Recently, a yeast two-hybrid approach was used to identify CD63, a member of the tetraspanin family, as a cell-binding partner for TIMP-1 in MCF10A human mammary epithelial cells (89). Confocal microscopy confirmed co-localization of TIMP-1 with CD63 and the β1 integrin subunit. TIMP-1 overexpression disrupted acinar formation in 3-dimensional matrigel cultures and inhibited MCF10A apoptosis as previously described. Downregulation of CD63 with shRNA resulted in reduced TIMP-1 binding, and restored acinar formation, cell polarization, and cell apoptosis. Furthermore, independent investigations have demonstrated that CD63 regulates PI3K, FAK, Src, and Akt signaling pathways that have been implicated in the anti-apoptotic activity of TIMP-1 (90). These investigators have also reported that CD63 interacts with TIMP-4, but not with TIMP-2 or TIMP-3, (91). As described above, TIMP-1 and TIMP-4 have both been reported to have pro-apoptotic functions, thus it is not surprising to find that they probably share cell surface receptors. Thus, TIMP-1 (and probably TIMP-4) also uses a previously undescribed signaling paradigm that is initiated by binding to CD63 (Fig. 2).

TIMP-3 also inhibits angiogenesis (92). Mutations in the *TIMP-3* gene are associated with Sorsby fundus dystrophy, a macular degenerative disease characterized by submacular neovascularization that occurs in the third and fourth decades of age. A recent report demonstrated that TIMP-3 bound to the vascular endothelial growth factor receptor-2, a principal receptor driving endothelial cell proliferation, and that this binding inhibited VEGF-A binding; thus TIMP-3 acts as a VEGF-A antagonist (75). This activity is completely independent of inhibition of MMP activity as demonstrated by the use of a TIMP-3 mutant, as well as the lack of an effect of TIMP-2 or synthetic MMP inhibitors. The IC_{50} for inhibition of VEGF binding to the VEGFR-2 was 3.3-4.5 μg/mL, which is approximately 10 fold higher than the IC_{50} for inhibition of endothelial cell proliferation and migration (IC_{50}= 0.3-3.0 μg/mL). Although this difference may be attributable to differences in the sensitivity of the read out of these assays, as the authors suggest, it is also possible that TIMP-3 inhibition of angiogenesis involves a dual mechanism. In either case, the 3.3-4.5 μg/mL TIMP-3 concentrations are very high compared with physiologic concentration of other TIMPs, and because TIMP-3 is matrix bound, it is difficult to assess the physiologic importance of these findings.

TIMP-3 may also interact with angiotensin II type 2 receptor to additively inhibit angiogenesis (74). This interaction was also identified using yeast two-hybrid screening. In the presence of both factors VEGF-induced growth of endothelial cells was additively inhibited, and the inhibition of Akt and endothelial nitric oxide synthetase phosphorylation was blocked;

unfortunately little detail about the involvement of specific signaling pathways is available at this early phase of these investigations.

TIMP Tissue Distribution: Clues to Functions?

A recent study using quantitative PCR demonstrated essentially ubiquitous and abundant expression of all four mammalian TIMPs in most mouse tissues (23). TIMP-2 was constitutively expressed in high abundance in all tissues of the adult mouse, with the expression of the other three TIMPs demonstrating more selective patterns of tissue distribution, as described above. Although TIMP concentrations may be substantial in some normal tissues, the expression of active MMP species in these normal tissues is usually very low or non-existent (23). TIMP-2 abundance is decreased in human cancers both through genetic polymorphisms associated with increased cancer risk and through epigenetic mechanisms involving hypermethylation of the TIMP-2 promoter (93-98). In addition, the increase in MMP active sites in the tumor microenvironment may further reduce the effective concentration of free, uncomplexed TIMP-2 (99,100). Thus the function of TIMP-2 is entirely dependent on the cellular context in which it is present.

The recent demonstration that TIMP-2 inhibits growth and promotes neurite differentiation in vitro via an α3β1 integrin dependent mechanism is consistent with this hypothesis (101). In this system, cell cycle arrest also occurs in G1 but appears to be mediated by enhanced expression of the cyclin-dependent kinase inhibitor $p21^{Cip}$, not $p27^{Kip1}$ as in the endothelial cell system. Furthermore, TIMP-2 expression correlates with the appearance of microfilament-positive neurons and live cell labeling experiments showed TIMP-2 association only with α3 integrin-positive cells (102). These observations suggest that up-regulation of TIMP-2 expression by proliferative stimuli implicates TIMP-2 expression in the transition from neuronal proliferation to promotion of terminal neuronal differentiation. This concept is further supported by the subsequent demonstration that TIMP-2 KO mice have motor deficits, and these motor deficits are the first demonstration of a significant phenotype for the TIMP-2 deficient mice (12).

In a similar fashion it has been proposed that TIMP-1 function is also influenced by cellular context, specifically in that MMPs, in particular MMP-9, may reduce the effective concentration of TIMP-1 and compete with TIMP-1 for binding to the cell surface receptor CD63 (91). In contrast to TIMP-2, TIMP-1 concentrations are increased in cancer patients, particularly in those with breast or colorectal carcinoma, and this increase is negatively associated with patient outcome (103-107). These recent studies have demonstrated the clinical utility of TIMP-1 as a biomarker and independent prognostic factor in breast, colorectal, and several hematological cancers. They are consistent with the anti-apoptotic activity of TIMP-1 mediated by CD63 binding.

The identification of cell surface receptors for TIMP family members is a first step in beginning to sort out the MMP-independent, cytokine-like functions of the TIMPs. Hopefully, this can serve as a starting point for the molecular dissection of signaling events associated with the various activities of these proteins and their function in both normal physiologic and pathologic processes. It is clear that the pleotropic activities of the TIMP family members are complex and depend upon subtle interactions with other extracellular components, as well as direct interactions with cell binding partners. Understanding these processes and how they are modulated during disease progression will be helpful in development of novel therapeutic interventions.

References

1. Khokha R, Waterhouse P, Yagel S, Lala PK, Overall CM, Norton G, Denhardt DT. Antisense RNA-induced reduction in murine TIMP levels confers oncogenicity on Swiss 3T3 cells. Science 1989;243:947–950. [PubMed: 2465572]
2. Albini A, Melchiori A, Santi L, Liotta LA, Brown PD, Stetler-Stevenson WG. Tumor cell invasion inhibited by TIMP-2. Journal of the National Cancer Institute 1991;83:775–779. [PubMed: 1645772]
3. Alvarez OA, Carmichael DF, DeClerck YA. Inhibition of collagenolytic activity and metastasis of tumor cells by a recombinant human tissue inhibitor of metalloproteinases. Journal of the National Cancer Institute 1990;82:589–595. [PubMed: 2156082]
4. DeClerck YA, Perez N, Shimada H, Boone TC, Langley KE, Taylor SM. Inhibition of invasion and metastasis in cells transfected with an inhibitor of metalloproteinases. Cancer Research 1992;52:701–708. [PubMed: 1732058]
5. Montgomery AM, Mueller BM, Reisfeld RA, Taylor SM, DeClerck YA. Effect of tissue inhibitor of the matrix metalloproteinases-2 expression on the growth and spontaneous metastasis of a human melanoma cell line. Cancer Research 1994;54:5467–5473. [PubMed: 7923181]
6. Brown PD. Matrix Metalloproteinase Inhibitors. Angiogenesis 1998;1:142–154. [PubMed: 14517381]
7. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002;295:2387–2392. [PubMed: 11923519]
8. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nature Reviews Cancer 2002;2:161–174.
9. Lambert E, Dasse E, Haye B, Petitfrere E. TIMPs as multifacial proteins. Critical Reviews in Oncology Hematology 2004;49:187–198.
10. Crocker SJ, Pagenstecher A, Campbell IL. The TIMPs tango with MMPs and more in the central nervous system. Journal of Neuroscience Research 2004;75:1–11. [PubMed: 14689443]
11. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochimica Biophysica Acta 2000;1477:267–283.
12. Jaworski DM, Soloway P, Caterina J, Falls WA. Tissue inhibitor of metalloproteinase-2(TIMP-2)-deficient mice display motor deficits. Journal of Neurobiology 2006;66:82–94. [PubMed: 16216006]
13. Zhang JS, Bai S, Tanase C, Nagase H, Sarras MP. The expression of tissue inhibitor of metalloproteinase 2 (TIMP-2) is required for normal development of zebrafish embryos. Development Genes and Evolution 2003;213:382–389. [PubMed: 12736828]
14. Gill SE, Pape MC, Khokha R, Watson AJ, Leco KJ. A null mutation for tissue inhibitor of metalloproteinases-3 (Timp-3) impairs murine bronchiole branching morphogenesis. Developmental Biology 2003;261:313–323. [PubMed: 14499643]
15. Fata JE, Leco KJ, Voura EB, Yu HY, Waterhouse P, Murphy G, Moorehead RA, Khokha R. Accelerated apoptosis in the Timp-3-deficient mammary gland. Journal of Clinical Investigation 2001;108:831–841. [PubMed: 11560952]see comment
16. Godenschwege TA, Pohar N, Buchner S, Buchner E. Inflated wings, tissue autolysis and early death in tissue inhibitor of metalloproteinases mutants of Drosophila. European Journal of Cell Biology 2000;79:495–501. [PubMed: 10961449]
17. Tuuttila A, Morgunova E, Bergmann U, Lindqvist Y, Maskos K, Fernandez-Catalan C, Bode W, Tryggvason K, Schneider G. Three-dimensional structure of human tissue inhibitor of metalloproteinases-2 at 2.1 A resolution. Journal of Molecular Biology 1998;284:1133–1140. [PubMed: 9837731]
18. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. Journal of Cell Science 2002;115:3719–3727. [PubMed: 12235282]
19. Amour A, Slocombe PM, Webster A, Butler M, Knight CG, Smith BJ, Stephens PE, Shelley C, Hutton M, Knauper V, Docherty AJ, Murphy G. TNF-alpha converting enzyme (TACE) is inhibited by TIMP-3. FEBS Letters 1998;435:39–44. [PubMed: 9755855]
20. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. Journal of Clinical Investigation 1999;103:1237–1241. [PubMed: 10225966]

21. Yu WH, Yu S, Meng Q, Brew K, Woessner JF Jr. TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix. Journal of Biological Chemistry 2000;275:31226–31232. [PubMed: 10900194]
22. Clark IM, Swingler TE, Sampieri CL, Edwards DR. The regulation of matrix metalloproteinases and their inhibitors. International Journal of Biochemistry & Cell Biology. 2008doi:10.1016/jbiocel.2007.12.006
23. Nuttall RK, Sampieri CL, Pennington CJ, Gill SE, Schultz GA, Edwards DR. Expression analysis of the entire MMP and TIMP gene families during mouse tissue development. FEBS Letters 2004;563:129–134. [PubMed: 15063736]
24. Blavier L, DeClerck YA. Tissue inhibitor of metalloproteinases-2 is expressed in the interstitial matrix in adult mouse organs and during embryonic development. Molecular Biology of the Cell 1997;8:1513–1527. [PubMed: 9285822]
25. Barasch J, Yang J, Qiao JZ, Tempst P, Erdjument-Bromage H, Leung W, Oliver JA. Tissue inhibitor of metalloproteinase-2 stimulates mesenchymal growth and regulates epithelial branching during morphogenesis of the rat metanephros. Journal of Clinical Investigation 1999;103:1299–1307. [PubMed: 10225973]
26. Derry JM, Barnard PJ. Physical linkage of the A-raf-1, properdin, synapsin I, and TIMP genes on the human and mouse X chromosomes. Genomics 1992;12:632–638. [PubMed: 1572636]
27. Dunham I, Shimizu N, Roe BA, Chissoe S, Hunt AR, Collins JE, Bruskiewich R, Beare DM, Clamp M, Smink LJ, Ainscough R, Almeida JP, Babbage A, Bagguley C, Bailey J, Barlow K, Bates KN, Beasley O, Bird CP, Blakey S, Bridgeman AM, Buck D, Burgess J, Burrill WD, O'Brien KP. The DNA sequence of human chromosome 22. Nature 1999;402:489–495. [PubMed: 10591208][see comment][erratum appears in Nature 2000 Apr 20;404(6780):904]
28. Pohar N, Godenschwege TA, Buchner E. Invertebrate tissue inhibitor of metalloproteinase: structure and nested gene organization within the synapsin locus is conserved from Drosophila to human. Genomics 1999;57:293–296. [PubMed: 10198170]
29. Hilfiker S, Pieribone VA, Czernik AJ, Kao HT, Augustine GJ, Greengard P. Synapsins as regulators of neurotransmitter release. Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences 1999;354:269–279.
30. Dean G, Young DA, Edwards DR, Clark IM. The human tissue inhibitor of metalloproteinases (TIMP)-1 gene contains repressive elements within the promoter and intron 1. Journal of Biological Chemistry 2000;275:32664–32671. [PubMed: 11032844]
31. Jaworski DM, Beem-Miller M, Lluri G, Barrantes-Reynolds R. Potential regulatory relationship between the nested gene DDC8 and its host gene tissue inhibitor of metalloproteinase-2. Physiological Genomics 2007;28:168–178. [PubMed: 16985004]
32. Caterina JJ, Yamada S, Caterina NCM, Longenecker G, Holmback K, Shi J, Yermovsky AE, Engler JA, Birkedal-Hansen H. Inactivating mutation of the mouse tissue inhibitor of metalloproteinases-2 (Timp-2) gene alters proMMP-2 activation. Journal of Biological Chemistry 2000;275:26416–26422. [PubMed: 10827176]
33. Wang Z, Juttermann R, Soloway PD. TIMP-2 is required for efficient activation of proMMP-2 in vivo. Journal of Biological Chemistry 2000;275:26411–26415. [PubMed: 10827175]
34. Kopitz C, Gerg M, Bandapalli OR, Ister D, Pennington CJ, Hauser S, Flechsig C, Krell HW, Antolovic D, Brew K, Nagase H, Stangl M, von Weyhern CW, Brucher BL, Brand K, Coussens LM, Edwards DR, Kruger A. Tissue inhibitor of metalloproteinases-1 promotes liver metastasis by induction of hepatocyte growth factor signaling. Cancer Research 2007;67:8615–8623. [PubMed: 17875701]
35. Mohammed FF, Pennington CJ, Kassiri Z, Rubin JS, Soloway PD, Ruther U, Edwards DR, Khokha R. Metalloproteinase inhibitor TIMP-1 affects hepatocyte cell cycle via HGF activation in murine liver regeneration. Hepatology 2005;41:857–867. [PubMed: 15726641]
36. Martin DC, Fowlkes JL, Babic B, Khokha R. Insulin-like growth factor II signaling in neoplastic proliferation is blocked by transgenic expression of the metalloproteinase inhibitor TIMP-1. Journal of Cell Biology 1999;146:881–892. [PubMed: 10459021]
37. Docherty AJ, Lyons A, Smith BJ, Wright EM, Stephens PE, Harris TJ, Murphy G, Reynolds JJ. Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity. Nature 1985;318:66–69. [PubMed: 3903517]

38. Gasson JC, Bersch N, Golde DW. Characterization of purified human erythroid-potentiating activity. Progress in Clinical & Biological Research 1985;184:95–104. [PubMed: 3931094]
39. Stetler-Stevenson WG, Bersch N, Golde DW. Tissue inhibitor of metalloproteinase-2 (TIMP-2) has erythroid-potentiating activity. FEBS Letters 1992;296:231–234. [PubMed: 1370805]
40. Larsen MB, Stephens RW, Brunner N, Nielsen HJ, Engelholm LH, Christensen IJ, Stetler-Stevenson WG, Hoyer-Hansen G. Quantification of tissue inhibitor of metalloproteinases 2 in plasma from healthy donors and cancer patients. Scandinavian Journal of Immunology 2005;61:449–460. [PubMed: 15882437]
41. Pasieka Z, Stepien H, Czyz W, Pomorski L, Kuzdak K. Concentration of metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 in the serum of patients with benign and malignant thyroid tumours treated surgically. Endocrine Regulations 2004;38:57–63. [PubMed: 15497929]
42. Bertaux B, Hornebeck W, Eisen AZ, Dubertret L. Growth stimulation of human keratinocytes by tissue inhibitor of metalloproteinases. Journal of Investigative Dermatology 1991;97:679–685. [PubMed: 1940438]
43. Corcoran ML, Stetler-Stevenson WG. Tissue inhibitor of metalloproteinase-2 stimulates fibroblast proliferation via a cAMP-dependent mechanism. Journal of Biological Chemistry 1995;270:13453–13459. [PubMed: 7768948]
44. Hayakawa T, Yamashita K, Ohuchi E, Shinagawa A. Cell growth-promoting activity of tissue inhibitor of metalloproteinases-2 (timp-2). Journal Of Cell Science 1994;107:2373–2379. [PubMed: 7844157]
45. Hayakawa T, Yamashita K, Tanzawa K, Uchijima E, Iwata K. Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. A possible new growth factor in serum. FEBS Letters 1992;298:29–32. [PubMed: 1544418]
46. Nemeth JA, Goolsby CL. TIMP-2, a growth-stimulatory protein from SV40-transformed human fibroblasts. Experimental Cell Research 1993;207:376–382. [PubMed: 8393803]
47. Nemeth JA, Rafe A, Steiner M, Goolsby CL. TIMP-2 growth-stimulatory activity: a concentration- and cell type-specific response in the presence of insulin. Experimental Cell Research 1996;224:110–115. [PubMed: 8612674]
48. Chesler L, Golde DW, Bersch N, Johnson MD. Metalloproteinase inhibition and erythroid potentiation are independent activities of tissue inhibitor of metalloproteinases-1. Blood 1995;86:4506–4515. [PubMed: 8541540]
49. Emmert-Buck MR, Emonard HP, Corcoran ML, Krutzsch HC, Foidart JM, Stetler-Stevenson WG. Cell surface binding of TIMP-2 and pro-MMP-2/TIMP-2 complex. FEBS Letters 1995;364:28–32. [PubMed: 7750537]
50. Yamashita K, Suzuki M, Iwata H, Koike T, Hamaguchi M, Shinagawa A, Noguchi T, Hayakawa T. Tyrosine phosphorylation is crucial for growth signaling by tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2). FEBS Letters 1996;396:103–107. [PubMed: 8906876]
51. Wang T, Yamashita K, Iwata K, Hayakawa T. Both tissue inhibitors of metalloproteinases-1 (TIMP-1) and TIMP-2 activate Ras but through different pathways. Biochemical & Biophysical Research Communications 2002;296:201–205. [PubMed: 12147251]
52. Yang TT, Hawkes SP. Role of the 21-kDa protein TIMP-3 in oncogenic transformation of cultured chicken embryo fibroblasts. Proceedings of the National Academy of Sciences of the United States of America 1992;89:10676–10680. [PubMed: 1438264]
53. Guedez L, Stetler-Stevenson WG, Wolff L, Wang J, Fukushima P, Mansoor A, Stetler-Stevenson M. In vitro suppression of programmed cell death of B cells by tissue inhibitor of metalloproteinases-1. Journal of Clinical Investigation 1998;102:2002–2010. [PubMed: 9835626]
54. Guedez L, Mansoor A, Birkedal-Hansen B, Lim MS, Fukushima P, Venzon D, Stetler-Stevenson WG, Stetler-Stevenson M. Tissue inhibitor of metalloproteinases 1 regulation of interleukin-10 in B-cell differentiation and lymphomagenesis. Blood 2001;97:1796–1802. [PubMed: 11238122]
55. Guedez L, McMarlin AJ, Kingma DW, Bennett TA, Stetler-Stevenson M, Stetler-Stevenson WG. Tissue inhibitor of metalloproteinase-1 alters the tumorigenicity of Burkitt's lymphoma via divergent effects on tumor growth and angiogenesis. American Journal of Pathology 2001;158:1207–1215. [PubMed: 11290537]see comment

56. Li G, Fridman R, Kim HR. Tissue inhibitor of metalloproteinase-1 inhibits apoptosis of human breast epithelial cells. Cancer Research 1999;59:6267–6275. [PubMed: 10626822]
57. Liu XW, Bernardo MM, Fridman R, Kim HR. Tissue inhibitor of metalloproteinase-1 protects human breast epithelial cells against intrinsic apoptotic cell death via the focal adhesion kinase/phosphatidylinositol 3-kinase and MAPK signaling pathway. Journal of Biological Chemistry 2003;278:40364–40372. [PubMed: 12904305]
58. Liu XW, Taube ME, Jung KK, Dong Z, Lee YJ, Roshy S, Sloane BF, Fridman R, Kim HR. Tissue inhibitor of metalloproteinase-1 protects human breast epithelial cells from extrinsic cell death: a potential oncogenic activity of tissue inhibitor of metalloproteinase-1. Cancer Research 2005;65:898–906. [PubMed: 15705888]
59. Han X, Sun Y, Scott S, Bleich D. Tissue inhibitor of metalloproteinase-1 prevents cytokine-mediated dysfunction and cytotoxicity in pancreatic islets and beta-cells. Diabetes 2001;50:1047–1055. [PubMed: 11334407]
60. Singla DK, McDonald DE. Factors released from embryonic stem cells inhibit apoptosis of H9c2 cells. American Journal of Physiology - Heart & Circulatory Physiology 2007;293:H1590–1595. [PubMed: 17545477]
61. Valente P, Fassina G, Melchiori A, Masiello L, Cilli M, Vacca A, Onisto M, Santi L, Stetler-Stevenson WG, Albini A. TIMP-2 over-expression reduces invasion and angiogenesis and protects B16F10 melanoma cells from apoptosis. International Journal of Cancer 1998;75:246–253.erratum appears in Int J Cancer 1999 Jan 29;80(3):485
62. Lim MS, Guedez L, Stetler-Stevenson WG, Stetler-Stevenson M. Tissue inhibitor of metalloproteinase-2 induces apoptosis in human T lymphocytes. Annals of the New York Academy of Sciences 1999;878:522–523. [PubMed: 10415761]
63. Baker AH, Zaltsman AB, George SJ, Newby AC. Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. TIMP-3 promotes apoptosis. Journal of Clinical Investigation 1998;101:1478–1487. [PubMed: 9502791]
64. Ahonen M, Poukkula M, Baker AH, Kashiwagi M, Nagase H, Eriksson JE, Kahari VM. Tissue inhibitor of metalloproteinases-3 induces apoptosis in melanoma cells by stabilization of death receptors. Oncogene 2003;22:2121–2134. [PubMed: 12687014]
65. Baker AH, George SJ, Zaltsman AB, Murphy G, Newby AC. Inhibition of invasion and induction of apoptotic cell death of cancer cell lines by overexpression of TIMP-3. British Journal of Cancer 1999;79:1347–1355. [PubMed: 10188875]
66. Mohammed FF, Smookler DS, Taylor SEM, Fingleton B, Kassiri Z, Sanchez OH, English JL, Matrisian LM, Au B, Yeh WC, Khokha R. Abnormal TNF activity in Timp3(-/-) mice leads to chronic hepatic inflammation and failure of liver regeneration. Nature Genetics 2004;36:969–977. [PubMed: 15322543]
67. Bian J, Wang Y, Smith MR, Kim H, Jacobs C, Jackman J, Kung HF, Colburn NH, Sun Y. Suppression of in vivo tumor growth and induction of suspension cell death by tissue inhibitor of metalloproteinases (TIMP)-3. Carcinogenesis 1996;17:1805–1811. [PubMed: 8824499]
68. Smith MR, Kung H, Durum SK, Colburn NH, Sun Y. TIMP-3 induces cell death by stabilizing TNF-alpha receptors on the surface of human colon carcinoma cells. Cytokine 1997;9:770–780. [PubMed: 9344510]
69. Jiang Y, Wang M, Celiker MY, Liu YE, Sang QX, Goldberg ID, Shi YE. Stimulation of mammary tumorigenesis by systemic tissue inhibitor of matrix metalloproteinase 4 gene delivery. Cancer Research 2001;61:2365–2370. [PubMed: 11289097]
70. Fata JE, Leco KJ, Moorehead RA, Martin DC, Khokha R. Timp-1 is important for epithelial proliferation and branching morphogenesis during mouse mammary development. Developmental Biology 1999;211:238–254. [PubMed: 10395785]
71. Taube ME, Liu XW, Fridman R, Kim HR. TIMP-1 regulation of cell cycle in human breast epithelial cells via stabilization of p27(KIP1) protein. Oncogene 2006;25:3041–3048. [PubMed: 16407831]
72. Moses MA, Sudhalter J, Langer R. Identification of an inhibitor of neovascularization from cartilage. Science 1990;248:1408–1410. [PubMed: 1694043]

73. Murphy AN, Unsworth EJ, Stetler-Stevenson WG. Tissue inhibitor of metalloproteinases-2 inhibits bFGF-induced human microvascular endothelial cell proliferation. Journal of Cellular Physiology 1993;157:351–358. [PubMed: 7693724]
74. Kang KH, Park SY, Rho SB, Lee JH. Tissue inhibitor of metalloproteinases-3 interacts with angiogtensin II type receptor and additively inhibits angiogensis. Cardiovascular Research. Apr 2;2008 2008, Epub ahead of print.
75. Qi JH, Ebrahem Q, Moore N, Murphy G, Claesson-Welsh L, Bond M, Baker A, Anand-Apte B. A novel function for tissue inhibitor of metalloproteinases-3 (TIMP3): inhibition of angiogenesis by blockage of VEGF binding to VEGF receptor-2. Nature Medicine 2003;9:407–415.
76. Celiker MY, Wang M, Atsidaftos E, Liu X, Liu YE, Jiang Y, Valderrama E, Goldberg ID, Shi YE. Inhibition of Wilms' tumor growth by intramuscular administration of tissue inhibitor of metalloproteinases-4 plasmid DNA. Oncogene 2001;20:4337–4343. [PubMed: 11466614]
77. Wingfield PT, Sax JK, Stahl SJ, Kaufman J, Palmer I, Chung V, Corcoran ML, Kleiner DE, Stetler-Stevenson WG. Biophysical and functional characterization of full-length, recombinant human tissue inhibitor of metalloproteinases-2 (TIMP-2) produced in Escherichia coli. Comparison of wild type and amino-terminal alanine appended variant with implications for the mechanism of TIMP functions. Journal of Biological Chemistry 1999;274:21362–21368. [PubMed: 10409697]
78. Hoegy SE, Oh HR, Corcoran ML, Stetler-Stevenson WG. Tissue inhibitor of metalloproteinases-2 (TIMP-2) suppresses TKR-growth factor signaling independent of metalloproteinase inhibition. Journal of Biological Chemistry 2001;276:3203–3214. [PubMed: 11042184]
79. Itoh Y, Ito A, Iwata K, Tanzawa K, Mori Y, Nagase H. Plasma membrane-bound tissue inhibitor of metalloproteinases (TIMP)-2 specifically inhibits matrix metalloproteinase 2 (gelatinase A) activated on the cell surface. Journal of Biological Chemistry 1998;273:24360–24367. [PubMed: 9733724]
80. Bernardo MM, Fridman R. TIMP-2 (tissue inhibitor of metalloproteinase-2) regulates MMP-2 (matrix metalloproteinase-2) activity in the extracellular environment after pro-MMP-2 activation by MT1 (membrane type 1)-MMP. Biochemical Journal 2003;374:739–745. [PubMed: 12755684]
81. Fernandez CA, Butterfield C, Jackson G, Moses MA. Structural and functional uncoupling of the enzymatic and angiogenic inhibitory activities of tissue inhibitor of metalloproteinase-2 (TIMP-2): loop 6 is a novel angiogenesis inhibitor. Journal of Biological Chemistry 2003;278:40989–40995. [PubMed: 12900406]
82. Seo DW, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R, Wei BY, Stetler-Stevenson WG. TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. Cell 2003;114:171–180. [PubMed: 12887919]
83. Seo DW, Li H, Qu CK, Kim YS, Diaz T, Wei B, Han JW, Stetler Stevenson WG. Shp-1 mediates the antiproliferative activity of tissue inhibitor of metalloproteinase-2 in human microvascular endothelial cells. Journal of Biological Chemistry 2006;281:3711–3721. [PubMed: 16326706]
84. Oh J, Seo DW, Diaz T, Wei B, Ward Y, Ray JM, Morioka Y, Shi S, Kitayama H, Takahashi C, Noda M, Stetler-Stevenson WG. Tissue inhibitors of metalloproteinase 2 inhibits endothelial cell migration through increased expression of RECK. Cancer Research 2004;64:9062–9069. [PubMed: 15604273]
85. Muraguchi T, Takegami Y, Ohtsuka T, Kitajima S, Chandana EP, Omura A, Miki T, Takahashi R, Matsumoto N, Ludwig A, Noda M, Takahashi C. RECK modulates Notch signaling during cortical neurogenesis by regulating ADAM10 activity. Nature Neuroscience 2007;10:838–845.
86. Oh J, Diaz T, Wei B, Chang H, Noda M, Stetler-Stevenson WG. TIMP-2 upregulates RECK expression via dephosphorylation of paxillin tyrosine residues 31 and 118. Oncogene 2006;25:4230–4234. [PubMed: 16491114]
87. D'Allesio S, Ferrari G, Cinnante K, Scheerer W, Galloway AC, Roses DF, Rozanov DV, Remacle AG, Oh E-S, Shiryaev SA, Strongin AY, Pintucci G, Mignatti P. Tissue Inhibitor of Metalloproteinases-2 Binding to Membrane -type 1 Matrix Metalloproteinases Induces MAPK Activation and Cell Growth by a Non-proteolytic Mechanism. Journal of Biological Chemistry 2008;283:87–99. [PubMed: 17991754]
88. Zhao WQ, Li H, Yamashita K, Guo XK, Hoshino T, Yoshida S, Shinya T, Hayakawa T. Cell cycle-associated accumulation of tissue inhibitor of metalloproteinases-1 (TIMP-1) in the nuclei of human gingival fibroblasts. Journal of Cell Science 1998;111:1147–1153. [PubMed: 9547291]

89. Jung KK, Liu XW, Chirco R, Fridman R, Kim HR. Identification of CD63 as a tissue inhibitor of metalloproteinase-1 interacting cell surface protein. EMBO Journal 2006;25:3934–3942. [PubMed: 16917503]

90. Berditchevski F, Odintsova E. Characterization of integrin-tetraspanin adhesion complexes: role of tetraspanins in integrin signaling. Journal of Cell Biology 1999;146:477–492. [PubMed: 10427099]

91. Chirco R, Liu XW, Jung KK, Kim HR. Novel functions of TIMPs in cell signaling. Cancer & Metastasis Reviews 2006;25:99–113. [PubMed: 16680576]

92. Anand-Apte B, Pepper MS, Voest E, Montesano R, Olsen B, Murphy G, Apte SS, Zetter B. Inhibition of angiogenesis by tissue inhibitor of metalloproteinase-3. Investigative Ophthalmology & Visual Science 1997;38:817–823. [PubMed: 9112976]see comment

93. Galm O, Suzuki H, Akiyama Y, Esteller M, Brock MV, Osieka R, Baylin SB, Herman JG. Inactivation of the tissue inhibitor of metalloproteinases-2 gene by promoter hypermethylation in lymphoid malignancies. Oncogene 2005;24:4799–4805. [PubMed: 15870703]

94. Kubben FJ, Sier CF, Meijer MJ, van den Berg M, van der Reijden JJ, Griffioen G, van de Velde CJ, Lamers CB, Verspaget HW. Clinical impact of MMP and TIMP gene polymorphisms in gastric cancer. British Journal of Cancer 2006;95:744–751. [PubMed: 16940985]

95. Pulukuri SM, Patibandla S, Patel J, Estes N, Rao JS. Epigenetic inactivation of the tissue inhibitor of metalloproteinase-2 (TIMP-2) gene in human prostate tumors. Oncogene 2007;26:5229–5237. [PubMed: 17325663]

96. Ivanova T, Vinokurova S, Petrenko A, Eshilev E, Solovyova N, Kisseljov F, Kisseljova N. Frequent hypermethylation of 5′ flanking region of TIMP-2 gene in cervical cancer. International Journal of Cancer 2004;108:882–886.

97. Vairaktaris E, Yapijakis C, Yiannopoulos A, Vassiliou S, Serefoglou Z, Vylliotis A, Nkenke E, Derka S, Critselis E, Avgoustidis D, Neukam FW, Patsouris E. Strong association of the tissue inhibitor of metalloproteinase-2 polymorphism with an increased risk of oral squamous cell carcinoma in Europeans. Oncol Rep 2007;17:963–968. [PubMed: 17342343]

98. Yang L, Gu H-J, Zhu H-J, Sun Q-M, Cong R-H, Zhou B, Tang N-P, Wang B. Tissue inhibitor of metalloproteinases-2 G-418C polymorphism is associated with an increased risk of gastric cancer in a Chinese Population. Eur. J. Surg. Oncol 2008;34:636–641. [PubMed: 17954022]

99. Stetler-Stevenson WG. The tumor microenvironment: regulation by MMP-independent effects of tissue inhibitor of metalloproteinases-2. Cancer Metastasis Rev 2008;27:57–66. [PubMed: 18058195]

100. Stetler-Stevenson WG, Seo DW. TIMP-2: an endogenous inhibitor of angiogenesis. Trends in Molecular Medicine 2005;11:97–103. [PubMed: 15760767]

101. Perez-Martinez L, Jaworski DM. Tissue inhibitor of metalloproteinase-2 promotes neuronal differentiation by acting as an anti-mitogenic signal. Journal of Neuroscience 2005;25:4917–4929. [PubMed: 15901773]

102. Jaworski DM, Perez-Martinez L. Tissue inhibitor of metalloproteinase-2 (TIMP-2) expression is regulated by multiple neural differentiation signals. Journal of Neurochemistry 2006;98:234–247. [PubMed: 16805810]

103. Holten-Andersen M, Christensen IJ, Nilbert M, Bendahl PO, Nielsen HJ, Brunner N, Fernebro E, E. O.-R. a. B. Group. Association between preoperative plasma levels of tissue inhibitor of metalloproteinases 1 and rectal cancer patient survival. a validation study. European Journal of Cancer 2004;40:64–72. [PubMed: 14687791]

104. Holten-Andersen MN, Fenger C, Nielsen HJ, Rasmussen AS, Christensen IJ, Brunner N, Kronborg O. Plasma TIMP-1 in patients with colorectal adenomas: a prospective study. European Journal of Cancer 2004;40:2159–2164. [PubMed: 15341992]

105. McCarthy K, Maguire T, McGreal G, McDermott E, O'Higgins N, Duffy MJ. High levels of tissue inhibitor of metalloproteinase-1 predict poor outcome in patients with breast cancer. International Journal of Cancer 1999;84:44–48.

106. Nakopoulou L, Giannopoulou I, Stefanaki K, Panayotopoulou E, Tsirmpa I, Alexandrou P, Mavrommatis J, Katsarou S, Davaris P. Enhanced mRNA expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) in breast carcinomas is correlated with adverse prognosis. Journal of Pathology 2002;197:307–313. [PubMed: 12115876]

107. Schrohl AS, Christensen IJ, Pedersen AN, Jensen V, Mouridsen H, Murphy G, Foekens JA, Brunner N, Holten-Andersen MN. Tumor tissue concentrations of the proteinase inhibitors tissue inhibitor of metalloproteinases-1 (TIMP-1) and plasminogen activator inhibitor type 1 (PAI-1) are complementary in determining prognosis in primary breast cancer. Molecular & Cellular Proteomics 2003;2:164–172. [PubMed: 12672830]

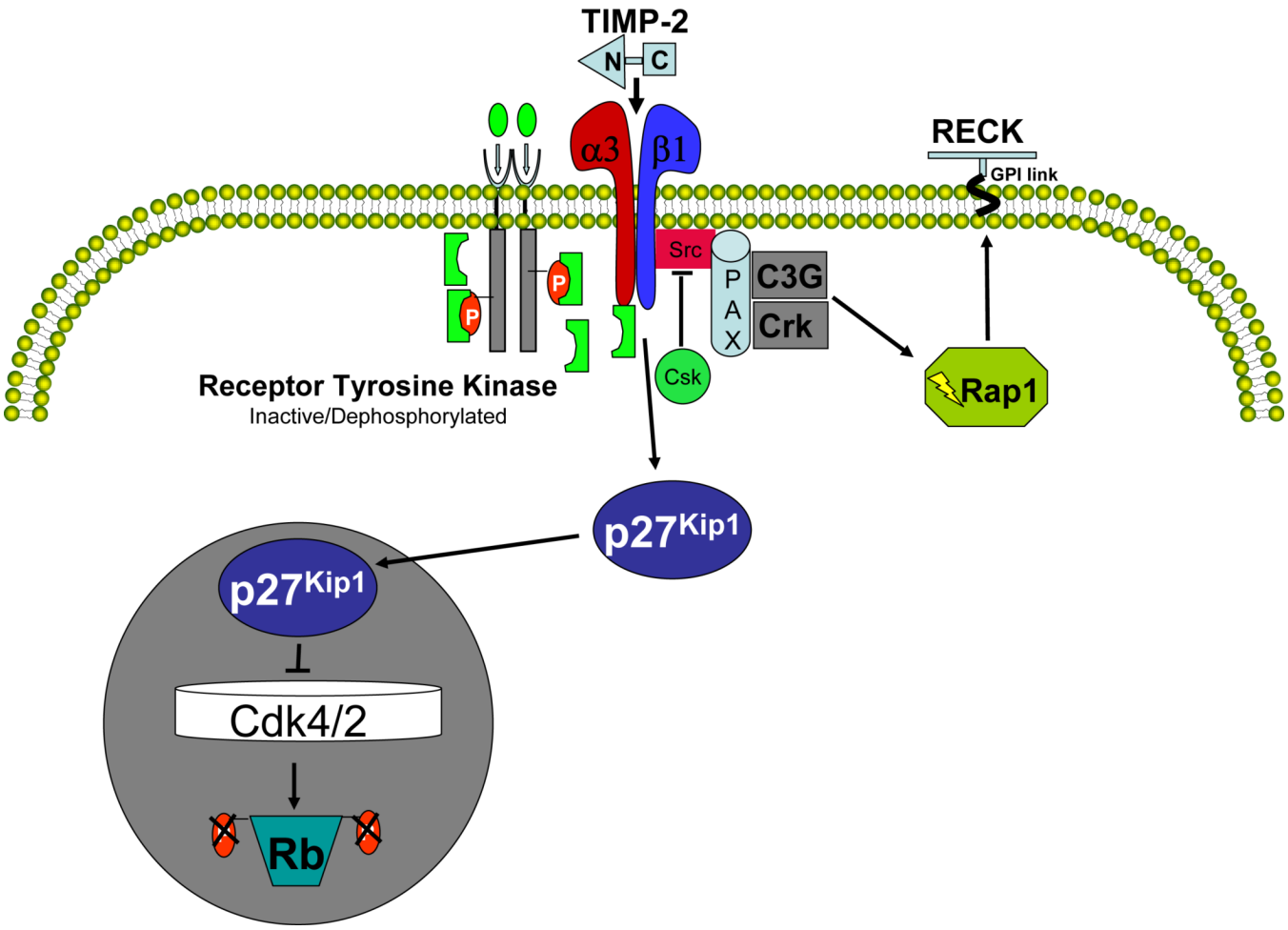

Fig. 1.

Multiple Pathways of TIMP-2-α3β1 signaling. TIMP-2 binding to α3β1 integrin initiates receptor tyrosine kinase inactivation through the action of the protein tyrosine phosphatase, Shp-1, which binds to SH2 sites created by phosphorylation of specific tyrosine residues. Cell cycle arrest is mediated by the de novo synthesis and nuclear translocation (gray circle) of $p27^{Kip1}$, which down-regulates activity of the cyclin-dependent kinases 4 and 2 (Cdk4/2). This results in hypophosphorylation of pRb and cell cycle arrest in G_1. Paxillin (PAX) is a scaffolding protein associated with integrin receptors that is phosphorylated downstream of activated Src. TIMP-2 binding to α3β1 activates c-terminal Src kinase (Csk) which is responsible for inactivation of Src kinase activity. TIMP-2 also mediates activation of the small GTPase Rap1 through a mechanism involving altered association of guanidine exchange factors (C3G and Crk) with PAX), ultimately resulting in increased abundance of RECK, a cell surface associate metalloproteinase inhibitor. Enhanced RECK expression at both the mRNA and protein level is associated with inhibition of cell migration. This suggests that, in addition to inhibiting cellular proliferation, TIMP-2 also seems to promote expression of cellular differentiation markers, i.e. RECK.

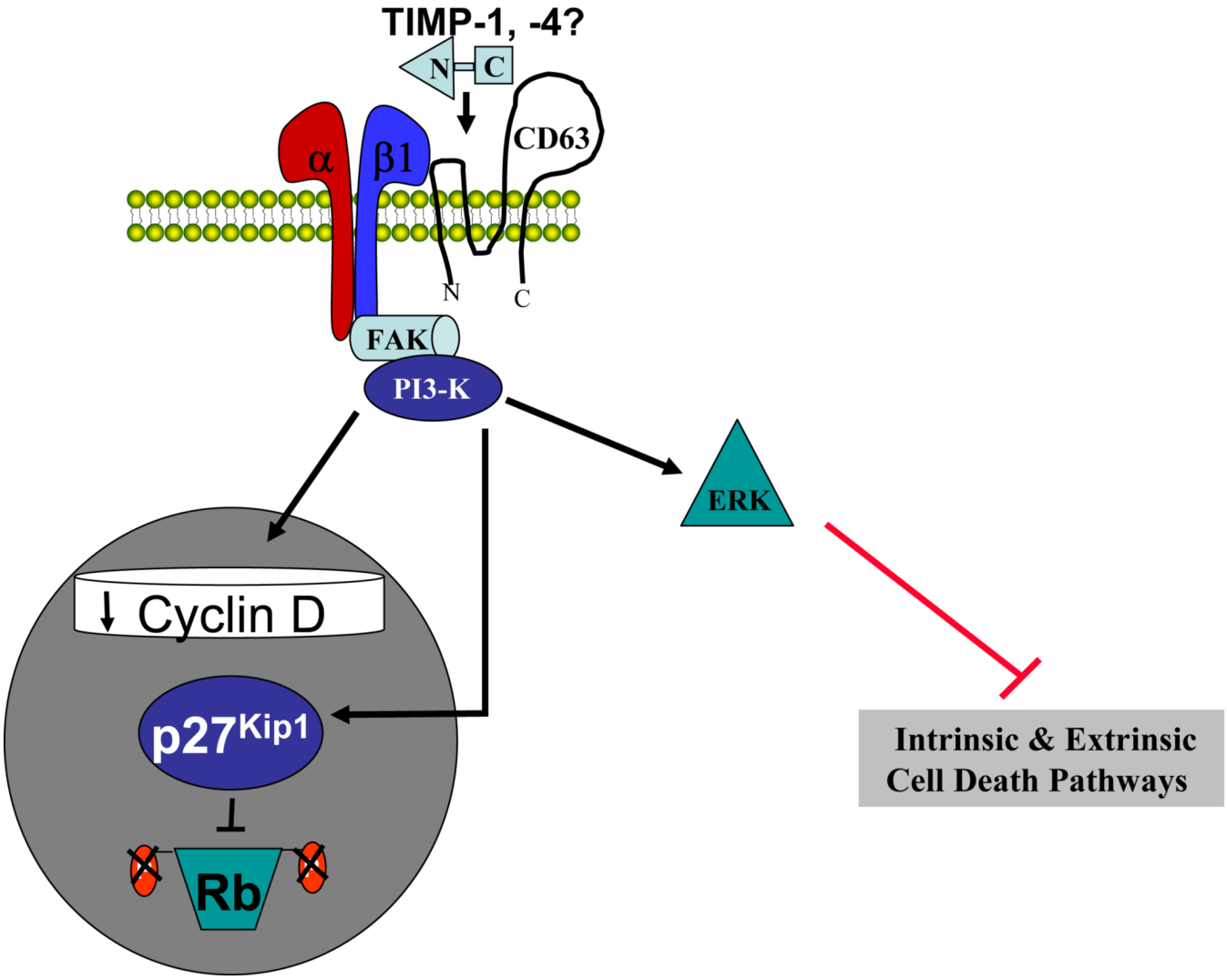

Fig. 2.

Multiple Pathways of TIMP-1-CD63 signaling. TIMP-1 and TIMP-4 interact with the tetraspanin CD63, which associates with the β1 subunit of integrin receptors. Downstream of CD63, TIMP-1 inhibits both the intrinsic and extrinsic cell death pathways through activation of the FAK-PI3K pathway. These effects are mediated by activation of the extracellular regulated kinase (ERK). In addition, TIMP-1 inhibits cell growth through a mechanism similar to that of TIMP-2, involving suppression of cyclin D_1 and up regulation of $p27^{Kip1}$. The net effect is hypophosphorylation of pRB and cell cycle arrest in the G_1 phase of the cell cycle.

Table 1

Metalloproteinase-independent biological activity of TIMPs

Inhibitor	Cellular Function	Putative Pathways or Molecular Effectors	Cell Types	References
TIMP-1	Promotion of Cell Growth	Ras, MAPK	Keratinocytes, fibroblasts, cancer cell lines, Erythroid precursors	(38,42,45,48)
TIMP-2	Promotion of Cell Growth	cAMP, PKA, Ras, MT1-MMP, MAPK	Fibroblasts, cancer cell lines, erythroid precursors, ureteric bud epithelia, mesangial mesenchyme,	(25,43,44,73,87)
TIMP-1	Inhibition of apoptosis (and anoikis)	CD63,Bcl-X_L, IκBα, FAK, PI3K	Burkitt's lymphoma, breast cancer cell lines, rat pancreatic islet cells, cardiomyocytes	(53,54,56-60,89, 91)
TIMP-1	Inhibition of cell growth	Cyclin D (downregulation), $p27^{Kip1}$	Breast cancer cell line	(71)
TIMP-2	Inhibition of cell growth	α3β1, Shp-1, $p27^{Kip1}$, $p21^{Cip1}$, Paxillin, FAK, C3G, Crk, Csk, Rap1, RECK	Endothelial cells, fibroblasts, neurons, tumor cell lines	(72,73,78,81-83, 86,101,102)
TIMP-3	Inhibition of cell growth	VEGFR2, Angiotensin II type 2 receptor	Endothelial cells	(74,75)
TIMP-4	Inhibition of cell growth		Wilm's tumor xenograft	(76)
TIMP-4	Inhibition of apoptosis	CD63, Bcl-X_L, Bcl-2,	Breast cancer cell lines	(69,91)