Tissue Inhibitor of Metalloproteinase 1 and Apoptosis Regulation

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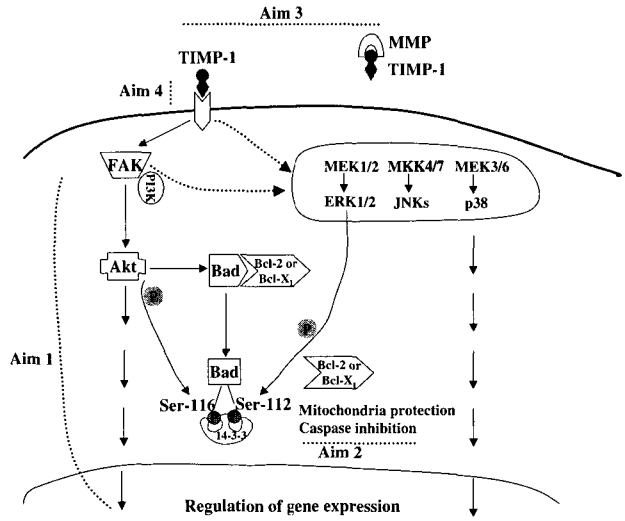
Tissue inhibitor of metalloproteinase (TIMP-1) is a natural protease inhibitor of matrix metalloproteinases (MMPs). Previous studies in our laboratory examined the role of bcl-2 in apoptosis using breast epithelial cells genetically engineered to overexpress bcl-2. Since ECM is critical for apoptosis regulation, particularly in breast epithelium, we hypothesized that the death-suppressing activity of bcl-2 is partly mediated by a complex regulation of matrix-degrading enzymes and/or their inhibitors, the TIMPs. From this study, we made the following novel observations: **(i)** Bcl-2 upregulates TIMP-1 expression in human breast epithelial cells (MCF10A ["normal" BE cell line], MCF10AneoT.TG3B [preneoplastic BE cell line] and MCF-7 [malignant breast carcinoma cell line]) **(ii)** TIMP-1, like bcl-2, is a potent inhibitor of apoptosis induced by a variety of apoptotic stimuli including hydrogen peroxide, radiation and adriamycin. Furthermore, both exogenous and endogenous expression of TIMP-1 has anti-apoptotic activity. **(iii)** TIMP-1 inhibits apoptosis independent of its effect on proliferation. **(iv)** TIMP-1 inhibits apoptosis in the absence of bcl-2 overexpression. **(v)** TIMP-1 inhibits a classical apoptotic pathway mediated by caspases, independent of its ability to stabilize cell-substrate or cell-cell interactions. **(vi)** Overexpression of TIMP-1 is associated with constitutive activation of focal adhesion kinase (FAK) in an anchorage-independent manner, suggesting that TIMP-1 regulates apoptosis through constitutive activation of cell survival signaling pathways. We further demonstrated that **(vii)** TIMP-1 overexpression or exposure of human breast epithelial cells to TIMP-1 activates cell survival signaling pathways involving focal adhesion kinase, PI 3-kinase and ERKs, and that **(viii)** inhibition of PI 3 kinase or ERKs abolishes TIMP-1 inhibition of apoptosis. **(ix)** TIMP-1-activated cell survival signaling downregulates caspase-mediated classical apoptotic pathways induced by a variety of stimuli including anoikis, staurosporine exposure and growth factor withdrawal. Consistently, **(x)** downregulation of TIMP-1 expression greatly enhances apoptotic cell death. In a previous study, substitution of the second amino acid residue threonine for glycine in TIMP-1, which confers selective MMP inhibition, was shown to obliterate its anti-apoptotic activity in activated hepatic stellate cells suggesting that the anti-apoptotic activity of TIMP-1 is dependent on MMP-inhibition. However, we show that **(xi)** TIMP-1 with the same mutation (T2G) inhibits apoptosis of human breast epithelial cells. **(xii)**

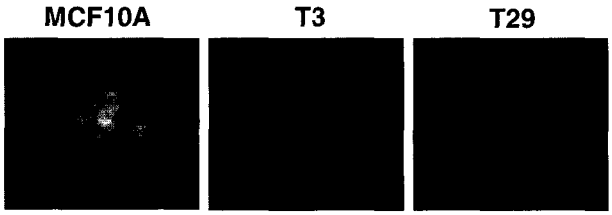
Neither TIMP-2 nor a synthetic MMP inhibitor protects breast epithelial cells from intrinsic apoptotic cell death. Furthermore, TIMP-1 enhances cell survival signaling pathways in the presence of the synthetic MMP inhibitor, suggesting TIMP-1 regulation of breast epithelial cell apoptosis is independent of its inhibition of MMPs.

Recently, we have examined whether TIMP-1 also regulates the extrinsic apoptosis. This is of particular interest considering the previous findings that TIMP-3 enhances extrinsic cell death by inhibiting the shedding of the cell surface death receptors mediated by tumor necrosis factor-α converting enzymes (TACE/ADAM-17). Surprisingly, we found that **(xiii)** TIMP-1 effectively protects breast epithelial cells from extrinsic apoptotic cell death induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, Apo-2L). The levels of endogenous TIMP-1 expression inversely correlates with TRAIL-induced cytotoxicity, and recombinant TIMP-1 protein effectively protects human breast epithelial cells from TRAIL-induced apoptosis, demonstrating opposite roles of TIMP-1 and TIMP-3 for the regulation of the extrinsic cell death pathway. We also showed that **(xiv)** TIMP-1 inhibition of TRAIL-induced apoptosis is independent of its MMP or ADAM inhibition, and not related to its ability to stabilize active or decoy death receptors. Importantly, **(xv)** downregulation of FAK expression using a small interfering RNA (siRNA) abolished TIMP-1 protection of human breast epithelial cells against TRAIL-induced extrinsic cell death. Taken together, our study unveils a novel function of TIMP-1 for the regulation of in trinsic and extrinsic cell death through TIMP-1-specific cell survival signal transduction. Thus, it becomes clear that TIMP-1 can function as a signaling molecule independent of its inhibition of MMPs. However, the cell surface binding protein that mediates TIMP-1 signaling has not been reported. Here, we present preliminary data showing that TIMP-1 interacts with a member of the tetraspanin family and modulates integrin-mediated signaling pathway. In addition, an *in vitro* three dimensional (3D) culture study showed that **(xvi)** apoptosis critical for the lumen formation during morphogenesis of MCF10A acini was effectively inhibited by TIMP-1.

Although biochemical studies as well as mice experiments clearly demonstrated the tumor suppressing activity of TIMP-1 through MMPs inhibition, immunohistochemical studies show that increased TIMP-1 expression is often associated with negative prognosis in many human solid tumors including distant metastasis of breast cancer. Taken together, our study suggests an "oncogenic activity" of TIMP-1 through inhibition of apoptosis in breast cancer, providing an explanation for the unexpected results of these clinical studies.



Working hypothesis of TIMP-1 inhibition of apoptosis.



TIMP-1 disrupts the formation of the hollow lumen structure in 3D culture. MCF10A, and TIMP-1 overexpressing MCF10A clone #3 and #29 (T3 and T29, respectively) were culture on Matrigel for 8 days. Confocal microscopic imaging of cross-sections through the middle of developing acini are shown. Cells were stained with anti-integrin •6 Ab/Texas red conjugated secondary Ab (red staining) to delineate the basement membrane, with anti-active caspase-3 Ab/FITC conjugated secondary Ab (green staining) to detect apoptotic cells, and with DAPI (blue) to counterstain the nuclei.