Tumor suppression by stromal TIMPs

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The tumor stroma has the capacity to drive cancer progression, although the mechanisms governing these effects are incompletely understood. Recently, we reported that deletion of tissue inhibitor of metalloproteinases (Timps) in fibroblasts unleashes the function of cancer-associated fibroblasts and identifies a novel mode of stromal–tumor communication that activates key oncogenic pathways invoving Notch and ras homolog gene family, member A (RhoA) via stromal exosomes.

Tumors are complex clusters of malignant cancer cells that are embedded in vasculature and surrounded by a dynamic tumor stroma consisting of various non-malignant cells, such as cancer-associated fibroblasts (CAFs) and immune cells, and dense connective tissue.[1] The local microenvironment of a cancer cell plays an important function during cancer progression, and the support of such niches can promote cancer stem cell (CSC) subpopulations. The loss of tumor suppressor genes has been observed in tumor stroma such as CAFs and is implicated in tumor progression, but cell alterations that promote the carcinogenic niche have not been well established. Understanding the critical regulators of this stromal environment and their mechanism of action may provide an untapped target for cancer treatment.

The 4 members of the tissue inhibitor of metalloproteinases (Timp) family control extracellular matrix (ECM) remodeling and the cell surface protein landscape through functional inhibition of several classes of metalloproteinases including MMPs (matrix metalloproteinases), ADAMs (a disintegrin and metalloproteinases), and ADAMTSs (a disintegrin and metalloproteinases with thrombospondin motifs). We have previously identified TIMPs as critical regulators of several pathways including tumor necrosis factor (TNF), transforming growth factor-β (TGF-β), and epidermal growth factor (EGF) signaling, which control immune cell recruitment, matrix deposition, and epithelial cell turnover.[2] Each TIMP has the capacity to inhibit several metalloproteinases; however, their unique expression and localization patterns allow each TIMP to regulate distinct cellular processes by inhibiting these enzymes in specific tissue compartments. TIMPs are predominantly synthesized by the tissue stroma, but the contribution of the TIMP–metalloproteinase axis at the tumor–stromal interface is not fully understood.

Recently, we generated quadruple TIMP-deficient (TIMPless) fibroblasts to unleash metalloproteinase activity within the tumor–stromal compartment and demonstrated that the complete loss of Timps allows the acquisition of hallmark CAF functions.[3] Loss of Timp in fibroblasts elicits a myofibroblastic phenotype that is associated with the conversion of stromal fibroblasts into CAFs. Similar to spontaneous human CAFs,[4] TIMPless fibroblasts promoted the growth and angiogenesis of human cancer xenografts. CAF-induced enhancement of cancer cell motility is a crucial step in cancer metastasis and it is notable that TIMPless fibroblasts enhance distant lung metastasis of breast and lung cancer. Detailed analysis revealed that increased metalloproteinase activity is involved in the induction of this activated fibroblast state, suggesting that the balance between TIMPs and metalloproteinases regulates a CAF-like cell state. Although previous work has described fibroblast-derived soluble factors as inducers of cancer progression, we uncovered fibroblast-derived ADAM10-rich exosomes as a vector for stromal–cancer communication, which underpins the enhanced migratory and metastatic capacities of lung and breast cancer cells in our system. It remains to be investigated whether TIMPless fibroblasts play a similar function in other cancers such as pancreatic cancer, where the stromal compartment is highly prominent but its role remains controversial.

Exosomes are small membrane vesicles (30–100 nm in size) that are derived from luminal membranes of multi-vesicular bodies and are constitutively released by endocytosis. Increased production of exosomes by tumor cells has been associated with tumorigenesis and metastasis,[5] but whether stromal cell-derived exosomes deliver oncogenic cargo is an open question. TIMPless fibroblasts produce proteomically distinct exosomes containing ADAM10, as well as Thy-1, Lysyl oxidase homolog 2, and Tenascin C (TNC). The ECM proteins TNC and periostin are upregulated in human CAFs and have attracted attention as factors
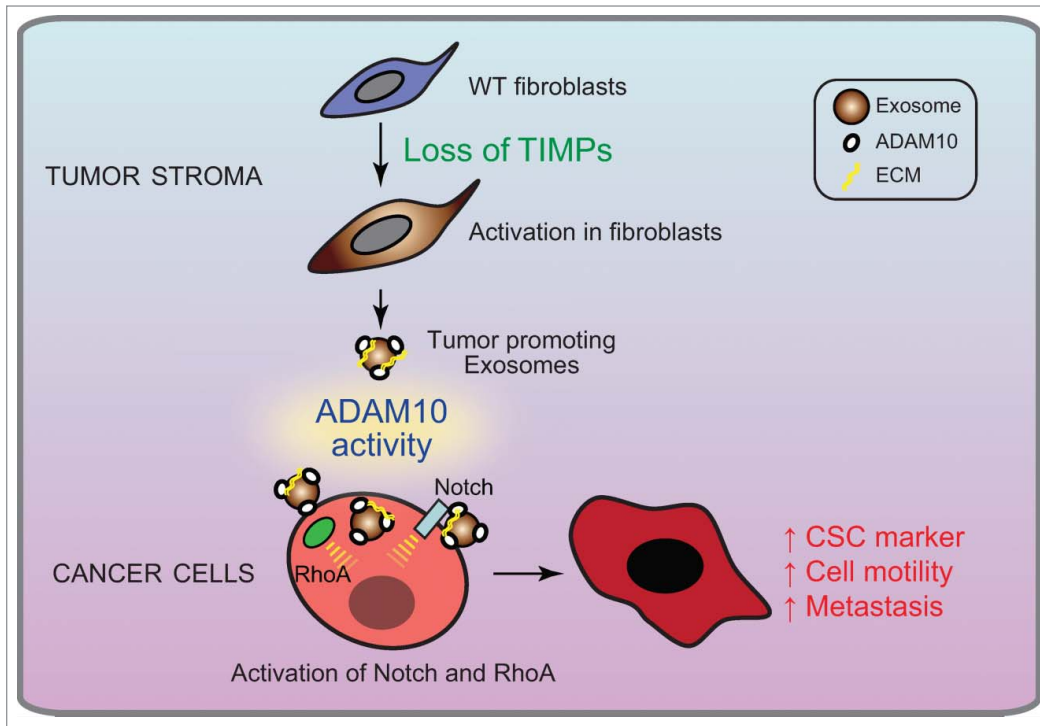
Figure 1. TIMP-deficient fibroblasts modify the tumor microenvironment to support tumor progression. Fibroblastic loss of the Timp family is sufficient for the acquisition of hallmark CAF functions. TIMPless fibroblasts produce tumor-promoting exosomes, which induce expression of CSC markers in cancer cells through Notch activation in addition to increasing cancer cell motility through activation of RhoA via a disintegrin and metalloproteinase 10 (ADAM10) activity. TIMPless fibroblasts also contain various ECM proteins, which may provide a CSC-metastatic niche for cancer cells. ADAM, a disintegrin and metalloproteinase; CAF, cancer-associated fibroblast; CSC, cancer stem cell; ECM, extracellular matrix; RhoA, ras homolog gene family, member A; Timp, tissue inhibitor of metalloproteinases.

that form a CSC or pre-metastatic niche,[6] and our analyses highlight the requirement of exosomal ADAM10 for fibroblast-mediated effects. ADAM10 maintains its proteolytic activity within exosomes[7] and inhibition of exosomal ADAM10 suppressed the ability of TIMPless exosomes to enhance breast cancer cell migration, xenograft growth, and metastasis.

Ectodomain shedding by ADAM10 activates Notch signaling, a pathway that regulates multiple cellular processes including stem cell maintenance, cell fate specification, differentiation, and CSC phenotypes. We found that ADAM10-rich TIMPless exosomes induce Notch activation in cancer cells, possibly after incorporation. Furthermore, expression of CSC markers was increased in TIMPless exosome-treated cancer cells in an ADAM10–Notch-dependent manner. On the other hand, exosome-induced cancer cell motility was Notch-independent, and was accompanied by activation of ras homolog gene family, member A (RhoA) in TIMPless-

exosome treated cancer cells. As inducers of actomyosin contraction and cell body translocation, RhoA and Rho-associated protein kinase (ROCK) have been linked to invasion and metastasis. Thus, the horizontal transfer of ADAM10 from stroma to tumor by exosomes stimulates tumorigenesis.

A mounting body of evidence has revealed the existence of metalloproteinases in exosomes,[8] which may provide a novel platform by which these proteases induce ectodomain shedding. Although some metalloproteinases are proteolytically active in exosomes, the biological functions of exosome-associated metalloproteinases and their clinical implications are poorly understood. Our study has advanced the understanding of exosome-associated metalloproteinases as a mode of stromal–cancer communication. Metalloproteinases such as MMP-9 have been shown to play a key role in pre-metastatic niche formation,[9] and delivery via exosomes may contribute to this and to the establishment of metastasis-prone sites at tumor-distant organs.

In addition to the recent findings by Luga et al. showing that fibroblast-secreted exosomes mediate breast cancer metastasis through wingless-type MMTV integration site family (Wnt)-planar cell polarity signaling,[10] our findings strengthen a role for fibroblast-derived exosomes in tumor progression. Taken together, tumor-promoting exosomal cargo from activated fibroblasts may create a specialized environment that promotes the mobilization of cancer cells in patients (**Fig. 1**).

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No potential conflicts of interest were disclosed.

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