Stromal TGFβR2 signaling: a gateway to progression for pancreatic cancer

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The function of transforming growth factor β (TGFβ) in the progression of pancreatic ductal adenocarcinoma (PDA) is complex and therapeutic targeting of this pathway is challenging. We showed that antibody-mediated inhibition of stromal Tgfβr2 prevented or reversed epithelial plasticity resulting in a potent reduction of metastasis in xenograft models of PDA.

Pancreatic cancer is the fourth leading cause of cancer-related mortality in the United States, and has proved to be a formidable challenge with regard to treatment.1 An elevated level of transforming growth factor β (TGFβ) is a negative prognostic indicator for patients diagnosed with pancreatic ductal adenocarcinoma (PDA) and over 50% of human PDAs have mutations in the TGFβ pathway.2 This makes the TGFβ pathway an attractive therapeutic target. However, the function of TGFβ in the development and progression of PDA is complex and severely dysregulated in advanced tumors. As in other epithelial tumors, TGFβ functions as a tumor suppressor early in the development of PDA, but switches to a tumor promoter function late in the disease process. This switch, together with findings that direct neutralization of Tgfβ2 accelerates tumor growth in some mouse models of PDA, makes targeting the TGFβ pathway challenging.3 In this study, we found that selected neutralization of murine Tgfβr2 with a monoclonal antibody (2G8) led to a differentiated epithelial tumor cell phenotype and potent anti-metastatic effects.4 These effects were seen in multiple mouse models of PDA including 4 xenograft models and syngeneic and genetic (LSL-KrasG12D; Cdkn2a<sup>−/−</sup>; p48-Cre) models. In the xenograft setting in particular, these effects can be attributed directly to inhibition of stromal Tgfβr2 because 2G8 is a mouse-specific monoclonal antibody.

In each PDA model, Tgfβr2 neutralization significantly reduced metastasis and cell proliferation, while increasing apoptosis in the primary tumor. Interestingly, the effect of 2G8 on primary tumor size was not predictive of the effect on metastasis. In vitro data corroborated these findings, as 2G8 had no effect on tumor cell viability. However, 2G8 reduced a stimulatory effect of conditioned media from murine stromal cells on tumor cell migration and anchorage-independent growth. Together, the in vivo metastasis data and in vitro characterization studies strongly implicate stromal Tgfβr2 as a critical driver of PDA dissemination. These results are consistent with the characterization of primary tumor tissue in each model after treatment with 2G8. 2G8 consistently reduced the level of activated fibroblasts, collagen deposition, and microvessel density associated with primary PDA. These observations are concordant with Tgfβr2 as a regulator of ECM deposition and the fibroblast phenotype in the PDA microenvironment. Arguably though, the most interesting finding was the effect on tumor cell epithelial to mesenchymal transition (EMT); tumors treated with 2G8 displayed a more epithelial or differentiated phenotype than tumors exposed to control or gemcitabine alone. This is demonstrated convincingly in the genetic and syngeneic models of PDA (Fig. 1). In the genetic model, 2G8-treated mice had significantly more pancreatic intraepithelial

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neoplasia (PanIN) lesions than those treated with saline control or gemcitabine. Additionally, in the syngeneic model using Pan02 cells, a highly mesenchymal murine pancreatic tumor cell line, 2G8 induced prominent expression of epithelial cadherin (E-cadherin) and epithelial membrane expression of β-catenin together with decreased vimentin expression, whereas Pan02 tumors treated with control or gemcitabine showed a less differentiated phenotype. β-catenin is expressed on the membrane of epithelial cells and translocates into the nucleus during the process of EMT, and vimentin is a
Pharmacologic blockade of TGFβR2 resulted in a proinflammatory immune cell phenotype, a decrease in mature/activated fibroblasts, and a decrease in collagen deposition. These microenvironmental changes occurred in concert with tumor cell epithelial differentiation and a reduction in metastasis, leading us to conclude that TGFβ signaling within stromal cells has a direct influence on pancreatic tumor cell phenotype and pancreatic cancer progression. Whether this impact is secondary to a distinct soluble factor released into the tumor microenvironment, or to effects on other cells in the microenvironment such as pancreatic stellate cells, mesenchymal stem cells, and endothelial cells, remains to be seen. We suspect that the complexity of TGFβ biology in cancer is intimately tied to the interplay between stromal cells and the tumor cells that recruit them. We propose that signaling through TGFβR2 on stromal cells is a requisite pathway for a subset of tumors that stimulates the release of prometastatic soluble factors from stromal cells. Identifying tumors that rely on this pathway and the factors that are produced by stromal cells in response to activation of TGFβR2 are remaining challenges.

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Overall, this study further defines the importance of stromal TGFβ signaling in the development and progression of PDA.