IN THE SPOTLIGHT

B Cells Promote Pancreatic Tumorigenesis

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Summary: Three recent studies, approaching the question from different angles and using different and/or overlapping models, provide compelling evidence for the involvement of tumor-infiltrating B cells in the initiation and progression of pancreatic ductal adenocarcinoma. These studies highlight the need for a better understanding of pancreatic tumor–immune system interactions and the immunologic mechanisms that promote or inhibit tumorigenesis, paving the way for better treatment strategies. *Cancer Discov; 6(3); 230–2. ©2016 AACR.*

See related article by Pylayeva-Gupta et al., p. 247 (8).

See related article by Lee et al., p. 256 (9).

See related article by Gunderson et al., p. 270 (10).

Treatment of pancreatic cancer has proven very difficult because of both the low/nonimmunogenic nature of the tumor and the late stages at diagnosis. This is reflected in the abysmal 5-year survival rate of ~7% and the harsh standard-of-care (SOC) for patients. The most common treatment, aside from palliative care or surgery in eligible patients, is gemcitabine, which is a general DNA-damaging agent. However, those patients healthy enough to handle strong treatment side effects are given a cocktail of four drugs called FOLFIRINOX in the hope of stalling tumor development. Other FDA-approved drug combinations may be tried as the first-line treatment, but all have significant side effects affecting patient quality of life without much measurable benefit. Although no immunotherapies are currently approved for treating pancreatic cancers, there are 16 clinical trials of various immunotherapies for this disease (clinicaltrials.gov). These trials fall into two categories: combination therapies and vaccines. Most of the combination therapies involve adding an immunotherapy-like checkpoint blockade, vaccine, or cytokines to the SOC, aiming to first make pancreatic ductal adenocarcinoma (PDAC) immunogenic and then promote antitumor immune responses (1).

Studies in both human and animal models have now demonstrated that the immune system plays a critical role in modulating the outcome of tumor development. In general, cytotoxic $CD8^+$ T cells, Th1-type $CD4^+$ T cells, and natural killer (NK) cells exhibit antitumor activity, whereas regulatory T cells, myeloid-derived suppressor cells (MDSC), and tumor-associated macrophages (TAM) suppress antitumor immune responses and promote tumor progression and metastasis (2). Increasingly, B cells are also found to play a significant role in modulating the growth and progression of solid tumors (2, 3). Tumor-infiltrating lymphocytic B cells (TIL-B) are a major component of TILs in breast and advanced ovarian cancers, and their presence correlates with improved survival. However, in multiple mouse models, tumor development is enhanced when B cells are present. Growth of EL4 thymoma, MC38 colon cancer, and EMT6 breast cancer is significantly inhibited in B cell–deficient mice. The absence of B cells is associated with increased infiltration of Th1 cells, $CD8^+$ T cells, and NK cells in the tumor. Conversely, adoptive transfer of B cells into immunodeficient mice results in increased tumor growth.

The role of B cells in neoplasia has also been evaluated in genetically modified mouse models. In K14-HPV16–induced epithelial carcinogenesis, tumor progression is decreased in immunodeficient mice with no mature B and T cells (4). Adoptive transfer of B cells into these immunodeficient mice results in increased malignancy. Interestingly, depleting B cells with anti-CD20 monoclonal antibody (mAb) alone does not inhibit tumor progression, but improves response in combination with chemotherapy (5). One suggested mechanism for TIL-B enhancement of tumor progression is through a subset of B cells that are suppressive, thereby inhibiting the antitumor immune responses (6). Similarly, in a transgenic metastatic, castration-resistant prostate cancer model (7), progression of the disease is associated with immune infiltrates, including B cells, which are recruited to the tumor by the chemokine CXCL13. Tumors are refractory to the chemotherapeutic agent oxaliplatin unless mice are depleted of B cells. B cells inhibited antitumor responses of $CD8^+$ T cells through regulating IL10 and PD-L1 in that model.

To date, the role of TIL-Bs in PDAC has not been investigated. By using different approaches in various preclinical models, the three reports in the current issue of *Cancer Discovery* provide compelling evidence for the involvement of TIL-Bs in supporting both early and more advanced stages of pancreatic tumorigenesis. This occurs via multiple mechanisms, including suppression of other immune cells (e.g., $CD8^+$ T cells and macrophages) in the tumor microenvironment and promoting pancreatic cancer cell proliferation (Fig. 1). Inhibition of B-cell infiltration into the tumor by blocking chemokine CXCL13, inhibition of B-cell activity using a Bruton tyrosine

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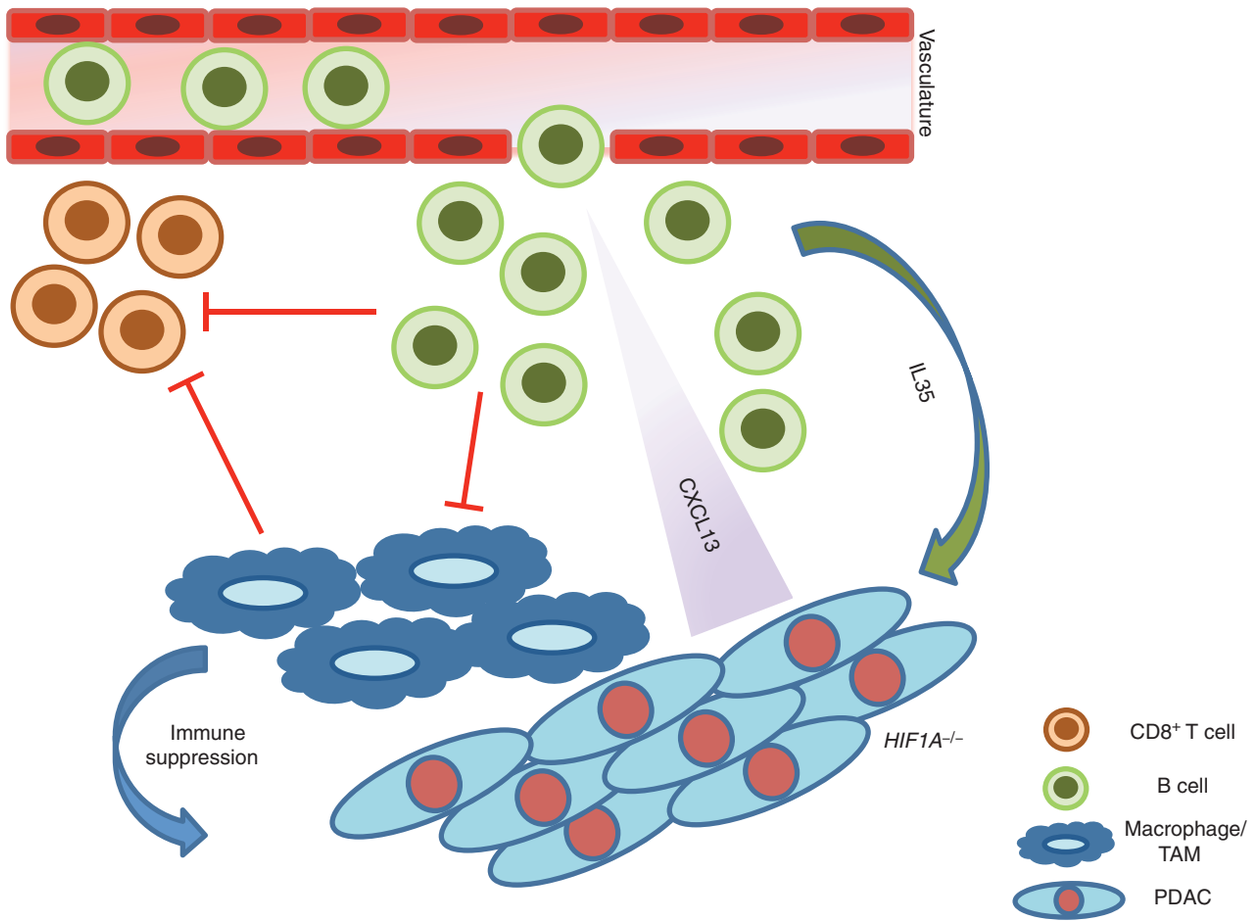

Figure 1. Contribution of TIL-Bs to pancreatic tumorigenesis. B cells infiltrate pancreatic tissues in response to the release of local chemokines, such as CXCL13. The TIL-Bs in turn secrete IL35 that stimulates tumor cell proliferation (8). On the other hand, pancreas-specific *HIF1A* deletion accelerates pancreatic neoplasia and results in increased secretion of B-cell chemokines and B-cell infiltration into the pancreas (9). Furthermore, PDAC growth depends on cross-talk between B cells and FcγR$^+$ TAMs, resulting in M2 programming via BTK activation in a PI3Kγ-dependent manner (10). Inhibiting B-cell infiltration, depleting B cells, inhibition of BTK, or upregulation of HIF1α in the pancreatic tumor microenvironment stimulates immune activation and inhibits tumorigenesis.

kinase (BTK) inhibitor, or simple depletion of B cells using a specific mAb all significantly reduced tumor progression.

Pylayeva-Gupta and colleagues (8) used primary human pancreatic intraepithelial neoplasia (PanIN) and the *LSL-Kras*^{G12D}; *Trp48*^{Cre} (KC) mouse model of PDAC to demonstrate a significant presence of B cells in the proximity of PanIN lesions. Using the KC model, they further demonstrated that CD1d^{hi}CD5$^+$ B-cell subsets contribute to pancreatic pathogenesis through a paracrine mechanism (IL35; heterodimer of subunits p35 and EB13) that promotes proliferation of the transformed epithelium. Importantly, the B-cell chemoattractant CXCL13, secreted by the fibroinflammatory stroma in human and mouse PanIN lesions, was shown to be responsible for the influx of B cells into the tumor. Treatment of tumor-bearing mice with a CXCL13-blocking mAb reduced B-cell infiltration in KC mice and mice orthotopically implanted with KRAS^{G12D}-PDEC cells.

On the other hand, Lee and colleagues (9) used primary human pancreatic tumors and the KC mouse model (crossed to *Hif1a*$^{fl/fl}$) to demonstrate that HIF1α is highly expressed during the preinvasive stage of PDAC and deletion of HIF1α accelerates PDAC development in KC mice. Unexpectedly, elimination of HIF1α resulted in an increased secretion of B-cell chemoattractants (CXCL13, CCL19, CCL20, CCL21, and CXCL12), which in turn promoted intratumoral accumulation of the CD19$^+$CD43$^+$IgM^{hi}CD5$^+$ subset of B1b cells during early pancreatic neoplasia. In line with these observations, depletion of B cells reduced progression of PanIN and development of invasive carcinomas in tumor-bearing mice. As above, immunohistochemical analysis of primary human pancreatic cancer tissues also revealed a significant presence of intrapancreatic B cells, supporting the involvement of human B cells in pancreatic tumorigenesis.

Lastly, Gunderson and colleagues (10) used primary human pancreatic tumors, public databases, and two syngeneic murine PDAC cell lines—derived from primary pancreatic carcinomas of transgenic *Kras*^{G12D}; *Pdx*^{Cre} mice harboring *p16*$^{Ink4\alpha}$ or *Trp53* deletion—to demonstrate that BTK regulates B-cell and macrophage-mediated T-cell suppression in PDAC development. Both human and murine PDACs were shown to exhibit high BTK activation in tumor-resident B cells and macrophages. BTK inhibition with the FDA-approved inhibitor

ibrutinib reduced PDAC growth and abated immunosuppression *in vivo* by reprogramming macrophages toward an M1 phenotype that promoted CD8 T-cell cytotoxicity.

One caveat to the three studies is that, in a number of the preclinical models used, the contribution of infiltrating B cells to pancreatic tumorigenesis was monitored in orthotopically engrafted tumors. As this involves a major surgery in the recipient mice, it could in turn create a local inflammatory environment that promotes a higher-than-normal infiltration of B cells into the tumor site, and hence the data obtained from these models should be interpreted with caution. Additionally, there are conflicting reports on the role of TIL-Bs in other tumor types, demonstrating the need for further study (2). Nevertheless, findings from the three studies provide compelling evidence for the involvement of tumor-infiltrating B cells in the initiation and progression of PDAC.

In addition to advancing the overall field of immuno-oncology, findings from the three studies point to novel approaches for treating PDAC. Increasing the success rate for treating PDAC is likely to require combination therapies that target both tumor cells and immune cells, as well as earlier detection and intervention. Currently, T cell–centric immunotherapies are being tested in late-stage PDAC. One new therapeutic strategy for PDAC would include targeted B-cell suppression, such as BTK inhibition, or depletion, as part of a combination therapy. Similarly, neutralizing IL35 and/or CXCL13 in combination therapies may increase therapeutic efficacy. Moreover, levels of B cells, IL35, and CXCL13 in the pancreas and/or PDAC may serve as biomarkers for early diagnosis, especially in higher-risk individuals who are predisposed to developing PDAC due to family history (e.g., *BRCA* mutation or diabetes) or tobacco usage, helping clinicians develop earlier interventions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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