

Breaking barriers for T cells by targeting the EPHA2/TGF- β /COX-2 axis in pancreatic cancer

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Pancreatic ductal adenocarcinoma is projected to become the second-leading cause of cancer-related death and is largely resistant to immunotherapies. The tumor microenvironment, largely composed of heterogeneous myeloid cells, creates a physical, metabolic, and immunosuppressive barrier that prevents T cells from infiltrating cancer beds. In this issue of the *JCI*, Markosyan and colleagues have reported a tumor-intrinsic mechanism that excludes T cells from the vicinity of tumor cells. They showed that a receptor tyrosine kinase, ephrin-A receptor 2 (EPHA2), regulates prostaglandin endoperoxide synthase 2 (*PTGS2*) (encodes COX-2) expression in a TGF- β signaling-dependent manner. Genetic ablation of *Epha2* or *Ptgs2* in preclinical models or pharmacological inhibition of COX-2 elicited the transformation of this immunosuppressive microenvironment into a T cell-permissive milieu. Consequent T cell relocation rendered this immunoresistant malignancy responsive to combinations of checkpoint blockers and CD40 agonists. Because the association between T cell infiltration and the EPHA2/TGF- β /COX-2 axis is supported by independent clinical data, these results provide a rationale for ensuing clinical trials aimed at incorporating pancreatic cancer into the range of immunotherapy-responsive tumors.

EPHA2 expression defines T cell exclusion

Although antibodies that block immune checkpoints have revolutionized the management of some tumors with tumor-infiltrating lymphocytes (TILs), most cancer patients still fail to respond to single-agent immunotherapies. While protective immune responses depend on the intrinsic antigenicity of the tumor, they are also influenced by T cell-recruiting chemokines at tumor beds and a physically permissive milieu that allows TILs to contact antigen-expressing tumor cells. In pancreatic ductal adenocarcinoma, a unique desmoplastic reaction impedes the accessibility of activated T cells to the vicinity of

tumor cells, where they should exert their effector function (1).

By combining the analysis of human pancreatic tumors with genetic studies in mouse models, Markosyan and colleagues found that ephrin-A receptor 2 (*EPHA2*) expression determines CD8⁺ T cell exclusion in pancreatic cancer (2). The authors focused on *EPHA2* by analyzing links to pathways among genes inversely correlated with the expression of *CD8A* and markers of cytolytic T cell activity in The Cancer Genome Atlas (TCGA) data sets. Validation analyses of independent patient cohorts and mouse models with different degrees of TILs further supported the association between *EPHA2* expression and the paucity

of TILs. Accordingly, *Epha2* ablation in murine pancreatic cancer tumors promoted TIL enrichment at the expense of immunosuppressive myeloid cells, with a prominent decrease of cells that express granulocytic lineage markers. Although the authors did not formally test the suppressive activity of these cells, granulocytic myeloid-derived suppressor cells (gMDSCs) accumulate in patients with cancer, where they coexist with conventional neutrophils and suppress the effector activity of tumor-reactive T cells (3). This includes pancreatic cancer desmoplasia, which generates a stromal compartment composed of hematopoietic cells, extracellular matrix, and fibroblasts, typically larger than the space occupied by pancreatic tumor cells. A significant component of this desmoplastic reaction is made by heterogeneous populations of immunosuppressive gMDSCs along with macrophages and immature MDSC precursors (4). Because *Epha2* ablation failed to change immunosuppressive mediator expression in tumor-associated gMDSCs on a per cell basis, Markosyan and colleagues suggest that granulocyte cell accumulation, rather than immunosuppressive activity, determines whether or not the microenvironment in pancreatic cancer will be T cell permissive or exclusive (2).

EPHA2/TGF- β /COX-2 axis governs T cell exclusion

Markosyan et al. went on to demonstrate that proinflammatory mediator COX-2 (encoded by prostaglandin endoperoxide synthase 2 [*Ptgs2*]) is differentially expressed in *Epha2*^{hi}TIL^{lo} compared with *Epha2*^{hi}TIL^{hi} tumors (2). Subsequent analyses of additional samples from human and mouse pancreatic cancer-bearing hosts with low or high TIL density again confirmed an association between COX-2 expression and T cell exclusion. COX-2 is the inducible form of cyclooxygenase, the enzyme responsible for converting fatty acids into prostanoids (5). A role for COX-2 in pancreatic cancer progression is

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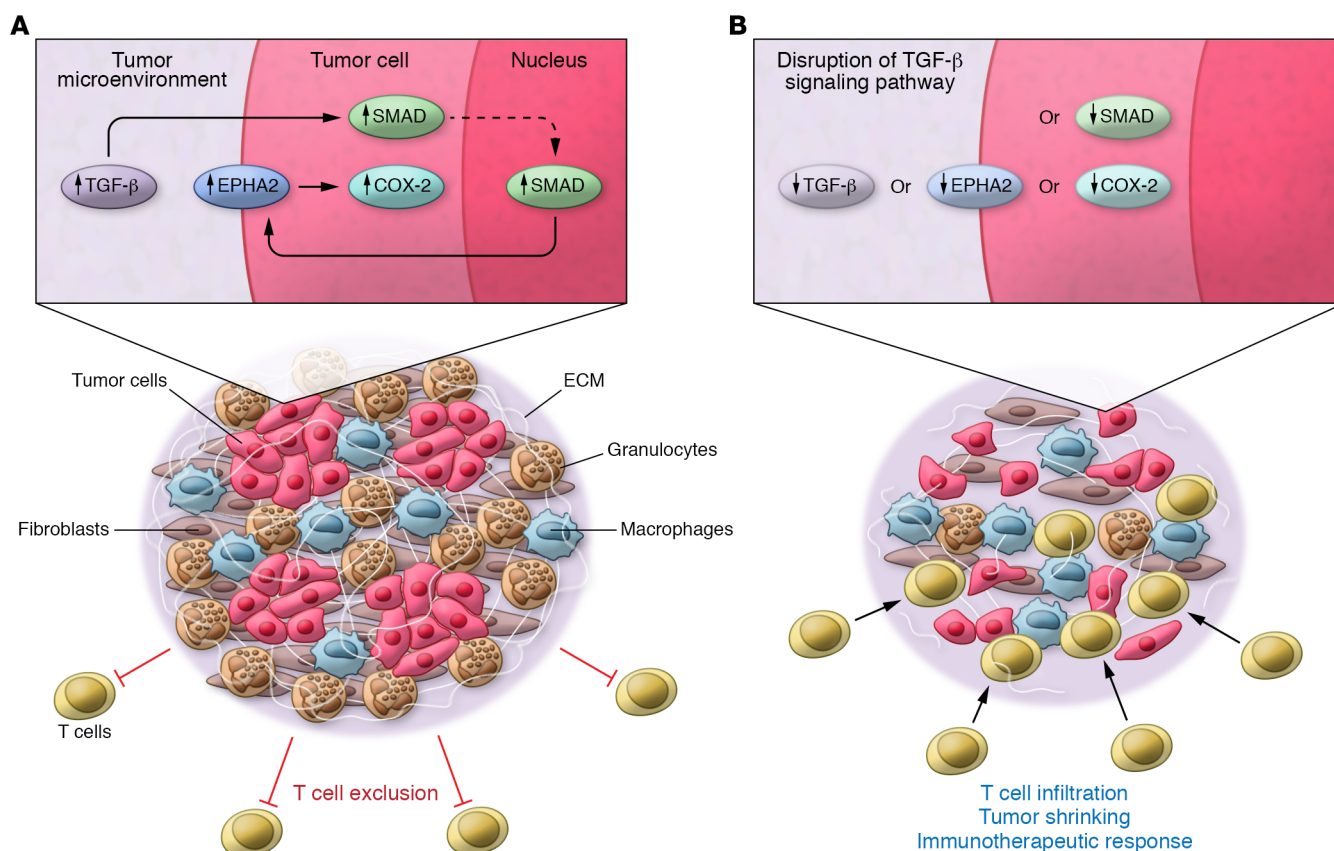


Figure 1. Tumor cell-intrinsic TGF- β signaling drives the upregulation of EPHA2 on the tumor cell surface, which promotes the overexpression of COX-2. COX-2 activity governs the orchestration of a pancreatic cancer microenvironment characterized by the accumulation of cells of the granulocytic lineage that, along with macrophages, fibroblasts, and the deposition of extracellular matrix (ECM), prevents T cell infiltration. Genetic ablation of TGF- β signaling, *Epha2*, or *Ptgs2* (COX-2) transforms this microenvironment into one permissive to T cell trafficking, which is primarily associated with a decrease in granulocytes (likely gMDSC). Accordingly, pharmacological inhibition of COX-2 renders highly resistant pancreatic tumors sensitive to immunotherapeutic interventions.

not surprising, as high COX-2 expression levels in both pancreatic cancer cells and stroma cells have been previously reported (6). In addition, COX-2 expression was shown to be positively regulated by TGF- β signaling; however, mediation via SMAD3 is likely indirect, as the *PTGS2* promoter region lacks SMAD3-binding sites.

Markosyan et al. have revealed the importance of the EPHA2/TGF- β /COX-2 axis in forging the pancreatic cancer microenvironment. When tumor cells were treated with TGF- β , EPHA2 increased on the tumor cell surface. Additionally, excision of TGF- β -dependent *Smad3/4* in tumor cells or ablation of *Ptgs2* recapitulated the increases in T cells and decreases in granulocytes in implanted tumors similarly to what was observed upon *Epha2* ablation. Furthermore, ectopic expression of COX-2 in tumor cells promoted the accumulation of granulocytes and possibly gMDSCs at

the expense of antigen-presenting cells, thereby phenocopying the effects induced by spontaneous *Epha2* overexpression. Collectively, these data indicate that an EPHA2/TGF- β /COX-2 axis governs the pancreatic cancer immune environment toward a myeloid-rich, T cell-poor setting (Figure 1).

PGE₂, which is the most abundant secreted prostaglandin, shows strong tumor-promoting activities (7), including a major role in immunosuppression in cancer that acts by driving MDSC proliferation (8). Further, PGE₂ may directly mediate COX-2-dependent T cell exclusion. Markosyan et al. found that minor reductions in COX-2 substantially reduced PGE₂ expression. Interestingly, PGE₂ also modulates chemokine production, enhancing local accumulation of both populations of MDSCs (9). While all these mechanisms can contribute to the observed effects,

the results of Markosyan and colleagues point to specific determinants of the selective accumulation of granulocytes and gMDSCs at tumor beds.

An attractive candidate for driving these effects could be the chemokine family member CXCL8. CXCL8 expression is increased by PGE₂ signaling in human epithelial cells (10) and could offer an additional therapeutic target for antibody blockade. Markosyan and colleagues did not find differences in the expression of the mouse counterpart of CXCL8 (*Cxcl1*) between WT and *Epha2*-KO tumors. Still, it will be interesting to determine whether a correlation between CXCL8 and EPHA2 could be identified in TCGA human pancreatic cancer data sets and other independent cohorts. Nevertheless, the results of Markosyan and colleagues suggest that EPHA2 and CXCL8 could represent different tumor cell-intrinsic mechanisms

driving T cell exclusion in pancreatic cancer. Future studies will determine whether other chemokines involved in neutrophil trafficking (i.e., CXCL2) are upregulated in an EPHA2-dependent manner (11).

COX-2 inhibition could overcome resistance to immunotherapy

Strikingly, Markosyan et al. showed that treating otherwise unresponsive models of murine pancreatic cancer with celecoxib, a COX-2 inhibitor, rendered tumors sensitive to combinations of checkpoint inhibitors and CD40 agonists (2). The same group previously demonstrated that CD40 stimulation of tumor-associated macrophages is required to transform macrophages into cytotoxic killers that, unlike conventional T cells, are able to navigate the physical barriers of the pancreatic cancer stroma. However, CD40-induced stromal remodeling was insufficient, and activated T cells remained blocked from the vicinity of tumor cells (1). By reversing T cell exclusion, pharmacological inhibition of COX-2 sensitizes tumors to immunotherapy. Interestingly, in mouse models, this sensitization process can occur in the absence of strong neopeptides (12).

COX-2 inhibitors have been previously shown to decrease stromal-dependent pancreatic cancer cell invasiveness (6), but the study by Markosyan et al. provides a mechanistic rationale for clinical trials on concurrently inhibiting COX-2 and PD-1 with existing drugs in pancreatic cancer patients. Although pancreatic cancers have relatively low mutational burdens compared with other human malignancies (13), some pancreatic cancers express cancer-associated antigens that are recognizable by bone marrow-resident T cells (14). In addition, the results presented by Markosyan et al. suggest that the immunogenicity of pancreatic cancer (and perhaps other tumors with low mutational burden) is independent of single-nucleotide mutations that are conclusively associated with immunotherapeutic responses in other tumors. Given that defects in p53 are a hallmark of this disease, gene fusions arising from genomic instability are other attractive antigenic drivers, although the role of conventional shared antigens, including cancer testis antigens, cannot be disregarded.

The work by Markosyan and colleagues underscores the importance of another appealing target for blockade, namely, TGF- β . In cancer, TGF- β suppresses CD8⁺ T cell effector activity (15) and TGF- β signaling drives PD-1 overexpression through a SATB1-dependent mechanism (16). In human pancreatic cancer, upregulation of TGF- β isoforms drives desmoplasia (17). However, while the results presented in this study deepen our understanding of TGF- β and SMAD signaling in antitumor immunity, long-term neutralization of TGF- β could have undesired effects; a note of caution is warranted. For example, TGF- β has positive effects in the acquisition of a tissue-resident memory (TRM) differentiation program (18). In other tumors, TRM T cells have been associated with effective protective immunity upon checkpoint blockade (19); therefore, these benefits could be lost in response to TGF- β inhibitors. On the other hand, blockade of TGF- β could synergize with other T cell-based immunotherapies.

Finally, other targetable tumor cell-intrinsic drivers of T cell exclusion have been previously identified in different tumors. For instance, a CDK4/6-dependent, tumor cell-intrinsic transcriptional program associated with checkpoint inhibitors was identified in melanoma (20). The studies of Markosyan and colleagues represent an important step in determining combinatorial interventions in pancreatic cancer patients with malignant progression to revert the restrictive microenvironment into a milieu that facilitates existing and future T cell-based immunotherapies.

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