

# The desmoplastic stroma of pancreatic cancer is a barrier to immune cell infiltration

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**Keywords:** B cells, CD8<sup>+</sup> T cells, natural killer cells, macrophages, neutrophils

**Abbreviations:** ATRA, all-trans retinoic acid; HSC, hepatic stellate cell; KPC, LSL-Kras<sup>G12D/+</sup>-LSL-Trp53<sup>R172H/+</sup>-Pdx-1-cre; NK, natural killer; MDSC, myeloid-derived suppressor cell; PDAC, pancreatic ductal adenocarcinoma; PSC, pancreatic stellate cell

A detailed map demonstrating the spatially restricted distribution of immune cells within the desmoplastic stroma of human pancreatic tumors opens up the field of pancreatic oncoimmunology. CD8<sup>+</sup> T cells located in the proximity of malignant lesions are associated with a survival advantage, suggesting the existence of immunoediting. Pancreatic stellate cells appear to dictate the infiltration of the juxtatumoral stroma by CD8<sup>+</sup> T cells.

Pancreatic ductal adenocarcinomas (PDACs) are characterized by a dense desmoplastic reaction, primarily reflecting the activation of pancreatic stellate cells (PSC) driven by cancer cells.<sup>1</sup> Non-transformed, healthy pancreatic tissues contain low amounts of PSCs in a quiescent state. In this setting, PSCs contain vitamin A droplets and have a limited ability to proliferate, migrate, and secrete extracellular matrix proteins.<sup>1,2</sup> As a result of tissue injury, PSCs become activated in response to growth factors, oxidative stress, and change in tissue plasticity. Such activated PSCs lose their cytoplasmic vitamin A droplets, adopt a myofibroblastic phenotype, produce large amounts of extracellular matrix proteins, and exhibit increased migratory and proliferative activity. In these conditions, PSCs not only stimulate the proliferation of pancreatic cancer cells and their invasive potential, but also inhibit their apoptotic demise through a multitude of signaling pathways.<sup>2</sup>

The activation of PSCs is reversible, even in presence of malignant cells, and hence potentially important from a therapeutic standpoint.<sup>3</sup>

LSL-Kras<sup>G12D/+</sup>-LSL-Trp53<sup>R172H/+</sup>-Pdx-1-cre (KPC) mice provide a spontaneous model of PDAC that mimics several features of the human disease, including the representative desmoplastic stroma.<sup>4</sup> When KPC mice were treated with all-trans retinoic acid (ATRA), the desmoplastic stroma collapsed along with the inhibition of tumor growth.<sup>3</sup> ATRA efficiently restores retinol depots within PSCs in vivo, rendering them quiescent.

The failure of many chemotherapeutics to provide benefits to pancreatic cancer patients despite their efficacy against epithelial cancer cells in vitro has been attributed to the fact that many researchers have failed to recognize the protective effects of the desmoplastic stroma.<sup>5</sup> This dense stroma is being recognized to comprise distinct morphological and functional compartments, as evidenced by independent work from different laboratories.<sup>6,7</sup> For example, the gene expression pattern of stromal components has been shown to differ depending on their proximity to malignant cells. In particular, the juxtatumoral stroma (the stromal component that is juxtaposed to malignant cells) exhibits a distinct gene expression profiles

as compared with other stromal compartments (panstroma).<sup>6</sup>

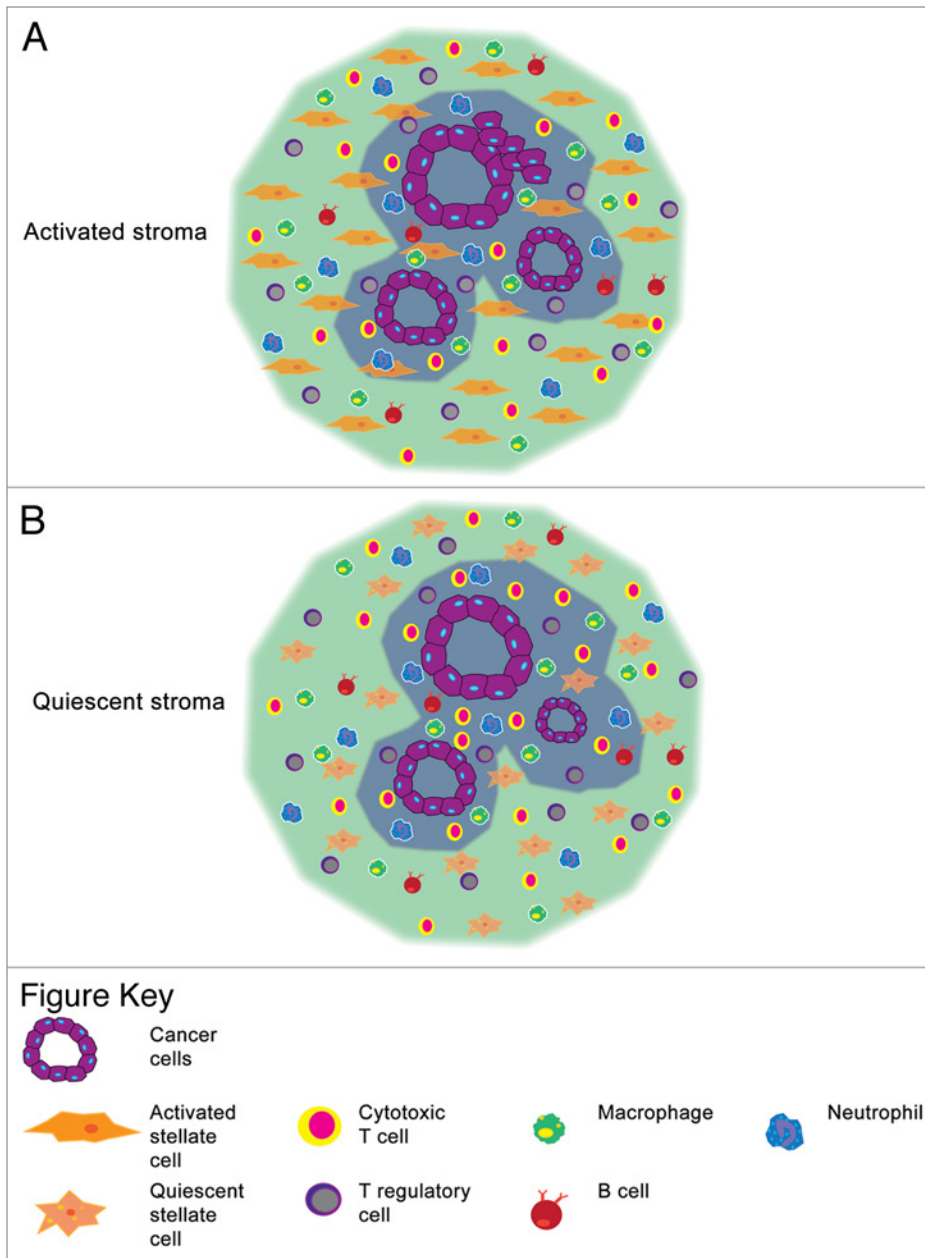
In the setting of inflammation and fibrosis, hepatic stellate cells (HSCs) have been shown to act as antigen-presenting cells and interact with CD1<sup>-</sup> as well as MHC class I- and MHC class II-restricted T cells.<sup>8</sup> Thus, HSCs can present lipid antigens to natural killer (NK) cells, stimulating their proliferation, as well as antigenic peptides to CD8<sup>+</sup> and CD4<sup>+</sup> T cells, resulting in the cross-priming of the former. However, to date no such interaction has been reported between PSCs and T cells.

Most often, the infiltration of neoplastic lesions by CD8<sup>+</sup> T lymphocytes is associated with improved prognosis.<sup>7</sup> We have recently investigated the global and compartment-specific immune infiltrate of human pancreatic cancer, as compared with other pancreatic disorders, using an unbiased automated system. We unveiled for the first time a PDAC-specific and compartment-specific immune cell infiltrative defect. In particular, neoplastic tissues, in particular those associated with poor prognosis (i.e., from cases of PDAC and cholangiocarcinoma), were infiltrated

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Submitted: 10/11/2013; Accepted: 10/11/2013; Published Online: 10/22/2013

Citation: Watt J, Kocher HM. The desmoplastic stroma of pancreatic cancer is a barrier to immune cell infiltration. *Oncoimmunology* 2013; 2:e26788; <http://dx.doi.org/10.4161/onci.26788>



**Figure 1.** Influence of ATRA on the immune cell subsets that infiltrate the stroma of pancreatic tumors. **(A)** The pancreatic ductal adenocarcinoma (PDAC) lesions developing in *LSL-Kras<sup>G12D/+</sup>-LSL-Trp53<sup>R172H/+</sup>-Pdx-1-cre* (KPC) mice exhibit the same pattern of infiltration by immune cells observed in human PDAC tissues. In particular, the distribution of neutrophils and macrophages is homogeneous throughout the stromal compartments, while regulatory T cells (Tregs), cytotoxic CD8<sup>+</sup> T cells and B cells are enriched in the panstromal compartment compared with the juxtatumoral stroma. **(B)** The administration of all-*trans* retinoic acid (ATRA) to these mice induces the quiescence of pancreatic stellate cell, resulting in an increased proportion of cytotoxic T cells within the juxtatumoral stroma.

by immune cells of all types, with the standalone exception of CD8<sup>+</sup> cytotoxic T cells, to greater extents than benign tissues. Indeed, CD8<sup>+</sup> T cells were found to infiltrate PDAC lesions in amounts that were comparable to those observed in chronic pancreatitis but significantly

lower than those detected in other malignant pancreatico-biliary (PB) conditions. Similar findings have been obtained with KPC mice.<sup>9</sup>

The most intriguing of our findings was that we observed a stromal compartment-specific, as opposed to generalized,

infiltrative defect. In particular, we found that CD8<sup>+</sup> T cells, CD20<sup>+</sup> B cells, CD56<sup>+</sup> NK cells, and FOXP3<sup>+</sup> regulatory T cells (T cells) are unable to sufficiently infiltrate the juxtatumoral stroma of PDAC lesions (Fig. 1). Conversely, other cells of the innate immune system such as CD68<sup>+</sup> macrophages and myeloperoxidase-expressing neutrophils could infiltrate this compartment. Of note, PDAC patients harboring high amounts of CD8<sup>+</sup> T cells in the juxtatumoral stroma exhibited improved survival. We could recapitulate most of these findings in KPC mice. Upon the administration of ATRA to KPC mice (resulting in the quiescence of PSCs), we observed a reversal of the juxtatumoral exclusion of CD8<sup>+</sup> T cells. Conversely, no difference in the distribution of other immune cell subsets was detected in response to ATRA. These observations suggest that activated PSCs in the panstroma selectively sequester immune cells. These findings fit nicely with the results obtained by Winau et al. relative to the interplay between CD8<sup>+</sup> T cells, NK cells and HSCs in the course of hepatic inflammation. While we did not exhaustively investigate all the functional interactions between PSCs and immune cells including NK cells, MDSCs (myeloid-derived suppressor cells) and others, our findings provide a vivid picture of the human PDAC microenvironment for other researchers to build their hypotheses and investigate such a complex crosstalk.

Our initial migration and adhesion experiments based on T cells and PSCs isolated from healthy individuals and PDAC patients confirmed the critical role of the activation state of PSC in altering the behavior of T cells. Chemokine C-X-C motif ligand 12 (CXCL12) was identified from our previous work as a potential inducer of such a functional alteration.<sup>8</sup> Using recombinant CXCL12 and RNA interference we could demonstrate that, at least in this setting, the migration of T cells depend on CXCL12 secreted from PSCs, which may explain the in vivo sequestration that we observed. A number of other adhesion molecules and chemokines should be investigated for their ability to influence

the spatial and temporal distribution of immune cells within PDAC lesions.

Altogether, our findings demonstrate that activated PSCs within the PDAC stroma have an important impact on the migration of immune cells toward malignant lesions, perhaps orchestrating a hierarchy of interactions between immune cells

and neoplastic cells. We have shown that CD8<sup>+</sup> T lymphocytes found in the proximity of malignant cells confer a survival benefit to PDAC patients, and that their abundance can be increased by ATRA, owing to its ability to disrupt the juxtatumoral stroma. Thus, PSCs play an important role in the modulation of the crosstalk

between neoplastic, stromal and immune cells, and need therefore to be considered as a therapeutic target in the context of combinatorial anticancer regimens.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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