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EDITORIAL

Finding Method in the Madness of Pancreatic Carcinogenesis

Two of the defining features of pancreatic ductal adenocarcinoma (PDAC) are activating mutations in KRAS and development of a dense, fibroinflammatory stroma. Inactivation of oncogenic KRAS in established PDAC,[1,2] or its downstream effector MYC,[3] leads to remodeling of the tumor microenvironment. Reciprocally, inflammatory or stroma-derived cues signal to the epithelial compartment to cooperate with oncogenic KRAS signaling and augment tumor-promoting transcriptional outputs.[4–7] Although these prior studies suggest links between KRAS and establishment of a fibrotic, immune-suppressive microenvironment, important questions and knowledge gaps remain, including the temporal dynamics of pancreatic inflammation following oncogenic KRAS activation, a detailed depiction of the phenotypic state of immune and stromal cell types in the context of epithelial KRAS signaling, and the relevant molecular mediators that establish a tumor-permissive milieu in the pancreas. In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Velez-Delgado et al[8] set out to address these questions and investigated the functional interaction between pancreatic epithelial cell-intrinsic KRAS signaling and the immune and stromal microenvironment during early stages of pancreatic tumorigenesis. Studies such as these are important because they may help ultimately identify the tissue alterations that permit, drive, or maintain the transition from indolent lesion to malignant disease, and to develop early intervention strategies.

Velez-Delgado et al[8] first set out to depict the cellular and molecular changes to the pancreas that evolve following introduction of an activating mutation in KRAS, and the consequences of KRAS inactivation, so as to assess dependency on this pathway for maintenance of the fibroinflammatory stroma.[8] Using an elegant mouse model enabling temporally controlled KRAS activation and inactivation,[1] the authors applied diverse approaches to analyze the microenvironment using single-cell RNA-seq, multiplex immunohistochemistry, and cytometry by time of flight to analyze tissue context at early time points (3, 7, and 14 days) after epithelial KRAS activation. After just 3 days of oncogenic KRAS signaling, macrophages infiltrated the pancreas, coincident with induction of phospho-ERK. Macrophage infiltration increased in abundance over time, but at the earliest time point examined, macrophage accumulation and phospho-ERK induction clearly preceded acinar cell transdifferentiation, one of the earliest steps of pancreas tumorigenesis. By 1 week after oncogenic KRAS activation, the still morphologically normal acini with elevated phospho-ERK were surrounded by macrophages and a thin layer of a-SMA+ fibroblasts, raising the possibility that these microenvironmental changes drive subsequent acinar transdifferentiation. To expand these analyses to PDAC precursor lesions, the authors activated oncogenic KRAS during experimentally induced acute pancreatitis, and then analyzed tissues at 3-day, 7-day, and 3-week time points. KRAS activation during pancreatitis induced acinar-to-ductal metaplasia and low-grade PanIN lesions, and extensive fibroblast activation and collagen deposition. To examine the requirement for KRAS signaling in this setting, the authors inactivated oncogenic KRAS in these established precursor lesions for 3 or 7 days, time points reflective of early and middle phases of pancreatic tissue remodeling. Surprisingly, although oncogenic KRAS signaling was required to maintain fibroblast activation and fibrosis, mutant KRAS was dispensable for regulation of myeloid cell abundance. Importantly, although total myeloid cell frequencies were not dependent on oncogenic KRAS signaling in these precursor lesions, KRAS inactivation dramatically altered myeloid cell polarization, with significant decreases in abundance of immunosuppressive ARG1+Ly6C+ macrophages, granulocytic myeloid-derived suppressor cells, and CCR1+CD206+ macrophages. STAT3 phosphorylation was reduced in stromal cell populations and the epithelial compartment at these time points, consistent with reduced inflammatory signaling on inactivation of oncogenic KRAS signaling.

To identify potential mechanistic regulators of these KRAS-dependent inflammatory processes, the authors analyzed pancreas tissues harboring precursor lesions after 3 weeks of pancreatitis and oncogenic KRAS signaling, and 3 days after inactivation of oncogenic KRAS in this setting at this time point by single-cell RNA-seq. These analyses identified fibroblasts as rich producers of immune-modulatory factors in early pancreatic neoplasia, including Cxcl1, Il33, Saa3, and Il6, all important mediators of pancreatic carcinogenesis. Furthermore, receptors for all of these factors, and other KRAS-dependent factors produced by inflammatory fibroblasts, are expressed by macrophages and other myeloid cell populations. These results implicate epithelial KRAS signaling in inflammatory programming of pancreatic fibroblasts, which then signal to infiltrating myeloid cells to promote an immunosuppressive phenotype. Although presently available KRAS inhibitors target mutants rarely detected in PDAC patients, this study highlights potential microenvironmental interactions downstream of oncogenic KRAS signaling that may form the basis for combination therapeutic intervention. For example, inhibition of STAT3 activation using a JAK inhibitor blocked inflammatory fibroblast programming. Interestingly, these inflammatory fibroblasts in the context of precursor lesions expressed immune-modulatory cytokines, associated with the previously established inflammatory cancer-associated fibroblast subtype, and a-SMA, a marker of the myofibroblastic cancer-associated fibroblast subtype,

which are largely nonoverlapping in established PDAC.[9,10] These results highlight differences between fibroblast populations in early lesions versus invasive cancer, such that transcriptionally distinct fibroblast populations in PDAC seem to evolve from fibroblastic cells with apparent wound-healing and inflammatory potential in precursor microenvironments.

In summary, the study by Velez-Delgado et al[8] establishes key features and dynamics of the pancreatic cancer precursor lesion microenvironment that are dependent on oncogenic KRAS signaling, and thus provides rich datasets for further investigation of communication among epithelial cells, fibroblasts, and immune cells that shape the tumor microenvironment.

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Conflicts of interest
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