Pancreatic fibroblasts smoothen their activities via AKT–GLI2–TGFα

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Pancreatic stromal fibroblasts provide structural support. Activated fibroblasts are critical in the tumor microenvironment. In this issue of *Genes & Development*, Liu and colleagues (pp. 1943–1955) unravel the finding that depletion of Smoothened (Smo) in pancreatic stromal fibroblasts results in AKT activation and noncanonical GLI2 activation with subsequent TGFα secretion, activation of EGFR in pancreatic epithelial cells, and augmentation of acinar–ductal metaplasia. Additionally, Smo-mediated signaling has proproliferative effects on pancreatic tumor cells.

Fibroblasts contribute to the structural integrity of the mesenchyme in part through the production of extracellular matrix proteins and collagen. Maintained in a quiescent state during homeostasis, fibroblasts may become activated during a variety of processes, such as inflammation, wound healing, and cancer. Activated fibroblasts demonstrate a different morphological identity and are annotated by markers such as vimentin, smooth muscle actin (SMA), fibroblast-activation protein (FAP), fibroblast-secretory protein (FSP), N-cadherin, and others. Furthermore, activated fibroblasts are known to secrete a variety of growth factors, cytokines, and chemokines that display both autocrine and paracrine effects—the latter on tumor cells but also inflammatory cells, immune cells, and blood vessels.

Certain cancers are highlighted by dense growth of connective tissue, referred to as desmoplasia. Striking examples are evident in esophageal cancer and pancreatic ductal adenocarcinoma (PDAC). Long felt to be permissive for tumor cell migration and invasion, the dichotomous nature of desmoplasia is being appreciated recently as harboring tumor-restraining properties related to Hedgehog signaling or cancer-associated fibroblasts (CAFs) (Lee et al. 2014; Özdemir et al. 2014; Rhim et al. 2014). To that end, pharmacological inhibition or genetic depletion of Hedgehog signaling or genetic depletion of CAFs results in reduced desmoplasia but favors tumor cell survival and dissemination.

A deep appreciation of the biological behavior of fibroblasts in PDAC has evolved with initial insights gained through studies of the pancreatic epithelium. One critical component is the Hedgehog pathway, already established to be important in the repression of pancreatic organogenesis [Hebrok et al. 1998; 2000]. Pancreata of *Pdx-Shh* mice harbor abnormal tubular structures reminiscent of human pancreatic intraepithelial neoplasia 1 (PanIN-1) and PanIN-2 (Thayer et al. 2003). When hedgehog signaling is activated in the pancreatic epithelium of transgenic mice, there is the emergence of undifferentiated carcinomas [Pasca di Magliano et al. 2006]. This group also found that concurrent activation of KRAS and Hedgehog signaling causes robust PanIN formation and accelerates mortality, thereby raising the specter of cooperation between KRAS and Hedgehog in pancreatic ductal epithelial cells and subsequent Smoothened (SMO) activation in fibroblasts. Insights into this were gained through the genetic finding in mice that Gli transcription is not linked to Shh–Ptc–Smo signaling and is regulated by TGFβ and KRAS [Nolan-Stevaux et al. 2009]. Furthermore, Gli is needed for the KRAS-mediated transformed phenotype in PDAC cells [Nolan-Stevaux et al. 2009].

In the current study by Liu et al. (2016), the investigators sought to elucidate how Hedgehog signaling from pancreatic stromal fibroblasts impacts pancreatic acinar–ductal metaplasia (ADM), which is a consequence of cellular plasticity in response to injury [e.g., cerulein-induced acute pancreatitis in mice] and is governed by a set of transcriptional factors, such as Sox9, Prx1, Mist1, and Ptf1a [Reichert et al. 2013]. ADM has been established in mice to be a precursor of PanIN and PDACs. The investigators ablated Smo in the fibroblasts of *Kras*<sup>G12D</sup> mice, resulting in increased ADM. This was found to be due to increased TGFα secretion from the fibroblasts and activation of EGFR signaling in acinar cells—the latter known to be an upstream regulator of ADM. Within the

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fibroblasts, Smo deletion results in AKT activation and noncanonical GLI2 activation; interestingly, there is no effect on GLI1 or GLI3. AKT signaling phosphorylates GLI2 Ser230, and this enhances TGFβ expression. This may be part of a non-cell-autonomous basis for the regulation of ADM, as other studies have provided evidence for cell-autonomous regulation of ADM mediated through a combination of EGFR signaling, Notch signaling, of course KRAS activation, and the aforementioned transcriptional factors. Nonepithelial effects are observed as well with Smo ablation in fibroblasts, including increased angiogenesis, decreased CD3-positive T-cell infiltration, and increased FoxP3-positive T regulatory cell infiltration, suggesting effects on the microenvironment.

The effects of Smo-deleted fibroblasts are not restricted to ADM, although regulation of ADM was the primary intent of the study. Smo-mediated signaling has proproliferative effects on KPC tumor cells. Coinjection of a KPC tumor cell line with Smo-deleted fibroblasts augments tumor burden when compared with coinjection with Smo-intact fibroblasts.

Overall, Hedgehog signaling has diverse and complex effects on pancreatic epithelial cells versus pancreatic stromal fibroblasts and helps to explain its impact on ADM, PanIN, and PDAC (Fig. 1). Targeting Hedgehog signaling is not a simple matter and may require combinatorial approaches with VEGFR antibody, as suggested recently (Rhim et al. 2014). The current study raises the possibility that Hedgehog signaling inhibition in concert with AKT inhibition (stromal fibroblasts) may be beneficial or Hedgehog signaling inhibition with EGFR inhibition as well (pancreatic epithelial cells), although EGFR inhibition alone has not proven efficacious in PDAC patients.

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