Fibroblasts are key players in inflammation and cancer, secreting growth factors, ligands, and extracellular matrix proteins that shape the microenvironment. In pancreatic ductal adenocarcinoma (PDAC), fibroblasts are one of the most abundant components and perform critical roles in therapy resistance and cancer progression. The implementation of single-cell RNA sequencing strategies and the development of novel in vitro co-culture models have shown that PDAC cancer-associated fibroblasts (CAFs) comprise distinct subtypes with different molecular and, likely, functional features. These findings raise the question of whether PDAC CAF subtypes have different cells of origin. Answering this question is necessary to advance our knowledge of fibroblast biology because a particular cell of origin could at least partially determine the molecular and functional properties of a specific CAF subtype. Importantly, single-cell RNA sequencing analysis of healthy pancreata has shown the presence of distinct fibroblast populations that may represent different precursors of PDAC myofibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAFs). However, whether PDAC CAF subtypes derive from distinct cell lineages is unknown. Indeed, until now, in vivo evidence of the cells of origin of PDAC CAFs has been lacking.

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Garcia et al. start to fill in this knowledge gap by performing in vivo lineage tracing of *Hoxb6*, a mesenchymal marker expressed mainly during pancreas development, and *Gli1*, a target gene of the Hedgehog signaling pathway, which is active in PDAC CAFs. To this end, Garcia et al. generated several genetically engineered mouse models that enabled inducible labeling of cells expressing these genes in the healthy adult pancreas and during PDAC development. By analyzing these new mouse models, Garcia et al. showed that *Gli1*- and *Hoxb6*-positive cells constitute largely nonoverlapping and spatially separated populations in the healthy adult pancreas and differentially contribute to CAFs in PDAC. In particular, Garcia et al. showed that only *Gli1*-positive fibroblasts expand dramatically during pancreatic carcinogenesis and partially give rise to the myCAF subtype. In addition, although most lineage-traced *Gli1*-positive fibroblasts in PDAC express the myCAF marker α-smooth muscle actin, approximately 30% do not. However, it remains to be determined whether these nonmyofibroblastic cells derived from *Gli1*-positive fibroblasts contribute to the inflammatory iCAF population. Addressing this point is key because previous studies have shown that distinct PDAC CAF subtypes are dynamic and can interconvert in vitro and in vivo, which may indicate a common cell of origin.

The study from Garcia et al. also challenges the common notion that pancreatic stellate cells, which are resident cells of the pancreas, are a major precursor of PDAC CAFs. Indeed, Garcia et al. showed that *Gli1*-positive fibroblasts of the healthy pancreas, which contribute significantly to the formation of CAFs in PDAC, do not have typical features of pancreatic stellate cells. Although pancreatic stellate cells co-cultured with PDAC organoids largely recapitulate the fibroblast composition of murine and human PDAC tumors, these new findings highlight the need to implement in vitro models with other cell types that could recapitulate different aspects of PDAC CAF heterogeneity.

The work from Garcia et al. sparks discussion on several additional points. As similarities in fibroblast composition are emerging between inflammatory and malignant states, it will be informative to evaluate whether *Gli1*-positive fibroblasts also contribute significantly to the fibrosis of pancreatitis models in the absence of oncogenic *Kras* mutations. In addition, the observation that not all resident fibroblasts of the pancreas expand during PDAC development raises the question of what determines the responsiveness to cancer cell–secreted ligands of certain fibroblast populations compared with others. It also remains to be assessed whether pancreatic fibroblasts that do not expand during carcinogenesis still play a role, and whether their depletion impacts PDAC progression. Finally, because the authors show that not all PDAC CAFs are derived from *Gli1*-positive fibroblasts, it remains to be determined what other contributors are responsible for PDAC CAF heterogeneity in vivo. This question relates not only to resident cell populations, but also to cell types that could be recruited from other sites during PDAC development.

Overall, this timely study from Garcia et al. advances our knowledge of fibroblast heterogeneity in pancreatic cancer and prompts new lines of investigation that could lead to the development of novel therapeutic strategies against tumor-promoting components of the PDAC microenvironment.

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**References**

1. Biffi G, Tuveson DA. Diversity and biology of cancer-associated fibroblasts. *Physiol Rev* 2020 May 28. doi: 10.1152/physrev.00048.2019. Online ahead of print.

2. Bernard V, Semaan A, Huang J, San Lucas FA, Mulu FC, Stephens BM, Guerrero PA, Huang Y, Zhao J, Kamyabi N, Sen S, Scheet PA, Taniguchi CM, Kim MP, Tzeng CW, Katz MH, Singhi AD, Maitra A, Alvarez HA. Single-cell transcriptomics of pancreatic cancer...
precursors demonstrates epithelial and microenvironmental heterogeneity as an early event in neoplastic progression. Clin Cancer Res 2019;25:2194–2205.

3. Dominguez CX, Moller S, Keerthivasan S, Koeppen H, Hung J, Gierke S, Breat B, Foreman O, Bainbridge TW, Castiglioni A, Senbabaoglu Y, Modrusan Z, Liang Y, Junttila MR, Klijn C, Bourgon R, Turley SJ. Single-cell RNA sequencing reveals stromal evolution into LRRC15(+) myofibroblasts as a determinant of patient response to cancer immunotherapy. Cancer Discov 2020;10:232–253.

4. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, Teinor JA, Belleau P, Biffl G, Lucito MS, Sivajothi S, Armstrong TD, Engle DD, Yu KH, Hao Y, Wolfgang CL, Park Y, Preall J, Jaffee EM, Califano A, Robson P, Tuveson DA. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. Cancer Discov 2019;9:1102–1123.

5. Hosein AN, Huang H, Wang Z, Parmar K, Du W, Maitra A, Olson E, Verma U, Brekken RA. Cellular heterogeneity during mouse pancreatic ductal adenocarcinoma progression at single-cell resolution. bioRxiv 2019;5:e129212.

6. Ohlund D, Handly-Santana A, Biffl G, Elyada E, Almeida AS, Ponz-Sarvise M, Corbo V, Oni TE, Hearn SA, Lee EJ, Chio II, Hwang CI, Tiriac H, Baker LA, Engle DD, Feig C, Kultti A, Egeblad M, Fearon DT, Crawford JM, Clevers H, Park Y, Tuveson DA. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med 2017;214:579–596.

7. Peng J, Sun BF, Chen CY, Zhou JY, Chen YS, Chen H, Liu L, Huang D, Jiang J, Cui GS, Yang Y, Wang W, Guo D, Dai M, Guo J, Zhang T, Liao Q, Liu Y, Zhao YL, Han DL, Zhao Y, Yang YG, Wu W. Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma. Cell Res 2019;29:725–738.

8. Biffl G, Oni TE, Spieelman B, Hao Y, Elyada E, Park Y, Preall J, Tuveson DA. IL1-induced JAK/STAT signaling is antagonized by TGFbeta to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. Cancer Discov 2019;9:282–301.

9. Garcia PE, Adoumie M, Kim EC, Zhang Y, Scales MK, El-Tawil YS, Shaikh AZ, Wen HJ, Bednar F, Allen BL, Wellik DM, Crawford HC, Pasca di Magliano M. Differential contribution of pancreatic fibroblast subsets to the pancreatic cancer stroma. Cell Mol Gastroenterol Hepatol 2020;10:581–599.

10. Tian H, Callahan CA, DuPree KJ, Darbonne WC, Ahn CP, Scales SJ, de Sauvage FJ. Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. Proc Natl Acad Sci U S A 2009;106:4254–4259.

11. Apte MV, Pirola RC, Wilson JS. Pancreatic stellate cells: a starring role in normal and diseased pancreas. Front Physiol 2012;3:344.