

variants from 3,020 healthy individuals so that researchers can use this database to filter population-specific polymorphisms and assess the pathogenicity of rare variants when studying families with East Asian ancestry who have high rates of cancer (Figure 1). More WGS of lung cancers, perhaps with a focus on early onset cases, will likely uncover more germline risk variants. Curiously, population-specific germline alleles may contribute to the long-established enrichment of *EGFR* mutations in lung adenocarcinoma patients of East Asian or American ancestry (Carrot-Zhang et al., 2021). Future studies will need more clinical and somatic data from diverse populations to elucidate the genetic etiology of different lung cancer histological subtypes with different somatic mutational landscapes and thus enable precision prevention for all.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Dissecting the intracellular pancreatic tumor microbiome at single-cell level

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Microorganisms play a role in the progression of various cancers. In this issue of *Cancer Cell*, Ghaddar et al. traced bacteria in pancreatic tumors at single-cell resolution and associated their intracellular presence with cell-type-specific transcriptional shifts, with links to clinical prognosis.

Pancreatic ductal adenocarcinoma (PDAC) tumors harbor a microbiome that is likely sourced from the gastrointestinal tract (Kartal et al., 2022; Thomas et al., 2018), may play a role in tumorigenesis (Aykut et al., 2019; Pushalkar et al., 2018) and progression (Riquelme et al., 2019), and has therefore been proposed as a therapeutic target (Vitiello et al., 2019). PDAC micro-

biome composition varies greatly between patients, while imaging data also suggest large intra-tumoral heterogeneity in microbial carriage within subjects (Nejman et al., 2020). Yet the causes and consequences of this heterogeneity remain poorly understood, and the mechanisms by which microbes affect infected tumor cells remain elusive.

In this issue of *Cancer Cell*, Ghaddar et al. (2022b) report an approach to probe microbial diversity and activity at the level of individual host cells (Figure 1). They traced microbial reads in single-cell RNA sequencing (scRNA-seq) data of PDAC tumors and healthy pancreatic tissue from control subjects using the newly developed tool SAHMI (single-cell analysis



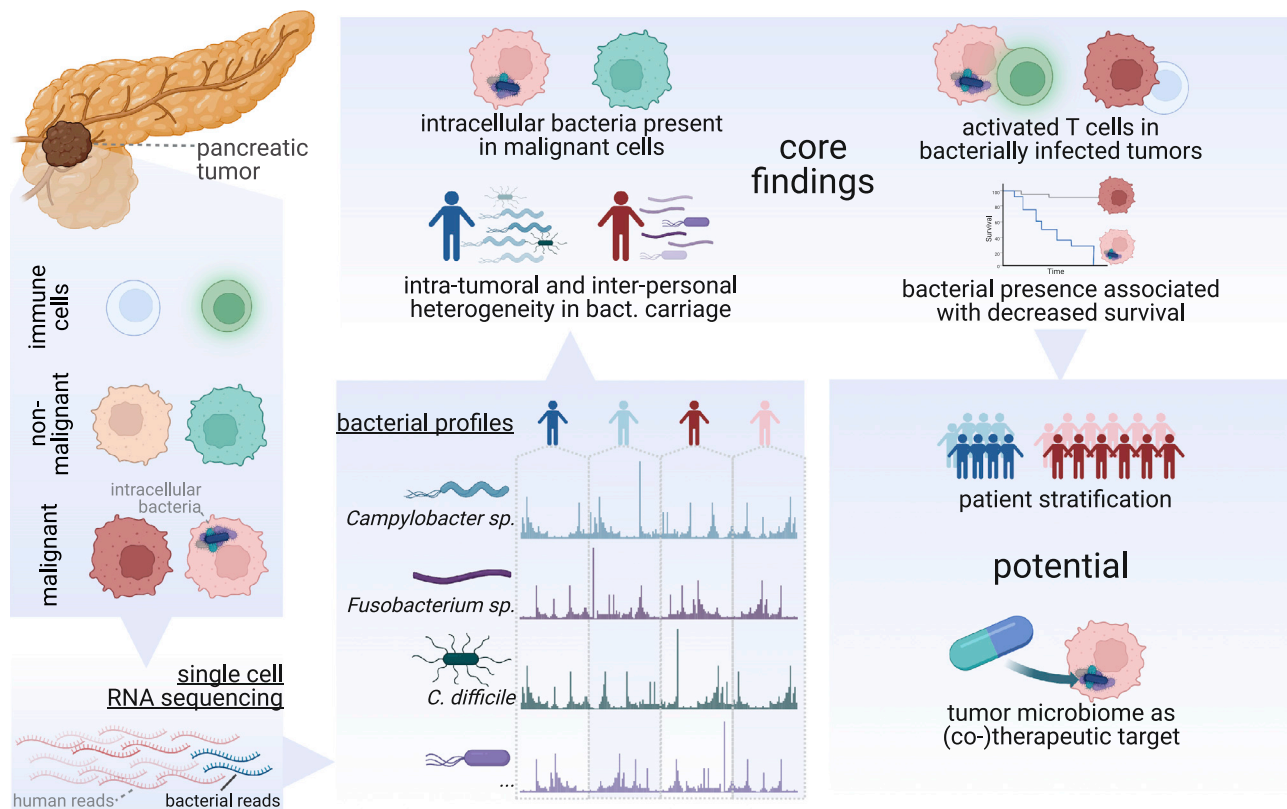


Figure 1. Pancreatic tumor microbiome profiling at single-cell resolution

Ghaddar et al. (2022b) developed an approach to profile tumor-dwelling bacterial populations based on single-cell RNA-sequencing data. They report that malignant cells harbor intracellular bacteria, with high intra-tumoral and inter-personal heterogeneity of colonization. High bacterial carriage in tumors was associated with higher levels of infiltration by activated T cells and with lower patient survival. In the future, single-cell tumor microbiome profiling may prove relevant in stratifying patient cohorts and may allow for (co)therapeutic interventions targeting individual microbial species. This figure was created using Bio-Render.com.

of host-microbiome interactions; (Ghaddar et al., 2022a). Their workflow detects relevant microbial signals with high specificity: thorough bioinformatic filters remove reads attributable to the human host or to putative bacterial contaminants introduced during sample processing and sequencing. Moreover, the authors distinguished intracellular from extracellular microbial carriage by matching barcodes in the raw sequencing data.

The authors detected intracellular bacteria in malignant tumor and immune cells, consistent with previous imaging-based results (Nejman et al., 2020), but not in healthy pancreatic tissue of control patients. While the majority of detected microbes were “usual suspects” with well-described PDAC associations, such as *Campylobacter* sp., *Leptotrichia* sp., or *Fusobacterium nucleatum*, the authors also report a remarkably high prevalence of *Clostridioides difficile*, a gut pathobiont that had not previously been observed in

the pancreas. The carriage of these bacteria in tumor cells was found to be highly heterogeneous but correlated with species-specific transcriptional patterns. This suggests that infection by different gastrointestinal bacteria shapes the activity of tumor cells in distinct ways, which may further hint at differential roles of these species in tumorigenesis and disease progression.

In particular, the authors observed that bacteria-infected tumors were more commonly infiltrated by activated T cells, providing further evidence that immune system function within the tumor may be (differentially) modulated by the local microbiome. Remarkably, both activated T cells and malignant cells in tumors they infiltrated had upregulated PD-1-signaling pathways. As the gut microbiome modulates the efficacy of anti-PD-1 therapeutic interventions in other cancer types (Gopalakrishnan et al., 2018), this suggests the tumor microbiome as a possible co-thera-

peutic target in PDAC. This notion is further corroborated by the authors’ observation in publicly available datasets that carriage of intracellular bacteria was associated with decreased patient survival.

This study addresses a recognized but so-far intractable problem in the field: while it is well established that bacterial carriage in tumors both varies between subjects and is heterogeneous within the tumor, this heterogeneity has been difficult to quantify with sufficient throughput using imaging-based techniques. The use of sequencing data at the resolution of single host cells is an elegant approach to overcome this challenge, and it translates seamlessly to the study of other cancer types in which a role for tumoral bacteria can be suspected, as well as to other body tissues where bacteria may be relevant but only present at low abundances. Although the generation of scRNA-seq data remains challenging and costly in practice, the present work adds a highly

relevant additional layer of interpretation that may make the effort worthwhile, in particular if bacterial contamination during sample preparation and processing is kept at a minimum and the study population size is sufficient for robust associations.

The study by Ghaddar et al. relies on a comparatively small patient cohort, and non-tumor “control” pancreatic tissue samples were obtained from different individuals, prohibiting matched comparisons within the same subject. Moreover, the authors point out that they did not detect fungal or viral signals above detection limits, indicating that other important yet low-abundant players in the local tumor microbiome may likewise be missed by the technique. Nevertheless, the work is an important step toward quantitatively studying host-microbe interactions in tumors at very high and biologically relevant resolutions.

Profiling bacteria at single-host-cell resolution thus offers exciting inroads for future studies of the (pancreatic) cancer microbiome. The work by Ghaddar et al. provides a pilot study that demonstrates possible lines of investigation: the species-specific association of intracellular infection with distinct transcriptional shifts in the host cell with further clinical parameters, and with prognosis. Yet while the present study remains observational and descriptive by design, the technique will arguably enable mechanistic work in animal or organoid models, in particular for understanding local modulation of immune functions or for elucidating possible *in situ* effects of tumoral bacteria on therapy response. While the presented method is fully invasive and tissues are

destroyed during sample preparation, the combination of quantitative scRNA-seq readouts with spatial information from imaging data may further inform spatially explicit models of tumorigenesis and progression. Similarly, the combination with data from other body sites (e.g., oral and fecal metagenomes or blood readouts) will allow tracing of the origin, route, and conditions of bacterial translocation and ectopic colonization in tumors.

As scRNA-seq readouts of tumors become increasingly popular and feasible, mining such data for bacterial signals will be a highly relevant addition to the existing toolbox for studying oncogenesis and tumor progression. The fine-scale association of heterogeneous microbial colonization in tumors may further elucidate whether specific microorganisms initiate and promote tumorigenesis, or whether microbial infection is mostly opportunistic. Either way, this approach promises clinically relevant insights, both with regard to disease etiology and possibly to stratify therapeutic interventions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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