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Correspondence

Intraparenchymal metastases as a cause for local recurrence of pancreatic cancer

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Sir: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal forms of cancer, with a dismal 5-year survival rate of 11%.[1] Of the one in five patients fortunate enough to qualify for surgery, approximately 80% will develop a recurrence, with 24% of recurrences involving the remnant pancreas.[2] Although PDAC is known to be highly invasive along ductal, neural and vascular networks, limitations in the resolution of diagnostic imaging (such as computed tomography) have hindered our ability to identify the microscopic processes that produce recurrences. Here, using serial tissue sectioning and deep learning, we report unique identification of a microscopic, intraparenchymal metastasis, providing evidence for one mechanism by which PDAC can recur in the remnant pancreas.

An elderly male was diagnosed with a 2.5-cm poorly differentiated pancreatic ductal adenocarcinoma. A Whipple procedure was performed and all surgical margins were pronounced microscopically negative for tumour. CODA, a recently developed tool for three-dimensional (3D) reconstruction of tissues, was applied to resected grossly uninvolved pancreatic tissue from the elderly male patient.[3] Briefly, grossly normal pancreatic parenchyma was formalin-fixed, paraffin-embedded (FFPE) and serially sectioned to a depth of 6.4 mm. Every third section was stained with haematoxylin and eosin (H&E) and digitised, while intervening sections were left unstained (Figure 1A). Scanned H&E sections were registered to create a digital volume using a non-linear histological image registration approach. A semantic segmentation algorithm was used to label seven pancreatic components in the tissue: islets of Langerhans, normal ductal epithelium, vasculature, fat, acini,

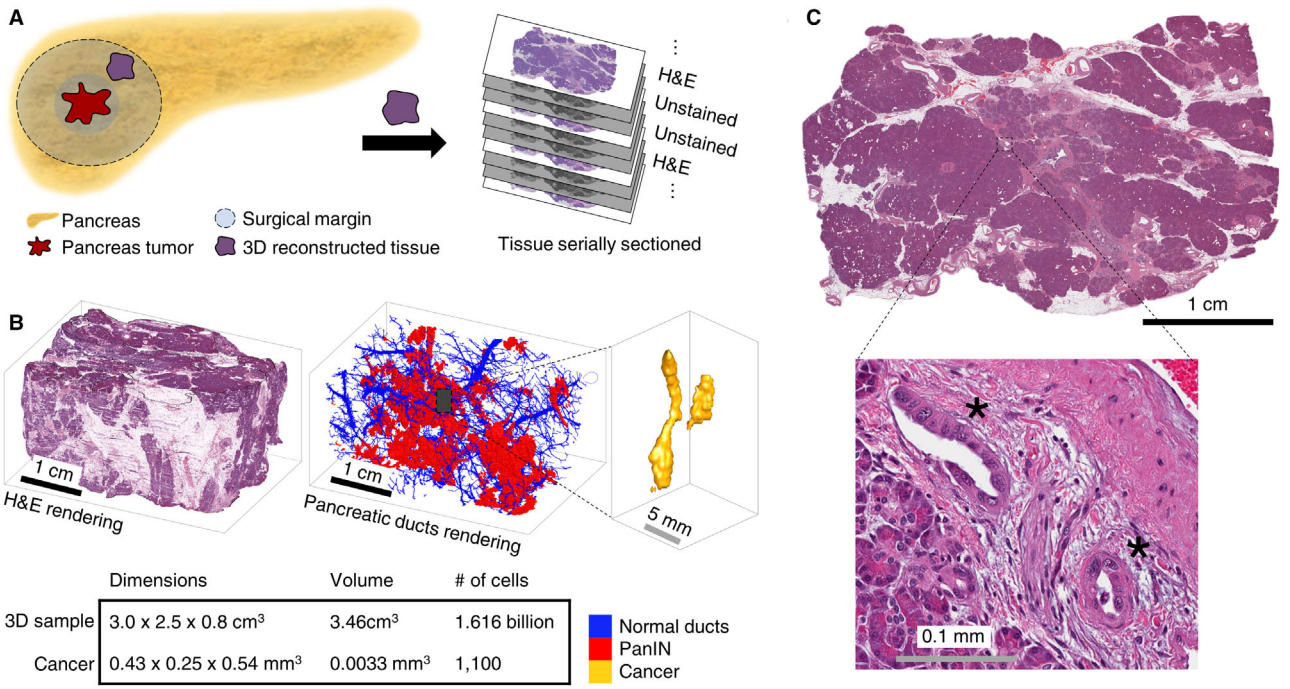

Figure 1. Microscopic intralobular metastasis revealed during the reconstruction of pancreatic tissue. **A,** Grossly normal pancreatic tissue from the surgical margin of a Whipple surgery was serially sectioned and every third slide stained with haematoxylin and eosin (H&E). **B,** Scanned sections were analysed using CODA, described in Kiemen *et al.*,[3] to reconstruct and label pancreatic microanatomy three-dimensionally. Shown are reconstructions of the H&E volume (left), normal and precancerous tissue (centre) and a zoom-in of the focus of cancer. Relevant dimensions, volumes and cell counts are given. **C,** Sample whole-slide image and zoom-in showing the venous invasion (cancer marked with *).

collagen and pancreatic neoplasia. The deep learning algorithm relies upon pathologist-guided manual annotations on a subset of the serial images and is validated upon an independent testing data set of annotated images. The deep learning algorithm achieved > 90% per-class precision and recall. Following image registration and tissue labelling, CODA allows 3D rendering and quantification of serial histological images at high resolution.

A grossly normal sample of 3.46 cm^3 was reconstructed using CODA, and was found to contain approximately 1.6 billion cells (Figure 1B). Deep learning detection of neoplasms was verified through examination of histological sections. Here, 92 structures were confirmed as pancreatic intra-epithelial neoplasia (PanIN). Two small structures, consisting of abnormal-appearing epithelial cells in communication with an endothelial lining, proved to be microscopic foci of invasive pancreatic cancer completely detached from the larger tumour (Figure 1C; Supporting information, Video S1). The two clusters contained approximately 1100 cancer cells and occupied a volume of 0.0033 mm^3, representing merely 0.00007% (or approximately one in 15 000) of the cells in the sample.

Separate foci of cancer in an organ can represent two independent primary tumours or intraparenchymal spread of one neoplasm.[4] To investigate the relationship of the microscopic intraparenchymal lesion to the patient's larger PDAC, we first attempted to use somatic DNA sequencing. However, the microscopic focus was so small that, after laser capture microdissection and DNA extraction, we recovered only 6 ng of DNA and so were unable to perform targeted sequencing.

Instead, we performed immunohistochemical labelling with antibodies to *Tp53* and *Smad4* on the remaining unstained serial sections to compare the labelling between the microscopic focus and the larger tumour. Gain of tumour protein *Tp53* is found in approximately 85% of pancreatic cancers, while loss of *Smad4* is found in approximately 55% of pancreatic cancers.[5] We found that both foci had wild-type labelling for *Tp53* and intact *Smad4* expression (Figure 2). Review of the literature suggests a co-dependence of *Tp53* and *Smad4* expression in pancreatic cancers.[6] As the exact probabilities of this co-dependence are not well established we cannot calculate the probability of two unrelated tumours with this combination of stains, but instead suggest that the foci are probably related, as only 15% of pancreatic cancers present wild-type labelling for *Tp53*. This conclusion is supported by the location of the microscopic focus, intimately associated with endothelial cells, suggesting intravascular spread from the nearby tumour.

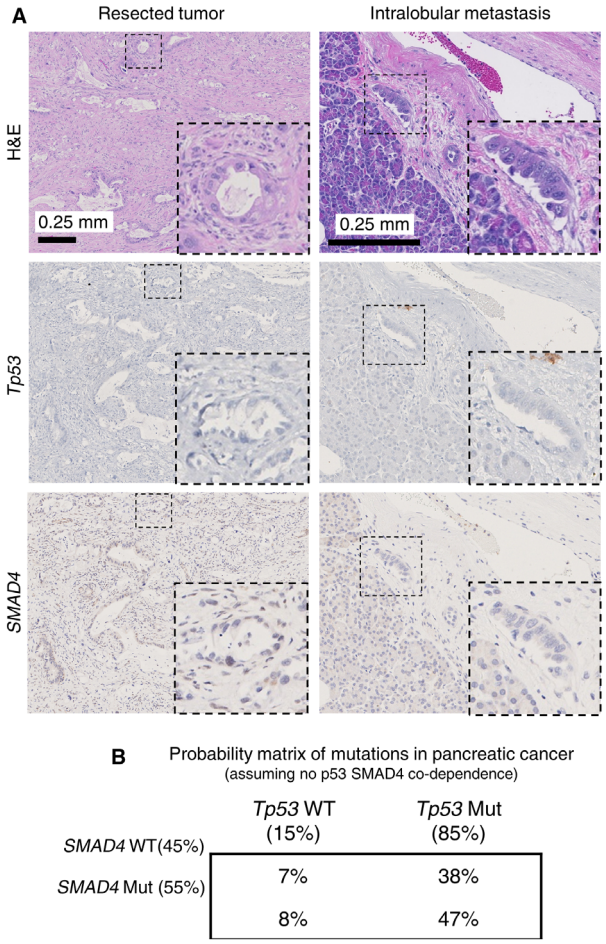

Figure 2. Histology informs the relationship between the tumour and the intralobular metastasis. Zoom-in of a section from the intralobular metastasis (top) and resected tumour (bottom) presented in haematoxylin and eosin, Tp53 and Smad4 staining (from left to right). We determined that the Tp53 stain is wild-type and the Smad4 stain is intact in both regions.

Our identification of a microscopic, intraparenchymal pancreatic cancer metastasis highlights one mechanism for tumour recurrence in the remnant pancreas after apparently curative surgery. It also underscores the power of artificial intelligence and 3D reconstruction in pathology research. The focus described in this paper was present on only 135 of 1600 serial histological sections analysed. At its largest, only 16 cancer cells were present on a single histological slide: a small number that could be easily missed without the aid of computer vision. Deep learning platforms such as CODA are capable of rapid screening of hundreds of sections and have the

potential to dramatically increase the power of histological imaging in biomedical research. In conclusion, we present here evidence of a microscopic intraparenchymal metastasis of pancreatic cancer cells in a grossly normal parenchyma in a patient who underwent surgical resection for a 2.5-cm primary lesion. We posit that such intraparenchymal metastases may contribute to local recurrence of pancreatic cancer.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

Data availability statement

The data presented in this study are available from the corresponding authors upon request.

Ashley L Kiemen[1,2,3,*]
YoungGeun Choi[4,*]
Alicia M Braxton[1]
Cristina Almagro Pérez[4]
Sarah Graham[1]
Mia P Grahn[2]
Neha Nanda[1]
Nicholas Roberts[1]
Laura Wood[1,3]
PeiHsun Wu[2]
Ralph H Hruban[1,3]
and Denis Wirtz[1,2,3,5]

[1]*Department of Pathology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, MD, USA,* [2]*Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, MD, USA,* [3]*Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA,* [4]*Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD, USA,* [5]*Department of Materials Science and Engineering, The Johns Hopkins University, Baltimore, MD, USA*

**These authors contributed equally to this study.*

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1.