Supplementary Methods

Genomic DNA extraction

Each of the tumor specimens from both the surgical and endoscopic biopsies were prepared as formalin-fixed paraffin embedded blocks and slides. Genomic DNA was then purified and isolated using the GeneRead DNA FFPE Kit (Qiagen; Hilden, Germany) according to the manufacturer's instructions and finally eluted with 30 μL of elution buffer. The sample was quantified using the Qubit dsDNA HS Assay Kit using a Qubit2.0 fluorometer (ThermoFisher Scientific; Waltham, MA, USA).

Targeted amplicon sequencing

Mutation profiles were determined by target amplicon sequencing using a next-generation sequencer as described previously [1]. Briefly, 20–60 ng of gDNA was amplified via PCR using a PDA-related gene panel that we designed; this panel consisted of 18 genes and 220 amplicons (Ion AmpliSeq™ Custom DNA Panel). Sequencing was performed using an IonPGM System (Thermo Fisher Scientific), and the sequence reads were demultiplexed, quality-filtered, and aligned to the human reference genome (GRCh37) using the Torrent Suite software (ver. 5.0.4; Thermo Fisher Scientific). Variants were identified using the Variant Caller software (ver. 5.0.4.0; Thermo Fisher Scientific). To identify somatic mutations, independent genotyping of each lesion and of a normal duodenum-derived sample was subtracted; variants found in the normal sample were excluded from molecular profiling. The variant-calling analysis was operated using the somatic variant-calling mode optimized to detect low-frequency variants set to a minimum allele frequency of 0.02 and minimum coverage of 100. We also excluded putative false-negative variants by evaluating the phred-scaled variation call quality calculated by this plugin, and by manually confirming the alignment using the IGV software version 2.3.59 (Broad Institute; Cambridge, MA, USA and Regents of the University of California, CA, USA).

Mutation detection by digital PCR

Mutant *KRAS* variants (codons 12 and 13) were analyzed using a QX200 Droplet Digital PCR System (Bio-Rad; Hercules, CA, USA) as described previously [1,2]. To detect the *SMAD4* W323X variant, a combination of the commercial primer/probe set (Bio-Rad, assay ID; dHsaMDS479854191) and custom designed probe (IDT, 56-FAM/T+GG+AAC+A+T+CA+ATA /3IABkFQ/, where "+N" represents locked nucleic acid base) was utilized. Purified DNA was partitioned into ~22,000 droplets per sample by mixing with 70 μL droplet-generation oil in

a QX200 droplet generator (Bio-Rad). Droplets were then subjected to thermal cycling, and the samples were transferred to a QX200 droplet reader (Bio-Rad) for fluorescence measurement of 6-fluorescein amidite and hexachlorofluorescein probes. Droplets were scored as positive or negative based on their fluorescence intensities; the intensity thresholds were gated using the following template: gBlocks Gene Fragments of approximately 120 bp (Integrated DNA Technologies, Inc.; Coralville, IA, USA) that were complementary to *KRAS* G12D (the target sequence) and *KRAS* G12V (negative control) were used to detect *KRAS* G12D. *SMAD4* W323X genomic DNA from the primary tumor was used as a positive control. Finally, the absolute copy number input in the reaction as well as the ratio of the mutated fragment were calculated by the QuantaSoft software (version 1.7; Bio-Rad) based on the Poisson distribution.

Immunohistochemistry

SMAD4 expression was analyzed via immunohistochemistry using anti-SMAD4 antibody (clone B-8, 1:100; Santa Cruz Biotech, Santa Cruz, CA, USA) as previously described [1]. Absent or weak SMAD4 expression (suggesting haploinsufficiency) was considered aberrant protein expression.

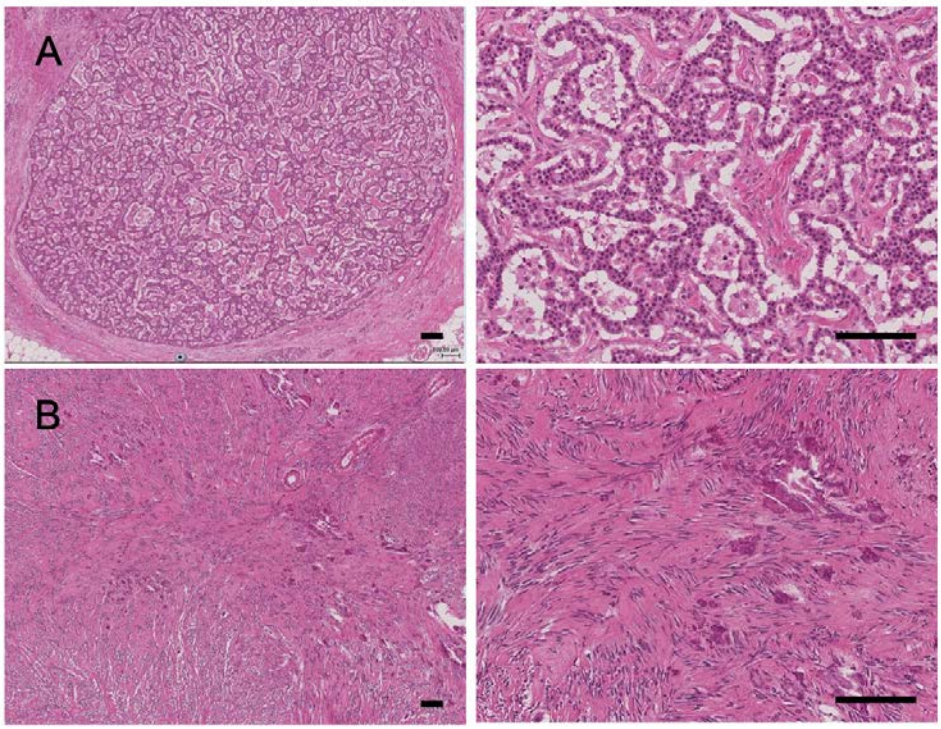
Supplementary References

1. Omori Y, Ono Y, Tanino M et al. Pathways of progression from intraductal papillary mucinous neoplasm to pancreatic ductal adenocarcinoma based on molecular features. Gastroenterology 2019; 156: 647–661 e642
2. Ono Y, Sugitani A, Karasaki H et al. An improved digital polymerase chain reaction protocol to capture low-copy KRAS mutations in plasma cell-free DNA by resolving 'subsampling' issues. Mol Oncol 2017; 11: 1448–1458.

Supplementary Figure legend

Supplementary Figure 1: Histological findings of pancreatic neuroendocrine and gastrointestinal stromal tumors.

(A) Well differentiated pancreatic neuroendocrine tumor, 4 mm in diameter. (B) Gastrointestinal stromal tumor, 7 mm in diameter. Scale bars: 100 μm.



Supplementary Figure 2: Droplet digital PCR confirming *KRAS* mutation in the biopsy/cytology samples collected via the transpapillary route.

KRAS G12D was selectively detected by a mutant-specific probe against mutant *KRAS* at codon 12/13 in the endoscopic pancreatic duct biopsy but not in the pancreatic juice. No signals for *SMAD4* W323X variant were detected. EPB, endoscopic pancreatic duct biopsy; Cy., cell pellet for pancreatic juice cytology.