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Molecular characterization of organoids derived from pancreatic intraductal papillary mucinous neoplasms

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Abstract

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Author contributions statement

FC, APK, MG, LDW, and NJR planned and designed study. BH, MAT, MQ, CG, AM-G, DH, S-MH, RHH, EDT, APK, MG, LDW, and NJR collected samples and clinicopathologic data. BH, MAT, MQ, MF, CZ, JRE, LDW, and NJR conducted experiments and generated sequence data. BH, MAT, KF, MS, LDW, and NJR analyzed data. BH, LDW, and NJR wrote the manuscript. All authors approved the final version of the manuscript.

Data availability statement

RNA-Seq data has been deposited in the European Genome-phenome Archive (EGA) with accession code XXupdate at proof stageXX. Whole genome sequence data is available on request from the corresponding authors.

Conflicts of interest

RHH has the right to receive royalty payments from Thrive Earlier Diagnosis for the GNAS in pancreatic cysts invention. LDW is an Associate Editor of The Journal of Pathology. No other conflicts of interest were declared.

Intraductal papillary mucinous neoplasms (IPMNs) are a commonly identified non-invasive cyst-forming pancreatic neoplasms with the potential to progress into invasive pancreatic adenocarcinoma. There are few *in vitro* models with which to study the biology of IPMNs and their progression to invasive carcinoma. Therefore, we generated a living biobank of organoids from 7 normal pancreatic ducts and 10 IPMNs. We characterized 8 IPMN organoid samples using whole genome sequencing and characterized 5 IPMN organoids and 7 normal pancreatic duct organoids using transcriptome sequencing. We identified an average of 11,344 somatic mutations in the genomes of organoids derived from IPMNs, with one sample harboring 61,537 somatic mutations enriched for T->C transitions and T->A transversions. Recurrent coding somatic mutations were identified in 15 genes, including *KRAS*, *GNAS*, *RNF43*, *PHF3*, and *RBM10*. The most frequently mutated genes were *KRAS*, *GNAS*, and *RNF43*, with somatic mutations identified in 6 (75%), 4 (50%), and 3 (37.5%) IPMN organoid samples respectively. On average, we identified 36 structural variants in IPMN derived organoids, and none had an unstable phenotype (>200 structural variants). Transcriptome sequencing identified 28 genes differentially expressed between normal pancreatic duct organoid and IPMN organoid samples. The most significantly upregulated and downregulated genes were *CLDN18* and *FOXA1*. Immunohistochemical analysis of FOXA1 expression in 112 IPMNs, 113 mucinous cystic neoplasms, and 145 pancreatic ductal adenocarcinomas demonstrated statistically significant loss of expression in low-grade IPMNs ($p < 0.0016$), mucinous cystic neoplasms ($p < 0.0001$), and pancreatic ductal adenocarcinoma of any histologic grade ($p < 0.0001$) compared to normal pancreatic ducts. These data indicate that FOXA1 loss of expression occurs early in pancreatic tumorigenesis. Our study highlights the utility of organoid culture to study the genetics and biology normal pancreatic duct and IPMNs.

Keywords

Intraductal papillary mucinous neoplasm; pancreas; pancreatic; precursor; cancer; organoids; DNA; RNA; mutations; expression

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a 5-year survival rate of only 9 percent, and PDAC is predicted to become the second leading cause of cancer-related death in the United States by 2030 [1,2]. PDAC develops from non-invasive precursor lesions in a process that takes many years, if not decades [3]. These pancreatic cancer precursor lesions can be microscopic, as is the case for pancreatic intraepithelial neoplasia (PanIN), or macroscopic, as is the case for intraductal papillary mucinous neoplasms (IPMNs). As IPMNs are macroscopic, and incidental pancreatic cystic lesions suggestive of IPMNs are identified in up to 2.6% of the population when imaged by contrast-enhanced multi-detector computed tomography, IPMNs present an ideal opportunity for early clinical intervention [4].

PDACs have been extensively characterized by high-throughput genomic, transcriptomic, and proteomic approaches. This has led to many new insights in pancreatic tumorigenesis, including the identification of genetic alterations driving tumorigenesis, molecular

subgroups that may respond to specific therapies, and evolutionary features underlying pancreatic cancer progression indicating a broad time window of opportunity for early detection [5–12]. However, comparatively little is known about the genetics and biology of pancreatic precursor lesions such as IPMNs. Previous genomic studies to characterize pathogenic germline variants and somatic alterations in IPMN have used whole exome or targeted gene panels and identified key drivers previously associated with PDAC tumorigenesis (*KRAS*, *CDKN2A*, *TP53*, *SMAD4*) as well as drivers of the IPMN pathway (*GNAS*, *RNF43*) [13–15]. Furthermore, microarray-based RNA expression analyses in IPMNs highlighted differentially expressed genes associated with histologic grade and putatively, therefore, IPMN progression [16,17]. Similarly, immunohistochemistry to detect expression of candidate genes has been used to identify differentially expressed proteins in IPMN compared to normal duct and invasive carcinoma [16,18]. While these studies provided important insights into the origin and development of IPMNs, they did not comprehensively characterize somatic alterations that occur in the genome and transcriptome of these earliest stages in pancreatic tumorigenesis.

The development of model systems to study pancreatic tumorigenesis is essential to understand the physiologic effects of genetic alterations and of changes in gene expression. Unfortunately, there are few reported examples of *in vitro* models of normal pancreatic duct and pancreatic precursor lesions such as IPMN, and those that do exist are either experimentally immortalized cells or derived from patients with concurrent invasive carcinoma [19,20]. Furthermore, while several genetically engineered mouse models of IPMN have been described, *in vivo* models are expensive and time consuming to generate and do not have the genetic complexity present in human IPMN [21,22]. This lack of disease appropriate models has contributed to the paucity of research into pancreatic cancer precursor lesions.

Three-dimensional organoid culture is a recently described *ex vivo* model system, in which small clusters of neoplastic cells are grown in matrix gels, maintaining similar appearance and functionality to the original tissue. From the pancreas, organoids can be generated from small amounts of tissue such as endoscopic fine-needle aspiration or biopsy samples in a short period of time, thereby allowing for additional testing (such as pharmacotyping) to complement the pathological diagnosis [23]. Organoid culture can also be used to enrich ductal or neoplastic cells for DNA or RNA analyses without contamination by associated mesenchymal or immune cells, which are typically eliminated in early passages [24]. With its combination of fidelity to three-dimensional structure and ease of experimental manipulation, the organoid model represents a valuable tool for several lines of investigation, including validation of genetic alterations required for cancer progression and correlation of *in vitro* behavior with treatment response and outcome *in vivo*. However, the ability to culture and propagate organoids from human pancreatic precursor lesions has not been systematically investigated.

In this study, we generate a living biobank of organoids derived from 7 normal human pancreatic ducts and 10 human IPMN samples. We also present the characterization of somatic alterations in these IPMN organoids using whole genome sequencing. Furthermore,

we use transcriptomic sequencing to identify genes differentially expressed between normal pancreatic duct and IPMN organoids.

Materials and methods

Additional details are provided in Supplementary material and methods

Study participants and ethics statement

This study was reviewed and approved by the Johns Hopkins University Institutional Review Board. All patients gave written informed consent. Seventeen patients were enrolled in this study. All patients had undergone pancreatic resection at the Johns Hopkins Hospital between January 2016 and September 2018 (supplementary material, Table S1). IPMN organoids were derived from 10 patients, designated H23 to H79. Normal pancreatic duct organoids were derived from macro-dissected, grossly normal, main pancreatic duct from 7 unmatched patients, designated N22 to N92.

Whole genome sequencing

Whole genome sequencing was performed as previously described [25]. DNA from eight IPMN organoids (in passages 1–4) and matched primary non-neoplastic duodenal tissue were whole genome sequenced by the Next Generation Sequencing Core of the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center as previously described [25]. In brief, sequence libraries were generated using the TruSeq DNA Nano Library Prep Kit (Illumina, San Diego, CA, USA; catalog no. 20015964) and sequenced on a HiSeq2500 (Illumina) to generate 2×150 bp reads.

Whole exome sequencing

Whole exome sequencing was performed as previously described [25]. Briefly, paired IPMN (tumor) and duodenum (normal) DNA from H55, H68, and H79 were extracted from FFPE tissue and whole exome sequenced by the Next Generation Sequencing Core of the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center. Exome capture and library preparation were performed with Agilent SureSelect Target Enrichment V5-post (Agilent, Santa Clara, CA, USA). DNA libraries were sequenced on an Illumina NovoSeq instrument to generate 2 × 150 bp PE sequence reads.

RNAseq

RNA-Seq was performed as previously described [25]. RNA from IPMN organoids and normal pancreatic duct organoids (in passages 2 or 3) were sequenced by Genewiz Inc. (Plainsfield, NJ, USA). In brief, mRNA was enriched by polyA selection before random priming, cDNA synthesis, A-tailing, adapter ligation, and PCR amplification of final sequence libraries. Libraries were sequenced on an Illumina HiSeq instrument to generate 2×150-base pair (bp) reads.

Immunohistochemistry

Tissue microarrays (TMAs) containing IPMNs, mucinous cystic neoplasms (MCNs), PDAC, and normal pancreatic ducts were used to verify RNAseq findings at the protein level by

immunohistochemistry (IHC). TMAs were grouped by lesion type and IPMNs were grouped by grade. TMAs included tissue samples from 112 patients with IPMN (61 IPMN-LG and 51 IPMN-HG), 113 patients with MCN (103 MCN-LG and 10 MCN-HG/CA), 145 patients with PDAC (77 well-moderately differentiated and 68 poorly differentiated). Normal pancreatic duct on IPMN, MCN, and PDAC TMAs were scored and used for comparisons, 49 normal pancreatic ducts were identified on the IPMN TMA, 99 on the MCN TMA, and 102 on the PDAC TMA. Immunolabeling for FOXA1 was performed on formalin-fixed, paraffin (FFPE) embedded sections on a Ventana Discovery Ultra autostainer (Roche Diagnostics, Indianapolis, IN, USA). In brief, following dewaxing and rehydration on board, epitope retrieval was performed using Ventana Ultra CC1 buffer (Roche Diagnostics; catalog no. 6414575001) at 96 °C for 64 min. The primary antibody, anti-FOXA1 (1:300 dilution; Abcam, Cambridge, MA, USA; catalog no. ab170933) was applied at 37 °C for 60 min. Primary antibodies were detected using an anti-rabbit HQ detection system (Roche Diagnostics; catalog no. 7017936001 and 7017812001) and Chromomax DAB IHC detection kit (Roche Diagnostics; catalog no. 5266645001). Sections were counterstained with Mayer's hematoxylin, dehydrated and mounted.

IHC results were evaluated using a H-score, a semi-quantitative, non-blinded approach. In brief, more than 100 cells were counted for each case, and the H-score was subsequently generated by adding the percentage of strongly stained nuclei (3X), the percentage of moderately stained nuclei (2X), and the percentage of weakly stained nuclei (1X), giving a possible range of 0–300 [26]. The score was independently obtained by two authors (BH and KF) and the mean of the two values used for statistical analysis. Inter-observer differences were < 5%.

Results

Whole genome sequencing

Eight IPMN derived organoids and matched normal tissue samples were analyzed by whole genome sequencing (supplementary material, Table S1). All IPMN organoids had either an oncogenic *KRAS* or an oncogenic *GNAS* mutation as determined by PCR amplification and Sanger sequencing prior to whole genome sequencing analysis to confirm the neoplastic origin of the samples (supplementary material, Table S2). The IPMN organoids sequenced included three from IPMNs with low-grade dysplasia (IPMN-LG) and five from IPMNs with high-grade dysplasia (IPMN-HG) (supplementary material, Table S1). The mean number of reads per sample was 356,581,475 (range: 304,627,462 – 434,765,537) and the mean alignment rate was 99.6% (range: 97.3% – 99.8%). The mean coverage was 33.8X (range: 27.2 – 41.8X). The mean of bases covered at 10X was 98.2% (range 97.8% – 98.9%) and covered at 20X was 93.7% (range: 88.5% – 96.9%) (supplementary material, Table S3).

A total of 90,751 somatic mutations (single base substitutions and INDELs) were identified across all eight IPMN organoids sequenced. The mean number of somatic mutations per sample was 11,344 (range: 1,419 – 61,537) (Figure 1A). One sample (H79) had a much higher number of somatic mutations than the rest of the cohort (61,537); when this sample was excluded, the mean number of somatic mutations per sample was 4,173 (range: 1,419 – 8,927). After excluding H79, there was no significant difference in the number of somatic

mutations in organoids derived from IPMN-LG and IPMN-HG (mean number of somatic mutations 2,932 and 5,104 respectively; $p = 0.3712$). The mutation signatures and transition/transversion patterns are similar among all IPMN derived organoids except for H79 (supplementary material, Figure S1, and Figure 1B). Furthermore, while the mutation signatures of the 7 IPMNs (when H79 was excluded) were predominated by putative age-related changes such as C->T transitions, the mutation signature of H79 was unique, with enrichment of T->C transitions and T->A transversions. To test for the possibility that the increased mutations were a result of a mismatch repair defect, we tested H79 organoid sample for microsatellite instability using a PCR-based assay, which did not demonstrate microsatellite instability in this sample.

The mean number of somatic mutations in coding regions was 52 (range: 29 – 90) excluding H79. The mean number of non-silent somatic mutations was 36 (range: 16 – 69) (supplementary material, Figure S2). We found that 16 genes had non-silent mutations in 2 IPMN organoid samples, including H79. The most commonly mutated genes were *KRAS* (6 IPMN organoid samples), *GNAS* (4 IPMN organoid samples), and *RNF43* (3 IPMN organoid samples). The remaining 13 genes (*CYP4Z1*, *DNAH9*, *HLA-DQB2*, *KIAA1109*, *MUC4*, *MUC12*, *PHF3*, *RBM10*, *RFXP2*, *SLC7A8*, *SLC9A3*, *ZNF260*, *ZNF835*) were somatically mutated in two IPMN organoid samples (Figure 1C). Somatic mutations were also identified in established pancreatic cancer driver genes *CDKN2A* p.H83Y (1 IPMN organoid sample) and *TP53* p.R174X (1 IPMN organoid sample) (supplementary material, Table S4). Moreover, inactivating somatic mutations were identified genes in that may play a role in pancreatic tumorigenesis (*AXIN2*, *INPPL1*, *HES1*, and *RREB1*). Importantly, average allele frequencies for coding and splice site somatic mutations ranged from 0.11 for H40 to 0.38 for H60 (supplementary material, Table S4), suggesting ongoing heterogeneity in organoid culture, an observation supported by previous studies [27].

Of note, IPMN organoid sample (H40) had a *KRAS* p.G12V mutation at 0.0455 allele fraction by whole genome sequencing in addition to a *KRAS* p.R73R mutation at 0.0968 allele fraction. This observation may be due to subclonal mutation or the presence of contaminating non-neoplastic cells in the organoid culture. Confirming the neoplastic origin of the organoids from H40, we identified 45 coding somatic mutations in this sample with a mean allele frequency of 0.11 (range: 0.06 – 0.26), including in *MUC4*, *MUC12*, and *PHF3*, genes that were somatically mutated in at least one other IPMN organoid sample (Figure 1C). As expected, somatic mutations included known oncogenic hotspot mutations such as *KRAS* p.G12D (4 IPMN organoid samples), *KRAS* p.Q61R (1 IPMN organoid sample) and *KRAS* p.Q61H (1 IPMN organoid sample), as well as *GNAS* p. R201H (3 IPMN organoid samples) and *GNAS* p.R201C (1 IPMN organoid sample) (supplementary material, Figure S3). The only other recurrent mutation was *SLC7A8* p.T79P in 2 IPMN organoid samples (supplementary material, Figure S3 and Table S4).

We identified a total of 250 somatic SVs in the 8 IPMN organoid samples. The mean number of somatic SVs per sample was 31 (range: 9 – 82). These included 40 duplications, 111 deletions, 58 inversions and 41 translocations (supplementary material, Figure S4 and Table S5). 16 SVs involving the same gene were identified in 2 samples, including 2 SVs occurring at the same positions in *CMPK1-CLIC5* and *DSTYK*. The most frequently

observed SVs were a duplication encompassing *CCNYL3* and *ANKRD26P1*, a deletion of *KIAA1671*, and an inversion involving *LINC02254*, each in three IPMN organoids. Another recurrent SV (2 IPMNs) with the most supporting reads (PR>20 and SR>20) was an inversion in the gene *DSTYK*, encoding a dual serine/threonine and tyrosine protein kinase that is thought to regulate cell death (supplementary material, Figure S5 and Table S5). As the number of SVs per IPMN organoid sample was < 100 and had no obvious pattern of distribution in the genome, none would meet the classification of unstable used in whole genome sequenced PDAC [8].

As pathogenic germline variants in pancreatic cancer susceptibility genes and hereditary cancer predisposition genes have been identified in patients with surgically resected IPMN, we assessed germline variants in sequenced IPMN organoids [15]. However, no pathogenic germline variants in 94 hereditary cancer predisposition genes were identified in this sample set.

Whole exome sequencing

We conducted whole exome sequencing of FFPE IPMN and non-tumor archival tissue from H55, H68, and H79. Identity between whole exome sequenced tissue samples and whole genome sequenced organoid samples was confirmed by comparing germline variant calls. Interestingly, no somatic mutations were shared between FFPE IPMN and organoid sample for H55 and H68, indicating that a different subclone was sequenced from FFPE tissue than grew out in organoid culture. FFPE IPMN and organoid from H79 were both hypermutated, an uncommon finding in IPMN, with 369 and 342 coding or splice site somatic mutations identified in each sample respectively (supplementary material, Table S4 and Table S6) [28]. 58 mutations identified in the FFPE IPMN were present in the organoid sample, highlighting that different subclone(s) of H79 were cultured than were whole exome sequenced.

RNA-Seq

RNA from five IPMN organoids and seven normal pancreatic duct organoids were sequenced. IPMN organoid samples include three from patients with IPMN-LG and two from patients with IPMN-HG (supplementary material, Table S1). Normal duct organoid samples were derived from two patients with branch duct IPMN, four patients with pancreatic neuroendocrine tumors, and one patient with metastatic clear cell renal cell carcinoma. In each case, the harvested pancreatic duct was grossly normal main duct, and analysis of sequence reads in IGV covering exon 2 and exon 3 of *KRAS*, and exon 8 and exon 9 of *GNAS*, did not identify any oncogenic mutations, confirming the non-neoplastic origin of the normal pancreatic duct organoids used for RNA-Seq. The mean number of reads per sample was 47,248,301 (30,775,100 – 60,882,990) and the mean quality score is 37.62 (37.52 – 37.75). 89.67% (89.33% – 90.15%) of bases with quality score is above 30 (≥ 30). The mean alignment rate is 97.72% (97.53% – 98.02%) (supplementary material, Table S7)

Differential gene expression analysis identified 28 genes (29 transcripts) as aberrantly expressed in IPMN derived organoids compared to normal pancreatic duct organoids. These included 15 transcripts representing 14 genes upregulated in IPMN organoids, and 14

transcripts representing 14 genes downregulated in IPMN organoids (Figure 2, Figure 3, and supplementary material, Table S8). Of these genes, *CLDN18* was the most significant upregulated gene ($p = 6.89\text{E-}06$, $q = 0.031$) and *FOXA1* was the most significant downregulated gene ($p = 6.10\text{E-}08$, $q = 0.0009$).

Fusion gene analysis identified 10 fusion events in the RNA-Seq data, including 4 putatively somatic fusion events present only in IPMN organoid samples and 1 putative germline fusion event present in both IPMN and normal pancreatic duct organoid samples. One IPMN organoid sample (H60) had both whole genome sequence and RNA-Seq data available for analysis. In this case, 3 fusions identified by RNA-Seq were not supported by whole genome sequence data, highlighting the discrepancy between fusion and rearrangement calling pipelines. (supplementary material, Table S9).

Immunohistochemistry

To validate FOXA1 expression observed in RNA-Seq data we conducted immunohistochemistry on FFPE IPMN samples from 4 patients. The IPMNs which showed decreased *FOXA1* expression in the RNA-Seq analysis also showed decreased protein expression by immunohistochemistry, with either diffuse moderate (rather than strong) expression or focal areas of loss of expression. In order to validate the most differentially expressed genes from our RNA-Seq analysis, we created a human tissue microarray containing IPMN tissue from 112 patients. Because expression of *CLDN18* has already been evaluated at the protein level in IPMNs [18], we focused on *FOXA1*, which was the most significantly downregulated gene in IPMN compared to normal pancreatic duct. In our TMA sections, immunohistochemistry demonstrated strong and consistent nuclear FOXA1 expression in both normal pancreatic ductal and acinar cells. When all IPMNs were considered together, FOXA1 expression (as determined by H-score) was not significantly lower in IPMNs of any grade compared to normal pancreatic ductal cells (Figure 4, $p = 0.68$). However, low-grade IPMNs had significantly lower expression compared to normal pancreatic duct (Figure 4, $p < 0.0016$). These results suggest that *FOXA1* downregulation at the RNA level leads to decreased protein expression in low-grade IPMNs.

To ascertain the role of FOXA1 in other pancreatic neoplasms, we evaluated FOXA1 expression in MCNs and PDACs, as determined by H-score. A statistically significant decrease in FOXA1 expression was observed for MCNs (supplementary material, Figure S6, $p < 0.0001$) and PDACs (supplementary material, Figure S7, $p < 0.0001$), compared to normal pancreatic duct irrespective of histologic grade. These data indicate that decreased FOXA1 expression is present in a broad range of pancreatic neoplasms.

Discussion

We created a unique set of normal pancreatic duct and IPMN organoids. We then used whole genome sequencing to identify recurrent somatic mutations and structural variants in these organoids. As expected, prevalent somatic mutations in *KRAS*, *GNAS*, *CDKN2A*, *TP53*, and *RNF43* were observed. We also identified 13 other genes with recurrent somatic mutations in IPMN (*CYP4Z1*, *DNAH9*, *HLA-DQB2*, *KIAA1109*, *MUC4*, *MUC12*, *PHF3*, *RBM10*, *RXFP2*, *SLC7A8*, *SLC9A3*, *ZNF260*, and *ZNF835*). *RBM10*, encoding an RNA

binding protein, has been shown to be mutated in multiple cancer types including IPMN [29]. Of note, several other genes with inactivating somatic mutations in our study may play a role in the development of IPMN. These include *AXIN2*, a Wnt pathway regulator, *INPPL1*, a component of FGFR signaling, *HES1*, a target of Notch signaling that can affect Hedgehog signaling, and *RREB1*, a regulator of zinc homeostasis recurrently mutated in PDAC [30–34]. Additional studies to determine the prevalence of somatic alterations in these genes in larger cohorts of IPMNs and their functional role in pancreatic tumorigenesis are warranted.

Interestingly, one high-grade IPMN organoid sample (H79) had an excess of somatic mutations relative to the other IPMNs, and a mutation spectrum with a predominance of T->C transitions and T->A transversions. Previous reports have suggested a higher prevalence of microsatellite instability in IPMNs compared to PDACs [28]. However, we did not identify pathogenic germline variants or somatic alterations in mismatch repair genes in this sample, and microsatellite testing indicated that the tumor was microsatellite stable. Exogenous carcinogens have previously been associated with specific somatic mutation spectra, for example alcohol and aristolochic acid [35,36]. Further investigation to determine the prevalence and etiology of hypermutated IPMNs, and whether this phenotype is associated with high-grade histomorphology, is warranted given the potential clinical implications of somatic mutation burden on therapy [37].

Our germline analysis of 94 hereditary cancer predisposition genes did not identify any pathogenic variants in this cohort, however, the reported prevalence of such variants in patients with surgically resected IPMN is 7.3% [15]. As such, this observation was not unexpected. Multiple variants of unknown significance were found in the assessed hereditary cancer susceptibility genes; however, as none were associated with somatic mutations or copy number alterations, their contribution to tumorigenesis is unknown.

Analysis of RNA-Seq data for gene fusions identified 4 putatively somatic fusion events present only in IPMN organoid samples (supplementary material, Table S8). Previous reports of intraductal oncocytic papillary neoplasms (IOPN) note frequent fusions between *ATP1B1-PRKACB*, *DNAJB1-PRKACA*, or *ATP1B1-PRKACA* [38]. Interestingly, our analysis did not identify any fusions involving these genes, confirming the previous finding that these fusions are a specific feature of IOPNs.

We identified 28 genes that were differentially expressed between IPMN organoids and normal duct organoids. Importantly, *CLDN18*, an integral component of the cell membrane and tight junctions, was the most statistically significant upregulated gene and had previously been shown to be over expressed in IPMNs using immunohistochemistry, validating our approach [18]. The most statistically significant downregulated gene was *FOXA1*, a regulator of apoptosis through downstream mediators including BCL2 and CDKN1B as well as a mediator of epithelial-to-mesenchymal transition [39–41]. We assessed FOXA1 expression using immunohistochemistry and confirmed a statistically significant decrease in protein expression in IPMN, MCN, and PDAC compared to normal pancreatic duct [41]. The association of FOXA1 and other differentially expressed genes with early pancreatic tumorigenesis is intriguing, and studies in larger cohorts will be

necessary to determine whether these genes differentiate IPMNs from other types of precursor lesions or are associated with progression to PDAC.

Our study also provides important data about the feasibility of organoid culture of normal and precancerous epithelial cells derived from human pancreatic resection specimens. We cultured several specimens of normal pancreatic duct and both low-grade and high-grade IPMNs in the organoid system. We were able to isolate adequate DNA and RNA at enriched neoplastic cellularity for genomic and transcriptomic analyses. This highlights the value of organoid culture as a source of enriched biological molecules (including nucleic acids and proteins) for analysis of uncommon, difficult to harvest, or paucicellular neoplastic lesions. In addition, the success of organoid culture of both normal and precancerous pancreatic epithelium highlights this system as a potential tool for experimental manipulation of such human cells in culture.

Though our study represents a unique combination of organoid culture and molecular analysis of human IPMN specimens, it has a number of limitations. First, we and others have demonstrated that organoid cultures of pancreatic precursor lesions and invasive carcinoma may contain many of the same mutations present in original lesion [23,25]. However, our organoid samples were derived from a single piece of resected IPMN and therefore subclones established in organoid culture may not capture the genetic heterogeneity of the whole lesion [42,43]. This was demonstrated by our observation of low concordance between somatic mutations identified in single pieces of FFPE IPMN and organoid samples and is consistent with previous studies demonstrating driver gene heterogeneity in IPMNs [42]. Of note, organoid culture maintains a diverse population of sub-clones. Our observation that average allele frequencies for coding and splice site somatic mutations in our organoid samples range from 0.11 to 0.38 indicate that our organoid cultures also show this diversity. Second, as pancreatic resections are always performed for some underlying pancreatic disease or neoplasm, the non-neoplastic duct samples may not be truly normal. In each case, the harvested pancreatic duct was grossly normal and derived organoids did not have a *KRAS* or *GNAS* mutation. Third, while our study provides insight into the genetics of IPMNs, it was not powered to identify somatic alterations that drive development of high-grade IPMN from low-grade lesion. In the future, larger studies of low-grade and high-grade lesions will be needed to deconvolute the genetic basis of this evolution.

In summary, our study documents a biobank of IPMN and normal pancreatic duct organoids, characterized by genomic and transcriptomic sequencing, a unique resource for the interrogation of these precancerous lesions. Using whole genome sequencing and RNA-Seq we identified potential drivers of IPMN tumor development. These included 12 genes with recurrent somatic mutations and 28 genes that were differentially expressed when compared to normal pancreatic duct. Our results demonstrate that organoid culture is an effective tool for analyzing small precursor lesions at -omics scale to identify critical molecular alterations in precious human tissue samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Somatic mutations identified in whole genome sequenced IPMN organoids. (A) The number of somatic mutations in coding and non-coding regions by sample. (B) Profile of transition/transversion in somatic mutations by sample. (C) Genes with recurrent somatic coding mutations by sample.

Figure 2.

Volcano plot showing expression profile for transcripts in IPMN organoids versus normal pancreatic duct organoids. Q value of 0.05 indicated by red dashed line. Transcripts with q value < 0.05 and fold-change ± 4 highlighted in red with gene symbol.

Figure 3.

Heatmap of 29 differentially expressed transcripts in IPMN organoids versus normal pancreatic duct organoids grouped by sample.

Figure 4.

The expression of FOXA1 in normal pancreas and IPMNs. (A) H&E and (B) immunohistochemical image of normal pancreatic duct and surrounding acinar cells showing strong nuclear expression of FOXA1. (C) H&E and (D) immunohistochemical image of IPMN showing variable weak-moderate expression of FOXA1. (E) H&E and (F) immunohistochemical image of IPMN showing strong expression of FOXA1. (G) H-score for immunohistochemical staining of FOXA1 in IPMN of any grade compared to normal duct and (H) IPMN-HG compared to IPMN-LG and normal duct.