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The oncocytic subtype is genetically distinct from other pancreatic intraductal papillary mucinous neoplasm subtypes

Olca Basturk[1,7], **Marcus Tan**[2,7], **Umesh Bhanot**[1], **Peter Allen**[3], **Volkan Adsay**[4], **Sasinya N Scott**[1], **Ronak Shah**[1], **Michael F Berger**[1], **Gokce Askan**[1], **Esra Dikoglu**[5], **Vaidehi Jobanputra**[5,6], **Kazimierz O Wrzeszczynski**[5], **Carlie Sigel**[1], **Christine Iacobuzio-Donahue**[1], and **David S Klimstra**[1]

[1]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, USA

[2]Department of Surgery, University of South Alabama, Mobile, AL, USA

[3]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, USA

[4]Department of Pathology, Emory University, Atlanta, GA, USA

[5]Department of Molecular Diagnostics, New York Genome Center, New York, USA

[6]Department of Pathology, Columbia University Medical Center, New York, USA

Abstract

In 2010, the World Health Organization reclassified the entity originally described as intraductal oncocytic papillary neoplasm as the ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm. Although several key molecular alterations of other intraductal papillary mucinous neoplasm subtypes have been discovered, including common mutations in *KRAS*, *GNAS*, and *RNF3*, those of oncocytic subtype have not been well characterized. We analyzed 11 pancreatic ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms. Nine pancreatic ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms uniformly exhibited typical entity-defining morphology of arborizing papillae lined by layers of cells with oncocytic cytoplasm, prominent, nucleoli, and intraepithelial lumina. The remaining two were atypical. One lacked the arborizing papilla and had flat oncocytic epithelium only; the other one had focal oncocytic epithelium in a background of predominantly intestinal subtype intraductal papillary mucinous neoplasm. Different components of this case were analyzed separately. Formalin-fixed, paraffin-embedded specimens of all cases were microdissected and subjected to high-depth-targeted next-generation sequencing for a panel of 300 key cancer-associated genes in a platform that enabled the identification of sequence mutations, copy number alterations, and select structural rearrangements involving all targeted genes. Fresh frozen specimens of two cases were also subjected to whole-

Correspondence: Dr DS Klimstra, MD, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York 10065, USA. klimstrd@mskcc.org.

[7]These authors contributed equally to this work.

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genome sequencing. For the nine typical pancreatic ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms, the number of mutations per case, identified by next-generation sequencing, ranged from 1 to 10 (median = 4). None of these cases had *KRAS* or *GNAS* mutations and only one had both *RNF43* and *PIK3R1* mutations. *ARHGAP26, ASXL1, EPHA8*, and *ERBB4* genes were somatically altered in more than one of these typical ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms but not in the other two atypical ones. In the neoplasm with flat oncocytic epithelium, the only mutated gene was *KRAS*. All components of the intestinal subtype intraductal papillary mucinous neoplasms with focal oncocytic epithelium manifested *TP53, GNAS*, and *RNF43* mutations. In conclusion, this study elucidates that ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm is not only morphologically distinct but also genetically distinct from other intraductal papillary mucinous neoplasm subtypes. Considering that now its biologic behavior is also being found to be different than other intraductal papillary mucinous neoplasm subtypes, ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm warrants being recognized separately.

‘Oncocytic subtype’ of intraductal papillary mucinous neoplasms of the pancreas was originally described as a separate variant of pancreatic intraductal neoplasms.[1] However, the current (2010) World Health Organization designated this neoplasm as a subtype of intraductal papillary mucinous neoplasm of the pancreas because oncocytic subtype has some overlapping features with other subtypes of intraductal papillary mucinous neoplasm.[2] For example, they all present as an at least partially cystic pancreatic mass.[3] The cystic appearance is due to dilation of the ducts by an intraductal neoplasm composed of tumor cells arranged in a papillary pattern.[1,4] These neoplasms also have less aggressive course than conventional pancreatic ductal adenocarcinoma, even if there is an associated invasive carcinoma.[1,3–10] In fact, the family of intraductal papillary mucinous neoplasms may contain ‘individuals’, which show overlapping features between the four so far recognized subtypes.

However, ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm also has several distinguishing pathologic characteristics such as the complex arborizing papillae with delicate fibrovascular cores and distinctive intraepithelial lumina formation, in addition to the oncocytic nature of the cells, due to abundant intracytoplasmic mitochondria.[1,3,4,9,11] In other organs, tumors that are prone to accumulate abundant intracytoplasmic mitochondria in the exclusion of other organelles appear to have distinct pathogenesis and different biology than their non-oncocytic counterparts, with the best example being renal oncocytic neoplasms or Hurthle cell tumors of the thyroid.[12,13] Recently, the studies have shown that despite being very complex lesions, ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms are also actually either noninvasive or minimally invasive, and although they may recur, their seldom lead to mortality of the patient.[1,9,10]

With the introduction of routine molecular genetic analyses, including next-generation sequencing,[14–16] various molecular alterations have been identified in other subtypes of intraductal papillary mucinous neoplasms. As in many ductal neoplasms including pancreatic ductal adenocarcinomas for which *KRAS* mutation appears to be a nearly pre-requisite baseline change, *KRAS* mutations are the most common mutations and have been

detected in the majority of intraductal papillary mucinous neoplasms (up to 100%).[8,15,17–22] More interestingly, activating *GNAS* mutations at codon 201 have been identified in approximately half (41–66%) of intraductal papillary mucinous neoplasms,[15,16,23–26] particularly in the intestinal subtype.[15,23,25] Inactivating mutations in the *RNF43* gene, a likely tumor suppressor and negative regulator of the Wnt signaling pathway,[27] are also seen in up to 75% of intraductal papillary mucinous neoplasms.[15,24] Mutations of *PIK3CA* are described in 3–11%.[28–30] Less common alterations involve *CDKN2A/p16* (loss of expression in 18% of non-invasive intraductal papillary mucinous neoplasms and 53% of associated invasive carcinomas), *SMAD4* (loss of expression in 3% of non-invasive intraductal papillary mucinous neoplasms and 30% of associated invasive carcinomas), *TP53* (10% of high-grade intraductal papillary mucinous neoplasms), *BRAF* (6% of high-grade intraductal papillary mucinous neoplasms), *CTNNB1/β-catenin* (4%), *IDH1* (4%), *STK11* (4%), *PTEN* (4%), *ATM* (2%), *CDH1* (2%), FGFR3 (2%), and *SRC* (2%).[17,24]

In contrast, the literature on the molecular features of ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm is fairly limited, partially due to relative rarity of the neoplasm. Emerging studies including case reports,[31] a few cases studied together with other subtypes of intraductal papillary mucinous neoplasms,[15,32,33] or studies analyzing selected gene mutations, such as *KRAS*,[34,35] have shown that ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm generally lacks the mutations commonly found in other subtypes of intraductal papillary mucinous neoplasms. This raises the questions of whether ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm is genetically distinct from the other subtypes of intraductal papillary mucinous neoplasm and furthermore, whether it should be considered as a separate diagnostic entity?[36] To further elucidate this question, we analyzed a series of ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms by targeted next-generation sequencing, using a broad panel of cancer-related genes, to investigate genes not previously assessed.

Materials and Methods

With approval of the Institutional Review Board, the surgical pathology databases of Memorial Sloan Kettering Cancer Center and Emory University were searched for patients with a diagnosis of pancreatic ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm between 1991 and 2013. Resections of 11 pancreatic neoplasms were identified for which the slides and tissue blocks were available. The diagnoses were confirmed by the authors. Medical records including pathology reports were reviewed to obtain clinical data including age, gender, treatment modalities, and outcome.

Targeted Next-Generation Sequencing

Twenty 10-micron-thick sections were cut from formalin-fixed, paraffin-embedded tissue blocks containing ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms. From these sections, areas of interest were needle microdissected. For each patient, extraction of DNA was performed on dissected tissue and where available on normal, non-pancreatic tissue (stomach, spleen or duodenum). Deep coverage, targeted next-generation sequencing was then performed on a panel of 300 genes, including *KRAS, GNAS*, and *RNF43*, listed in

the supporting information (Supplementary Information File 1), known to undergo somatic genomic alterations in cancer, as previously described.[37,38] Briefly, massively parallel sequencing libraries (Kapa Biosystems, New England Biolabs) that contain barcoded universal primers were generated from 115 to 250 ng genomic DNA from the tumor material and matched normal tissue. After library amplification and DNA quantification, equimolar pools were generated consisting of up to 24 barcoded libraries. These DNA pools were subjected to solution-phase hybrid capture with synthetic biotinylated DNA probes (Nimblegen SeqCap) targeting all protein-coding exons from the selected 300 cancer genes as well as introns known to harbor recurrent translocation breakpoints. Genes were selected to include commonly implicated oncogenes, tumor suppressor genes, and members of pathways deemed actionable by targeted therapies. Each hybrid capture pool was sequenced to deep coverage in a single paired-end lane of an Illumina flow cell. Subsequently, the sequencing data were deconvoluted to match all high-quality barcoded reads with the corresponding tumor samples, and genomic alterations (single-nucleotide sequence variants, small insertions/deletions, and DNA copy number alterations) were identified. For matched tumor/normal tissue pairs ($n = 9$), somatic single-nucleotide variants and insertions and deletions were called using MuTect and the SomaticIndelDetector tools in GATK, respectively.[39,40] For unmatched tumors ($n = 2$), MuTect was run against a pool of unrelated DNAs from normal formalin-fixed, paraffin-embedded tissue blocks, and variants were filtered out if they were present in the 1000 Genomes project at a population frequency of >1%. All candidate mutations, insertions, and deletions were reviewed manually using the Integrative Genomics Viewer.[41]

Whole-Genome Sequencing

Fresh frozen tumor material and matched normal tissues of two cases (Cases 4 and 5) were also subjected to whole-genome sequencing, which was performed using Illumina paired-end chemistry on a HiSeqX sequencer, which yielded coverage of at least 80 × for tumor samples and 40 × for normal samples, with more than 95% of the target bases having at least 5 × coverage.

Genomic DNA isolation—Tissue was extracted using Qiagen AllPrep DNA/RNA Mini Kit. DNA was quantified using the Qubit 2.0 Fluorometer, Invitrogen, and quality was determined by using Agilent Bioanlyzer.

Illumina whole-genome sequencing and genotyping—DNA libraries were prepared using the KAPA Hyper Prep Kit (Kapa, Kapa Biosystems, Wilmington, MA, USA). For each sample library preparation, 100 ng of high molecular weight genomic DNA was fragmented using the Covaris LE220 system to an average size of 350 bp. Fragmented samples were end repaired and adenylated using Kapa's end-repair and a-tailing enzymes. The samples were then ligated with Biooscientific adapters and PCR amplified using KAPA Hifi HotStart Master Mix (Kapa, Kapa Biosystems, Wilmington, MA, USA). The DNA libraries were clustered onto flowcells using Illumina's cBot and HiSeq Paired End Cluster Generation kits as per manufacturer protocol (Illumina, San Diego, CA, USA). Sequencing was performed using 2 × 150 Illumina HiSeqX platform with v2.5 chemistry reagents. Genotyping was performed using HumanOmni2.5 M BeadChips (Illumina).

Whole-genome sequencing and data analysis was performed at the New York Genome Center (NYGC, New York, NY, USA). Paired-end 2 × 150 bp reads were aligned to the GRCh37 human reference using the Burrows-Wheeler Aligner (BWA aln v.0.7.8)[42] and processed using the best-practices pipeline that includes marking of duplicate reads by the use of Picard tools and realignment around indels and base recalibration via Genome Analysis Toolkit (GATK) ver. 2.7.4.[43] We employ the following variant callers: muTect v1.1.4,[39] LoFreq v2.0.0[44] (single-nucleotide variants only), Strelka v1.0.13[45] (both single-nucleotide variants and indels), Pindel[46] and Scalpel[47] (indels only) and return the union of calls, filtered using the default filtering criteria as implemented in each of the callers. Single-nucleotide variants and indels were annotated via snpEff, snpSift,[48] and GATK VariantAnnotator using annotation from ENSEMBL, COSMIC,[49] Gene Ontology, and 1000 Genomes.

Structural variants, such as copy number variants as well as complex genomic rearrangements, were detected by the use of multiple tools: NBIC-seq[50] for copy number variants/structural variants calling, Delly,[51] Crest,[52] and BreakDancer[53] for structural variant calling. We prioritize structural variants in the intersection of callers and structural variants for which we can find additional split-read evidence using SplazerS.[54] Structural variants for which there is split-read support in the matched normal or that are annotated as known germline variants (1000 Genomes call set, DGV) were removed as likely remaining germline variants. The predicted sets of somatic structural variants were annotated with gene overlap (RefSeq, Cancer Gene Census) including prediction of potential effect on genes (eg, disruptive/exonic, intronic, intergenic, fusion candidate). In addition, copy number variants and loss of heterozygosity were also analyzed from the genotyping chip using Nexus (Biodiscovery) software.

Results

Clinicopathologic Features

Eleven patients were identified. The clinicopathologic features of the cases are summarized in Table 1. Half of these patients were male and the mean age was 60 years (range, 45–78). No patients received neoadjuvant chemotherapy or chemoradiation. One patient with an associated invasive colloid carcinoma received adjuvant chemotherapy, but not chemoradiation. The majority (67%) of tumors was located in the head and patients underwent pancreatoduodenectomy. Tumor size varied from 1 to 10 cm (median, 5.5 cm).

Of 11 cases, 9 exhibited typical entity-defining characteristics. Grossly, these tumors were characterized by large, soft tan friable nodules associated with cystic spaces (Figure 1). In contrast to other subtypes of intraductal papillary mucinous neoplasm, the intraductal location was difficult to recognize. Microscopically, the tumors were architecturally complex, with arborizing papillae growing into the lumens of massively dilated ducts. The neoplastic epithelial cells had abundant eosinophilic granular cytoplasm and nuclei with single prominent nucleoli. Intraepithelial and intracellular lumina were also identified (Figure 2). Only one of the nine typical ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm had an associated invasive carcinoma, in the form of multiple

microscopic foci of small micropapillary clusters of oncocytic cells infiltrating the periductal stroma. The invasive component was too small for molecular analysis.

Two ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms had atypical morphology. Case 10 (Table 2) was non-papillary and revealed a flat cyst-lining of neoplastic epithelial cells with oncocytic features (Figure 3). Case 11 (Table 2) was predominantly intestinal subtype intraductal papillary mucinous neoplasm with a distinct focus of oncocytic epithelium on H&E (Figure 4). Of note, a previously performed MUC6 immunohistochemical stain was negative in the intestinal component and positive only at the base of the papillae of the oncocytic focus. This case also had an associated invasive colloid carcinoma (Figure 5). All components of this case (intestinal subtype intraductal papillary mucinous neoplasm, oncocytic, and invasive carcinoma) were analyzed separately.

The follow-up period ranged from 2 to 190 months (median, 61 months). Two of nine patients with available follow-up died; the remaining seven (77%) were alive without disease. Case 11 (with heteregenous epithelium and an associated invasive colloid carcinoma) died of perioperative complications 2 months after the surgery, and Case 2 (typical ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm) died of unrelated causes after 190 months.

Molecular Features

Targeted next-generation sequencing—A total of 13 tumor samples from 11 patients underwent targeted deep sequencing. The results of these genetic studies are summarized in Table 2, and details of the individual cases are described in the supporting information (Supplementary Information File 2).

A total of 41 mutations were identified in the 11 cases, ranging 1–10 mutations per neoplasm (median = 4, Figure 6). In the nine typical ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms, the following four genes were mutated in at least two neoplasms: *ARHGAP26* (missense mutations at K592N or P4Q), *ASXL1* (missense mutation at V119L or a frameshift deletion at M341fs), *EPHA8* (missense mutations at R375H or R384H), and *ERBB4* (missense mutations at L939F or L1163M). None of the nine typical ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms had *KRAS* or *GNAS* mutations. One typical ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm (Case 2) had *RNF43* (frameshift/deletion at L61fs), *PIK3R1* (a missense mutation at P194T), and *PIK3R3* (missense mutation at R49Q) mutations.

In case 10 (with flat oncocytic epithelium), the only somatic mutation found was *KRAS* (missense mutation at G12V). Case 11 (with heteregenous epithelium and an associated invasive colloid carcinoma) manifested *GNAS* (missense mutation at R201C and R201S), *RNF43* (missense mutation at M1I), and *TP53* (missense mutations at R248W and S241F as well as nonsense mutation at W146) mutations in all three components. Whereas, *PDGFRA* (missense mutation at T230M) and *ATRX* (missense mutation at E1492G) mutations were exclusive to the oncocytic component.

Whole-genome sequencing—Fresh frozen tumor samples from two patients (Cases 4 and 5) also underwent whole-genome sequencing. Details of the individual cases are described in the supporting information (Supplementary Information Files 3 and 4).

Copy number analysis revealed that both samples had multiple copy number gains and losses, the vast majority of which including a contiguous set of genes, in multiple chromosomes (Case 4: chromosomes 8, 12, 17, and 21; Case 5: chromosomes 1, 2, 3, 7, 8, 9, 13, 14, 15, and 22). Also both specimens had clonal loss of heterozygosity of an entire chromosome (Case 4: chromosome 12; Case 5: chromosome 20).

A total of 91 mutations within 87 genes were identified among the 2 cases, 40 mutations in Case 4 and 51 mutations in Case 5. Among the five gene mutations in Cases 4 and 5 identified by targeted next-generation sequencing, only *TEK* gene mutation (missense mutations at T401M) was also detected by whole-genome sequencing. Whole-genome sequencing also failed to reveal mutations in any of the well-recognized intraductal papillary mucinous neoplasm or pancreatic ductal adenocarcinoma genes.

Discussion

Recent studies have helped to better characterize the histologic subtypes of intraductal papillary mucinous neoplasm[15,24,32,55–60] and have confirmed that they have differences in genetic progression patterns compared with conventional pancreatic ductal adenocarcinoma.[19,30,61–63] However, a focused study of ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm has not been reported to date. Our results show that typical ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms do not harbor previously reported intraductal papillary mucinous neoplasm-related mutations (*KRAS, GNAS, PIK3CA, CDKN2A/p16, SMAD4, TP53*, etc.). Only one of our typical intraductal papillary mucinous neoplasms (Case 2) had an *RNF43* mutation, which is a different mutation than previously reported intraductal papillary mucinous neoplasm-related *RNF43* mutations.

Our results provide support for the proposition that ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm is a distinct entity with not only different morphologic features and biologic behavior, but also a different genotype. The most important genotypic difference from other subtypes of intraductal papillary mucinous neoplasm is the lack of *KRAS* mutations. *KRAS* gene mutations are frequent in intraductal papillary mucinous neoplasms without oncocytic differentiation. For example, Jang *et al.*[22] investigated 37 intraductal papillary mucinous neoplasms and found mutations in codons 12 and 13 of the *KRAS* gene in 50% of pancreatobiliary subtype, 36% of gastric subtype, and of 21% of intestinal subtype. A review of relevant literature provided in their manuscript also shows that *KRAS* gene mutations were reported in up to 100% of intraductal papillary mucinous neoplasms.[22] Similarly, in a prior study, our group identified mutations in codons 12 and 13 of the *KRAS* gene in 18 of 26 (69%) intraductal papillary mucinous neoplasm cases of other subtypes although there were no *KRAS* mutations in eight ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm cases.[34] Patel *et al.*[31] also found no activating point mutations in codons 12 and 13 of the *KRAS* gene in a single case of ‘oncocytic subtype’ of intraductal papillary mucinous neoplasm with associated invasive carcinoma. With possible evidence to

the contrary, Xiao *et al.*[35] identified somatic *KRAS* gene mutations in codon 12 in 3 of 18 (17%) 'oncocytic subtype' of intraductal papillary mucinous neoplasms. However, the authors acknowledged they included 'oncocytic subtype' of intraductal papillary mucinous neoplasms exhibiting heterogeneous epithelium.[35] Therefore, it is quite possible that the three cases they reported as *KRAS* mutated may have exhibited heterogeneous epithelium, for which the oncocytic features were a morphologic variation within a non-oncocytic intraductal papillary mucinous neoplasm. As evidenced by our Case 11, the genotype of the intestinal and oncocytic components manifested similar mutations that are more typical of other intraductal papillary mucinous neoplasm subtypes.

We found further support of 'oncocytic subtype' of intraductal papillary mucinous neoplasm as an entity distinct from other subtypes of intraductal papillary mucinous neoplasm by the absence of *GNAS* mutations. Only Case 11 with heteregenous epithelium and an associated invasive colloid carcinoma manifested a *GNAS* mutation in all its components. *GNAS* gene mutations are common in other subtypes of intraductal papillary mucinous neoplasm, particularly in the intestinal subtype. Dal Molin *et al.*[23] reported that 100% of intestinal subtype, 71% of pancreatobiliary subtype, and 51% of gastric subtype intraductal papillary mucinous neoplasms harbored a codon 201 *GNAS* mutation. The authors also analyzed two 'oncocytic subtype' of intraductal papillary mucinous neoplasm cases, which were found to be *GNAS* wild type,[23] in accordance with our findings. Similarly, in a recent study, our group has identified frequent *GNAS* gene mutations in non-oncocytic intraductal papillary mucinous neoplasms.[26] Of note, no *GNAS* mutations have been identified in other pancreatic cystic neoplasms; nor were they identified in pancreatic ductal adenocarcinoma, suggesting that *GNAS* mutations are specific for the non-oncocytic intraductal papillary mucinous neoplasm phenotype.[15,23,25]

The lack of involvement in 'oncocytic subtype' of intraductal papillary mucinous neoplasms of the typical genetic underpinnings that occur in other subtypes of intraductal papillary mucinous neoplasm should not come as a surprise considering the distinctive pathologic manifestations of this tumor type. Also, it is becoming clear in other organs that oncocytic neoplasms characterized by abundant mitochondrial accumulation have different identity than their non-oncocytic counterparts of the respective organs.[12,13] Similarly, the absence of the genetic alterations that underlie pancreatic ductal adenocarcinoma may explain the incomparably better clinical behavior of 'oncocytic subtype' of intraductal papillary mucinous neoplasms.[1,9] More importantly, these differences suggest that pancreatic 'oncocytic subtype' of intraductal papillary mucinous neoplasm has distinct pathways of tumor progression, possibly through different underlying mechanisms causing different genetic alterations. In fact, the majority of the pancreatic cystic neoplasms (intraductal papillary mucinous neoplasms, mucinous cystic neoplasms, serous cyst adenomas and solid-pseudopapillary neoplasms) are associated with recurrent mutations in components of ubiquitin-dependent pathways.[15] However, 'oncocytic subtype' of intraductal papillary mucinous neoplasm does not seem to be associated with ubiquitin-dependent pathways. In contrast, four specific genes (*ARHGAP26, ASXL1, EPHA8*, and *ERBB4*) were found to be mutated by targeted next-generateion sequencing in more than one of our cases (due to smaller sampling of target regions with whole-genome sequencing, compared to targeted next-generation sequencing, whole-genome sequencing may not detect variants of low

allelic frequency). Excluding reports of potential involvement of the *EPHA8* gene in pancreatic carcinogenesis,[64–67] these genes have not otherwise been previously associated with intraductal papillary mucinous neoplasms. Therefore, they are worth further scrutiny as being more than mere epiphenomena for their potential role in tumorigenesis of 'oncocytic subtype' of intraductal papillary mucinous neoplasms.

The *ARHGAP26* gene encodes Rho GTPase activating protein 26 (ARHGAP26), also known as GTPase regulator associated with focal adhesion kinase (GRAF), in humans. It is recognized as a tumor suppressor gene that binds to focal adhesion kinase. Mutations and deletions of the *GRAF* gene are strongly implicated in leukemia development.[68–70] The *GRAF* gene has also recently been shown to be mutated in gastric cancer.[71,72] The function of additional sex comb-like 1 (ASXL1) protein is not fully delineated,[73] but it has been postulated that it may be involved in DNA and/or histone modification.[74] Similar to *ARHGAP26*, mutations in *ASXL1* have been identified in myelodysplastic syndromes[75] and other myeloid malignancies, like acute myeloid leukemia, chronic myelomonocytic leukemia, and myeloproliferative neoplasia.[76] Both *EPHA8* and *ERBB4* genes are members of human receptor tyrosine kinase family. *EPHA8* encodes ephrin type-A receptor 8 protein. EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system.[77] Genetic studies suggest that *EPHA8* is involved in regulating cell adhesion[78] and apoptosis.[79] *ERBB4* encodes receptor tyrosine-protein kinase erbB-4 enzyme, a member of the epidermal growth factor receptor subfamily. Mutations in this gene have been associated with diverse cancers including esophagus,[80] gallbladder,[81] and colon.[82]

In conclusion, although its intraductal nature and somewhat overlapping clinicopathologic features have led to classification of 'oncocytic subtype' of intraductal papillary mucinous neoplasms as a variant of intraductal papillary mucinous neoplasm, our results suggest that, when defined strictly, 'oncocytic subtype' of intraductal papillary mucinous neoplasms are genetically distinct and thus should be recognized separately. Further analysis of molecular alterations in biologically distinct pathway(s) including *ARHGAP26, ASXL1, EPHA8*, and *ERBB4* genes will likely shed new light on the mechanisms of intraductal tumor formation in the pancreas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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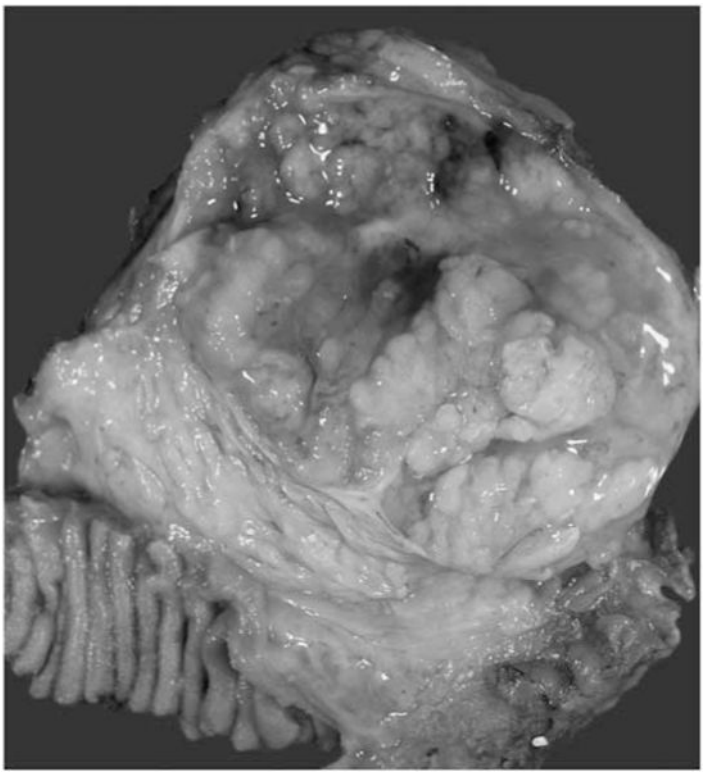

Figure 1.
Macroscopically, typical pancreatic ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms were characterized by tan, friable, and large papillary excrescences filling cystic structures.

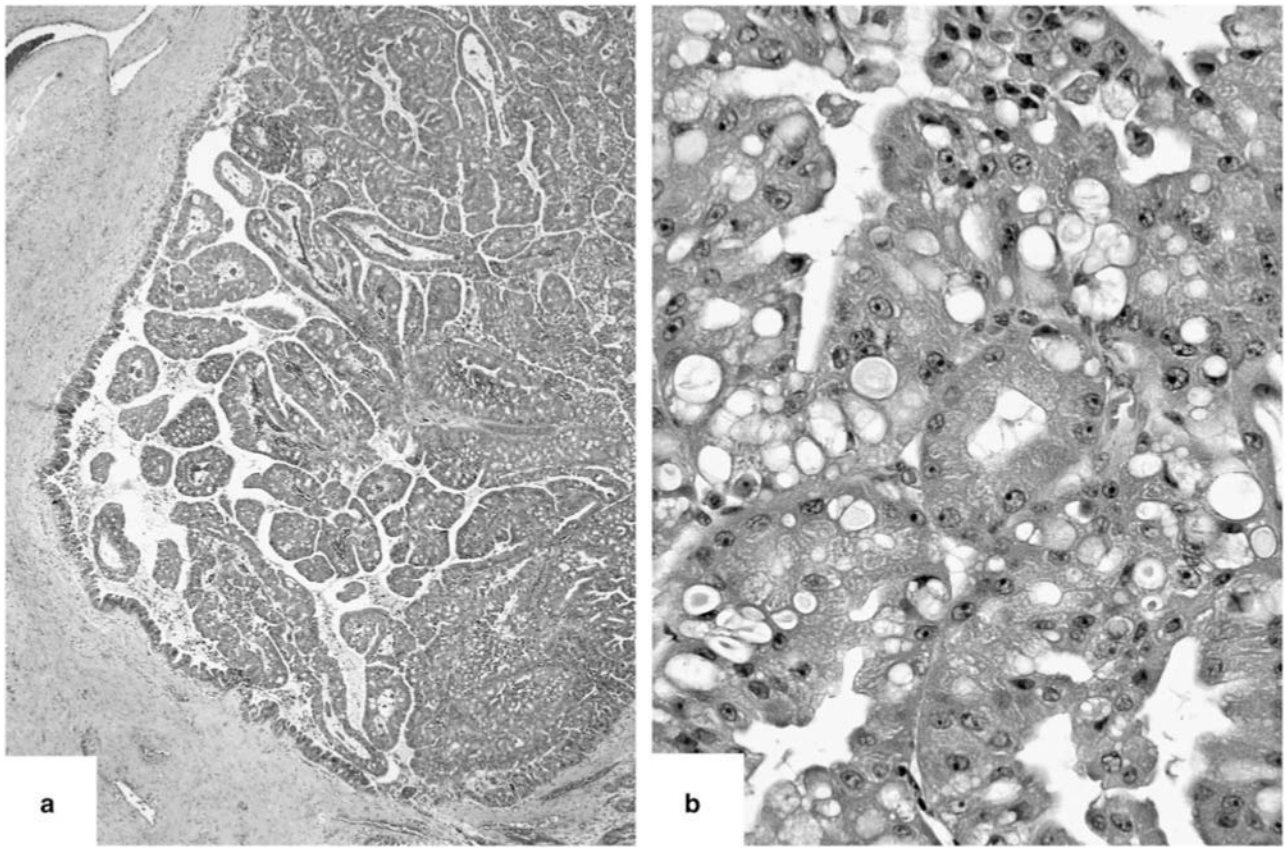

Figure 2.

Papillae of typical pancreatic ‘oncocytic subtype’ of intraductal papillary mucinous neoplasms were often very delicate and arborizing (**a**), and the cells revealed distinctive oncocytic appearance with voluminous acidophilic granular cytoplasm and single prominent nucleoli. (**b**) Multiple intracellular lumina were also present.

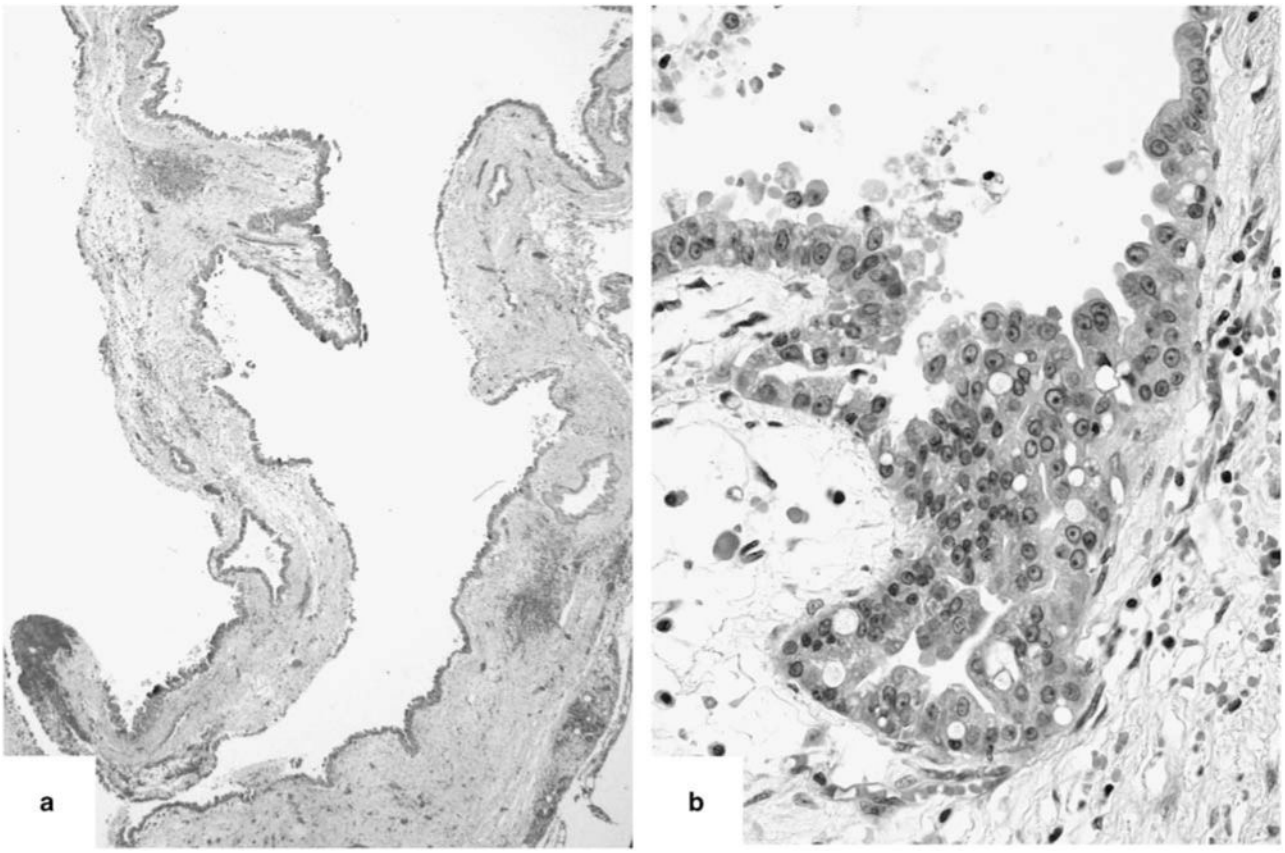

Figure 3.

Case 10 lacked the arborizing papillae (**a**) but the neoplastic epithelial cells revealed oncocytic features (**b**).

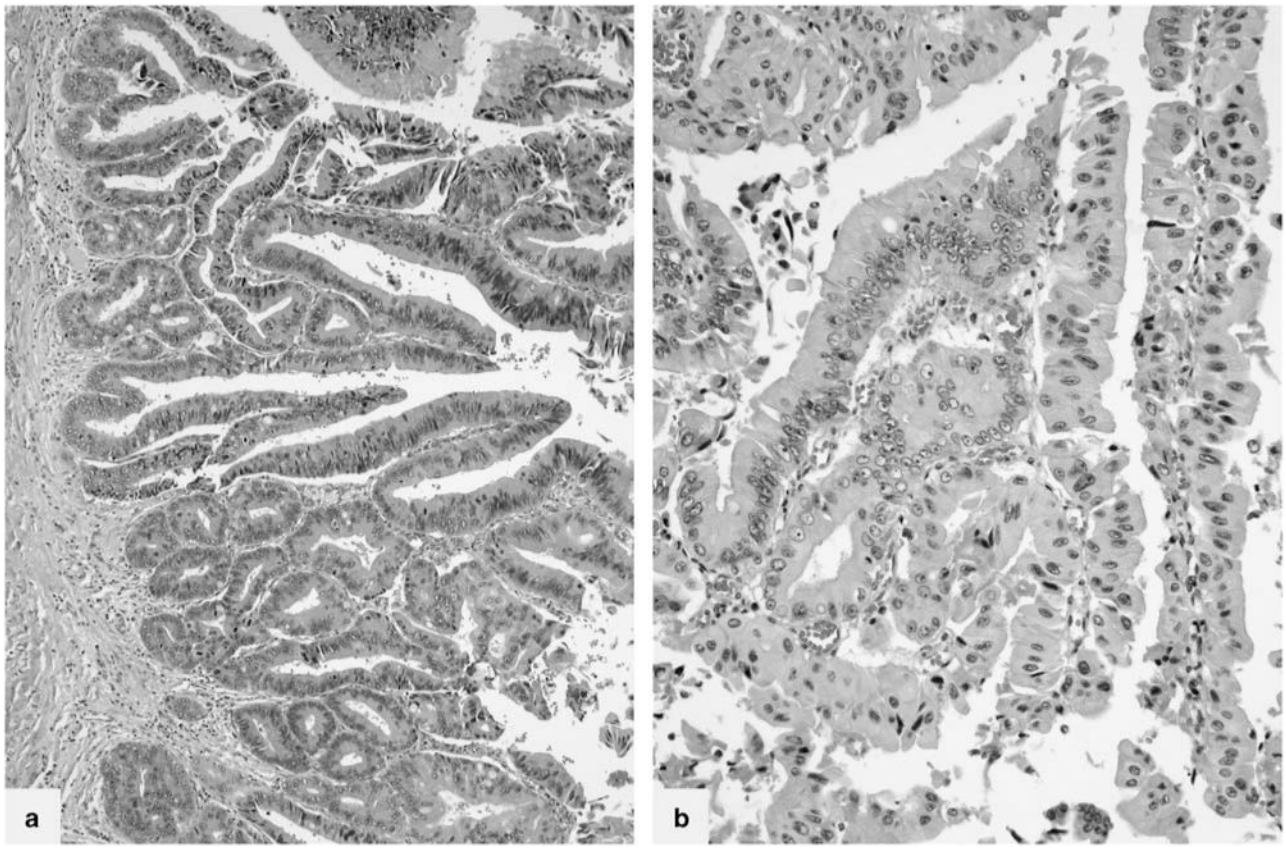

Figure 4.

Case 11 was a predominantly intestinal subtype intraductal papillary mucinous neoplasm (**a**) with a distinct focus of oncocytic epithelium (**b**). Intestinal and oncocytic components were analyzed separately.

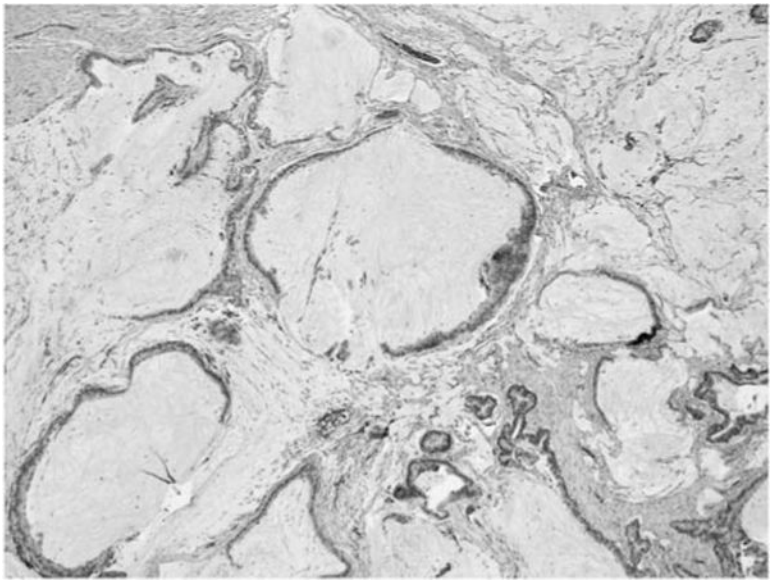

Figure 5.
Case 11 also revealed an associated invasive carcinoma of colloid type characterized by pools of mucin that are partially lined by carcinoma cells.

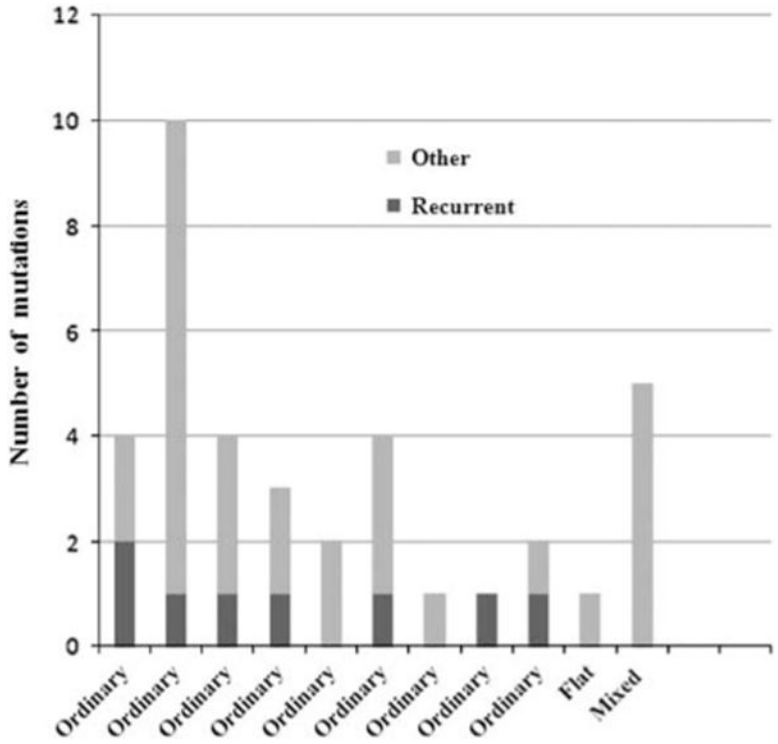

Figure 6.
Number of mutations seen in each case analyzed.

Table 1

Clinicopathologic features of the cases

Gender	
Male	50%
Female	50%
Mean age	60 years
Type of resection	
Whipple	67%
Distal pancreatectomy	33%
Tumor location	
Head	67%
Tail	33%
Mean tumor size	5 cm
Histologic type	
Pure	91%
Mixed	9%
Invasive component	
Yes	18%
No	82%
Lymphovascular invasion	
No	100%
Perineural invasion	
No	100%
Resection margin	
Positive	29%
Negative	71%
Lymph node status	
No	100%
Median follow up	61 months
Survival	
Alive	77%
Dead	23%

Table 2

Distinct mutations identified by next-generation sequencing

Case	*Gene*	*Type of mutation*	*Protein change*
Case 1 (Typical IOPN)	***ARHGAP26***	Missense mutation	p.K592N
	EPHA8	Missense mutation	p.R384H
	MLL2	Missense mutation	p.G1281R
	PTPN11	Missense mutation	p.F438V
Case 2 (Typical IOPN)	***EPHA8***	Missense mutation	p.R375H
	EPHB1	Missense mutation	p.P457L
	ERBB2	Missense mutation	p.R47H
	JAK3	Missense mutation	p.V718L
	PIK3R1	Missense mutation	p.P194T
	PIK3R3	Missense mutation	p.R49Q
	MLL	Missense mutation	p.P1840S
	NOTCH1	Missense mutation	p.R2272H
	NOTCH1	Missense mutation	p.N104S
	RNF43	Frameshift deletion	p.L61fs
Case 3 (Typical IOPN)	***ERBB4***	Missense mutation	p.L1163M
	EPHA10	Missense mutation	p.A453V
	GLI3	Missense mutation	p.S1137R
	RB1	Frameshift deletion	p.T5fs
Case 4 (Typical IOPN)[a]	***ARHGAP26***	Missense mutation	p.P4Q
	NTRK3	Missense mutation	p.L629H
	RICTOR	Missense mutation	p.D1357Y
Case 5 (Typical IOPN)[a]	*NKX2-1*	Frameshift deletion	236_237GG>G
	TEK	Missense mutation	p.T401M
Case 6 (Typical IOPN)	***ASXL1***	Missense mutation	p.V119L
	ABL2	Missense mutation	p.S1000P
	NOTCH2	Missense mutation	p.R1372W
	RET	Missense mutation	p.T244I
	RET	Missense mutation	p.K1011E
Case 7 (Typical IOPN)	*PAX5*	Nonsense mutation	p.R377
Case 8 (Typical IOPN)	***ASXL1***	Frameshift deletion	p.M341fs
Case 9 (Typical IOPN)[b]	***ERBB4***	Missense mutation	p.L939F
	KDM6A	Frameshift deletion	p.L416fs
Case 10 (Intraductal neoplasm with flat oncocytic epithelium)	*KRAS*	Missense mutation	p.G12V
Case 11 (Predominantly intestinal subtype IPMN with a distinct focus of oncocytic epithelium)[b]	*Intestinal subtype IPMN*		
	TP53	Missense mutation	p.S241F
	TP53	Missense mutation	p.R248W
	TP53	Nonsense mutation	p.W146
	GNAS	Missense mutation	p.R844C
	RNF43	Missense mutation	p.M1I

Case	*Gene*	*Type of mutation*	*Protein change*
	Oncocytic epithelium		
	ATRX	Missense mutation	p.E1492G
	PDGFRA	Missense mutation	p.T230M
	TP53	Missense mutation	p.S241F
	TP53	Nonsense mutation	p.W146
	GNAS	Missense mutation	p.R844C
	RNF43	Missense mutation	p.M1I
	Invasive colloid carcinoma		
	TP53	Missense mutation	p.S241F
	TP53	Missense mutation	p.R248W
	GNAS	Missense mutation	p.R844C
	RNF43	Missense mutation	p.M1I

[a] Cases 4 and 5 were also subjected to whole-genome sequencing.

[b] Cases 9 and 11 had an associated invasive carcinoma component. The invasive component of case 9 was too small for molecular analysis. Recurrent mutations are written in bold.