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GNAS sequencing identifies IPMN-specific mutations in a subgroup of diminutive pancreatic cysts referred to as “incipient IPMNs”

Hanno Matthaei, MD^{1,7,*}, Jian Wu, PhD^{4,*}, Marco dal Molin, MD¹, Chanjuan Shi, MD⁶, Sven Perner, MD^{8,9}, Glen Kristiansen, MD⁸, Philipp Lingohr, MD⁷, Jörg C. Kalff, MD⁷, Christopher L. Wolfgang, MD³, Kenneth W. Kinzler, PhD⁴, Bert Vogelstein, MD^{4,5}, Anirban Maitra, MBBS^{1,2}, and Ralph H. Hruban, MD^{1,2}

¹Department of Pathology, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, Maryland ²Department of Oncology, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, Maryland ³Department of Surgery, The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, Maryland ⁴Ludwig Center for Cancer Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland ⁵Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland ⁶Department of Pathology, Microbiology, Immunology, Vanderbilt University Medical Center, Nashville, Tennessee ⁷Department General, Visceral, Thoracic and Vascular Surgery, University of Bonn, Bonn, Germany ⁸Institute of Pathology, University of Bonn, Bonn, Germany ⁹Department of Prostate Cancer Research, University of Bonn, Bonn, Germany

Introduction

Through steady improvement, widespread availability and decreasing cost of modern imaging, asymptomatic pancreatic cysts are increasingly being detected, and these cysts pose a growing clinical management problem (1). Studies of autopsy material suggest that pancreatic cysts are present in as many as one fourth of individuals (2) of which a majority can now be detected on high resolution cross-sectional imaging (3). Fortunately, a majority of such cysts is entirely benign and they do not have to be treated unless they become symptomatic or develop worrisome features on imaging. Numerous comprehensive investigations have been performed to characterize and classify these larger pancreatic cysts on a clinicopathologic and molecular basis with possible important translational impact on patient treatment (4–9).

The purpose of the present study is to examine the grey-zone occupied by “incipient IPMNs” (10). IPMNs are *by definition* larger than 1 cm in maximum diameter while PanINs are microscopic. The term “incipient IPMNs” has been used for cystic lesions that fall in between these two lesions. Incipient IPMNs are smaller than 1 cm in maximum diameter but, in contrast to PanINs have a number of epithelial features similar to IPMNs, including characteristic long finger-like papillae and prominent mucin production. These lesions are poorly characterized with respect to clinical relevance, pathology and molecular alterations. Incipient IPMNs are occasionally found in association with familial pancreatic cancer

Corresponding author, Ralph Hruban, MD, Weinberg Room 2242, 401 North Broadway, The Sol Goldman Pancreatic Cancer Research Center, Baltimore MD 21231, Ph: 410 955 2163; Fax: 410 955 0115; rhruban@jhmi.edu.
*equal contribution

syndromes (10) or in the context of multifocal IPMNs (11). But they also can be observed in pancreata resected for other indications such as chronic pancreatitis or nonepithelial neoplasms. Based on the histologic appearance of the papillae and epithelium, we hypothesized that incipient IPMNs may belong to the IPMN pathway.

Mutations in *GNAS* at codon 201 have recently been identified as a hallmark molecular alteration of IPMNs (prevalence ~66%) (6). Interestingly, *GNAS* mutations seem to be an early event in IPMN development for several reasons. First, a majority of histologically low-grade IPMNs harbor this mutation (6). Secondly, a recent study demonstrated that *GNAS* mutations can be identified in duodenal fluid samples even before the IPMN lesion can be identified on radiologic imaging (8). We therefore used *GNAS* mutations as a marker of the “IPMN pathway,” to study incipient IPMNs. Our long-term goal is to improve the clinical and pathogenetic understanding of small pancreatic cysts.

Material and Methods

Patients and tissues

This study was approved by the Johns Hopkins Review Board and the review boards of collaborating institutions. In order to identify relevant tissue specimens the prospectively maintained surgical pathology databases of Johns Hopkins University, Vanderbilt University and University of Bonn were queried for incipient IPMNs per diagnosis according to final histology report of patients who underwent pancreatic surgery. Additional lesions were acquired by meticulous random review of pancreatectomy specimens.

Histology

The histologies of all tumors included were reassessed by two experts in pancreas pathology (RHH and AM) based on the most recent World Health Organization (WHO) guidelines (12). Briefly, incipient IPMNs were recognized as small cyst-forming lesions composed of tall papillae lined by mucin-producing columnar cells. By definition, all were smaller than 1 cm in maximum diameter. The epithelial lining was classified as gastric-foveolar, pancreatobiliary, intestinal or oncocytic using previously described criteria (12). The incipient IPMNs were further stratified according to the degree of cellular dysplasia into low-grade, intermediate-grade, and high-grade dysplasia (a.k.a. carcinoma *in situ*).

Manual microdissection

Formalin-fixed and paraffin-embedded (FFPE) tissue blocks containing lesional tissue were sectioned (10–30 sections at 10 µm) were deparaffinized, stained and microdissected on a stereomicroscope using a sterile needle (SMZ1500, Nikon, Tokyo, Japan). An average of 1,000 – 2,000 cells were collected from each lesion and the neoplastic cellularity was estimated to be >80%. Afterwards, genomic DNA was extracted using the QIAamp DNA Micro Kit (Qiagen Inc., Valencia, CA, USA).

Digital ligation PCR

The DNA amplification and ligation assays were performed as described in previous publications (13–17).

Results

Clinicopathologic parameters

This study was performed using FFPE tissues from surgical pathology archives of three centers with focus on hepatopancreatobiliary diseases. Detailed patient information is shown in Table 1.

We included lesions from 7 male and 12 female patients who had a median age of 63 years (range, 40–76 years). Most lesions (N=8) were observed in association with a resected PDAC or were found in pancreata resected for ampullary adenocarcinomas (AC; N=3). One of the incipient IPMNs included arose in a patient with a strong family history of pancreatic cancer.

The median size of incipient IPMNs was 4 mm (range, 2 – 7 mm). The lining epithelium showed mostly intermediate-grade dysplasia (N=14) and predominantly a gastric-foveolar epithelial subtype (N=11).

GNAS and KRAS mutations in incipient IPMNs

We assessed the mutational status of *KRAS* and *GNAS* using a sensitive PCR/ligation method. Genomic DNA was extracted for this purpose from the 21 incipient IPMNs.

Sequencing studies revealed that all incipient IPMNs lesions had at least one *KRAS* codon 12 mutation. Most incipient IPMNs had a *KRAS* G12D (N=8) or a *KRAS* G12V (N=7) mutation. Four incipient IPMNs had a *KRAS* G12R mutation – one of them with an additional *KRAS* G12D allele. *GNAS* mutations were identified in 7 incipient IPMNs (33%) (Table 1). Five had a solitary *GNAS* R201H and one a solitary *GNAS* R201C mutation. One incipient IPMN harbored a *GNAS* R201H as well as a *GNAS* R201C mutation. On histopathologic assessment incipient IPMNs with a *GNAS* mutation had a median size of 5 mm (range, 2–7) cm and displayed in majority a gastric-foveolar subtype (N=5) with intermediate dysplasia (N=5).

Discussion

Small pancreatic cysts are increasingly diagnosed. IPMN is the most frequent cystic neoplasm of the pancreas and IPMNs are now well understood clinically and on a molecular level (4–9). An IPMN has been defined as a grossly visible cyst larger than 1 cm in maximum diameter with characteristic papillary mucin-producing epithelium (12). IPMNs can be differentiated from microscopic PanINs not only based on morphology and size but also based upon distinct molecular pathways (18, 19). For instance, *GNAS* codon 201 mutations have been identified as a hallmark molecular alteration occurring in a majority of IPMNs (5, 6, 20). This genetic hit is virtually absent in PanINs as well as in PDAC arising without associated IPMN (18, 21).

The term “incipient IPMN” has been used for intraductal lesions smaller than 1 cm that are composed of an IPMN-like epithelium. Lesions of this histopathologic “grey-area” are occasionally seen in the context of sporadic multifocal IPMNs (11) or in pancreata of familial pancreatic cancer kindred (10). They can also be seen in pancreata resected for other tumors or nontumorous diseases, these tiny cysts are found with entirely unknown clinical relevance. It is unclear if the pathogenetic pathway for incipient IPMNs is along the PanIN-associated or the IPMN-associated pathways. That is, are incipient IPMNs small IPMNs or are they large PanIN lesions? An understanding of incipient IPMNs is also clinically relevant, as even small IPMNs can have a substantial risk for malignant transformation (22).

In the pancreas, *GNAS* codon 201 mutations appear to be a highly specific marker of IPMNs. Hence, we assessed the prevalence of these molecular alterations in a series of well-characterized incipient IPMNs. *GNAS* (20q13.3) is a gene that encodes for a protein functioning as a subunit of a stimulatory G-protein alpha-subunit (Gs- α), a crucial component of numerous signal transduction pathways. Mutations in this gene result in the McCune Albright Syndrome with its characteristic pubertas precox, Café-au-lait spots and polyostotic fibrous dysplasia (23). Patients with the fibrous dysplasia disorder named Mazabrauds syndrome present with intramuscular myxomas that frequently show activating *GNAS* mutations (24). Furthermore, somatic *GNAS* mutations have been observed in various neoplasms such as renal cell carcinoma (25), thyroid tumors (26) pituitary adenomas (27), inflammatory liver tumors (28), rarely in colorectal cancer (29), and interestingly quite prevalent in villous adenomas of the colorectum (30).

We found that a large fraction of incipient IPMNs carry a *GNAS* mutation and that all had *KRAS* mutations as expected from previous sequencing studies (5, 6). Incipient IPMNs therefore appear to be part of the IPMN pathway of pancreatic neoplasia. The fact that most lesions were of low- or intermediate-grade dysplasia suggests that *KRAS* and *GNAS* mutations are both early events in the development of IPMNs. This conclusion is supported by the previous observation that *GNAS* mutations can be detected in duodenal juice samples even before the patient has a radiologically discernible lesion(8).

Our study has several relevant limitations. We included only a small series of incipient IPMNs since these lesions are only rarely observed in pancreatectomy specimens. According to expert estimates incipient IPMNs occur in merely 5% of all resected pancreata. Hence, the present cohort was too small to draw final conclusions about associations with demographic or clinicopathologic data and it is not possible to assess their prognostic impact of incipient IPMNs.

In summary, 33% of incipient IPMNs analyzed harbored a *GNAS* mutation, suggesting that a large proportion of incipient IPMNs are, as the name suggests, part of the IPMN pathway and these mutations occur early in this process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Panel A) Incipient intraductal papillary mucinous neoplasm (IPMN) localized in the pancreatic head. Note the papillary projections and the cystic appearance of the lesion measuring 7 mm in maximum diameter. The epithelium shows a gastric-foveolar subtype mainly exhibiting intermediate-grade dysplasia. This patient did not have an associated larger pancreatic neoplasm. The incipient IPMN harbored both a *KRAS* codon 12 and a *GNAS* codon 201 mutation characteristic of IPMN. **Panel B)** shows a 5 mm incipient IPMN with intestinal-type differentiation commonly seen in main duct IPMNs. This lesion had intermediate-grade dysplasia and a *KRAS* mutation but no *GNAS* mutation was identified. This lesion arose in a patient who underwent resection for a PDAC of 2.8 cm in the pancreatic head. **Panel C)** shows an incipient IPMN of 5 mm that was observed also in association with a PDAC of 3 cm of the pancreatic head. This small cyst had also intermediate-grade dysplasia and demonstrated a mixed gastric-foveolar and intestinal epithelial subtype. This lesion harbored a *KRAS* mutation, but no *GNAS* mutation.