HHS Public Access

Author manuscript

Ann Surg. Author manuscript; available in PMC 2017 July 01.

Published in final edited form as:

Ann Surg. 2017 July ; 266(1): 133–141. doi:10.1097/SLA.0000000000001817.

Targeted DNA Sequencing Reveals Patterns of Local Progression in the Pancreatic Remnant Following Resection of Intraductal Papillary Mucinous Neoplasm (IPMN) of the Pancreas

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Abstract

Objective—The aim of this study was to characterize patterns of local progression following resection for pancreatic intraductal papillary mucinous neoplasms (IPMN) using targeted next-generation sequencing (NGS).

Background—Progression of neoplastic disease in the remnant pancreas following resection of IPMN may include development of a new IPMN or ductal adenocarcinoma (PDAC). However, it is not clear whether this progression represents recurrence of the same neoplasm or an independent second neoplasm.

Methods—Targeted-NGS on genes commonly mutated in IPMN and PDAC was performed on tumors from (1) 13 patients who developed disease progression in the remnant pancreas following

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.annalsofsurgery.com).

The authors report no conflicts of interest.

resection of IPMN; and (2) 10 patients who underwent a resection for PDAC and had a concomitant IPMN. Mutations in the tumors were compared in order to determine the relationship between neoplasms. In parallel, clinical and pathological characteristics of 260 patients who underwent resection of noninvasive IPMN were reviewed to identify risk factors associated with local progression.

Results—We identified 3 mechanisms underlying local progression in the remnant pancreas: (1) residual microscopic disease at the resection margin, (2) intraparenchymal spread of neoplastic cells, leading to an anatomically separate but genetically related recurrence, and (3) multifocal disease with genetically distinct lesions. Analysis of the 260 patients with noninvasive IPMNs showed that family history of pancreatic cancer ($P = 0.027$) and high-grade dysplasia (HGD) ($P = 0.003$) were independent risk factors for the development of an IPMN with HGD or an invasive carcinoma in the remnant pancreas.

Conclusions—Using NGS, we identify distinct mechanisms for development of metachronous or synchronous neoplasms in patients with IPMN. Patients with a primary IPMN with HGD or with positive family history are at an increased risk to develop subsequent high-risk neoplasms in the remnant pancreas.

Keywords

completion pancreatectomy; intraductal papillary mucinous neoplasms; next-generation sequencing; pancreatic cancer; targeted sequencing

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas can progress from low to high-grade dysplasia, and ultimately to invasive carcinoma.[1–3] In addition to the progression of individual IPMNs, synchronous and metachronous development of neoplasms in the pancreas is a significant clinical problem.[4–15] Interestingly, development of invasive cancer in the remnant pancreas can manifest as either an invasive carcinoma that appears to have arisen from the IPMN, or as a de novo pancreatic ductal adenocarcinoma (PDAC) with no identifiable associated or antecedent cystic lesion. In both IPMN and conventional PDAC, it is not clear whether these neoplasms represent disease progression of the resected primary neoplasm or independent neoplasms.[16,17] In order to answer this question, we characterized the genetic alterations of pancreatic neoplasms in patients who underwent surgical resection for IPMN and who had subsequent completion pancreatectomy for progressive disease in the remnant pancreas. We also independently sequenced IPMNs and concomitant but morphologically separate PDACs resected within a single specimen. In order to define the genetic relationship of the lesions, we compared the genetic changes in the resected primary neoplasm with those in the separate synchronous or metachronous lesions.

Comparing the genetic alterations in multifocal pancreatic neoplasms revealed distinct mechanisms of progression within the remnant pancreas. In order to define whether these mechanisms are consistent with the natural history of IPMNs, we corroborated these genetic findings by analyzing disease progression with clinical data from one of the largest single institutional cohorts of resected IPMNs.

METHODS

Study Population for Genetic Analysis

This study was approved by the Institutional Review Board of The Johns Hopkins Hospital. A retrospective review of a prospectively collected pancreatic resection database and the surgical pathology database of the Johns Hopkins Hospital (JHH) was performed to identify 2 groups of patients for the genetic analysis: (1) patients who underwent a completion pancreatectomy for either an IPMN or PDAC following resection of an IPMN and (2) patients who underwent a pancreatic resection for a conventional PDAC who had a morphologically separate IPMN in their resected pancreas. Conventional PDAC has been defined as PDAC that at the pathological evaluation do not arise from an IPMN. In the second instance, separation was defined pathologically as PDAC that was physically separated from IPMN by uninvolved pancreatic parenchyma. We required that there was no histologic transition from IPMN with high-grade dysplasia to the PDAC in the specimen and that both lesions did not occur in same tissue block. IPMNs were assigned to 1 of 3 categories consisting of low-grade dysplasia (LGD) or high-grade dysplasia (HGD) based on the highest grade of dysplasia encountered in the specimen, or IPMN with invasive carcinoma.[18]

The study included a total of 23 patients for genetic analysis: 13 patients who underwent a completion pancreatectomy for progression in the remnant following the resection of an IPMN (Supplementary Figure 1, http://links.lww.com/SLA/B35), and 10 cases with separate PDAC and IPMN in the same resection.

Next-Generation Sequencing

Next-generation sequencing (NGS) assays were performed blinded to patient's clinical information. An Ion AmpliSeq Custom Panel was designed by the Ion AmpliSeq Designer (Pipeline version 4.2; Life Technologies) to perform multiplex PCR and sequencing of 11 genes (142 amplicons in 2 primer pools) known to be targeted in pancreatic ductal neoplasms and IPMNs (*KRAS, GNAS, TP53, SMAD4, CDKN2A, RNF43, TGFBR2, ARID1A, BRAF, MAP2K4*, and *PIK3CA*).[19–21] The targeted regions are described in Supplementary Table 1, http://links.lww.com/SLA/B35. Genomic DNA was extracted using QIAamp DNA Micro Kit (Qiagen) from matched formalin-fixed paraffin-embedded (FFPE) tumor/normal cores or 5 μm tissue sections that were manually microdissected to enrich for neoplastic cellularity. Genomic DNA was quantified by Quantifiler Human DNA Quantification kit (Applied Biosystems) before performing the NGS. Two nanograms of genomic DNA was amplified using Ampliseq reagents for the library preparation, loaded and sequenced onto a 318v2 chips using an Ion Torrent Personal Genome Machine (PGM; Life Technologies) following the manufacturer's protocols. Postsequencing data analyses, including alignment to the hg19 human reference genome and variant calling, were performed using NextGENe software (v2.4; SoftGenetics, Chicago, IL). Alignments and putative mutations were visually verified using the Integrative Genomics Viewer (IGV, v2.3; Broad Institute) and the NextGENeViewer (v2.4; SoftGenetics, Chicago, IL).

Relatedness Assessment

On the basis of the genetic alterations, we assessed the genetic relatedness of the separately dissected neoplasms from each patient. We assigned the patterns of genetic alterations to 1 of the 3 categories: “likely independent” when discordant mutations in driver genes were present in the 2 lesions; “likely related” when the 2 lesions from the same patient shared mutations in genes other than *KRAS*; and “indeterminate” when only the same *KRAS* mutation was present in both lesions. In fact, the main limit of this approach is the difficulty in interpreting the significance of *KRAS* gene mutations when they are present in both lesions. Because *KRAS* codon 12 hotspot mutations were very common in all of the neoplasms assayed, 2 independent lesions could indeed by chance harbor the same mutation.

Clinical Validation Study Population

Using the JHU database, we identified 634 consecutive patients who underwent a pancreatectomy for an IPMN between January 1996 and January 2014 at JHU. Patients with an unspecified IPMN grade of dysplasia, patients who had a total pancreatectomy and those with less than 6 months of follow-up after surgical resection were excluded from the study.

Patients with a primary resection of IPMN-associated invasive carcinoma were excluded from the clinical analysis, as these patients experience distant metastatic recurrence rather than local progression in the remnant pancreas. Therefore, only patients undergoing pancreatic resection for noninvasive IPMN were included.

A positive margin was defined as presence of IPMN, regardless of the grade of dysplasia, at a final resection margin. A positive family history was defined as pancreatic cancer diagnosis in a first-or second-degree relative.[14,22,23] Patients with initial noninvasive IPMN were classified into 3 different radiological categories of local progression (Fig. 1): (1) “BD progression,” defined as a radiological finding of a new branch-duct IPMN, a clinically significant increase in size of an existing BD-IPMN, or development of a new solid component within an existing BD-IPMN; (2) “MD progression,” defined as a radiological finding of main duct dilation suspicious for IPMN; (3) “separate solid mass progression,” defined as the development of a new PDAC in the remnant pancreas or the development of a locally unresectable or metastatic pancreatic cancer confirmed by imaging or biopsy. In the second category, postoperative main duct dilation stable on follow-up imaging in patients who underwent a pancreaticoduodenectomy was not considered progression but likely postoperative stricture of the pancreatic anastomosis. The time to disease progression was calculated from the time of pancreatic resection to diagnosis of progression on follow-up imaging.

Statistical Analyses

Continuous variables are presented as median and interquartile range (IQR) and were compared using a Wilcoxon-Mann-Whitney test. A Chi-squared test or a Fisher exact test was used to compare the categorical variables. Possible risk factors associated with disease progression in the remnant pancreas were evaluated using univariate and multivariate regression models. A backward stepwise elimination method with a threshold of $P = 0.20$ was performed for the final multivariate model. Kaplan-Meier survival estimates were used

to estimate the progression rates in IPMNs with different grades of dysplasia. A *P* value <0.05 was considered statistically significant. Stata/MP version 12.1 (StataCorp, College Station, TX) was used for statistical analysis.

RESULTS

Pathological Evaluation and Genetic Analyses

Completion Pancreatectomy Following Resection of IPMN—Targeted mutational analysis was performed for the primary IPMN and the progressive lesion of 13 patients who underwent completion pancreatectomy for progression in the remnant pancreas following IPMN resection. The results are summarized in Table 1 and detailed in Supplementary Table 2, http://links.lww.com/SLA/B35. The primary neoplasm of these 13 patients included 9 noninvasive IPMNs (3 LGDs and 6 HGDs) and 4 IPMN-associated invasive carcinomas. One of the latter was an IPMN-associated microinvasive cancer (0.4 cm focus of cancer). All resections for IPMN-associated invasive carcinoma were node negative (N0).

Relatedness was determined on the basis of the comparison of the mutations found in the 2 lesions from the same patient. Moreover, in patients who had IPMN at the margin available for DNA extraction, the genetic relationship of both lesions was compared with the lesion at the margin. Invasive and noninvasive components of an IPMN were separately sequenced.

Eight of the 13 patients had margin-negative resections. On the basis of the genetic analysis, the primary and progressive neoplasms were “likely related” in only 1 of these 8 patients (case #2), while 5 cases had evidence of independent genetic alterations in the secondary lesion and were classified as “likely independent.” The remaining 2 cases were “indeterminate,” as they only shared the same *KRAS* mutation (cases #3 and #5). The single patient with “likely related” lesions developed a poorly differentiated PDAC after an initial resection for an IPMN with an associated invasive colloid carcinoma. In this case, the primary and progressive neoplasms shared both *KRAS* and *GNAS* mutations, while a *TP53* mutation was unique to the primary neoplasm.

Five of the 13 IPMN patients had a positive margin resection. In 2 cases, the 2 neoplasms sequenced were classified as “likely independent” (cases #9 and #10) due to the discordant genetic alterations in the 2 tumors. Two cases (cases #12 and #13) were classified as “likely related,” based on identical driver gene mutations in the original tumors and subsequent resections. Of note, the “likely independent” progressions occurred in patients with LGD at the margin, while there was HGD at the margin in the “likely related” progressions. The last case was classified as “indeterminate” (case #11) because the primary and the progressive neoplasms shared the same *KRAS* mutation. Intriguingly, in the “indeterminate” (case #11), the subsequent tumor shared the same *KRAS* mutation with the patient’s original invasive IPMN at the resection margin that was different from that seen in the invasive IPMN at the pancreatic head.

IPMNs With Concomitant PDAC—The results of the analysis of 10 patients with IPMN and separate synchronous PDAC are summarized in Table 2 and detailed in Supplementary Table 3, http://links.lww.com/SLA/B35. In 3 cases (cases #1, #2, and #4), the IPMN and the

concomitant PDAC did not share the same mutations, suggesting that the IPMN and the PDAC were “likely independent.” In 2 cases (cases #5 and #7), the PDAC and the IPMN were “likely related,” as the two neoplasms shared multiple driver gene mutations. Of interest, both “likely related” PDACs had *GNAS* mutations, which are uncommon in PDACs not associated with IPMN.[19] In 4 cases (cases #3, #6, #8, and #9), results were indeterminate, as the 2 lesions in each case shared a same KRAS mutation. The last case (case #10) had 2 different IPMNs in the head of the pancreas, 1 with LGD and 1 with HGD; the mutational analysis showed that the invasive PDAC was “likely independent” from the IPMN with LGD, but its relationship with the IPMN with HGD was “indeterminate” on the basis of the presence of the same *KRAS* mutation.

Clinical Validation

Local Progression Following Resection of Noninvasive IPMNs—A total of 260 patients who underwent partial pancreatectomy for a noninvasive IPMN and had more than 6 months of follow-up were identified. Of these, 50 (19%) patients developed disease progression in the remnant pancreas: BD-progression in 32 (64%), MD-progression in 7 (14%), and progression to PDAC in 11 (22%) patients (Table 3). Of the 11 patients with progression to PDAC, 4 underwent a completion pancreatectomy, while 7 were diagnosed with metastatic or unresectable disease during follow-up and did not undergo an additional pancreatic resection. The median time to disease progression from the original surgical resection was 27 months (IQR 14–53) for patients with BD-progression, 52 months (IQR 38–69) for patients with MD-progression, and 59 months (IQR 32–78) for patients who developed a solid mass in the remnant.

We then evaluated the risk factors associated with development of an IPMN with HGD or an invasive carcinoma (both IPMN-associated invasive carcinoma and conventional PDAC) in the remnant pancreas of patients with surgically resected noninvasive IPMN (Table 4). By univariate analysis, family history of pancreatic cancer ($P = 0.029$), HGD in the initially resected IPMN ($P = 0.004$) (Fig. 2), and presence of any grade of dysplasia at the pancreatic neck resection margin ($P = 0.032$) were associated with developing neoplasia in the remnant. On multivariate analysis, only family history [odds ratio (OR) 5.82; 95% confidence interval (95% CI) 1.49–22.78; $P = 0.011$] and primary resected IPMN with HGD (OR = 9.22; 95% CI = 2.76–20.71; $P < 0.001$) were confirmed to be independent risk factors.

DISCUSSION

The progression of neoplastic disease within a remnant pancreas following resection of an IPMN is now a well-documented and challenging aspect of the management of IPMN patients.[10–14] Progression within the remnant pancreas may include the development of a new IPMN, an IPMN-associated cancer, or a conventional PDAC.[14,15,24,25]

Our targeted NGS comparing primary resected IPMN and progressive neoplasia in the remnant pancreas of 13 patients permitted us to assess the genetic relationship between the 2 lesions in 10 cases. The metachronous lesions were genetically unrelated in 5 of 8 patients with a negative resection margin and in 2 of 5 patients with a positive margin, while they were genetically related in 1 of 8 negative margin case and 2 of 5 positive margin cases. In

both margin-positive cases with genetically unrelated primary/progressive neoplasms, the IPMN at the resection margin harbored LGD and showed genetic discordance with both the primary and the progressive lesions, suggesting that it was an independent multifocal IPMN. On the contrary, in the 2 margin-positive cases with genetically related primary/progressive lesions, the margins harbored HGD. In 1 case, the IPMN at the margin was genetically identical to both the primary and the progressive lesions, suggesting direct extension at the microscopic level. In the remaining 3 cases (2 negative margin and 1 positive margin), the genetic relationship could not be determined.

Among the 10 patients with an IPMN and a separate concomitant PDAC in the same resected specimen, the lesions were genetically independent in 3 cases, while in 2 cases, the IPMN and the PDAC showed the same *KRAS* and *GNAS* mutations, suggesting that the 2 lesions were genetically related. In the remaining 5 cases, the genetic relationship could not be determined.

On the basis of these results, we propose that 3 different mechanisms account for the development of neoplastic lesions in the remnant pancreas following resection of IPMN (Fig. 3). The first mechanism is conceptually the simplest and consists of the transection of the neoplasm at the margin with resulting residual disease. In this mechanism, the primary, secondary, and microscopic margin lesions are all genetically related (Fig. 3A). The second mechanism consists of intraductal or intraparenchymal metastases. In these cases, no identifiable tumor is present at the margin between the 2 lesions, but the physically separate lesions are genetically related (Fig. 3B). Finally, the third mechanism is through the independent development of 2 separate “primary” lesions. In these cases, the 2 lesions are genetically unrelated (Fig. 3C).

Other studies have attempted to assess the genetic relatedness of multifocal IPMN, and their results support the mechanisms described above. Tamura et al,[17,26] based only on *KRAS* and *GNAS* mutations, have suggested that multisegmental MD-IPMNs can be clonally related, as described in our proposed second mechanism. However, they did not consider the limit of assessing the genetic relationship when only 1 of the drivers presents the same mutation in both lesions. In addition, previous molecular analyses demonstrated that the majority of multifocal BD-IPMNs are genetically unrelated,[16] as in our proposed third mechanism (Fig. 3).

However, our proposed mechanisms do not represent the only explanation of our data. An alternative explanation for our “likely independent” lesions is that the progressive lesion was seeded by subclones of IPMN cells that originated in the primary tumor but were not present in the sample used for genetic analysis. Other studies have already demonstrated polyclonality of IPMN epithelium within the same lesion.[20,27] Although we identified several neoplasms with multiple different mutations in the same driver gene, in most cases, we observed a predominant mutation in each gene with the highest concentration of mutant reads. This suggests that each neoplasm did have some clonal mutations that would be shared if the primary and progressive neoplasms were related. As such, the possibility of the progressive neoplasm arising from unsampled genetically distinct subclones of the primary tumor is less consistent with our data than the other models proposed.

One proposed explanation for the multifocal nature of IPMN and the related observation of progression in the remnant following resection is that a “field defect” exists that predisposes the entire pancreas to the development of neoplasia.[28] In 2 of the 3 mechanisms described above, the primary lesion serves as the source of neoplastic cells and progression is by dissemination of this lesion—either directly or indirectly by “skipping” throughout the pancreas. These mechanisms do not require a field defect, as a single neoplasm accounts for the primary and progressive disease. In the third mechanism, 2 independent neoplasms arise in the same pancreas, which could support the proposed “field defect” model. However, the absence of shared detectable driver mutations among the lesions suggests that, if an underlying field defect exists, it does not involve the driver genes that we used for genetic analysis.

Some limitations of our cohort for genetic analysis should be noted. First, this cohort is based on progressive pancreatic neoplasms that reached clinical criteria for a completion pancreatectomy, and as such may not be representative of all patients with progressive disease. In addition, the majority of patients with invasive carcinoma after surgery for IPMN were not resected because of an advanced disease stage; therefore, it is possible that lesions included in this study were characterized by a less aggressive biology.

In order to provide information that would be useful in clinical decision-making, we investigated a cohort of 260 patients who underwent surgical resection of noninvasive IPMNs for risk factors associated with development of metachronous disease in the remnant pancreas. The rate of local progression in our cohort was 19%, which is consistent with our previous studies.[14,15] We report that a family history of pancreatic cancer is a significant risk factor for the development of any type of progression in the remnant pancreas, and in particular of a high-risk neoplasm (Supplementary Table 4, http://links.lww.com/SLA/B35). This is consistent with previous data on patients with familial pancreatic cancer, as more precursor lesions are observed in their pancreata than in patients without a family history.[29–31] These results highlight the likelihood of multifocal neoplasia in patients with a family history of pancreatic cancer.[9,14,32,33] Although our study was underpowered to capture any differences in the strength of this association between first- and second- degree relatives, it does suggest that second-degree relatives also harbor an increased risk of progression. We also observed that HGD in the primary IPMN is an independent risk factor for development of a high-risk neoplasm in the remnant pancreas. Accordingly, several other studies have also suggested the possibility of HGD as risk factor for progression, and others have reported cases of systemic recurrence after resection of IPMN with HGD.[4,5,8,15,17,32]

Our study has important implications for clinical management, as we demonstrate that an IPMN harboring HGD predicts an overall more aggressive biology, with a relatively high risk (13%) of developing a subsequent HGD or PDAC lesions, particularly when other risk factors such as a positive family history are present. For these high-risk patients, interventions may prevent progression, including both chemoprevention and complete pancreatic resection. In order for either of these approaches to be implemented, further investigation is necessary to understand the risks and benefits, including studies to examine the natural history of a large population of patients with resected HGD and studies to better understand the long-term effects of the apancreatic state.

Our clinical analysis of 260 patients showed the expected higher tendency of noninvasive IPMNs with HGD at the resection margin to develop a high-risk lesion in the remnant pancreas. These results are corroborated by our genetic analysis that confirms that resection margins harboring HGD are a potential source of disease progression in the remnant pancreas. On the basis of these findings, we speculate that while an IPMN with LGD at the resection margin might not necessitate additional resection, achieving a negative margin when HGD is present may be of importance. These data, along with the international consensus guidelines and the European guidelines, suggest that an additional resection is necessary only if HGD is found at the pancreatic margin during surgical resection.[34–36]

In summary, we suggest 3 distinct mechanisms for metachronous pancreatic disease following resection of IPMN: tumor transected at a margin, intraparenchymal spread of neoplastic cells, and independent multifocal lesions. Our results, together with a growing body of literature, highlight the need for careful long-term follow-up of patients after surgical resection of an IPMN. Future efforts should confirm these findings in larger patient cohorts as well as identify additional patient groups at an increased risk for metachronous disease who would benefit from additional interventions. Large multicenter trials and consequent expert meetings are necessary to address the best frequency and modality to follow these patients.

Acknowledgments

Funding was received from NIH grants P50CA62924, R01CA176828, 3P30CA006973; Pancreatic Cancer Action Network Research Acceleration Network grant; Sol Goldman Pancreatic Cancer Research Center; Buffone Family Gastrointestinal Cancer Research Fund; Kaya Tuncer Career Development Award in Gastrointestinal Cancer Prevention; AGA-Bernard Lee Schwartz Foundation Research Scholar Award in Pancreatic Cancer; Sidney Kimmel Foundation for Cancer Research Kimmel Scholar Award; Rolfe Pancreatic Cancer Foundation; The Gerald O Mann Charitable Foundation (Harriet and Allan Wulfstat, Trustees); Sigma Beta Sorority; Tampa Bay Fisheries Inc; Susan Wojcicki and Dennis Troper; Fondazione Italiana Malattie Pancreas (FIMP- Ministero Salute, CUP_J33G13000210001); Associazione Italiana Ricerca sul Cancro (Grant no. 12182); Italian Cancer Genome Project (FIRB RBAP10AHJB); European Community 7 Framework Programme Cam-Pac (Grant no. 602783).

The authors wish to thank Corrine Sandone from the Department of Art as Applied to Medicine at The Johns Hopkins University for her assistance in preparation of Fig. 3.

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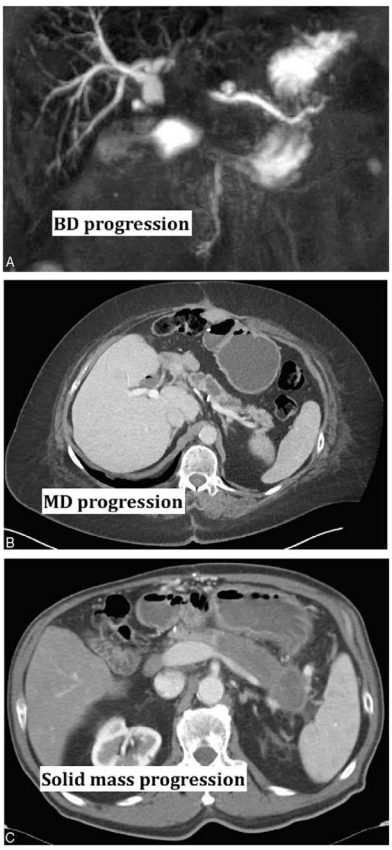

FIGURE 1.

Radiological patterns of progression in the remnant pancreas following resection of noninvasive IPMNs. “BD progression,” radiological finding of a new BD-IPMN, an increase in size of an existing BD-IPMN, or development of a new solid component within an existing BD-IPMN; “MD progression,” main duct dilatation suspicious for IPMN; “solid mass progression,” development of a solid mass suspicious for PDAC in the remnant pancreas (respectively cases #6, #7 and #1 of the genetic analysis).

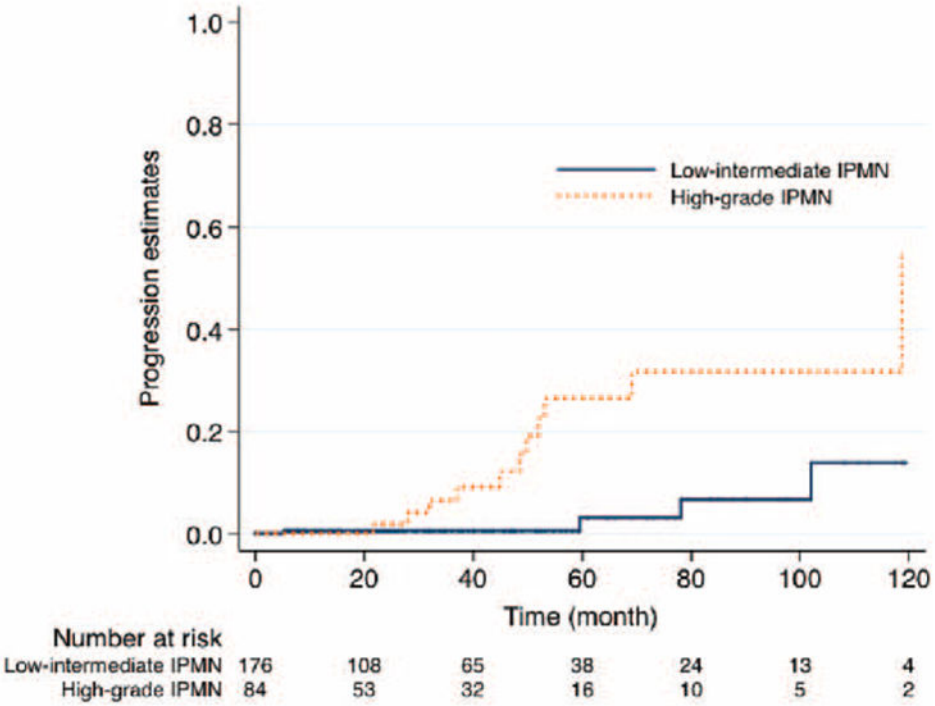

FIGURE 2.

Estimated risk of developing IPMN with high-grade dysplasia or invasive carcinoma (high-risk neoplasm), adjusted for family history of pancreatic cancer, following resection of noninvasive IPMN. The risk after resection of IPMN with LGD at 1, 5, and 10 years was 0%, 1%, and 6% and increased to 0%, 23%, and 52% for IPMNs harboring HGD.

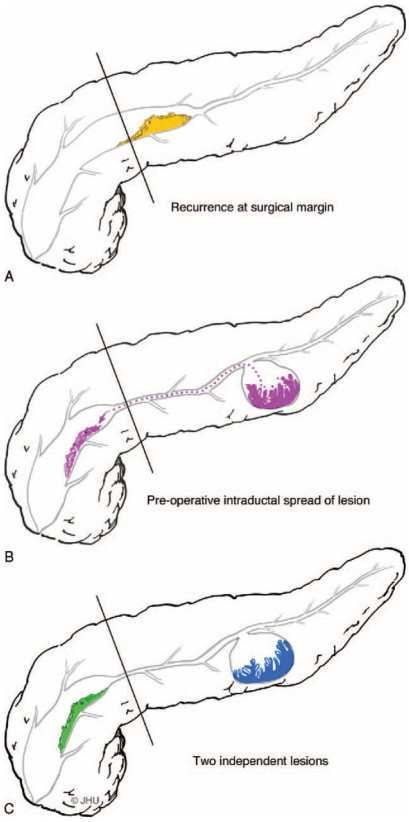

FIGURE 3.

Mechanisms of local progression after resection of pancreatic IPMNs. A, Residual microscopic disease at the surgical margin recurring in the remnant pancreas. B, Intraductal spread of neoplastic cells, leading to an anatomically separate but genetically related recurrence. C, Multifocal disease with genetically distinct lesions.

TABLE 1

Clinical, Pathological, and Genetic Characteristics of Cases With Primary IPMN That Underwent Completion Pancreatectomy for Development of New Neoplasm in the Remnant Pancreas

Patient, Age, Gender	Family history	1st Lesion			Neck Margin		Months After 1st op	2nd Lesion		Pattern of Progression
		Operation	Diagnosis	Mutations	Diagnosis	Mutations		Diagnosis	Mutations	
1) 76, m	no	Whipple	BD –IPMN HGD	*KRAS*: p.G12D *GNAS*: p.R201H	neg	/	46	PDAC G2	*KRAS*: p.G12R	Likely independent
2) 44, m	no	Whipple	MD – IPMN HGD	*KRAS*: p.G12D *GNAS*: p.R201C *TP53*: p.G245D	neg	/	69	PDAC G3	*KRAS*: p.G12D *GNAS*: p.R201C	Likely related
			INV (Colloid)	*KRAS*: p.G12D *GNAS*: p.R201C *TP53*: p.G245D						
3) 74, m	no	Whipple	MIXED – IPMN HGD	*KRAS*: p.G12D	neg	/	39	PDAC G2	*KRAS*: p.G12D	Indeterminate
4) 47, m	yes	Whipple	BD – IPMN LGD	*KRAS*: p.G13D *GNAS*: p.R201C	neg	/	103	PDAC G2	*KRAS*: p.G12V *SMAD4*: p.Y95N	Likely independent
5) 56, f	no	Whipple	BD – IPMN LGD	*KRAS*: p.G12D	neg	/	15	BD – IPMN LGD	*KRAS*: p.G12D	Indeterminate
6) 57, m	yes	Whipple	MIXED – IPMN LGD	*KRAS*: p.G12V	neg	/	48	BD – IPMN LGD CYST 1 BD – IPMN LGD CYST 2 BD – IPMN LGD CYST 3	*KRAS*: p.G12D *GNAS*: p.R201C *KRAS*: p.G12V *KRAS*: p.G12D *GNAS*: p.R201H	Likely independent
7) 67, f	no	Whipple	MIXED – IPMN HGD	*KRAS*: p.G13D *GNAS*: p.R201H *SMAD4*: p.C345Y	neg	/	53	MIXED– IPMN HGD	*KRAS*: p.Q61H *GNAS*: p.R201H	Likely independent
								INV	*KRAS*: p.G12V* p.G12D *GNAS*: p.R201H	
8) 64, f	no	Whipple	MIXED – IPMN HGD	*KRAS*: p.G12D *GNAS*: p.R201C	neg	/	134	MIXED– IPMN HGD	*KRAS*: p.G12V *GNAS*: p.R201H	Likely independent
9) 69, m	no	Whipple	MIXED – IPMN HGD	*KRAS*: p.G12V	IPMN LGD	KRAS: p.G12V	13	MIXED– IPMN LGD	*KRAS*: p.G12D *GNAS*: p.R201C *TP53*: p.G245D	Likely independent

Patient, Age, Gender	Family history	1st Lesion			Neck Margin		Months After 1st op	2nd Lesion		Pattern of Progression
		Operation	Diagnosis	Mutations	Diagnosis	Mutations		Diagnosis	Mutations	
				GNAS: p.R201H* p.R201C *RNF43*: p.A649T						
10) 66, f	no	Whipple	MIXED – IPMN HGD	*KRAS*: p.G12C	IPMN LGD	KRAS: p.G12D	50	MIXED– IPMN HGD	*KRAS*: p.G12V *GNAS*: p.R201H	Likely independent
11) 77, f	yes	Whipple	MD – IPMN HGD	*KRAS*: p.G12V	IPMN INV	KRAS: p.G12D	90	PDAC	*KRAS*: p.G12D	Indeterminate
			INV	*KRAS*: p.G12V						
12) 60, f	no	Whipple	MD-IPMN HGD	*KRAS*: p.G12D *TP53*: p.C176Y *SMAD4*: p.E330K	IPMN HGD	KRAS: p.G12D TP53: p.C176Y	53	MD-IPMN HGD	*KRAS*: p.G12D *TP53*: p.C176Y *SMAD4*:p.E330K	Likely related
			INV	*KRAS*: p.G12D *TP53*: p.C176Y				INV	*KRAS*: p.G12D *TP53*: p.C176Y *SMAD4*:p.E330K	
13) 66, m	no	Distal p	MD-IPMN HGD (0.4 cm focus of cancer)	*KRAS:* p.G12D *CDKN2A*: p.R80R *RNF43*: p.W200X	IPMN HGD	N/A	34	MD-IPMN HGD	*KRAS*: p.G12D *CDKN2A*: p.R80R *RNF43*: p.W200X	Likely related

BD indicates branch duct IPMN; distal p, distal pancreatectomyHGD, high-grade dysplasia; LGD, low-grade dysplasia; MD, main duct IPMN; MIXED, mixed-type IPMN.

*Mutation with the highest concentration.

TABLE 2

Clinical, Pathological, and Genetic Characteristics of Cases With IPMN With Concomitant PDAC

Patient, Age, Gender	IPMN				PDAC					Relation
	Loc	Type	Grad	Mutations	Loc	G	T	N	Mutations	
1) 73, m	Tail	MIXED	LGD	*KRAS*: p.G12V *GNAS*: p.R201H *TP53*: p.G325R *ARID1A*: p.Q386X *MAP2K4*: p.S90F	Head	3	3	1	*KRAS*: p.G12V p.G12D p.Q61R *TP53*: p.Y220C	Likely independent
2) 84, f	Tail	BD	LGD	*KRAS*: p.G12V *TGFBR2*: p.T264I *MAP2K4*: p.S90F	Head				*KRAS*: p.G12V *TP53*: p.H179L	Likely independent
3) 59, f	Multifocal	BD	LGD	*KRAS*: p.G12D	Uncinate	3	2	1	*KRAS*: p.G12D	Indeterminate
4) 55, m	Head	BD	LGD	*KRAS*: p.G12V *GNAS*: p.R201C	Head/neck	3	3	1	*KRAS*: p.G12D *TP53*: p.R213X	Likely independent
5) 65, m	Body	BD	LGD	*KRAS*: p.G12D *GNAS*: p.R201C	Head	2	3	0	*KRAS*: p.G12D *GNAS*: p.R201C	Likely related
6) 67, m	Neck	MIXED	LGD	*KRAS*: p.G12D *PIK3CA*: p.H047Y	Head	2	2	1	*KRAS*: p.G12D	Indeterminate
7) 65, m	Uncinate	BD	LGD	*KRAS*: p.G12D *GNAS*: p.R201H	Head	2	2	1	*KRAS*: p.Q61L* p.G12D *GNAS*: p.R201H* p.R201C	Likely related
8) 87, m	Head	BD	LGD	*KRAS*: p.G12V *GNAS*: p.R201C	Uncinate	2	1	0	*KRAS*: p.G12V	Indeterminate
9) 55, m	Multifocal	BD	LGD	*KRAS*: p.G12D		3	3	0	*KRAS*: p.G12D *TP53*: p.R342X	Indeterminate
10) 74, m	Head	Mixed	LGD	*KRAS*: p.G12R p.G12V *GNAS*: p.Q227H	Head/body	2	2	1	*KRAS*: p.G12D *TP53*: p.Y220C	Likely independent
	Head	MD	HGD	*KRAS*: p.G12D						Indeterminate

BD indicates branch duct IPMN; G, cellular grading; HGD, high-grade dysplasia; LGD, low-grade dysplasia; MD, main duct IPMN; MIXED, mixed-type IPMN; N, N stage; N/A FFPE blocks not available for DNA extraction; T, T stage.

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* Mutation with the highest concentration.

TABLE 3

Radiological and Pathological Patterns of Progression in Noninvasive IPMNs

	Radiologic Pattern of Progression in Noninvasive IPMNs				
	No Progression 210 (81%)	**BD Progression 32 (12%)**	**MD Progression 7 (3%)**	**Progression With Separate Solid Mass/Systemic 11 (4%)**	***P***
Characteristics of the primary IPMN					
IPMN duct type					
BD-IPMN	131 (81%)	22 (14%)	1 (1%)	7 (4%)	0.060
MD/Mixed-type IPMN	79 (80%)	10 (10%)	6 (6%)	4 (4%)	
Dysplasia					
Low grade	147 (84%)	24 (14%)	1 (1%)	3 (1%)	**0.001**
High grade	63 (75%)	8 (10%)	5 (6%)	8 (10%)	
Resection margin					
Negative	178 (82%)	27 (12%)	5 (2%)	8 (4%)	0.183
LGD	28 (80%)	5 (14%)	1 (3%)	1 (3%)	
HGD *	4 (57%)	0	1 (14%)	2 (29%)	
Family history	20 (61%)	8 (24%)	2 (6%)	3 (9%)	0.36
Time to progression Median (IQR)	—	27 (14–53)	52 (38–69)	49 (32–78)	
Completion pancreatectomy	0	4 (28%)	6 (42%)	4 (28%)	—
Pathology of disease progression in second operation					
IPMN low grade	—	3 (60%)	2 (40%)	0	
IPMN high grade	—	1 (25%)	3 (75%)	0	
IPMN with invasive carcinoma	—	0	1 (100%)	0	
Conventional PDAC	—	0	0	4 (100%)	

* Seven patients had a resection margin positive for HGD at the final histological examination. In 3 cases, the surgeon decided not to proceed with a completion pancreatectomy based on the patients' advanced age and medical problems, balancing the risk of residual neoplasia with potential disability from brittle diabetes following completion pancreatectomy. In 4 cases, although the definitive margin status on the final pathological report was positive for HGD, the intraoperative margin was called negative, precluding additional resection.

TABLE 4

Univariate and Multivariate Analyses of Risk Factors Associated With Development of IPMN With High-Grade Dysplasia or an Invasive Carcinoma (High-Risk Neoplasm) in the Remnant Pancreas

	Univariate			Multivariate		
	Progression to PDAC - HGD	**No Progression to PDAC -HGD**	***P***	**OR**	**95% CI**	***P***
	16 (6%)	**244 (94%)**				
Age, Median (IQR)	68 (56–75)	69 (61–76)	0.593	—	—	—
Male gender	9 (56%)	122 (50%)	0.628	—	—	—
Family history	5 (33%)	28 (12%)	0.029	5.82	1.49–22.78	0.011
IPMN duct-type						
Main/Mixed-type	7 (44%)	92 (38%)	0.608	—	—	—
IPMN						
LGD IPMN	5 (31%)	171 (70%)	0.004	9.22	2.76–20.71	<0.001
HGD IPMN	11 (69%)	73 (30%)				
Positive margin *	6 (38%)	37 (15%)	0.032	3.10	0.94–10.88	0.064
HGD at the margin	3 (18%)	4 (2%)	0.006			

HGD, high-grade dysplasia; LGD, low-grade dysplasia.

*Any grade of dysplasia.