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GNAS and KRAS Mutations Define Separate Progression Pathways in Intraductal Papillary Mucinous Neoplasm-Associated Carcinoma

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Abstract

Background—Intraductal papillary mucinous neoplasms (IPMN) are being increasingly recognized as important precursors to pancreatic adenocarcinoma. Elucidation of the genetic changes underlying IPMN carcinogenesis may improve the diagnosis and management of IPMN. We sought to determine whether different histologic subtypes of IPMN would exhibit different frequencies of specific genetic mutations.

Study Design—Patients with resected IPMN-associated invasive carcinoma (IPMN-INV) between 1997 and 2012 were reviewed. Areas of carcinoma, high-grade dysplasia and low-grade dysplasia were micro-dissected from each pathologic specimen. Targeted, massively-parallel sequencing was then performed on a panel of 275 genes (including KRAS, GNAS and RNF43).

Results—38 patients with resected IPMN-INV and sufficient tissue for microdissection were identified. Median follow-up was 2.6 years. GNAS mutations were more prevalent in colloid-type IPMN-INV than tubular-type IPMN-INV, 89% vs 32% respectively ($p=0.0003$). Conversely, KRAS mutations were more prevalent in tubular-type than colloid-type IPMN-INV, 89% vs 52%, respectively ($p=0.01$). For non-invasive IPMN subtypes, GNAS mutations were more prevalent in intestinal (74%) compared with pancreatobiliary (31%) and gastric (50%) subtypes ($p=0.02$). The presence of these mutations did not vary according to the degree of dysplasia (GNAS: invasive 61%, high-grade 59%, low-grade 53%, KRAS: invasive 71%, high-grade 62%, low-grade 74%), suggesting that mutations in these genes occur early in IPMN carcinogenesis.

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Conclusions—IPMN-associated colloid carcinoma and its intestinal-type pre-invasive precursor are associated with high frequencies of *GNAS* mutations. The mutation profile of tubular carcinoma resembles conventional pancreatic adenocarcinoma. Preoperative determination of mutational status may assist with clinical treatment decisions.

Intraductal papillary mucinous neoplasms of the pancreas (IPMN) are mucin-producing neoplasms of the pancreas first categorized as a distinct entity by the World Health Organization in 1996. Since this first categorization, pathologists have recognized that this disease exhibits significant heterogeneity. Histopathologically, four subtypes of non-invasive IPMN have been described: gastric, pancreatobiliary, intestinal and oncocytic(1,2). Macroscopic lesions often contain several of these histopathologic sub-types, as well as varying levels of dysplasia. Invasive IPMN carcinoma (IPMN-INV) is found in approximately 40-60% of resected IPMN lesions, and there are two distinct histopathologic subtypes of IPMN-INV(3). Colloid carcinoma typically arises in the setting of intestinal-type IPMN, and tubular carcinoma typically arises in the setting of pancreatobiliary-type IPMN. The survival outcomes following resection of IPMN-INV have been found to be associated with the type of invasive cancer: patients resected for colloid carcinoma have five-year survival rates of almost 75%, compared with 20-40% for those resected for tubular carcinoma.

This heterogeneity, and in particular the inability to determine degree of dysplasia or pathologic subtype preoperatively, has made IPMN a very challenging clinical problem. Operative treatment recommendations are currently based on radiographic and endoscopic findings(4). In the absence of a mass lesion suspicious for IPMN-INV, the strongest factor associated with high-grade dysplasia is dilation of the main pancreatic duct(5,6). There are currently no radiographic or endoscopic means of differentiating IPMN-INV subtype (colloid vs. tubular).

Recently, mutations in *KRAS*, *GNAS*, and *RNF43* have been identified in the tissue and cyst fluid of patients with IPMN(7,8). Importantly, these mutations were not identified in the cyst fluid of patients with serous cystadenoma, and other common types of benign pancreatic cysts. The association of these mutations with the pathway of progression to IPMN-INV has not been clearly defined. While mutation in *KRAS* has been shown to be an early genetic event(9), recent studies identifying mutations in *GNAS* and *RNF43* suggest that IPMN carcinogenesis may be distinct from that of conventional pancreatic adenocarcinoma(7,8,10–12).

In this study, we hypothesized that different histologic subtypes of IPMN would exhibit different mutation patterns of *KRAS*, *GNAS* and *RNF43*. This study was also designed to explore the timing of these mutations within the progression pathway. Our results confirm that mutations in these genes occur early in IPMN carcinogenesis, that *GNAS* mutations predominate in colloid carcinoma, and *KRAS* mutations predominate in tubular carcinoma. Importantly, these findings may allow us to identify those with colloid carcinoma from those with tubular carcinoma prior to operation.

Methods

Patients

With approval of the Institutional Review Board and in accordance with Health Insurance Portability and Accountability Act regulations, a prospectively maintained pancreatic database was used to identify all patients who underwent resection of IPMN-associated carcinoma (IPMN-INV) at Memorial Sloan-Kettering Cancer Center between 1997 and 2012. A retrospective review of each patient's medical record recorded demographic information, preoperative variables, operative therapy, long-term outcome, and recurrence patterns.

Histopathology

Tissue blocks from these patients were retrieved from the Department of Pathology archive and reviewed independently by two expert pancreatic pathologists (OB, DK). Areas of low- and high-grade dysplasia, as well as carcinoma, were identified and marked within each pathologic specimen. Dysplastic areas were also sub-classified by IPMN subtype: gastric, intestinal and pancreatobiliary. Carcinomas were classified as colloid or tubular type, as previously described(3). Briefly, an IPMN-INV was classified as tubular if it exhibited conventional ductal morphology, similar to that of pancreatic ductal adenocarcinoma with neoplastic cells arranged in small tubular glands with an infiltrative desmoplastic stroma. An IPMN-INV was classified as colloid if more than 80% of the invasive component consisted of extensive stromal pools of acellular mucin either lined by neoplastic epithelial cells or containing floating neoplastic epithelial cells. None of the tumors had a mixed histologic subtype. Ten-micron sections were then cut from tissue blocks containing areas of interest. From these sections, areas of dysplasia or carcinoma were needle micro-dissected. DNA extraction was performed on dissected tissue, as well as normal, non-pancreatic tissue (stomach, spleen or duodenum) for each patient (Figure 1).

High Throughput, Targeted Deep Sequencing

Targeted, massively-parallel sequencing was then performed on a panel of 275 genes (including *KRAS*, *GNAS* and *RNF43*; see **Appendix (available online)** known to undergo somatic genomic alterations in cancer, as previously described(13,14). Briefly, massively parallel sequencing libraries (Kapa Biosystems, New England Biolabs) that contain barcoded universal primers were generated from 78-250 ng genomic DNA of the micro-dissected, formalin-fixed, paraffin-embedded 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 275 cancer genes. 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. Somatic single

nucleotide variants and indels were called using MuTect and the SomaticIndelDetector tools in GATK, respectively(15,16). All candidate mutations and indels were reviewed manually using the Integrative Genomics Viewer(17). Because the tumor purity of the invasive samples was typically lower than the high-grade and low-grade samples, sites of mutations called in high-grade and low-grade samples but not in invasive samples were inspected and called positive if present in at least 10 unique reads and 2% of reads.

Statistical Analyses

Statistical analyses were performed with the software package JMP (JMP, Cary, NC, USA). Disease-specific survival (DSS) was calculated from the time of pancreatic resection until cancer-related death. Survival curves were generated using the Kaplan-Meier method, with patients censored when lost to follow-up or upon death from non-IPMN causes. Kaplan-Meier estimates of survival and chi-square tests were used to identify variables associated with DSS, histologic subtypes and presence of specific genetic mutations.

RESULTS

There were 74 patients who underwent resection of IPMN-INV between 1997 and 2012, and 61 of these patients had sufficient tissue for microdissection. Sufficient DNA for targeted sequencing of IPMN-INV was present in 38 of the 61 patients and these 38 patients comprise the final study population (Table 1). The majority of these patients were male and over 70 years of age. Median follow-up was 2.6 years. The vast majority (90%) of patients had main duct involvement. Pancreaticoduodenectomy was performed in 79% of the patients. Colloid carcinoma was present in 50%, and the other 50% had tubular type carcinoma. No patients received neoadjuvant chemotherapy or chemoradiation. All patients received adjuvant chemotherapy, but not chemoradiation. There was no difference in disease-specific survival according to macroscopic type (main-duct, branch-duct, or mixed).

Within this population of patients with IPMN-INV, adjacent areas of dysplasia were micro-dissected (Table 2). Low-grade dysplasia was identified in 19 patients: gastric subtype in 10 (53%), intestinal subtype in 9 (47%). There were no areas of low-grade pancreatobiliary subtype IPMN. High-grade dysplasia was identified in 34 patients: intestinal subtype in 18 (53%), pancreatobiliary subtype in 16 (47%). There were no areas of high-grade gastric subtype IPMN.

Spectrum of genetic mutations

A total of 91 tumor samples from the 38 patients underwent mutational profiling alongside matched normal tissue from each patient to filter out inherited variants. Low-grade dysplasia was present in 19 samples, high-grade dysplasia in 34 samples, and carcinoma in 38 samples (Figure 1).

Figure 2 shows all the genes mutated in 5% of specimens, stratified by level of dysplasia. In the 18 low grade specimens, there were a total of 49 mutations. The median number of mutations per specimen was 3, range 0-5.

In the 34 high grade specimens, there were a total of 175 mutations. The median number of mutations per specimen was 4.5, range 0-40. One patient (patient 46) had 40 mutations in his high grade specimen – this patient had a 1.3cm, node-positive tubular carcinoma on a background of pancreatobiliary high-grade dysplasia that recurred 2.5 years after distal pancreatectomy. If this patient is excluded, then the remainder of the high grade specimens had 0-8 genetic mutations.

In the 38 invasive specimens, there were a total of 162 mutations. The median number of mutations per specimen was 4, range 0-40. Just as for the high grade specimen, patient 46's invasive specimen also had 40 mutations; when this patient is excluded, the remainder of the invasive specimens had 0-8 genetic mutations. The number of mutations was not proportional to the size of the invasive IPMN component.

KRAS was the most commonly mutated gene, being mutated in 14 of 18 (78%), 21 of 34 (62%) and 23 of 38 (61%) of low grade, high grade and invasive specimens, respectively. *GNAS* was the next most commonly mutated gene, being mutated in 10 (56%), 20 (59%) and 19 (50%) of low grade, high grade and invasive specimens, respectively. Two (11%) low grade specimens had an *RNF43* mutation, compared with 9 (26%) high grade specimens and seven (18%) invasive specimens. *TP53* was mutated in 5% of low grade specimens, and in 21% of both high grade and invasive specimens (NS). Frequencies of mutation in *KRAS*, *GNAS* and *RNF43* did not vary according to tumor size, nodal status or macroscopic IPMN type.

Distribution of mutations in *KRAS*, *GNAS* and *RNF43*

KRAS mutations were predominantly at codon 12 (G12D/R/V; 47 specimens (81%)). There were 2 instances of G13D, 6 instances of Q61R and 2 of A146G mutations.

Of the 49 detected *GNAS* mutations, 45 (92%) were at codon 201 (R201H or R201C), the remaining four mutations were Q227L mutations. Three of these Q227L mutations were identified in the low-grade (intestinal-type), high-grade (intestinal-type) and colloid carcinoma specimens of a single patient.

Unlike *GNAS* and *KRAS*, there was no hotspot mutation for *RNF43*. *RNF43* mutations were identified in 19 samples: eight were frame-shift deletions, two were frame-shift insertions, six were missense mutations and three were nonsense mutations (**Supplemental Table 1, online only**).

There was no statistical difference in the frequency of mutations of these *KRAS*, *GNAS* and *RNF43* by level of dysplasia. For non-invasive IPMN (low-grade and high-grade combined, n=53), *KRAS*, *GNAS* and *RNF43* mutations were detected in 66%, 56% and 21%, respectively. For IPMN-INV (n=38), *KRAS*, *GNAS* and *RNF43* mutations were detected in 61%, 50% and 18%, respectively. 13 of 23 (56%) of *GNAS*-mutated IPMN-INV also harbored a *KRAS* mutation. Three invasive tumors contained mutations for all three genes (but none had recurred at an average follow-up of 3 years).

Analyzing the distribution of mutations by histologic type of invasive and non-invasive IPMN revealed that *GNAS* mutations were more prevalent in colloid-type IPMN-INV than tubular-type IPMN-INV, 89% vs 32% respectively ($p=0.0003$). Conversely, *KRAS* mutations were more prevalent in tubular-type than colloid-type IPMN-INV, 89% vs 52%, respectively ($p=0.01$, see Figure 3). Of the 17 *GNAS*-mutant colloid carcinomas, eight (47%) were also *KRAS* mutant. Of the 17 *KRAS*-mutant tubular carcinomas, five (29%) also harbored a *GNAS* mutation. For noninvasive IPMN subtypes (Table 3), *GNAS* mutations were more prevalent in intestinal (74%) compared with pancreatobiliary (31%) and gastric (50%) subtypes ($p=0.02$). No differences between the subtypes were observed with respect to *KRAS* or *RNF43* mutations.

On univariate analysis, factors associated with disease-specific survival were histologic subtype, *GNAS* status, nodal status and positive resection margin (Table 4). Small sample size precluded multivariate analysis. Figure 4 illustrates outcome of patients stratified by histology (colloid or tubular) and *GNAS* status. When considering both *KRAS* and *GNAS* mutational status, patients with *GNAS* mutant, *KRAS* wild type tumors showed a non-significant trend towards improved survival compared with patients with *KRAS* mutant tumors (Figure 4C).

Polyclonality

In 36 of 38 patients, at least one gene was found to be mutated in two specimens of varying dysplasia from that same patient. For ten patients (28%), the site and/or type of these mutations were different between specimens from the same patient (Table 5). For example, in patient 11, the low grade specimen had an R844H *GNAS* mutation and a G12V *KRAS* mutation, but the paired high grade specimen had a Q870L *GNAS* mutation and a G13D *KRAS* mutation. For 7 of these ten patients, one of the differentially mutated genes was *KRAS*.

There were 18 patients where a low grade specimen could be paired with a higher grade specimen (either high grade dysplasia or invasive). In 12 of these 18 patients (67%), there was at least one mutation (median 2, range 1-4) present in the low grade specimen that was NOT present in the higher grade specimen.

Similarly, there were 32 patients where a high grade specimen could be paired with an invasive specimen. In 25 of these 32 patients (78%), there was at least one mutation (median 3, range 1-7) present in the high grade specimen that was NOT present in the invasive specimen.

In summary, the presence of different mutations in the *same* gene between specimens from the same patient, as well as the absence of mutations in specimens of higher levels of dysplasia that were present in specimens of lesser dysplasia, is consistent with a multifocal neoplastic process within the pancreatic parenchyma.

Discussion

The results of this study provide two important findings regarding IPMN carcinogenesis. First, *KRAS* and *GNAS* likely represent markers for two distinct progression pathways. *GNAS* was predominant in colloid carcinoma, as well as within its intestinal subtype precursor lesion. Similarly, *KRAS* was predominant in tubular carcinoma, as well as within the pancreatobiliary precursor lesion. This finding may allow us to identify those with colloid carcinoma from those with tubular carcinoma, and make inferences regarding disease specific outcome, prior to operation. Second, these events occur very early within the progression pathway, and likely will not be useful for differentiating between low-grade and high-grade dysplasia. *KRAS* and *GNAS* mutations were identified equally in the early gastric subtype precursor lesions, which suggests that although these lesions appear similar pathologically, their future progression pathway may have already been determined.

Our work extends the studies of Wu et al(7) investigating the polyclonality of IPMN. In their study of IPMN cyst fluid, they quantified the levels of mutations in cyst samples containing more than one mutation of the same gene (*KRAS* or *GNAS*). There was a predominant mutation in four of the 11 IPMNs that had more than one mutation in either *KRAS* or *GNAS*. Since IPMN cysts, by definition, communicate with the remainder of the pancreatic ductal network, this finding may be explained by the presence of multiple, macroscopically-separated foci of IPMN arising independently, *or* by the presence of distinct clones within a single macroscopic focus of IPMN.

In our study, for each patient, microdissected lesions of all levels of dysplasia were all obtained from a single macroscopic focus of IPMN. We found that in 28% of gross tumor lesions, the site of mutation of a specific gene (most commonly *KRAS*) was different across varying levels of dysplasia. Combined with our finding that the prevalence of *GNAS* and *KRAS* mutations did not increase with increasing dysplasia, it would appear that these disparate mutations occur very early in IPMN tumorigenesis. Thus, these data suggest that the microscopic regions of differing dysplasia within a single macroscopic region may either diverge from a single clone at a very primitive stage, or arise completely independently. Thus, a gross tumor lesion may represent a “focal point” of genomic instability within an at-risk pancreas. Further work remains to be done to elucidate the underlying molecular mechanisms triggering this field instability.

The focus of the published literature studying mutational events in IPMN has been on *noninvasive* IPMN(7,8,10,11,18–20). The current study differs from these previous reports by focusing on a cohort of resected patients with *invasive* IPMN and by evaluating both the invasive and non-invasive components of these complex lesions.

Our finding of a higher frequency of *GNAS* mutations in colloid IPMN-INV, compared to tubular IPMN-INV, is novel. Dal Molin et al(19) found *GNAS* mutations more frequently in intestinal type IPMN, compared with gastric or pancreatobiliary subtypes. Thus, our observation is consistent with our understanding of IPMN carcinogenesis, as colloid carcinoma typically arises from intestinal IPMN, whereas tubular carcinoma typically arises from pancreatobiliary IPMN(21).

The higher frequency of *GNAS* mutations in colloid IPMN-INV and *KRAS* mutations in tubular IPMN-INV may have important clinical implications. For the first time we may have a marker that can define colloid from tubular carcinoma pre-operatively. In this study, within the subgroup of patients with *GNAS* mutation only (colloid carcinoma), not a single patient died of disease within four years of follow-up. Within the *KRAS* mutation-only group (tubular carcinoma) only 30% of patients were alive after four years of follow-up. Although the numbers are very small, if these findings can be validated, this information may be very helpful in clinical decision making. Many patients who present with IPMN are at an advanced age, and in the setting of multiple comorbidities, one may choose to not proceed with resection in the setting of a *GNAS*-only mutant. Conversely, in a young patient, one may choose more extensive resection in the setting of *GNAS* only as the long-term survival outcomes are likely excellent. From a practical standpoint, mutations in *GNAS* (as well as other genes) can be identified in DNA extracted from small volumes (0.25ml) of pancreatic cyst fluid(7).

Yamada et al(22) recently identified *GNAS*-activating mutations in 83% of colonic villous adenomas, but only rarely (3%) from tubulovillous adenomas or adenocarcinomas. The same group also reported *GNAS*-activating mutations in 63% of pyloric gland adenomas of the stomach and duodenum, but none in foveolar- or intestinal-type adenomas or gastric adenocarcinomas(23). These two studies suggest that intestinal-type IPMN and colloid carcinoma share not only morphologic features with tubulovillous adenomas and pyloric gland adenomas, but a distinct genetic background.

In tumors with *GNAS*-activating mutations, codon 201 mutations predominate(10,12,22–28). Although all series of *GNAS*-mutated tumors are small (<25 patients), in all but one study, codon 201 mutations comprise >90%. Therefore, while codon 227 mutations have not previously been described in IPMN, our finding of Q227L mutations in 8% of *GNAS* mutations is consistent with mutation patterns in other tumors.

KRAS is mutated in >90% of conventional pancreatic adenocarcinoma, with the vast majority of mutations occurring in codon 12. Mutations in *GNAS* and *RNF43* have not been found. Thus, the presence of *KRAS* mutations in 89% of IPMN-associated tubular carcinoma, all of which occurred at codon 12, along with low frequencies of *GNAS* (32%) and *RNF43* (11%) mutations, is consistent with previous work demonstrating that this type of IPMN-INV has a similar biologic behavior to conventional PDAC(3).

There are several limitations to this study. Only patients who had undergone resection for IPMNINV were included due to the need for sufficient tissue for precise histologic sub-classification, microdissection and DNA extraction. Therefore, our findings may not reflect the frequency and distribution of gene mutations in all patients with IPMN. The number of patients in this study is relatively small (38), but due to separate microdissection of distinct areas of dysplasia from each patient's tumor, there were a total of 91 sequenced specimens. Of these, 38 were of invasive tumors, which represents the largest number of IPMN-associated carcinomas sequenced thus far. This small sample size, especially of low grade lesions, limits further statistical analysis and identification of patterns of genetic mutations between histologic subtypes and across varying levels of dysplasia. The inherent limitation

of this type of study on IPMN is the possibility of contamination during microdissection, and that in higher grade lesions there is an abundance of stroma which may make identification of low-level mutations difficult.

In conclusion, this study of 38 patients with invasive IPMN demonstrated that mutations in *KRAS* and *GNAS* occur early in IPMN carcinogenesis. *GNAS* mutations predominated in colloid IPMN-INV, and *KRAS* mutations predominated in tubular IPMN-INV. *GNAS* status was associated with outcome, and this finding suggests that *KRAS* and *GNAS* mutations may represent prognostic biomarkers that could provide valuable information for clinical decision making. These preliminary findings will require validation with larger patient- and tissue-sample sets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Précis

In this mutational analysis of patients who underwent resection of carcinoma arising from intraductal papillary mucinous neoplasms (IPMN), we found high frequencies of *GNAS* mutations in colloid carcinoma and its intestinal-type pre-invasive precursor. Mutations in *GNAS* and *KRAS* appear to occur early in IPMN carcinogenesis.

FIGURE 1.
Study workflow.

FIGURE 2.

Genetic mutations and degree of dysplasia. Genes were included if mutation occurred in 5% of the population.

FIGURE 3. *GNAS* and *KRAS* mutations in IPMN-associated carcinoma. Blue bar, colloid (n=19); red bar, tubular (n=19). *p=0.0003; **p=0.01.

FIGURE 4.

Kaplan-Meier estimates of DSS in IPMN-associated carcinoma. The effect of (A) histologic subtype (colloid versus tubular, $p=0.007$); (B) *GNAS* mutational status ($p=0.03$); and (C) considering mutational status of both *KRAS* and *GNAS* ($p=0.3$).

TABLE 1

Patient Demographics

Patient Characteristic	n	%
Sex		
Male	24	63
Female	14	37
Age, y		
70	15	39
>70	23	61
Location of tumor		
Head	30	79
Other	8	21
Size of invasive component		
Microscopic, <0.5cm	6	16
Macroscopic, 0.5cm	32	84
Macroscopic type		
Main-duct	20	53
Mixed	14	37
Branch-duct	4	10
Type of resection		
Standard Whipple	28	74
Distal pancreatectomy	5	13
Total pancreatectomy	5	13
Resection margin		
Positive	7	18
Negative	31	82
Lymph node status		
Positive	14	37
Negative	24	63
Histologic subtype		
Colloid	19	50
Tubular	19	50
<i>GNAS</i> status		
Wild-type	15	39
Mutant	23	61
<i>KRAS</i> status		
Wild-type	11	29
Mutant	27	71
<i>RNF43</i> status		
Wild-type	31	82
Mutant	7	18

TABLE 2**IPMN Subtypes and Histologies**

Grade and subtype	n (%)
Low (n=19)	
Gastric	10 (53)
Intestinal	9 (47)
Pancreato-biliary	0
High (n=34)	
Gastric	0
Intestinal	18 (53)
Pancreato-biliary	16 (47)

TABLE 3

Distribution of Common Mutations by IPMN Subtype

	Gastric (n=10)	Intestinal (n=27)	Pancreato-biliary (n=16)	p Value
<i>KRAS</i> mutant	8 (80%)	17 (63%)	10 (63%)	0.6
<i>GNAS</i> mutant	5 (50%)	20 (74%)	5 (31%)	0.02
<i>RNF43</i> mutant	1 (10%)	5 (19%)	5 (31%)	0.4

TABLE 4

Factors Associated with Disease-Specific Survival

Factor	3-year DSS, %	Univariate p Value
Resection margin	33	0.05
Positive (n=7)	69	
Negative (n=31)		
Lymph node status	39	0.04
Positive (n=14)	72	
Negative (n=24)		
Histologic subtype	81	0.007
Colloid (n=19)	42	
Tubular (n=19)		
<i>GNAS</i> status	45	0.03
Wild-type (n=15)	71	
Mutant (n=23)		
<i>KRAS</i> status	31	0.2
Wild-type (n=11)	68	
Mutant (n=27)		
<i>RNF43</i> status	59	0.5
Wild-type (n=31)	33	
Mutant (n=7)		

TABLE 5

Distinct Mutations in Genes across Varying Levels of Dysplasia but from the Same Macroscopic IPMN Lesion

Pt No.	Level of dysplasia	Histologic subtype	Gene	Type of mutation	Protein change
8	High grade	Intestinal	RNF43	Frame Shift Insertion	p.R117fs
	High grade	Intestinal	RNF43	Frame Shift Deletion	p.K560fs
	Invasive	Tubular	RNF43	Frame Shift Deletion	p.L7fs
10	High grade	Pancreatobiliary	KRAS	Missense Mutation	p.G12D
	Invasive	Tubular	KRAS	Missense Mutation	p.G12V
11	High grade	Intestinal	GNAS	Missense Mutation	p.Q870L
	Low grade	Gastric	GNAS	Missense Mutation	p.R844H
	High grade	Intestinal	KRAS	Missense Mutation	p.G13D
	Low grade	Gastric	KRAS	Missense Mutation	p.G12V
13	Invasive	Tubular	KRAS	Missense Mutation	p.G12D
	Low grade	Gastric	KRAS	Missense Mutation	p.G12R
17	High grade	Intestinal	KRAS	Missense Mutation	p.G12D
	Low grade	Intestinal	KRAS	Missense Mutation	p.G12V
20	High grade	Pancreatobiliary	KRAS	Missense Mutation	p.G12R
	Invasive	Tubular	KRAS	Missense Mutation	p.G12D
44	Invasive	Tubular	KRAS	Missense Mutation	p.G12D
	Low grade	Gastric	KRAS	Missense Mutation	p.G12V
46	High grade	Pancreatobiliary	MLL2	Frame Shift Deletion	p.P2354fs
	Invasive	Tubular	MLL2	Frame Shift Insertion	p.P647fs
	High grade	Pancreatobiliary	PTEN	Frame Shift Deletion	p.L265fs
	Invasive	Tubular	PTEN	Frame Shift Deletion	p.T321fs