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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

GNAS but Not Extended *RAS* Mutations Spectrum are Associated with a Better Prognosis in Intraductal Pancreatic Mucinous Neoplasms

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ABSTRACT

Background. The management of intraductal papillary mucinous neoplasms (IPMNs) is mainly based on imaging features and clinical symptoms, and remains challenging.

Objective. The aim of this study was to assess *GNAS, RAS* family (*KRAS, NRAS* and *HRAS*), *BRAF,* and *PIK3CA* mutation status in resected IPMNs and correlate it with clinicopathological characteristics and patient survival.

Methods. Overall, 149 consecutive unselected patients who underwent pancreatectomy for IPMNs were included. After dissection from formalin-fixed and paraffin-embedded tumors, *GNAS* mutational screening was assessed by allelic discrimination using Taqman® probes and confirmed by SNaPshot analysis. *RAS* family, *BRAF,* and *PIK3CA* mutational screening was assessed by high resolution melt and Sanger sequencing.

Results. Gastric- and intestinal-type IPMNs were the most frequent lesions (52% and 41%, respectively). Intestinal-type IPMNs were more frequently associated high-grade dysplasia (49%) and were the only IPMNs associated with colloid-type carcinoma. All pancreatobiliary IPMNs were invasive lesions, located in the main pancreatic duct. *GNAS*-activating mutations were strongly associated with the intestinal phenotype ($p < 10^{-4}$), while *RAS* pathway mutations were not associated with any particular phenotype. Mutations within other members of the epidermal growth factor receptor (EGFR) pathway were very rare (2%). *GNAS*-mutated IPMNs were rarely invasive (11%) and almost exclusively (83%) of the colloid type. For invasive lesions, multivariate analyses determined that only node negativity was associated with improved cancer-specific survival, but, in univariate analysis, *GNAS* mutation was associated with prolonged survival.

Conclusion. In patients selected for surgery, *GNAS* mutation analysis and tumor phenotype can help to better predict patient prognosis. In the near future, a more precise mutational analysis of IPMNs might help to better tailor their management.

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Pancreatic intraductal papillary mucinous neoplasms (IPMNs) are one of the precursor lesions of pancreatic adenocarcinoma. Histologically, they are categorized according to their localization (main duct, branch duct involvement, or both), their grade of dysplasia (low or high), and their phenotype (gastric, intestinal, pancreaticobiliary, or oncocytic). Main duct localization, high-grade dysplasia, and pancreaticobiliary phenotype have been associated with a worse prognosis,[1,2] and their clinical management remains controversial and challenging, mainly based on imaging features and clinical symptoms.[3,4] With the increasing number of IPMNs detected, three main questions must be addressed:

(1) Which patient should be surgically managed?
(2) What are the risks of relapse after surgery?
(3) What is the risk of cancer progression in patients followed-up.[5,6]

To date, despite several national and international guidelines, answers remain elusive.

Somatic activating mutations of the G-protein α-stimulatory subunit (Gsα subunit) encoded by the *GNAS* gene (*GNAS*) have been reported in up to 70% of pancreatic IPMNs, with an important discrepancy shown between studies (33–79%).[7–10] *KRAS* is therefore one of the two most prevalent mutations in these tumors. In this setting, *GNAS* mutations, known to lead to elevated intracellular cyclic adenosine monophosphate (cAMP) levels and activation of downstream dependent pathways,[11] could open new clinical insights into IPMNs. As an example, the IPMN intestinal pattern of differentiation is associated with *GNAS* mutation,[12] underlining the functional consequences of *GNAS*-activating mutations in the pancreatic tract.

If *KRAS* mutations are well-documented in IPMNs, the incidence of other gene mutations implicated in the epidermal growth factor receptor (EGFR) pathway has been rarely studied and, overall, the clinical significance of these genetic alterations has been poorly documented. This is of particular interest as several studies have shown that these mutations can be reliably assessed in the cyst liquid acquired during an endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) procedure, or even by collecting the pancreatic juice in the duodenum after secretin stimulation.[13,14]

In the present study, we examined the mutation status of *GNAS, RAS* family mutation spectrum (*KRAS, NRAS,* and *HRAS*), *BRAF,* and *PIK3CA* genes in a large series of consecutive, unselected IPMN patients who underwent pancreatic resection, and correlated mutational status with clinicopathological characteristics and patient survival.

PATIENTS AND METHODS

Patients

After Institutional Review Board (IRB) approval (IRB 12-055), we reviewed the medical records of 149 consecutive unselected patients who underwent a pancreatic resection for IPMN, between 2007 and 2011, in the Department of Hepatobiliary and Pancreatic Surgery, Beaujon Hospital, Clichy, France. Demographic variables, clinical presentation, intraoperative data, and a definitive pathologic diagnosis were obtained from a prospective database with additional retrospective medical record review. All surgical indications were discussed in a multidisciplinary pancreatic tumor board including surgeons, pathologists, radiologists, and gastroenterologists. Surgical indications were decided according to the most recent guidelines of the International Association of Pancreatology (IAP) for IPMNs.[15,16]

Tumor Pathology

All IPMN cases were reviewed and the diagnosis confirmed by an experienced pathologist in pancreatic pathology (JC). The type of duct involvement was determined by macro- and microscopic examinations, and was classified into main duct, branch duct, or mixed-type IPMNs. Dysplasia was graded as low (previous mild and moderate dysplasia), high-grade dysplasia (carcinoma in situ) and invasive carcinoma, according to World Health Organization criteria[17] and the recent Baltimore Consensus.[18] Patients with minimally invasive carcinoma as defined by Nara et al.[19] were categorized as high-grade dysplasia in view of their comparable prognosis. If an IPMN displayed several grades of dysplasia, the highest grade was recorded for this study. Tumors were classified into four distinct epithelial subtypes, i.e. gastric, intestinal, pancreatobiliary, and oncocytic, on the basis of their epithelial morphology on routine hematoxylin–eosin–safran staining and mucin profile on immunochemistry (MUC1, MUC2 and MUC5AC; BD Bioscience, San Diego, CA, USA; 1/400). Representative pictures of each phenotype are presented in Fig. 1. In the rare cases with two distinct phenotypes (only gastric plus intestinal in this series), the most abundant subtype was recorded. Of note, in all cases, the most abundant subtype always displayed the highest grade of dysplasia. Invasive carcinomas were classified as tubular carcinoma, i.e. as usual classical pancreatic carcinoma or colloid carcinoma in which extracellular mucin comprises at least 80% of the tumor volume.

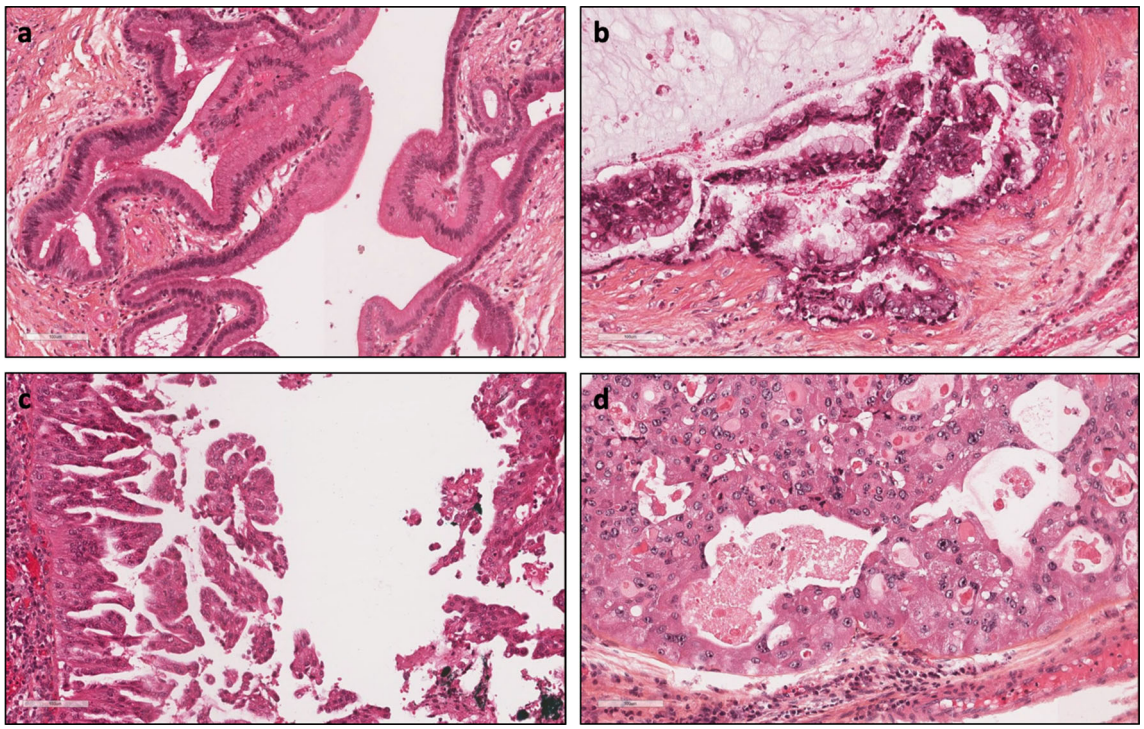

FIG. 1 Representative pictures of the four distinct epithelial subtypes, i.e. **a** gastric, **b** intestinal, **c** pancreatobiliary, and **d** oncocytic

GNAS Mutational Screening

As previously reported by our group,[20] DNA from tumor tissue was extracted after macrodissection from formalin-fixed and paraffin-embedded (FFPE) tumor specimens using a Qiagen® (Courtaboeuf, France) QIAamp FFPE tissue kit according to the manufacturer's instructions. Briefly, in order to ensure the best macrodissection, the area displaying the highest grade of dysplasia was chosen by an experienced pathologist (JC) on one hematoxylin and eosin slide. The area was marked with an adapted pen and reported on five serial unstained sections of 10 microns each. This area was then scratched with a clean scalpel and put into an Eppendorf tube for DNA extraction (electronic supplementary Fig. 1). Since all *GNAS*-activating mutations previously described in IPMNs were located in exon 8, codon 201 (GenBank accession no. NM_001077488.2), we consequently limited the mutational analysis to this hotspot. *GNAS* status was assessed by allelic discrimination using Taqman® probes and confirmed by SNaPshot analysis (see electronic supplementary Table 1 for primer details). All sequence variants identified were confirmed by two independent experiments.

RAS Mutational Screening

As previously reported,[21] the primer sequences used for both high resolution melt (HRM) and Sanger sequencing are shown in electronic supplementary Table 1. The majority of HRM primers were designed to span the entire exons with product sizes under 200 bp. Primers were designed for *KRAS* (exons 2–4), *HRAS* (exons 2 and 3), *NRAS*, (exons 2 and 3), *BRAF* (exon 15), and *PIK3CA* (exons 9 and 20). The polymerase chain reaction (PCR) for HRM and Sanger sequencing analysis was performed on a 384-well plate in the presence of the fluorescent DNA intercalating dye LC green (Idaho Technology, Salt Lake City, UT, USA) in a LightCycler480 (Roche Diagnosis, Meylan, France). The reaction mixture in a 15 ml final volume contained LC green, UDP-glycosylase (Roche), and Roche Master Mix (Roche). The cycling and melting conditions were as follows: an initial cycle of 10 min at 40 1C, one cycle of 95 1C for 10 min; 50 cycles of 95 1C for 10 s, 55–65 1C for 10 s, 72 1C for 30 s; one cycle of 97 1C for 1 min; and a melt from 70 1C to 95 1C rising 0.2 1C per second. Depending on the melting temperature, a touchdown approach was used for some primers. All samples were tested in duplicate. The HRM data were analyzed using the Genescan software (Roche). All samples including the wild-type (WT) exons were plotted according to their melting profiles on the differential plot graph. Any difference in the horizon line based on the WT sample was sequenced using Sanger sequencing. The reaction mixture in a total of 50 ml was made using 1 ml of PCR products without first purification, followed by a sequencing reaction with Big Dye Terminator v3.1

(Thermofisher, Courtaboeuf, France) according to the manufacturer's protocol. The sequencing products were purified with a Sephadex gel (GE Healthcare, Velizy-Villacoublay, France) before running on a 3500 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The sequencing data were visualized using Finch TV (Geospiza, Inc., Seattle, WA, USA) with detection sensibility of 10% mutated cells.

Statistical Analysis

Values are expressed as median and interquartile range, or percentage, as appropriate. The Chi square or Fisher's exact tests were used to compare differences in discrete or categorical variables. According to the distribution of variables, the *t* test or Wilcoxon rank-sum test were used for continuous variables. All preoperative clinical and radiological variables achieving statistical significance at a 0.1 level in univariate analysis were considered for multivariate analysis. A backward variable selection procedure was used to identify the independent predictive factors. Odds ratios (ORs) with 95% confidence intervals (CIs) are reported. Cancer-specific survival was measured from the date of surgery to the date of cancer-related death. Surviving patients were censored at the final follow-up. Cancer-specific survival was estimated using the Kaplan–Meier method, and survival was compared between the two groups using the log-rank test. All tests were two-sided. For all tests, statistical significance was defined as $p < 0.05$. Data were analyzed using STATA 12 statistical software (StataCorp LP, College Station, TX, USA; 2011. Stata Statistical Software: Release 12).

RESULTS

Patient and Tumor Characteristics

Patient and tumor characteristics according to IPMN phenotype are summarized in Table 1. Briefly, in this cohort of 149 resected lesions, low-grade IPMNs were the most prevalent lesions ($n = 78$; 52%), with a non-statistically different rate of involvement of the main and branch ducts ($n = 84$ [56%] vs. $n = 65$ [44%]). Dysplasia grade was significantly different according to the phenotype ($p < 10^{-4}$), and, overall, 21% ($n = 31$) were invasive. Gastric-type IPMNs were the most frequent lesions ($n = 77$; 52%), mainly of low-grade dysplasia ($n = 55$; 71%), while intestinal-type IPMNs were almost as frequent ($n = 61$; 41%), but were more frequently associated high-grade dysplasia ($n = 30$; 49%). All pancreatobiliary IPMNs were invasive lesions, located in the main pancreatic duct. While the rate of invasive carcinoma was comparable in intestinal and gastric-type IPMNs ($n = 8$ [13%] vs. $n = 14$ [18%]), colloid-type carcinoma was only seen in intestinal-type IPMNs ($p < 10^{-4}$).

GNAS and RAS Pathway Analysis

DNA was available for 135 patients (90.1%) for *GNAS* analysis, and 117 patients (78.5%) for *RAS* pathway analysis. Patient and tumor characteristics according to *GNAS* and *RAS* mutational status are summarized in Table 1 and electronic supplementary Table 2. Briefly, *GNAS*-activating mutations were strongly associated with the intestinal phenotype ($p < 10^{-4}$), while *RAS* pathway mutations were not associated with a particular phenotype. In addition, IPMNs displaying only the *GNAS* mutation were almost exclusively of the intestinal phenotype ($p < 10^{-4}$), and none were of the pancreatobiliary phenotype. Mutations within other members of the EGFR pathway were very rare (*NRAS* [$n = 1$] 0.9%; *BRAF* [$n = 1$] 0.9%), and mutually exclusive with *KRAS* mutations. Interestingly, the distribution of dysplasia grade and invasive IPMNs were different according to the *GNAS* mutational status ($p = 0.004$) [Table 2]. *GNAS* WT IPMNs were either of low grade ($n = 43$; 52%) and mostly of the gastric phenotype, or invasive ($n = 25$; 30%), while *GNAS*-mutated IPMNs were rarely invasive ($n = 6$; 12%) and almost exclusively of the colloid type in these cases, a rare occurrence in *GNAS* WT invasive IPMNs ($n = 2$; 8%) [$p = 0.001$]. In contrast, *RAS* mutations were not associated with any clinical or pathological variable (Table 2). There was no association between *GNAS* and *KRAS* status.

Long-Term Outcome of Intraductal Papillary Mucinous Neoplasms

Four patients deceased during the 90-day postoperative period were excluded from the survival analysis. In the remaining population, after a median follow-up of 104 months (77–123), 16 patients died from pancreatic cancer; the median cancer-specific survival was not reached. The 1-, 3-, and 5-year cancer-specific survival was 98% (94–99), 91% (85–95), and 90% (83–94), respectively. In the 31 patients with invasive cancers, the median survival was 43 months. The 1-, 3-, and 5-year cancer-specific survival was 89% (69–96), 56% (35–72), and 48% (29–65), respectively.

Univariate and multivariate analyses of prognostic factors are shown in Table 3 and Fig. 2. For all resected lesions, the phenotype in univariate analysis correlated with prognosis (Fig. 2a, c, e). In multivariate analyses, only branched-duct lesions and *GNAS* mutations were significantly associated with improved cancer-specific survival.

TABLE 1 Patient and tumor characteristics according to IPMN phenotype

	IPMN phenotype					
	Overall	Intestinal	Gastric	Pancreatobiliary	Oncocytic	*p* value
Patients						
% (*N*)	100 (149)	41 (61)	52 (77)	6 (9)	1 (2)	–
Male	54 (81)	69 (42)	45 (35)	33 (3)	50 (1)	0.01
Age, years (IQRs)	61 (54–68)	59 (54–67)	62 (54–69)	67 (64–71)	69.5 (63–76)	0.15
Cyst size, mm (IQRs)	20 (15–30)	25 (15–32.5)	20 (12–30)	30 (25–50)	40 (20–60)	0.02
Dysplasia						
Low-grade	52 (78)	38 (23)	71 (55)	0 (0)	0 (0)	0.000
High-grade	27 (40)	49 (30)	11 (8)	0 (0)	100 (2)	
Invasive carcinoma	21 (31)	13 (8)	18 (14)	100 (9)	0 (0)	
Cancer phenotype						
Colloid	23 (7)	87 (7)	0 (0)	0 (0)	0 (0)	0.000
Tubular	77 (24)	13 (1)	100 (14)	100 (9)	0 (0)	
Node (in invasive lesions)						
Negative	39 (12)	50 (4)	29 (4)	44 (4)	0 (0)	0.64
Positive	61 (19)	50 (4)	71 (10)	56 (5)	0 (0)	
Duct involvement						
Main or mixed	56 (84)	62 (38)	45 (35)	100 (9)	100 (2)	0.002
Branch	44 (65)	38 (23)	55 (42)	0 (0)	0 (0)	
Mutation (n = 135 for *GNAS* and n = 117 for *RAS*)						
GNAS	39 (52)	60 (34)	25 (17)	11 (1)	0 (0)	0.000
GNAS only	17 (22)	35 (18)	6 (4)	0 (0)	0 (0)	0.000
RAS	56 (66)	45 (22)	66 (38)	66 (6)	0 (0)	0.07
KRAS	55 (64)	41 (20)	66 (38)	66 (6)	0 (0)	0.03
NRAS	1 (1)	2 (1)	0 (0)	0 (0)	0 (0)	0.5
HRAS	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	–
BRAF	1 (1)	2 (1)	0 (0)	0 (0)	0 (0)	0.5
PIK3CA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	–

Data are expressed as % (*n*) unless otherwise specified

IPMNs intraductal papillary mucinous neoplasms, *IQR* interquartile range

For invasive lesions in multivariate analysis, only node negativity was associated with improved cancer-specific survival. In univariate analysis, *GNAS* mutations were associated with prolonged survival (Fig. 2b), while *RAS* mutational status did not impact survival. IPMN phenotype was not associated with prognosis, but the colloid cancer phenotype was associated with a non-significant trend of improved cancer-specific survival (Fig. 2f).

DISCUSSION

The present study involves 149 consecutive unselected patients who underwent a pancreatic resection for IPMN, with long-term follow-up (median follow-up of 104 months) and complete pathological analysis, in addition to *GNAS* and *RAS* pathway sequencing. First, we confirmed previous pathological observations, i.e. the clinical impact of the IPMN phenotype. Pancreatobiliary IPMNs have an aggressive behavior and are frequently invasive. On the contrary, gastric and intestinal lesions have a more indolent behavior and are less frequently invasive, with colloid-type carcinoma only associated with intestinal-type IPMNs. More interestingly, it is likely that part of these differences are driven by *GNAS* mutations, present overall in approximately 40% of lesions, and strongly associated with the IPMN intestinal phenotype. In the meantime, RAS pathway mutations, mainly represented by *KRAS* mutations, were not associated with any significant clinical consequences. Mutations of other members of the EGFR pathway (*NRAS*, n = 1; *BRAF*, n = 1) were very rare.

TABLE 2 Patient and tumor characteristics according to *GNAS* and RAS mutational status

	Mutational status							
	Overall	*GNAS* WT	*GNAS* mutation	*p* value	Overall	*RAS* WT	*RAS* mutation	*p* value
Patients								
% (*n*)	100 (135)	61 (83)	39 (52)	–	100 (117)	44 (51)	56 (66)	–
Male	56 (75)	53 (44)	60 (31)	0.48	53 (62)	59 (30)	48 (32)	0.35
Age, years (IQR)	62 (55–68)	62 (55–69)	61 (54–67)	0.25	62 (55–68)	60 (51–67)	62.5 (57–69)	0.09
Cyst size, mm (IQR)	25 (15–30)	25 (15–30)	25 (12–30)	0.69	25 (15–30)	20 (12–30)	25 (15–35)	0.12
Dysplasia								
Low-grade	50 (68)	52 (43)	48 (25)	0.004	50 (58)	47 (24)	52 (34)	0.13
High-grade	27 (36)	18 (15)	40 (21)		26 (31)	35 (18)	20 (13)	
Invasive carcinoma	23 (31)	30 (25)	12 (6)		24 (28)	18 (9)	29 (19)	
Phenotype (in invasive lesions)								
Colloid	23 (7)	8 (2)	83 (5)	0.001	21 (6)	33 (3)	16 (3)	0.35
Tubular	77 (24)	92 (23)	17 (1)		79 (22)	67 (6)	84 (16)	
Duct involvement								
Main or mixed	58 (79)	63 (52)	52 (27)	0.28	54 (63)	53 (27)	55 (36)	1
Branch	41 (56)	37 (31)	48 (25)		46 (54)	47 (24)	45 (30)	
Node (in invasive lesion)								
Negative	39 (12)	32 (8)	67 (4)	0.17	39 (12)	39 (11)	33 (1)	1
Positive	61 (19)	68 (17)	33 (2)		61 (19)	61 (17)	67 (2)	
Genotype mutation								
GNAS	–	–	–	–	39 (45)	43 (22)	35 (23)	0.44
GNAS only	17 (22)	0 (0)	49 (22)	–	–	–	–	–
RAS	56 (65)	59 (42)	51 (23)	0.44	–	–	–	–
KRAS	55 (64)	58 (41)	51 (23)	0.57	–	–	–	–
NRAS	0 (0)	0 (0)	0 (0)	–	0 (0)	0 (0)	0 (0)	–
HRAS	0 (0)	0 (0)	0 (0)	–	0 (0)	0 (0)	0 (0)	–
BRAF	1 (1)	1 (1)	0 (0)	1	1 (1)	1 (1)	0 (0)	–
PIK3CA	0 (0)	0 (0)	0 (0)	–	0 (0)	0 (0)	0 (0)	–

Data are expressed as % (*n*) unless otherwise specified

WT wild-type, *IQR* interquartile range

From a pathological point of view, gastric and intestinal IPMNs represent more than 90% of resected IPMNs in the present cohort, and were associated with a significantly better cancer-specific survival than pancreatobiliary IPMNs. These latter are mainly located in the main pancreatic duct, are more frequently invasive, and, when invasive, their long-term survival is comparable with that of pancreatic ductal adenocarcinoma patients because of their ductal differentiation, as previously reported.[1,22] Distler et al.[1] showed that most cancer recurrences are observed in the pancreatobiliary subtype, i.e. in cancer with a tubular differentiation. Interestingly, at cancer recurrence, patients with an intestinal subtype cancer (i.e. with colloid differentiation) had a significantly better prognosis when compared with the pancreatobiliary subtype. This advocated for a specific biological behavior of pancreatic adenocarcinoma with colloid differentiation,[23] either more indolent or more chemosensitive.

The results of our genetic analysis underlined the clinical consequences of *GNAS* mutations in IPMNs. As previously reported,[24–26] and in view of our results, it seems likely that *GNAS* and *KRAS*-only mutations define separate progression pathways in IPMN-associated carcinomas. In the present studies, *GNAS* was strongly associated with intestinal-type IPMNs, and, when invasive, with a colloid carcinoma phenotype. These observations are consistent with recent reports showing the specificity of *GNAS*-driven IPMN tumorigenesis,[26–28] which alters various gene expression, including expression of mucin genes, that may determine the IPMN phenotype.[29] In the present series, *GNAS* mutational status, but not tumor phenotype,

TABLE 3 Univariate and multivariate Cox regression analyses of risk of death from cancer in patients with IPMNs

Variable		Univariate analysis		Multivariate analysis	
		HR (95%CI)	*p* value	HR (95%CI)	*p* value
All resected IPMNs					
Clinical factors					
Age	Per unit	1.04 (0.98–1.09)	0.1	1.03 (0.96–1.08)	0.6132
Lesion size	Per mm	1.02 (1–1.04)	0.006	1.02 (1–1.04)	0.015
IPMN localization	Main/mixed type	1 (reference)	0.016	1 (reference)	0.048
	Branch duct	0.16 (0.03–0.71)		0.17 (0.04–0.75)	
Pathological factors					
IPMN phenotype	Intestinal	1 (reference)	0.001	1 (reference)	0.51
	Gastric	2.6 (0.7–9.67)		2.1.72 (0.45–6.6)	
	PB	14.9 (3.32–66.85)		7.96 (0.85–34.1)	
Genetic factors					
GNAS mutation	Absent	1 (reference)	0.022	1 (reference)	0.014
	Present	0.09 (0.01–0.71)		0.1 (0.01–0.79)	
RAS mutation	Absent	1 (reference)	0.363	1 (reference)	0.6156
	Present	1.6 (0.56–4.81)		1.64 (0.56–4.8)	
Invasive IPMNs					
Clinical factors					
Age	Per unit	1.02 (0.97–1.07)	0.32	1.02 (0.95–1.1)	0.48
Lesion size	Per mm	1.01 (0.99–1.04)	0.058	1.02 (0.99–1.05)	0.076
IPMN localization	Main/mixed type	1 (reference)	0.76	1 (reference)	0.414
	Branch duct	0.8 (0.18–3.5)		0.5 (0.1–2.57)	
Pathological factors					
IPMN phenotype	Intestinal	1 (reference)	0.489	1 (reference)	0.192
	Gastric	2.6 (0.7–9.7)		2.52 (0.67–9.49)	
	PB	1.7 (0.37–7.6)		2.61 (0.56–12.2)	
Cancer phenotype	Colloid	1 (reference)	0.12	1 (reference)	0.142
	Tubular	3.25 (0.7–14.5)		2.52 (0.73–8.7)	
Node involvement	Absent	1 (reference)	0.013	1 (reference)	0.013
	Present	5 (1.4–18)		5 (1.41–18)	
Genetic factors					
GNAS mutation	Absent	1 (reference)	0.047	1 (reference)	0.095
	Present	0.16 (0.02–1.25)		0.28 (0.06–1.24)	
RAS mutation	Absent	1 (reference)	0.66	1 (reference)	0.367
	Present	1.26 (0.43–3.7)		1.6 (0.57–4.52)	

IPMNs intraductal papillary mucinous neoplasms, *HR* hazard ratio, *CI* confidence interval, *PB* pancreatobiliary

lesion size, and IPMN location (branch vs. main/mixed type duct), are independent prognostic factors in resected patients. This might be explained by the fact that *GNAS* mutational status might represent a good surrogate marker for tumor phenotype, especially intestinal-type IPMNs, and was associated with a favorable long-term evolution. In addition, it was reported that approximately 20% of carcinomas co-arising with IPMNs are not genetically related and are independent events.[30] This was not the case with colloid carcinomas in which concordant mutational profiles were observed between the IPMNs and the carcinomas in almost all cases. This suggests that in colloid carcinomas arising in intestinal IPMNs, *GNAS* mutations are more frequently conserved through tumor progression, and probably correspond to the sequential model described by Omori et al.[31] This also suggests that while *KRAS* mutations may be seen in colloid carcinomas, although at a low frequency compared with ductal adenocarcinoma, the intestinal differentiation program and the carcinogenesis are mainly *KRAS*-independent. On the other hand, it may be hypothesized that *KRAS* mutations 'override' the *GNAS*-associated intestinal differentiation program in a subset of

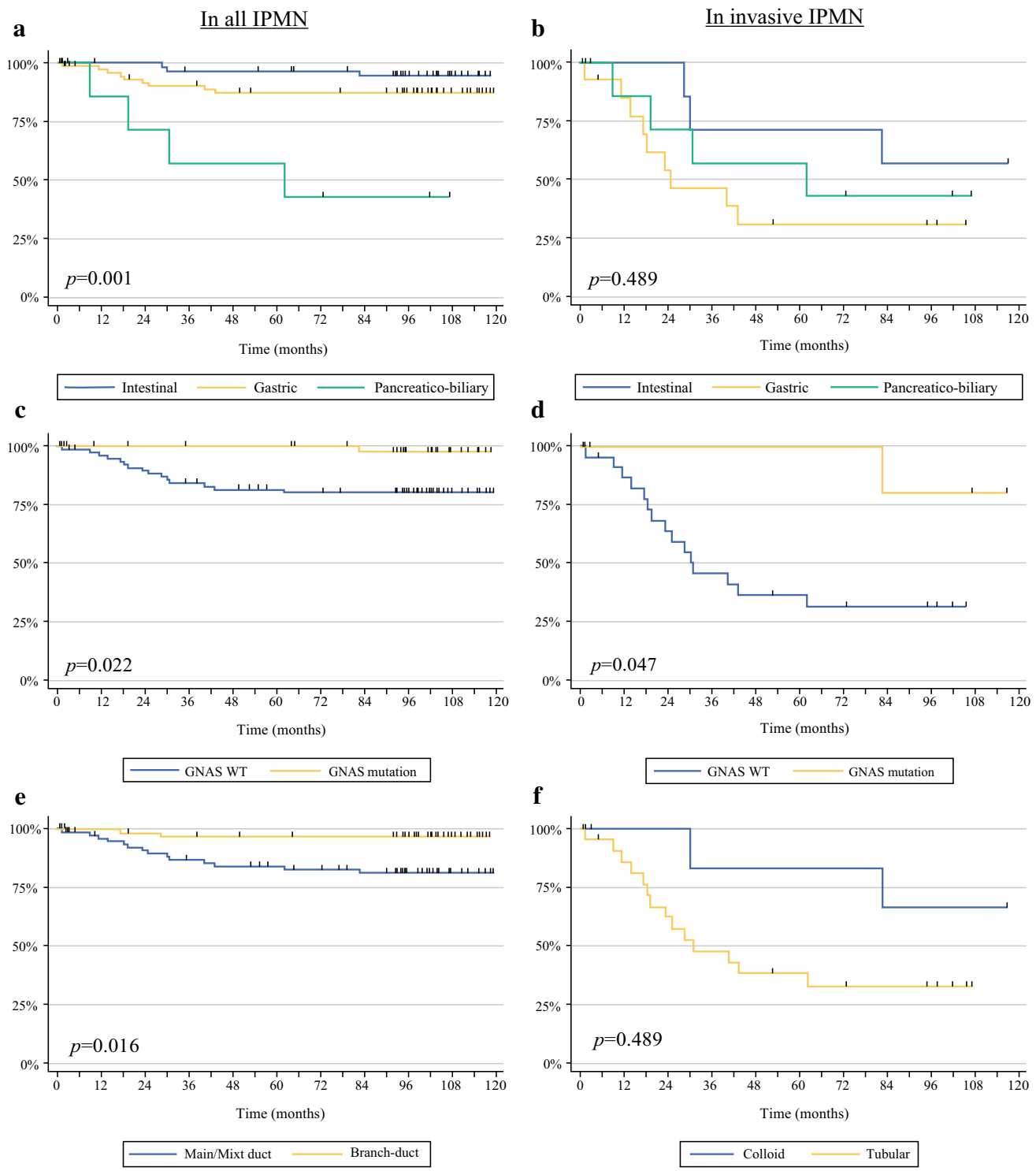

FIG. 2 Patients' overall survival according to IPMN characteristics: **a** grade of dysplasia, **b** phenotype, **c** carcinoma phenotype in invasive IPMNs, **d** *GNAS* mutation status in all IPMNs, **e** *GNAS* mutation status in invasive IPMNs, and **e** *RAS* mutation status in all IPMNs. *IPMNs* intraductal papillary mucinous neoplasms, *WT* wild-type

IPMN-associated carcinomas. Accordingly, *GNAS*-only mutations were almost exclusively seen in intestinal IPMNs (35% vs. 6 and 0% in gastric and pancreatobiliary IPMNs, respectively) [Fig. 3]. Finally, as proposed by Omori et al., most carcinomas, whether they are truly independent of the adjacent IPMN (de novo subtype) or

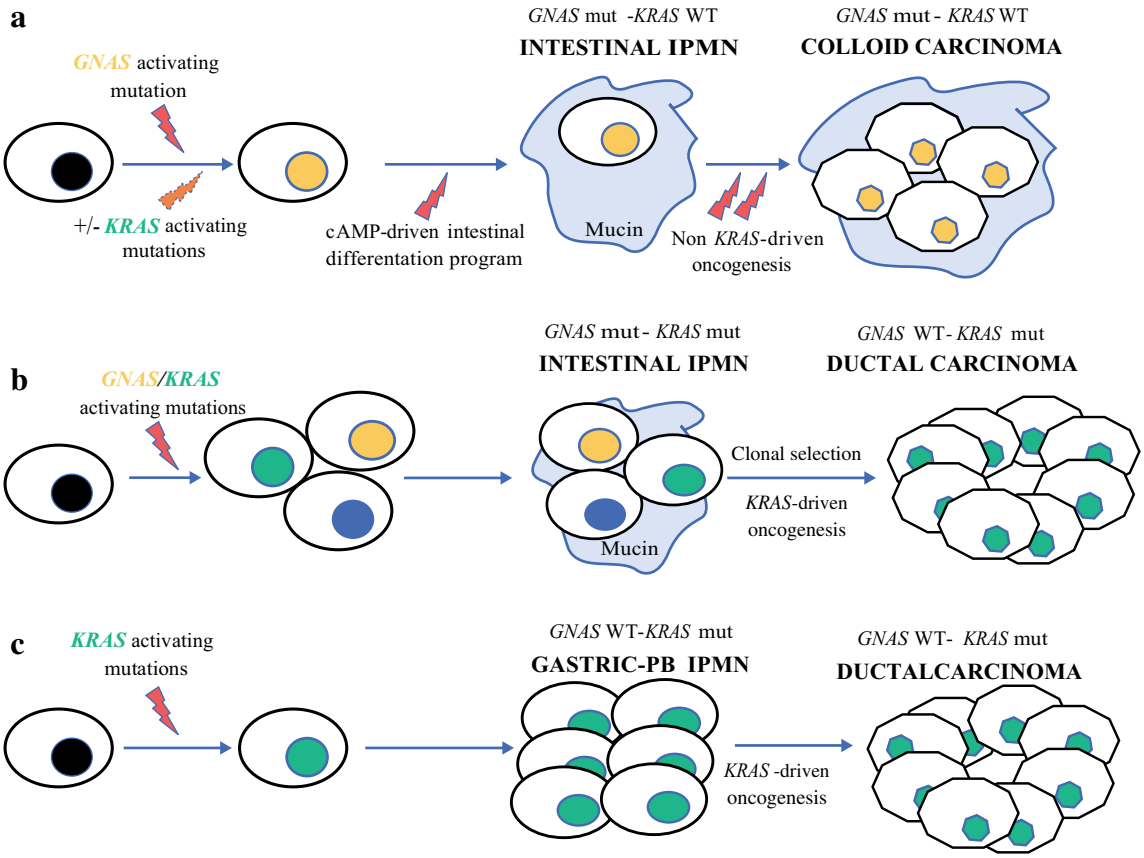

FIG. 3 Proposed carcinogenesis pathways in IPMNs. **a** *GNAS* mutation, especially when alone, favors an cAMP-driven program toward intestinal differentiation. Progression toward the mucinous type of carcinoma may be *KRAS*-independent and may rely on other carcinogenesis pathways. **b** *GNAS* and *KRAS* mutations may be heterogeneous in IPMNs, leading to the selection of clones with only *KRAS* mutation that may possess a growth advantage, explaining the drop in the rate of *GNAS* mutations between IPMN and IPMN-associated ductal carcinomas. Alternatively, some IPMN-associated carcinomas are in fact not genetically related to the IPMN and are arising *de novo*. **c** *KRAS*-only mutation favors progression toward gastric and pancreaticobiliary-type IPMNs. *KRAS* mutation together with other (epi)genetic events when associated with *GNAS* mutation may 'override' the later, explaining the *GNAS*-mutated gastric IPMN. *IPMNs* intraductal papillary mucinous neoplasms, *WT* wild-type, *mut* mutation, *PB* pancreatobiliary, *cAMP* cyclic adenosine monophosphate

have an early clonal relationship (branch-off subtype), have lost the *GNAS*-bearing clone through expansion of aggressive *KRAS*-driven clones.

Cystic pancreatic lesions, especially IPMNs, are diagnosed with an increasing incidence,[32] but routine resection of all lesions is no longer advocated. Surgical indications are based on symptoms and risk factors of malignant transformation,[3,4] but they remain insufficient, as illustrated by the numerous published and sometimes contradictory guidelines.[33] More accurate risk factors than clinical symptoms and radiologic features are urgently needed to best select patients for surgery, ideally before invasive carcinoma appears. Preoperative assessment of the IPMN phenotype or mutational status appears a promising area of research. What the true diagnostic or prognostic added value of histological subtypes and *GNAS/KRAS* mutations is remains to be more clearly determined. From a clinical point of view, it would be relevant to challenge the predictive value of clinical and radiological factors such as mural nodules, which are now considered reliable predictors of invasive cancer and high-grade dysplasia in IPMNs, as proposed by the 2016 IAP guidelines,[34] with the genetic factors assessed in the present work. Unfortunately, our database has not been built to predict IPMN invasiveness, and 'presence of absence of mural nodules' has not been captured in our database. Additionally, it seems that a combination of molecular markers, including *GNAS* and clinical features, improves the classification of pancreatic cysts.[35] *GNAS*-only, present in IPMNs,[7,10] is a highly specific diagnostic tool, even if its sensibility remains low at approximately 40–60%, and, in view of the present results and others,[24] it could also represent an interesting prognostic tool. If preoperative determination of *GNAS* mutational status by FNA is problematic because of the potential morbidity[36] of the procedure, *GNAS* mutations can also be detected in duodenal collections of secretin-

stimulated pancreatic juice,[9,13,37] or, even easier, in circulating cell-free DNA isolated from blood samples.[38] The present study is the first to investigate, in such a large number of resected IPMNs, the mutational status of the extended *RAS* family. Unlike in other carcinomas, we found a very low rate of *NRAS* and *HRAS* mutations, suggesting that they will have little impact on tumorigenesis, and almost no utility in pancreas-targeted diagnostic panel tools.

We are aware of some limitations of the present study. In the present surgical series, as in all other series, patients have been selected according to clinical and morphological criteria, and our study population does not represent all diagnosed IPMNs, with most of them being indolent.[39] Consequently, if *GNAS* status is a good diagnostic tool, it cannot be used at this moment to tailor management of patients without obvious criteria for surgery.

CONCLUSIONS

In patients selected for surgery, *GNAS* mutation analysis and tumor phenotype help to better predict patient prognosis. *GNAS* mutation status, tumor size, and IPMN location (branch vs. main/mixed type duct) are independent prognostic factors in resected patients. In the near future, with the diffusion of circulating cell-free DNA isolated from blood samples, a more precise mutational analysis of IPMNs might help to better tailor their management.

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