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PIK3CA, *KRAS*, and *BRAF* mutations in intraductal papillary mucinous neoplasm/carcinoma (IPMN/C) of the pancreas

Frank Schönleben,
Department of General Surgery, Friedrich-Alexander-University of Erlangen-Nuremberg, Krankenhausstrasse 12, 91054 Erlangen, Germany; Department of Otolaryngology/Head and Neck Surgery, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Wanglong Qiu,
Department of Otolaryngology/Head and Neck Surgery, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Helen E. Remotti,
Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Werner Hohenberger, and
Department of General Surgery, Friedrich-Alexander-University of Erlangen-Nuremberg, Krankenhausstrasse 12, 91054 Erlangen, Germany

Gloria H. Su
Department of Otolaryngology/Head and Neck Surgery, College of Physicians and Surgeons, Columbia University, New York, NY, USA; Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Frank Schönleben: frank.schoenleben@gmx.de

Abstract

Background and aims—Recent studies have reported high frequencies of somatic mutations in the phosphoinositide-3-kinase catalytic-α (*PIK3CA*) gene in various human tumors. Three hot-spot mutations in the exons 9 and 20 have been proven to activate the Akt signalling pathway. The Raf/MEK/ERK (mitogen-activated protein kinase) signal transduction is an important mediator of a number of cellular fates including growth, proliferation, and survival. The BRAF gene is activated by oncogenic RAS, leading to cooperative effects in cells responding to growth factor signals. Here we evaluate the mutational status of PIK3CA, KRAS, and BRAF in intraductal papillary mucinous neoplasm/carcinoma (IPMN/IPMNC) of the pancreas.

Materials and methods—Exons 1, 4, 5, 6, 7, 9, 12, 18, and 20 of *PIK3CA*, exons 1 of *KRAS*, and exons 5, 11, and 15 of *BRAF* were analyzed in 36 IPMN/IPMC and two mucinous cystadenoma specimens by direct genomic DNA sequencing.

Results—We identified four somatic missense mutations of PIK3CA within the 36 IPMN/IPMC specimens (11%). One of the four mutations, H1047R, has been previously reported to be a hot-spot mutation. Furthermore, we found 17 (47%) KRAS mutations in exon 1 and one missense mutation (2.7%) in exon 15 of BRAF.

Conclusion—This data is the first report of *PIK3CA* mutation in pancreatic cancer and it appears to be the first oncogene to be mutated in IPMN/IPMC but not in conventional ductal

Correspondence to: Frank Schönleben, frank.schoenleben@gmx.de.

adenocarcinoma of the pancreas. Our data provide evidence that *PIK3CA* and *BRAF* contribute to the tumorigenesis of IPMN/IPMC, but at a lower frequency than *KRAS*.

Keywords

IPMN; IPMC; Pancreas; *PIK3CA*; *KRAS*; *BRAF*; Mutation

Introduction

Intraductal papillary mucinous neoplasms (IPMN) are pancreatic exocrine lesions composed of dilated main or branch ducts lined by mucin producing atypical epithelium, which usually proliferates in a papillary fashion [1]. Based on their increasing architectural and nuclear atypia, IPMN are divided into three groups: intraductal papillary mucinous adenoma (IPMA), borderline IPMN (IPMB), and intraductal papillary mucinous carcinoma (IPMC) [1]. According to the absence or presence of neoplastic cells invading the pancreatic tissue surrounding the involved ducts, IPMC are separated into invasive and noninvasive types [2]. IPMN are precancerous lesions and disclose a progression pattern similar to the “adenoma–carcinoma sequence” in colorectal cancer [3–6]. Most IPMN are slow growing and less aggressive compared with conventional ductal adenocarcinoma. Borderline lesions and carcinoma are accompanied by less atypical lesions in the vicinity, and transition from adenoma to adenocarcinoma has been described. The overall incidence of invasive carcinoma associated with an IPMN is 20% to 40% [7] and invasiveness seems to be the strongest prognostic factor [4]. The prognosis of patients with noninvasive IPMN consisting of adenoma, adenocarcinoma in situ, or minimally invasive adenocarcinoma is excellent, and the 5-year survival rate was reported to be 77% to 100% [4, 8–10]. However, invasive IPMC that macroscopically involves the pancreatic parenchyma comprises 16% to 43% of all IPMN lesions, and the 5-year survival rate for patients with these lesions varied widely from 0% to 64% in several reported series [4, 8, 9, 11–13]. A significant proportion of the patients with completely resected noninvasive IPMN may develop pancreatic adenocarcinoma in the pancreatic remnant and die of disseminated disease. Other studies have also reported recurrences of invasive carcinoma in completely resected noninvasive IPMN [5, 6], some of which demonstrated only moderate dysplasia (borderline IPMN).

Phosphatidylinositol-3 kinases (PI3Ks) constitute a large and complex family of lipid kinases encompassing three classes with multiple subunits and isoforms [14–16]. They play an important role in several cellular functions, such as proliferation, differentiation, chemotaxis, survival, trafficking, and glucose homeostasis [14]. Class IA PI3Ks are heterodimeric proteins composed of a p110 catalytic subunit and a p85 regulatory subunit [17], which can be activated through interaction with phosphotyrosine residues of receptor tyrosine kinases [18, 19] or through the binding of active RAS to the p110 catalytic subunit [16, 19–21]. P85 lacks kinase activity and acts as an adaptor, coupling with the p110 subunit to activate protein tyrosine kinases [22]. Activated PI3Ks phosphorylate the inositol ring 3′-OH group in inositol phospholipids to generate the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP_3) [23], which in turn activates diverse cellular target proteins such as the survival signalling kinase Akt/protein kinase B [14, 15, 24]. A tumorigenic role has been proposed for the *PIK3CA* gene that encodes the catalytic p110α subunit of phosphatidylinositol 3-kinase belonging to the class IA of PI3Ks [14, 16]. Previously Samuels et al. reported mutations in *PIK3CA* in several tumor types, namely colorectal cancer, gastric cancer, glioblastoma, and breast and lung cancer [25]. Other independent studies in hepatocellular carcinomas, breast carcinomas, lung cancers, ovarian carcinomas, brain tumors, acute leukemias, and head and neck squamous cell carcinomas have since supported and emphasized the oncogenic potential of *PIK3CA* in the development of cancer [26–30]. In the study by Samuels et al. [25], three *PIK3CA*

mutational hot-spots were described and found to affect the helical (exon 9) and catalytic (exon 20) protein domains. Mutations were also described in exons 1, 2, 4, 7, 12, 14 and 18 of *PIK3CA*, but only in a minority of cases [25, 26]. Similar to colon tumors, *PIK3CA* mutations also clustered in the three hot-spot regions (exons 9 and 20) in gastric carcinomas [25, 27, 31]. No *PIK3CA* mutations have been previously reported in IPMN, IPMC, or conventional pancreatic ductal adenocarcinoma [25].

Since the discovery of the role of *RAS* oncogenes in tumorigenesis, an increasing focus has been set to define its oncogenic signal transduction pathway [32]. In trying to understand how Ras proteins transmit extracellular growth signals, the mitogen-activated protein (MAP) kinase pathway has emerged as an important link between membrane-bound Ras proteins and the nucleus [33, 34]. This key Ras effector pathway involves the kinase cascade Raf/MEK/ERK (*MEK*, MAPK/ERK kinase; *ERK*, extracellular signal-related kinase) [35]. Signalling through the MAPK cascade is transduced by guanosine triphosphate loading of Ras leading to the activation of Raf kinase. In mammalian cells, there are three isoforms of *RAF: ARAF*, *BRAF*, and *CRAF/RAF1*[36]. Although all three of the Raf isoforms share a common function with respect to MEK phosphorylation, studies have shown that these proteins might be differentially activated by oncogenic Ras [33, 37]. Recently activating *BRAF* mutations, in particular the V600E “hot-spot” mutation in *BRAF's* exon 15, have been described in about 15% of all human cancers, especially in malignant melanomas, papillary thyroid cancer as well as lung and ovarian cancer [38–43]. Reported genetic alterations in IPMN include mutations in the *KRAS* [44–49], *TP53* [47], and *STK11/LKB1* genes [50, 51] as well as loss of heterozygosity of several chromosomal loci [50, 52]. In addition to these genetic alterations, aberrant DNA methylation may contribute to the inactivation of a subset of tumor-suppressor genes in IPMN [53, 54]. Previous studies have found mutations in the exon 1 of *KRAS* in 31% to 86% of IPMNs [44–49]. Here, we analyzed the status of *PIK3CA*, *KRAS*, and *BRAF* to elucidate a possible role of these genes in the tumorigenesis of IPMN and IPMC.

Materials and methods

Patients and tissue samples

Surgical paraffin-embedded IPMN/IPMC and mucinous cystadenoma samples from 38 patients (female, $n = 14$; male, $n = 24$; median age, 68.1years; range, 41–84years) were obtained from the archival tissue collection of the Columbia University Medical Center. Acquisition of the tissue specimens was approved by the Institutional Review Board of Columbia University Medical Center and performed in accordance with Health Insurance Portability and Accountability Act regulations. In detail, we analyzed three IPMN, adenoma (female, $n = 1$; male, $n = 2$; median age, 62.7years; range, 53–77years), four IPMN, borderline (female, $n = 1$; male, $n = 3$; median age, 66.3years; range, 62–72years), five IPMC without invasion (male, $n = 5$; median age, 69.2years; range, 59–81years), 24 IPMC with invasive carcinoma (male, $n = 14$; female, $n = 10$; median age, 68.9years; range, 41–84years), and two mucinous cystadenomas (female, $n = 2$; median age, 57.5years; range, 53–62years). Thirty-two of these lesions arose in the pancreatic head, one in the uncinate process, four within the transition from pancreatic head to the body, and one within the body. The maximum diameter of the lesions ranged from 0.4 to 7cm (mean, 4.2cm) (for a more detailed register, see Table 1).

DNA samples for mutation analysis

All tissue and genomic DNA samples were handled in an environment free of polymerase chain reaction (PCR) products. All samples were coded, and the investigator was unaware of the patients' clinical data. Paraffin-embedded tumor samples were microdissected to ensure

the highest possible amount of tumor cells. Surrounding nontumorous tissue or tissue derived from a tumor-free block of each patient served as corresponding normal control. Genomic DNA was extracted using the QIAmp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions for paraffin-embedded tissues. Exons 1, 4, 5, 6, 7, 9, 12, 18, and 20 of *PIK3CA*, exon 1 of *KRAS*, and exons 5, 11, and 15 of *BRAF* were analyzed by PCR amplification of genomic DNA and direct sequencing. Genomic DNA (40ng per sample) was amplified with primers that had been designed to amplify each exon and its exon/intron boundaries of *PIK3CA* [25, 30] the codons 12 and 13 of *KRAS* or each of the three exons and its exon/intron boundaries in the *BRAF* locus [39, 40, 55]. All PCR products were purified (QIAquick PCR Purification Kit; Qiagen, Valencia, CA, USA) prior to sequencing. The PCR primers also served as the sequencing primers. Sequencing was performed with Applied Biosystems' 3100 capillary automated sequencer at the DNA Core Facility of Columbia University Medical Center. Each sample found to have a genetic alteration in the target gene was subsequently sequenced in the reverse direction to confirm the mutation and further verified by sequencing of a second PCR product derived independently from the original template.

Results

In our study, four of the 36 specimens (11%) contained a somatic mutation of the *PIK3CA* gene (Fig. 1 and Table 1) —one in exon 4 (T324I in an IPMC with invasion), one in exon 9 (W551G in an IPMB), and two in exon 20 (S1015F in an IPMC and H1047R in an IPMC with invasion)— whereas the samples showing the W551G and S1015F mutations were also harboring *KRAS* mutations. One of the missense mutations in exon 20 of *PIK3CA*, H1047R, is a previously described hot-spot mutation [25]. The other mutations in exons 4 and 9 are novel. We furthermore identified 17 (47%) mutations within the *KRAS* gene at codon 12 and one mutation (2.7%) in the exon 15 of *BRAF* (S616F in an IPMC with invasion) in the 36 IPMN/IPMC specimens. All mutations were sporadic, since none of the mutations was observed in the matching normal tissues (Fig. 1). Of the 17 samples that harbored *KRAS* mutations, five were IPMN cases without associated invasive carcinoma (5/12 samples, 41.7%) and 12 were IPMC with associated invasive carcinoma (12/24 samples, 50%). The *KRAS* codon 12 mutation was detected in IPMA (1/3), IPMB (2/4), IPMC without invasion (2/5), and IPMC with invasion (12/24) (Table 1). The spectrum of *KRAS* codon 12 mutations was G12D (7/17), G12V (6/17), and G12R (4/17). The exon 15 *BRAF* mutation was found in an IPMC sample with associated invasive carcinoma, which also harbored a *KRAS* (G12R) mutation (Table 1).

Discussion

Recently, much attention has been given to the significance of the *PIK3CA* gene mutations identified in several human tumors. Mutational analysis of the *PIK3CA* gene has revealed that genetic alterations at its locus occur in a wide spectrum of human neoplasms [25–30]. *PIK3CA* mutations preferentially occur in exons 9 and 20 (>75%), affecting the functionally important helical and kinase domains of the protein [25–27, 29, 31]. Functional studies have shown that PI3Ks carrying anyone of the three hot-spot mutations are able to induce transformation in cultures of chicken embryo fibroblasts and that the transforming activity of the mutant is correlated with increased lipid kinase activity and activation of the Akt signaling pathway [25, 56]. Although two of our mutations in exons 9 and 20 are not hot-spot mutations, the mutations are likely to have affected the kinase activity of the PIK3CA protein. The mutation within exon 4, nucleotide 971 C→T, which leads to an alteration of codon 324 ACA (T) → ATA (I), has not been described before. Although the significance of the novel mutation T324I, which belongs to the C2 domain, is unclear, a recent study found that the C2 domain of protein kinase C δ could be a phosphotyrosine-binding domain [57].

Since 7% of *PIK3CA* mutations have been detected within the C2 domain [25], it might be of value to study whether the C2 domain also plays a critical role in *PIK3CA* activity in the future. The frequency of *PIK3CA* mutations has been reported to be 32% in colon cancer, 4–25% in gastric cancer, 8–40% in breast cancer, 5–27% in brain tumors, 4% in lung cancer, and 4–7% in ovarian cancer [25, 28, 31, 58]. Samuels et al. screened 11 pancreatic ductal adenocarcinoma cell lines and found no mutation in the entire coding region of the *PIK3CA* gene [25]. A negative finding was also reported by Gallmeier et al. who examined the exons 9 and 20 of *PIK3CA* for mutation using direct genomic sequencing on the genomic DNA from 91 pancreatic cancer xenografts [59]. In the present study, we report 11% (4/36) of IPMN/IPMC to contain *PIK3CA* mutations. Two of these mutations (W551G and S1015F) were found in IPMN with nuclear grade 3 (IPMC) and nuclear grade 2 (IPMB), respectively, without associated invasive carcinoma. The other two (T324I and H1047R) were observed in IPMC with associated invasive carcinoma. The findings in colorectal cancers indicate that *PIK3CA* mutations generally arise just before or coincident with invasion [25]. Our data show that, in IPMN, mutations of the *PIK3CA* gene seem to be a rather late event on the transition of these lesions to malignancy.

Frequent *KRAS* gene mutations at codon 12 have been reported in several cancers, including those from colonic and pancreatic tissues [60–63]. Previous studies have found *KRAS* mutations, mainly at codon 12 in exon 1, in 31% to 86% of IPMN (47% in our study) [44–49]. The wide variety of the reported frequencies most likely is due to the ongoing better definition of these lesions [1, 64, 65] and might also be dependent on the sensitivity of a chosen screening methodology. In the present study, the distribution of *KRAS* mutation showed a single mutation in all observed cases. *KRAS* mutation is an early event in the tumorigenesis of IPMN—*KRAS* mutation was observed in IPMN; adenoma (1/3) and its mutation frequency remain consistent as IPMN progresses (2/4 in IPMN, borderline; 2/5 in IPMC; and 12/24 in IPMC with invasion). There was no tumor size, gender, or age bias observed associated with *KRAS* mutation. Unlike pancreatic ductal adenocarcinoma where *KRAS* is mutated at a frequency close to 100% [62, 63], 14–69% (53% in our study) of IPMN do not harbor an active *KRAS* mutation. This suggests that a relatively large percentage of IPMN/IPMC might use alternative ways other than *KRAS* mutation to stimulate this Ras-Raf-MEK-ERK-MAP kinase pathway. *BRAF*, a serine/threonin kinase located immediately downstream in Ras signalling, has been examined in a variety of human malignant neoplasms and found to be mutated frequently in malignant melanomas, thyroid cancer, and low-grade ovarian cancer and at lower frequencies in other cancer types [38–40, 42, 55, 66, 67]. Here we report a somatic *BRAF* mutation out of the 36 cases of IPMN/IPMC examined (2.7%). While *BRAF* contributes to the tumorigenesis of IPMN, it is not a frequent event and certainly does not entirely explain the lower mutation rate of *KRAS* in IPMN/IPMC than in pancreatic ductal adenocarcinoma. The *BRAF* mutation occurred at nucleotide 1850, a C to T change at codon 616 of the *BRAF* gene, leading to an amino acid change from serine to phenylalanine (S616F). Although located at exon 15, the S616F mutation is not the previously described hot-spot mutation at exon 15 (V600E) of the *BRAF* gene [38, 39, 55]. This mutation was also found to coexist with a G12R mutation of *KRAS* in the same sample. It has been observed previously in colon and lung cancers that *BRAF* mutations, other than *BRAF* V600E, coexisted with *RAS* mutations [39]. The *BRAF* V600E mutation seems to uncouple cells from their proliferation requirement of *RAS*, and therefore mutation of *RAS* was not detected in any of the tumors carrying *BRAF* V600E mutation [39]. In vitro data indicated that *BRAF* V600E mutants can be further activated by mutant *RAS*, whereas other *BRAF* mutants remain dependent on *RAS* function [39]. A previous study on pancreatic ductal adenocarcinoma revealed that the *BRAF* hot-spot mutation was observed in two of nine tumors retaining wild-type copies of the *KRAS*, *NRAS*, and *HRAS* genes, but none in 72 adenocarcinomas with *KRAS* mutations within exons 11 and 15 [68]. In contrast,

another study found both *KRAS* and *BRAF* V600E mutations coexisting in two cases of pancreatic ductal adenocarcinoma [69]. These two cases did not exhibit different clinicopathological characteristics from pancreatic cancers with *KRAS* mutation alone [69]. The novel S616F mutation observed here is also in the B-Raf activation segment [70], but its functional effect is unknown. Cells with activating mutations in both *KRAS* and *BRAF* had a substantially higher B-Raf kinase activity and ERK 1/2 phosphorylation activities than those with *BRAF* mutation alone [39]. It is possible that tumors with both *BRAF* and *KRAS* mutations have an accelerated course in the development or progression. Together, these observations suggest that different *BRAF* mutations can have distinct transforming potential in tumorigenesis, which would be worthy of further investigations in future studies.

So far, genetic analyses of IPMN have disclosed abnormalities in many of the same genes altered in conventional ductal adenocarcinoma, including mutations of *KRAS* [44], *TP53/p53* [71], and *CDKN2A/p16* genes [72]. In addition, as is true for pancreatic ductal carcinomas, a number of genes, including *CDKN2A/p16*, may be epigenetically inactivated in IPMN through aberrant DNA methylation [53, 54, 73, 74]. The Peutz-Jeghers gene *STK11/LKB1* is inactivated more frequently in IPMN (up to one third) than in ductal adenocarcinoma (4%) [51, 75], and some patients with the Peutz-Jeghers Syndrome develop IPMN [50]. In contrast to ductal adenocarcinomas and pancreatic intraepithelial neoplasia-3 lesions, abnormalities in the *MADH4/SMAD4/DPC4* gene seem to be rare in IPMN [76]. *PIK3CA* is the first gene to be found mutated in IPMN that had not been reported in ductal adenocarcinoma. Although the *BRAF* mutation frequency in IPMN/IPMC is low compared with those observed in malignant melanoma and colon cancers, our data suggest that alteration of the Ras-Raf-MEK-ERK-MAP kinase pathway by *BRAF* mutation together with *RAS* mutation may play an important role in the tumorigenesis of IPMN/IPMC.

Conclusion

In summary, this is the first report of *PIK3CA* mutation in pancreatic cancer and it appears to be the first oncogene to be mutated in IPMN/IPMC and not in conventional ductal adenocarcinoma of the pancreas. Our data provide evidence that oncogenic properties of *PIK3CA* and *BRAF* contribute to the tumorigenesis of IPMN/IPMC, but at a lower frequency than *KRAS*.

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References

1. Hruban RH, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Biankin AV, Biankin SA, Compton C, Fukushima N, Furukawa T. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol. 2004; 28(8):977–987. [PubMed: 15252303]
2. Kloppel, GSE.; Longnecker, DS. Histological typing of tumours of the exocrine pancreas. New York: 1996.
3. D'Angelica M, Brennan MF, Suriawinata AA, Klimstra D, Conlon KC. Intraductal papillary mucinous neoplasms of the pancreas: an analysis of clinicopathologic features and outcome. Ann Surg. 2004; 239(3):400–408. [PubMed: 15075659]
4. Sohn TA, Yeo CJ, Cameron JL, Hruban RH, Fukushima N, Campbell KA, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. Ann Surg. 2004; 239(6): 788–797. discussion 797–789. [PubMed: 15166958]

5. Adsay NV, Conlon KC, Zee SY, Brennan MF, Klimstra DS. Intraductal papillary-mucinous neoplasms of the pancreas: an analysis of in situ and invasive carcinomas in 28 patients. Cancer. 2002; 94(1):62–77. [PubMed: 11815961]
6. Salvia R, Fernandez-del Castillo C, Bassi C, Thayer SP, Falconi M, Mantovani W, Pederzoli P, Warshaw AL. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. Ann Surg. 2004; 239(5):678–685. discussion 685–677. [PubMed: 15082972]
7. Adsay NV, Merati K, Andea A, Sarkar F, Hruban RH, Wilentz RE, Goggins M, Iocobuzio-Donahue C, Longnecker DS, Klimstra DS. The dichotomy in the preinvasive neoplasia to invasive carcinoma sequence in the pancreas: differential expression of MUC1 and MUC2 supports the existence of two separate pathways of carcinogenesis. Mod Path. 2002; 15(10):1087–1095. [PubMed: 12379756]
8. Raimondo M, Tachibana I, Urrutia R, Burgart LJ, DiMagno EP. Invasive cancer and survival of intraductal papillary mucinous tumors of the pancreas. Am J Gastroenterol. 2002; 97(10):2553–2558. [PubMed: 12385438]
9. Maire F, Hammel P, Terris B, Paye F, Scoazec JY, Cellier C, Barthet M, O'Toole D, Rufat P, Partensky C. Prognosis of malignant intraductal papillary mucinous tumours of the pancreas after surgical resection. Comparison with pancreatic ductal adenocarcinoma. Gut. 2002; 51(5):717–722. [PubMed: 12377813]
10. Hermanova M, Lukas Z, Nenutil R, Brazdil J, Kroupova I, Kren L, Pazourkova M, Ruzicka M, Dite P. Amplification and overexpression of HER-2/neu in invasive ductal carcinomas of the pancreas and pancreatic intraepithelial neoplasms and the relationship to the expression of p21(WAF1/CIP1). Neoplasma. 2004; 51(2):77–83. [PubMed: 15190415]
11. Yamao K, Ohashi K, Nakamura T, Suzuki T, Shimizu Y, Nakamura Y, Horibe Y, Yanagisawa A, Nakao A, Nimuara Y. The prognosis of intraductal papillary mucinous tumors of the pancreas. Hepatogastroenterology. 2000; 47(34):1129–1134. [PubMed: 11020896]
12. Chari ST, Yadav D, Smyrk TC, DiMagno EP, Miller LJ, Raimondo M, Clain JE, Norton IA, Pearson RK, Petersen BT. Study of recurrence after surgical resection of intraductal papillary mucinous neoplasm of the pancreas. Gastroenterology. 2002; 123(5):1500–1507. [PubMed: 12404225]
13. Nakagohri T, Konishi M, Inoue K, Tanizawa Y, Kinoshita T. Invasive carcinoma derived from intraductal papillary mucinous carcinoma of the pancreas. Hepatogastroenterology. 2004; 51(59): 1480–1483. [PubMed: 15362782]
14. Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol. 2001; 17:615–675. [PubMed: 11687500]
15. Domin J, Waterfield MD. Using structure to define the function of phosphoinositide 3-kinase family members. FEBS Lett. 1997; 410(1):91–95. [PubMed: 9247130]
16. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer. 2002; 2(7):489–501. [PubMed: 12094235]
17. Carpenter CL, Duckworth BC, Auger KR, Cohen B, Schaffhausen BS, Cantley LC. Purification and characterization of phosphoinositide 3-kinase from rat liver. J Biol Chem. 1990; 265(32): 19704–19711. [PubMed: 2174051]
18. Wymann MP, Pirola L. Structure and function of phosphoinositide 3-kinases. Biochim Biophys Acta. 1998; 1436(1–2):127–150. [PubMed: 9838078]
19. Vanhaesebroeck B, Waterfield MD. Signaling by distinct classes of phosphoinositide 3-kinases. Exp Cell Res. 1999; 253(1):239–254. [PubMed: 10579926]
20. Kodaki T, Woscholski R, Hallberg B, Rodriguez-Viciana P, Downward J, Parker PJ. The activation of phosphatidylinositol 3-kinase by Ras. Curr Biol. 1994; 4(9):798–806. [PubMed: 7820549]
21. Rodriguez-Viciana P, Warne PH, Vanhaesebroeck B, Waterfield MD, Downward J. Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation. EMBO J. 1996; 15(10): 2442–2451. [PubMed: 8665852]

22. Hiles ID, Otsu M, Volinia S, Fry MJ, Gout I, Dhand R, Panayotou G, Ruiz-Larrea F, Thompson A, Totty NF. Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. Cell. 1992; 70(3):419–429. [PubMed: 1322797]
23. Whitman M, Downes CP, Keeler M, Keller T, Cantley L. Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. Nature. 1988; 332(6165): 644–646. [PubMed: 2833705]
24. Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. Biochem J. 2000; 346(Pt 3):561–576. [PubMed: 10698680]
25. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004; 304(5670):554. [PubMed: 15016963]
26. Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, Cristiano BE, Pearson RB, Phillips WA. Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res. 2004; 64(21):7678–7681. [PubMed: 15520168]
27. Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, Kim SH, Lee JY, Yoo NJ, Lee SH. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene. 2005; 24(8):1477–1480. [PubMed: 15608678]
28. Broderick DK, Di C, Parrett TJ, Samuels YR, Cummins JM, McLendon RE, Fults DW, Velculescu VE, Bigner DD, Yan H. Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. Cancer Res. 2004; 64(15):5048–5050. [PubMed: 15289301]
29. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, Konishi H, Karakas B, Blair BG, Lin C. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol Ther. 2004; 3(8):772–775. [PubMed: 15254419]
30. Qiu W, Schonleben F, Li X, Ho DJ, Close LG, Manolidis S, Bennett BP, Su GH. PIK3CA mutations in head and neck squamous cell carcinoma. Clin Cancer Res. 2006; 12(5):1441–1446. [PubMed: 16533766]
31. Li VS, Wong CW, Chan TL, Chan AS, Zhao W, Chu KM, So S, Chen X, Yuen ST, Leung SY. Mutations of PIK3CA in gastric adenocarcinoma. BMC Cancer. 2005; 5(1):29. [PubMed: 15784156]
32. Laud K, Kannengiesser C, Avril MF, Chompret A, Stoppa-Lyonnet D, Desjardins L, Eychene A, Demenais F, Lenoir GM, Bressac-de Paillerets B. BRAF as a melanoma susceptibility candidate gene? Cancer Res. 2003; 63(12):3061–3065. [PubMed: 12810628]
33. Peyssonnaux C, Eychene A. The Raf/MEK/ERK pathway: new concepts of activation. Biol Cell. 2001; 93(1–2):53–62. [PubMed: 11730323]
34. Dhillon AS, Meikle S, Peyssonnaux C, Grindlay J, Kaiser C, Steen H, Shaw PE, Mischak H, Eychene A, Kolch W. A Raf-1 mutant that dissociates MEK/extracellular signal-regulated kinase activation from malignant transformation and differentiation but not proliferation. Mol Cell Biol. 2003; 23(6):1983–1993. [PubMed: 12612072]
35. Aguirre-Ghiso JA, Estrada Y, Liu D, Ossowski L. ERK (MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). Cancer Res. 2003; 63(7):1684–1695. [PubMed: 12670923]
36. Rul W, Zugasti O, Roux P, Peyssonnaux C, Eychene A, Franke TF, Lenormand P, Fort P, Hibner U. Activation of ERK, controlled by Rac1 and Cdc42 via Akt, is required for anoikis. Ann N Y Acad Sci. 2002; 973:145–148. [PubMed: 12485852]
37. Smalley KS. A pivotal role for ERK in the oncogenic behaviour of malignant melanoma? Int J Cancer. 2003; 104(5):527–532. [PubMed: 12594806]
38. Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Gerrero R, Einhorn E, Herlyn M, Minna J, Nicholson A. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res. 2002; 62(23):6997–7000. [PubMed: 12460918]
39. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W. Mutations of the BRAF gene in human cancer. Nature. 2002; 417(6892):949–954. [PubMed: 12068308]
40. Naoki K, Chen TH, Richards WG, Sugarbaker DJ, Meyerson M. Missense mutations of the BRAF gene in human lung adenocarcinoma. Cancer Res. 2002; 62(23):7001–7003. [PubMed: 12460919]

41. Dong J, Phelps RG, Qiao R, Yao S, Benard O, Ronai Z, Aaronson SA. BRAF oncogenic mutations correlate with progression rather than initiation of human melanoma. Cancer Res. 2003; 63(14): 3883–3885. [PubMed: 12873977]
42. Singer G, Oldt R 3rd, Cohen Y, Wang BG, Sidransky D, Kurman RJ, Shih Ie M. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst. 2003; 95(6):484–486. [PubMed: 12644542]
43. Vasko V, Ferrand M, Di Cristofaro J, Carayon P, Henry JF, de Micco C. Specific pattern of RAS oncogene mutations in follicular thyroid tumors. J Clin Endocrinol Metab. 2003; 88(6):2745–2752. [PubMed: 12788883]
44. Z'Graggen K, Rivera JA, Compton CC, Pins M, Werner J, Fernandez-del Castillo C, Rattner DW, Lewandrowski KB, Rustgi AK, Warshaw AL. Prevalence of activating K-ras mutations in the evolutionary stages of neoplasia in intraductal papillary mucinous tumors of the pancreas. Ann Surg. 1997; 226(4):491–498. discussion 498–500. [PubMed: 9351717]
45. Satoh K, Shimosegawa T, Moriizumi S, Koizumi M, Toyota T. K-ras mutation and p53 protein accumulation in intraductal mucin-hypersecreting neoplasms of the pancreas. Pancreas. 1996; 12(4):362–368. [PubMed: 8740403]
46. Satoh K, Sawai T, Shimosegawa T, Koizumi M, Yamazaki T, Mochizuki F, Toyota T. The point mutation of c-Ki-ras at codon 12 in carcinoma of the pancreatic head region and in intraductal mucin-hypersecreting neoplasm of the pancreas. Int J Pancreatol. 1993; 14(2):135–143. [PubMed: 8283077]
47. Sessa F, Solcia E, Capella C, Bonato M, Scarpa A, Zamboni G, Pellegata NS, Ranzani GN, Rickaert F, Kloppel G. Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms: an investigation of tumour cell differentiation and K-ras, p53 and c-erbB-2 abnormalities in 26 patients. Virchows Arch. 1994; 425(4):357–367. [PubMed: 7820300]
48. Tada M, Omata M, Ohto M. Ras gene mutations in intraductal papillary neoplasms of the pancreas. Analysis in five cases. Cancer. 1991; 67(3):634–637. [PubMed: 1845953]
49. Yanagisawa A, Kato Y, Ohtake K, Kitagawa T, Ohashi K, Hori M, Takagi K, Sugano H. c-Ki-ras point mutations in ductectatic-type mucinous cystic neoplasms of the pancreas. Jpn J Cancer Res. 1991; 82(10):1057–1060. [PubMed: 1955373]
50. Sato N, Rosty C, Jansen M, Fukushima N, Ueki T, Yeo CJ, Cameron JL, Iacobuzio-Donahue CA, Hruban RH, Goggins M. STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas. Am J Pathol. 2001; 159(6):2017–2022. [PubMed: 11733352]
51. Sahin F, Maitra A, Argani P, Sato N, Maehara N, Montgomery E, Goggins M, Hruban RH, Su GH. Loss of Stk11/Lkb1 expression in pancreatic and biliary neoplasms. Mod Path. 2003; 16(7):686–691. [PubMed: 12861065]
52. Fujii H, Inagaki M, Kasai S, Miyokawa N, Tokusashi Y, Gabrielson E, Hruban RH. Genetic progression and heterogeneity in intraductal papillary-mucinous neoplasms of the pancreas. Am J Pathol. 1997; 151(5):1447–1454. [PubMed: 9358771]
53. Sato N, Ueki T, Fukushima N, Iacobuzio-Donahue CA, Yeo CJ, Cameron JL, Hruban RH, Goggins M. Aberrant methylation of CpG islands in intraductal papillary mucinous neoplasms of the pancreas. Gastroenterology. 2002; 123(1):365–372. [PubMed: 12105864]
54. House MG, Guo M, Iacobuzio-Donahue C, Herman JG. Molecular progression of promoter methylation in intraductal papillary mucinous neoplasms (IPMN) of the pancreas. Carcinogenesis. 2003; 24(2):193–198. [PubMed: 12584167]
55. Yuen ST, Davies H, Chan TL, Ho JW, Bignell GR, Cox C, Stephens P, Edkins S, Tsui WW, Chan AS. Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia. Cancer Res. 2002; 62(22):6451–6455. [PubMed: 12438234]
56. Kang S, Bader AG, Vogt PK. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. Proc Natl Acad Sci USA. 2005; 102(3):802–807. [PubMed: 15647370]
57. Benes CH, Wu N, Elia AE, Dharia T, Cantley LC, Soltoff SP. The C2 domain of PKCdelta is a phosphotyrosine binding domain. Cell. 2005; 121(2):271–280. [PubMed: 15851033]
58. Wang Y, Helland A, Holm R, Kristensen GB, Borresen-Dale AL. PIK3CA mutations in advanced ovarian carcinomas. Hum Mutat. 2005; 25(3):322. [PubMed: 15712344]

59. Gallmeier E, Calhoun ES, Kern SE. No mutations in PIK3CA identified in pancreatic carcinoma. NOGO. 2004; 8:2.
60. Andreyev HJ, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, Young J, Walsh T, Ward R, Hawkins N. Kirsten ras mutations in patients with colorectal cancer: the "RASCAL II" study. Br J Cancer. 2001; 85(5):692–696. [PubMed: 11531254]
61. Motojima K, Urano T, Nagata Y, Shiku H, Tsurifune T, Kanematsu T. Detection of point mutations in the Kirsten-ras oncogene provides evidence for the multicentricity of pancreatic carcinoma. Ann Surg. 1993; 217(2):138–143. [PubMed: 8439212]
62. Moskaluk CA, Hruban RH, Kern SE. p16 and K-ras mutations in the intraductal precursors of human pancreatic adenocarcinoma. Cancer Res. 1997; 57:2140–2143. [PubMed: 9187111]
63. Rozenblum E, Schutte M, Goggins M, Hahn SA, Lu J, Panzer S, Zahurak M, Goodman SN, Hruban RH, Yeo CJ. Tumor-suppressive pathways in pancreatic carcinoma. Cancer Res. 1997; 57:1731–1734. [PubMed: 9135016]
64. Longnecker DS, Adsay NV, Fernandez-del Castillo C, Hruban RH, Kasugai T, Klimstra DS, Kloppel G, Luttges J, Memoli VA, Tosteson TD. Histopathological diagnosis of pancreatic intraepithelial neoplasia and intraductal papillary-mucinous neoplasms: interobserver agreement. Pancreas. 2005; 31(4):344–349. [PubMed: 16258368]
65. Furukawa T, Kloppel G, Volkan Adsay N, Albores-Saavedra J, Fukushima N, Horii A, Hruban RH, Kato Y, Klimstra DS, Longnecker DS. Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study Virchows Arch. 2005; 447(5):794–799.
66. Sommerer F, Hengge UR, Markwarth A, Vomschloss S, Stolzenburg JU, Wittekind C, Tannapfel A. Mutations of BRAF and RAS are rare events in germ cell tumours. Int J Cancer. 2005; 113(2): 329–335. [PubMed: 15386408]
67. Weber A, Langhanki L, Sommerer F, Markwarth A, Wittekind C, Tannapfel A. Mutations of the BRAF gene in squamous cell carcinoma of the head and neck. Oncogene. 2003; 22(30):4757–4759. [PubMed: 12879021]
68. Calhoun ES, Jones JB, Ashfaq R, Adsay V, Baker SJ, Valentine V, Hempen PM, Hilgers W, Yeo CJ, Hruban RH. BRAF and FBXW7 (CDC4, FBW7, AGO, SEL10) mutations in distinct subsets of pancreatic cancer: potential therapeutic targets. Am J Pathol. 2003; 163(4):1255–1260. [PubMed: 14507635]
69. Ishimura N, Yamasawa K, Karim Rumi MA, Kadowaki Y, Ishihara S, Amano Y, Nio Y, Higami T, Kinoshita Y. BRAF and K-ras gene mutations in human pancreatic cancers. Cancer Lett. 2003; 199(2):169–173. [PubMed: 12969789]
70. Zhang BH, Guan KL. Activation of B-Raf kinase requires phosphorylation of the conserved residues Thr598 and Ser601. EMBO J. 2000; 19(20):5429–5439. [PubMed: 11032810]
71. Sasaki S, Yamamoto H, Kaneto H, Ozeki I, Adachi Y, Takagi H, Matsumoto T, Itoh H, Nagakawa T, Miyakawa H. Differential roles of alterations of p53, p16, and SMAD4 expression in the progression of intraductal papillary-mucinous tumors of the pancreas. Oncol Rep. 2003; 10(1):21–25. [PubMed: 12469138]
72. Biankin AV, Biankin SA, Kench JG, Morey AL, Lee CS, Head DR, Eckstein RP, Hugh TB, Henshall SM, Sutherland RL. Aberrant p16(INK4A) and DPC4/Smad4 expression in intraductal papillary mucinous tumours of the pancreas is associated with invasive ductal adenocarcinoma. Gut. 2002; 50(6):861–868. [PubMed: 12010891]
73. Fukushima N, Sato N, Sahin F, Su GH, Hruban RH, Goggins M. Aberrant methylation of suppressor of cytokine signalling-1 (SOCS-1) gene in pancreatic ductal neoplasms. Br J Cancer. 2003; 89(2):338–343. [PubMed: 12865927]
74. Matsubayashi H, Sato N, Fukushima N, Yeo CJ, Walter KM, Brune K, Sahin F, Hruban RH, Goggins M. Methylation of cyclin D2 is observed frequently in pancreatic cancer but is also an age-related phenomenon in gastrointestinal tissues. Clin Cancer Res. 2003; 9(4):1446–1452. [PubMed: 12684418]
75. Su GH, Hruban RH, Bansal RK, Bova GS, Tang DJ, Shekher MC, Westerman AM, Entius MM, Goggins M, Yeo CJ. Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. Am J Pathol. 1999; 154(6):1835–1840. [PubMed: 10362809]

76. Iacobuzio-Donahue CA, Klimstra DS, Adsay NV, Wilentz RE, Argani P, Sohn TA, Yeo CJ, Cameron JL, Kern SE, Hruban RH. Dpc-4 protein is expressed in virtually all human intraductal papillary mucinous neoplasms of the pancreas: comparison with conventional ductal adenocarcinomas. Am J Pathol. 2000; 157(3):755–761. [PubMed: 10980115]

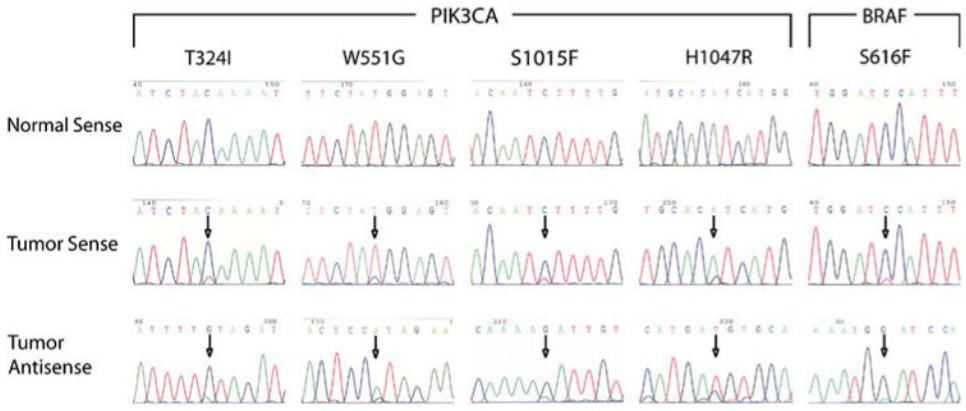

Fig. 1.

PIK3CA and *BRAF* mutations found in IPMN/IPMC. One of the four *PIK3CA* mutations (H1047R) was a hot-spot mutation. The other mutations were novel. All mutations were confirmed to be somatic

Table 1

Summary of the clinical data and mutation status of the lesion investigated

Case no.	Sex	Age	Lesion analyzed	IPMN nuclear grade	Differentiation of invasive carcinoma	Location within pancreas	Maximum dimension[a] (cm)	PIK3CA mutation	KRAS mutation	BRAF mutation
1	M	62	IPMB	2	N/A	Body	–	W551G	G12D	
2	M	73	IPMC/inv	3	Moderate	Head/body	4		G12D	
3	M	67	IPMC/inv	3	Moderate	Head	–		G12V	
4	M	69	IPMC	3	N/A	Head	3.5	S1015F	G12V	
5	F	75	IPMC/inv	3	Moderate	Head	5.5	T324I		
6	F	68	IPMC/inv	3	Moderate	Head	5		G12V	
7	M	65	IPMB	2	Poor[b]	Head	5			
8	F	66	IPMB	2	N/A	Uncinate process	2		G12V	
9	M	84	IPMC/inv	3	Moderate	Head	5			
10	M	53	IPMA	1	N/A	Head	3.5		G12V	
11	M	71	IPMC/inv	2–3	N/A	Head	–			
12	M	81	IPMC	3	N/A	Head	2.5			
13	M	63	IPMC	3	Moderate/poor[b]	Head	2.3			
14	M	66	IPMC/inv	3	Moderate/poor	Head	6	H1047R		
15	F	70	IPMC/inv	3	Moderate/poor	Head/body	7		G12R	S616F
16	F	70	IPMC/inv	3	Moderate	Head	1.5			
17	M	72	IPMB	2	N/A	Head	0.4			
18	F	53	mucinous cystadenoma	1	N/A	Head	3			
19	M	79	IPMC/inv	3	Moderate/poor	Head	6		G12R	
20	M	63	IPMC/inv	3	Moderate/poor	Head	3.5		G12R	
21	M	77	IPMA	1	N/A	Head/body	2.2			
22	F	62	mucinous cystadenoma	1	N/A	Head	2			
23	M	41	IPMC/inv	3	Moderate/poor	Head	5			
24	M	71	IPMC/inv	3	Moderate/poor	Head	1.5			
25	F	58	IPMA	1	N/A	Head	1.5			
26	M	49	IPMC/inv	3	Moderate	Head	4.5			
27	M	71	IPMC/inv	3	Moderate/poor	Head	5.5		G12D	
28	M	74	IPMC	3	Well[b]	Head/body	–			
29	M	59	IPMC	3	Poor[b]	Head	7		G12V	
30	M	81	IPMC/inv	3	Moderate/poor	Head	3			

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Case no.	Sex	Age	Lesion analyzed	IPMN nuclear grade	Differentiation of invasive carcinoma	Location within pancreas	Maximum dimension[a] (cm)	PIK3CA mutation	KRAS mutation	BRAF mutation
31	F	80	IPMC/inv	3	Moderate/poor	Head	5		G12R	
32	F	66	IPMC/inv	3	Poor	Head	3			
33	F	77	IPMC/inv	3	Poor	Head	3			
34	M	73	IPMC/inv	3	Poor	Head	5.5		G12D	
35	F	77	IPMC/inv	3	Well	Head	3.2		G12D	
36	F	61	IPMC/inv	3	Well	Head	1		G12D	
37	M	62	IPMC/inv	3	Moderate	Head	2.2			
38	F	59	IPMC/inv	3	Moderate	Head	3.4		G12D	

IPMA IPMN adenoma, *IPMB* IPMN borderline, *IPMC/inv* IPMC with invasion, *N/A* not applicable, – not available

[a] Maximum tumor size includes both invasive and noninvasive components of tumor.

[b] Invasive carcinoma was associated with IPMN/IPMC in pancreatic resection, but the lesion analyzed did not sample invasive carcinoma.