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PIK3CA Mutations in Mucinous Cystic Neoplasms of the Pancreas

Dario Garcia-Carracedo, PhD[*], **Zong-Ming Chen, MD, PhD**[†], **Wanglong Qiu, MD, PhD**[*], **Alicia S. Huang, BS**[‡], **Sophia M. Tang, BS**[‡], **Ralph H. Hruban, Ralph H.**[†], and **Gloria H. Su, PhD**[*,§]

[*]Department of Pathology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY

[†]Department of Pathology, The Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins Medical Institutions, Baltimore, MD

[‡]Department of Otolaryngology/Head and Neck Surgery, Columbia University Medical Center

[§]Department of Otolaryngology/Head and Neck Surgery, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY

Abstract

Objectives—Mucinous cystic neoplasms (MCNs) are rare, potentially curable, mucin-producing neoplasms of the pancreas. We have previously reported *PIK3CA* (phosphoinositide-3-kinase catalytic subunit, p110α) mutations in intraductal papillary mucinous neoplasms, another mucin-producing neoplasm of the pancreas. In this study, we analyzed the presence of *PIK3CA* and *AKT1/PKB* (V-akt murine thymoma viral oncogene homolog 1) hot-spot mutations in MCN specimens.

Methods—Using the genomic DNA sequencing of tumor tissues isolated by laser capture microdissection, we evaluated 15 well-characterized MCNs for the E542K, E545K(exon 9), and H1047R (exon 20) hot-spotmutations in the *PIK3CA* gene and the E17K mutation in the *AKT1* gene.

Results—A hot-spotmutation (E545K) of the *PIK3CA* gene was detected in 1 of the 15 MCNs and further confirmed by a mutant-enriched method. Interestingly, this mutation was found to be present only in the high-grade but not in low-grade dysplastic epithelium obtained from this neoplasm and coexisted with a $KRAS^{G12D}$ mutation. No mutations were identified in the *AKT1* gene.

Conclusions—Our data, when combined with previous reports on intraductal papillary mucinous neoplasms, indicate that oncogenic activation of the PI3K pathway involving *PIK3CA* gene mutations can contribute to the progression of mucin-producing neoplasms but not pancreatic

Reprints: Gloria H. Su, PhD, Department of Pathology, Columbia University Medical Center, 1130 St Nicholas Ave, Irving Cancer Research Center 1004, New York, NY 10032 (gs2157@columbia.edu).

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intraepithelial neoplasia. *PIK3CA* status could be useful for understanding their progression to malignancy.

Keywords

PIK3CA; *AKT1*; mucinous cystic neoplasm; oncogene; somatic mutations

Mucinous cystic neoplasms (MCNs) of the pancreas are rare cystic tumors, characterized by mucin-producing cuboidal to columnar cells surrounded by ovarian-like stroma.[1] The presence of this ovarian-like stroma is a defining feature that distinguishes MCNs from the more common intraductal papillary mucinous neoplasm (IPMN). Mucinous cystic neoplasms constitute approximately 2% of all pancreatic tumors and have distinctive clinicopathologic characteristics; they occur almost exclusively in perimenopausal women and almost always arise in the body and the tail of the pancreas. The cysts of MCNs do not communicate with the pancreatic ductal system. The lining epithelium is mucin producing, and these lesions resemble mucinous cystic tumors encountered in the ovary and liver. Noninvasive MCNs are classified as having low-grade dysplasia, intermediate-grade dysplasia, and high-grade dysplasia based on the degree of cytologic and architectural atypia.[2] Although noninvasive MCNs are curable if completely resected, MCNs can be a precursor to an invasive carcinoma, and up to one-third of MCNs are associated with an invasive adenocarcinoma.[3] Because of their grossly cystic nature and mucinous cyst content, early detection is now facilitated by improved radiologic techniques and cyst fluid examination, including cytology. The increased use of imaging has led to an increased detection of MCNs and thus to a remarkable increase in the exposure of pathologists to these lesions and the possibility of studying possible genetic alterations.

Little is known about the molecular mechanisms underlying progression of this neoplasm.[4,5] In fact, only 1 cell line derived from pancreatic MCNs has been established to date.[6] Reported molecular and genetic alterations in MCN of the pancreas include mutations in *KRAS* (*V-Ki-ras2*, Kirsten rat sarcoma viral oncogene homolog) oncogene[7] and *DPC4/SMAD4* (deleted in pancreatic cancer locus 4 protein) gene,[8,9] promoter hypermethylation of *p16^{ink4a}/CDKN2A* (inhibitor of cyclin-dependent kinase 4/cyclin-dependent kinase inhibitor 2A), and aberrant p53 protein expression.[10,11]

The PI3K pathway is genetically deregulated in human cancers at various levels. The tumor suppressor *PTEN* (phosphatase and tensin homolog), which dephosphorylates PIP_3 (phosphatidylinositol (3,4,5)-triphosphate) to PIP_2 (phosphatidylinositol 4,5-bisphosphate), thus antagonizing PI3K activity, is commonly mutated in prostate cancer, endometrial cancer, and glioblastoma among others.[12,13] The amplification of genomic regions containing *AKT1/PKB* (V-akt murine thymoma viral oncogene homolog 1) or *PIK3CA* (phosphoinositide-3-kinase catalytic subunit, p110α) genes has also been reported.[14–16] Recent studies have reported high frequencies of somatic mutations in the *PIK3CA* gene in several cancer types, including colorectal, gastric, thyroid, breast, ovary, certain brain tumors, and head and neck squamous cell carcinoma.[17–21] In the study by Samuels et al,[22] 75% of the mutations found in the *PIK3CA* gene were clustered within the helical (exon 9) and catalytic (exon 20) protein domains. Three hot-spot mutations, E542K, E545K (exon 9),

and H1047R (exon 20), were identified. Moreover, we have previously reported somatic *PIK3CA* gene mutations in 4 (11%) of 36 IPMNs.[23] The hot-spot mutations detected in the *PIK3CA* gene have been shown to elevate the PI3K oncogenic activity via PI3K signaling pathway, providing transforming properties in vitro and in vivo.[24–26] Mutations have also been described in exons 1, 2, 4, 7, 12, 14, and 18 of the *PIK3CA* gene but only in a minority of cases.[22,27] Similar to colon tumors, *PIK3CA* gene mutations are also clustered in exons 9 and 20 in gastric carcinomas.[22,28,29]

Rare or absent activating somatic mutations in the *AKT1* gene have also been recently described. The E17K mutation in the pelckstrin homology domain of the *AKT1* gene can result in PI3K-independent membrane recruitment of *AKT1*, mimicking the effects of the *AKT8* murine leukemia retrovirus GAG-AKT fusion protein. The E17K-*AKT1* exhibits transforming activity in vitro and in vivo, although at lower level than the myristoylated Akt.[30,31] The E17K mutation can lead to a constitutive activation of Akt, turning it into a potential therapeutic target. Thus far, the mutational status of the oncogene *AKT1* has not been evaluated in MCNs.

In this study, we evaluated 15 MCNs for hot-spot mutations in the *AKT1* and *PIK3CA* genes.

MATERIALS AND METHODS

Patients and Tissue Samples

Fifteen surgically resected formalin-fixed and paraffin-embedded MCNs were obtained from the archival tissue collection of the Johns Hopkins Hospital. The acquisition of the tissue specimens was approved by the institutional review board and performed in accordance with Health Insurance Portability and Accountability Act regulations. In detail, these MCNs came from 15 women with ages ranging from 25 to 71 years (median age, 54.0 years). Mucinous cystic neoplasm of the pancreas was diagnosed in all patients using standard criteria.[2] The specimens all contained the characteristic ovarian-type stroma, and the cysts did not communicate with the pancreatic duct system. Seven MCNs had low-grade dysplasia and 8 MCNs presented high-grade dysplasia. Among the 8 patients with high-grade dysplasia, 1 patient had an associated invasive adenocarcinoma, 1 patient had a microscopic focus invasive adenocarcinoma, and 2 patients had multifocal invasive adenocarcinomas. None of the patients recurred after follow-up, and only 1 patient developed breast carcinoma after 2 years of follow-up. For more detailed information see Table 1.

Preparation of DNA Extracts

For the study of *PIK3CA* and *AKT1* gene mutations, laser capture microdissection (LCM) was performed on the 15 MCN cases to ensure the highest possible concentration of neoplastic cells. Five to ten 5-μm serial sections were microdissected for each case. Paraffin-embedded tumor samples were deparaffinized by incubating the slides in xylene for 2 minutes and rehydrating in 99.9% ethanol for 2 × 10 minutes, in 96% ethanol for 2 × 10 minutes, and in 70% ethanol for 2 × 10 minutes. Slides were stained with hematoxylin and eosin (H&E). The regions containing the MCN neoplastic cell populations were microscopically defined and labeled by the pathologists on our team (Z.C. and R.H.H.). For

those cases in which high- and low-grade dysplasia were identified in the H&E slides (n = 3), we microdissected these areas separately. Microdissection was carried out using a LCM microscope (P.A.L.M., Bernried, Germany). Approximately between 10,000 and 14,000 cells were collected into 50 μL of ATL buffer, (Animal Tissue Lysis buffer from QIAamp DNA Mini Kit; QIAGEN, Valencia, CA). The surrounding non–neoplastic tissue served as a corresponding normal control for each sample. Cell lysis and DNA extraction were performed according to manufacturer's instructions.

Mutational Analysis of *PIK3CA* Gene

Mutations in exons 9 and 20 of the *PIK3CA* gene were analyzed by direct genomic sequencing methods and confirmed by mutant-enriched sequencing method.[21] Polymerase chain reaction (PCR) amplification of genomic DNA (40 ng each) and direct sequencing of the PCR products were performed using the same primers and conditions as previously described.[21] Briefly, the primers were designed to allow an efficient amplification of genomic DNA from paraffin-embedded tissues and to avoid the interference of a homologous pseudogene located on chromosome 22q11.2 in the eye cat syndrome region (in the case of exon 9).[32]. All PCR fragments were purified using ExoSAP-IT kit (USB Scientific, Cleveland, OH) by GENEWIZ (GENEWIZ Inc, South Plainfield, NJ) technical staff, and sequencing was performed with ABI Prism 3730xl DNA analyzers (Applied Biosystems, Foster City, CA) using the PCR primers.[21] Any alteration detected was further verified by sequencing of a second PCR product derived independently from the original template.

Mutational Analysis of *AKT1* Gene

The point mutation G > A at nucleotide 49 of the *AKT1* gene was analyzed by direct genomic sequencing. Genomic DNA was amplified with primers designed to amplify exclusively the hot spot (*AKT1*-F1 5′-ACATCTGTCCTGGCACAC-3′: *AKT1*-R1 5′-GCCAGTGCTTGTTGCTTG-3′[30]); All PCR fragments were purified using PureLink PCR purification kit (Invitrogen Life Technologies, Carlsbad, CA), and sequencing was carried out with ABI 3730xl DNA analyzers by GENEWIZ technical staff.

RESULTS

Mutational Analysis

Mucinous cystic neoplasms were screened for hot-spot point mutations in the *AKT1* and *PIK3CA* genes using LCM to enrich for neoplastic cellularity and direct genomic sequencing. No mutations were identified in the *AKT1* gene. One of the 15 cases was found to harbor an E545K (G1633A) mutation in the *PIK3CA* gene (Fig. 1). Interestingly, this mutation was found in the area of high-grade dysplasia but was not present in an area of low-grade dysplasia in the same MCN. Direct reverse sequencing and mutant-enriched sequencing method of the *PIK3CA* gene confirmed the E545K (G1633A) mutation identified (Fig. 1E–F). The mutation is somatic because it was not observed in the normal tissues from the patient.

DISCUSSION

In this study, we evaluated a series of 15 well-characterized MCNs for hot-spot mutations in the *PIK3CA* and *AKT1* genes. We found a somatic E545K mutation in the *PIK3CA* gene in 1 (6.6%) of the 15 MCNs. This *PIK3CA* gene mutation was found in an area of high-grade dysplasia (Fig. 1). The E545K mutation was in exon 9 of the gene and alters the helical domain of the p110α subunit of the protein.[17] This mutation has been shown to confer an increased lipid kinase activity, leading to the activation of PI3K signaling pathway in the absence of growth factors[25,26] and to induce oncogenic cell transformation of chicken embryo fibroblasts and NIH 3T3 cells.[24,33] It has been described that p110α mutants induce in vivo angiogenesis and malignant cell growth in chorioallantoic membrane of chicken embryo and cause hemangiosarcomas in young chickens.[24]

The frequency of *PIK3CA* gene mutations has been reported to be 32%in colon cancer, 8%to 40%in breast cancer, 4%to 25% in gastric, 5% to 27% in some brain tumors, 4% to 7% in ovarian cancer, and 4% in lung cancer.[22,29,34,35] None has been reported for conventional pancreatic ductal adenocarcinoma (PDA) to date. Pancreatic ductal adenocarcinoma arises from precursor lesions called *pancreatic intraepithelial neoplasia* (PanIN), which is distinct from IPMN and MCN. Negative findings in pancreatic cancer cell lines have been reported in exons 9 and 20 of the *PIK3CA* gene.[22] More recently, Jones et al[36] performed a comprehensive genetic analysis of 24 PDA specimens and did not identify the PI3K signaling pathway as 1 of the 12 core signaling pathways altered at genomic level in PDA. Although it remains possible that the PI3K signaling pathway can be dysregulated epigenetically in PDA indirectly through 1 or more of the 12 core pathways altered in PDA,[36] genetic alteration is not the major molecular mechanism regulating the PI3K pathway in PDA. We have previously reported *PIK3CA* gene mutations in 11% of IPMNs of the pancreas, suggesting that the *PIK3CA* gene and its pathway may have a role in IPMN but not in PanIN/PDA tumorigenesis.[23] In our previous study, *PIK3CA* gene mutations were detected only in IPMN with high-grade dysplasia, with the caveat that most of the cases examined had high-grade dysplasia.[23] In contrast to our results, Kuboki et al[37] reported absence of *PIK3CA* hot-spot mutations in 33 manually dissected formalin-fixed and paraffin-embedded MCNs. The negative result might have been due to the high content of ovarian-type stroma supporting the mucinous epithelium in this type of lesion along with the relatively low sensitivity of the assay. The detection of these mutations in MCN specimens might require the use of LCM and/or mutant-enriched techniques.

The fact that *PIK3CA* gene mutations are present in IPMN and MCN and not in PanIN/PDA also suggests that although all of these neoplasms share a good deal of molecular alterations, pancreatic carcinoma associated with MCN and IPMN may arise through a different molecular pathway from PanIN/PDA. The investigation of activating point mutations in *PIK3CA*, *AKT*, and other cell signaling pathways in pancreatic carcinomas associated with MCN or IPMN might be instructive to characterize the divergent molecular and histologic pathways of pancreatic cancer evolution.

There are a number of limitations in the present study. Because of the rarity of these tumors, the number of samples analyzed here is limited; in addition, we were able to microdissect

low- and high-grade dysplasia separately in just 3 of the 8 cases analyzed with high-grade dysplasia. However, it is remarkable that we identified the *PIK3CA* gene mutation in an area of high-grade dysplasia but not in an area of low-grade dysplasia from the same MCN, suggesting that mutations of the *PIK3CA* gene could be a rather late event in the transition to malignancy. This case also carried a $KRAS^{G12D}$ mutation in the area of high-grade dysplasia (Table 1, Supplemental Digital Content 1, http://links.lww.com/MPA/A255 and Supplementary Material 1, Supplemental Digital Content 2, http://links.lww.com/MPA/A256). In our set of samples, *KRAS* mutation was frequently detected in the MCN cases with high-grade dysplasia (5/7 patients) but was not found in those with no dysplasia. This might reflect the accumulation of genetic alterations because MCN progress from low- to high-grade dysplasia.[38] The coexistence of *PIK3CA* and *KRAS* mutations has been found to be a frequent event in advanced cancers,[39] and it might have clinical implications because simultaneous activation of PI3K and KRAS pathways can be associated with resistance to PI3K/AKT/ mTOR inhibitors.[40,41] Notably, we have also previously reported coexisting *PIK3CA* and *KRAS* mutations in IPMN samples.[42] This finding supports the concept that the progression from MCN with low-grade dysplasia to MCN associated with invasive carcinoma is linked to the progressive accumulation of genetic alterations in cancer-related genes, including *KRAS*, *TP53* (tumor protein p53), *p16*, and *SMAD4/DPC4*.[8,38] Furthermore, the probable late acquisition of the *PIK3CA* mutation in tumorigenesis suggests that the *PIK3CA* gene and/or its pathway could play a role in tumor progression and be a drug target for therapies. The feasibilities of these potentials would be worthy of further explorations to show if the presence of PI3K alterations are prevalent in more advanced types of MCN lesions. To summarize, this is the first report providing evidence for the existence of somatic mutations of the *PIK3CA* gene in MCN despite the limitations and albeit the low frequency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Galanis C, Zamani A, Cameron JL, et al. Resected serous cystic neoplasms of the pancreas: a review of 158 patients with recommendations for treatment. J Gastrointest Surg. 2007; 11:820–826. [PubMed: 17440789]
2. Hruban RH, Takaori K, Canto M, et al. Clinical importance of precursor lesions in the pancreas. J Hepatobiliary Pancreat Surg. 2007; 14:255–263. [PubMed: 17520200]
3. Adsay NV. Cystic neoplasia of the pancreas: pathology and biology. J Gastrointest Surg. 2008; 12:401–404. [PubMed: 17957438]
4. Wilentz RE, Albores-Saavedra J, Hruban RH. Mucinous cystic neoplasms of the pancreas. Semin Diagn Pathol. 2000; 17:31–42. [PubMed: 10721805]
5. Sarr MG, Carpenter HA, Prabhakar LP, et al. Clinical and pathologic correlation of 84 mucinous cystic neoplasms of the pancreas: can one reliably differentiate benign from malignant (or premalignant) neoplasms? Ann Surg. 2000; 231:205–212. [PubMed: 10674612]

6. Sorio C, Capelli P, Lissandrini D, et al. Mucinous cystic carcinoma of the pancreas: a unique cell line and xenograft model of a preinvasive lesion. Virchows Arch. 2005; 446:239–245. [PubMed: 15688169]
7. Bartsch D, Bastian D, Barth P, et al. K-*ras* oncogene mutations indicate malignancy in cystic tumors of the pancreas. Ann Surg. 1998; 228:79–86. [PubMed: 9671070]
8. Iacobuzio-Donahue CA, Wilentz RE, Argani P, et al. Dpc4 protein in mucinous cystic neoplasms of the pancreas: frequent loss of expression in invasive carcinomas suggests a role in genetic progression. Am J Surg Pathol. 2000; 24:1544–1548. [PubMed: 11075857]
9. Gerdes B, Wild A, Wittenberg J, et al. Tumor-suppressing pathways in cystic pancreatic tumors. Pancreas. 2003; 26:42–48. [PubMed: 12499916]
10. Kawahira H, Kobayashi S, Kaneko K, et al. p53 protein expression in intraductal papillary mucinous tumors (IPMT) of the pancreas as an indicator of tumor malignancy. Hepatogastroenterology. 2000; 47:973–977. [PubMed: 11020860]
11. Yamaguchi K, Chijiiwa K, Noshiro H, et al. Ki-*ras* codon 12 point mutation and p53 mutation in pancreatic diseases. Hepatogastroenterology. 1999; 46:2575–2581. [PubMed: 10522044]
12. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer. 2002; 2:489–501. [PubMed: 12094235]
13. Sansal I, Sellers WR. The biology and clinical relevance of the *PTEN* tumor suppressor pathway. J Clin Oncol. 2004; 22:2954–2963. [PubMed: 15254063]
14. Bellacosa A, de Feo D, Godwin AK, et al. Molecular alterations of the *AKT2* oncogene in ovarian and breast carcinomas. Int J Cancer. 1995; 64:280–285. [PubMed: 7657393]
15. Shayesteh L, Lu Y, Kuo WL, et al. *PIK3CA* is implicated as an oncogene in ovarian cancer. Nat Genet. 1999; 21:99–102. [PubMed: 9916799]
16. Pedrero JM, Carracedo DG, Pinto CM, et al. Frequent genetic and biochemical alterations of the PI 3-K/AKT/PTEN pathway in head and neck squamous cell carcinoma. Int J Cancer. 2005; 114:242–248. [PubMed: 15543611]
17. Samuels Y, Velculescu VE. Oncogenic mutations of *PIK3CA* in human cancers. Cell Cycle. 2004; 3:1221–1224. [PubMed: 15467468]
18. García-Rostán G, Costa AM, Pereira-Castro I, et al. Mutation of the *PIK3CA* gene in anaplastic thyroid cancer. Cancer Res. 2005; 65:10199–10207. [PubMed: 16288007]
19. Or YY, Hui AB, To KF, et al. *PIK3CA* mutations in nasopharyngeal carcinoma. Int J Cancer. 2006; 118:1065–1067. [PubMed: 16114017]
20. Kozaki K, Imoto I, Pimkhaokham A, et al. *PIK3CA* mutation is an oncogenic aberration at advanced stages of oral squamous cell carcinoma. Cancer Sci. 2006; 97:1351–1358. [PubMed: 17052259]
21. Qiu W, Tong GX, Manolidis S, et al. Novel mutant-enriched sequencing identified high frequency of *PIK3CA* mutations in pharyngeal cancer. Int J Cancer. 2008; 122:1189–1194. [PubMed: 17990317]
22. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the *PIK3CA* gene in human cancers. Science. 2004; 304:554. [PubMed: 15016963]
23. Schönleben F, Qiu W, Ciau NT, et al. *PIK3CA* mutations in intraductal papillary mucinous neoplasm/carcinoma of the pancreas. Clin Cancer Res. 2006; 12:3851–3855. [PubMed: 16778113]
24. Bader AG, Kang S, Vogt PK. Cancer-specific mutations in *PIK3CA* are oncogenic in vivo. Proc Natl Acad Sci U S A. 2006; 103:1475–1479. [PubMed: 16432179]
25. Kang S, Bader AG, Vogt PK. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. Proc Natl Acad Sci U S A. 2005; 102:802–807. [PubMed: 15647370]
26. Samuels Y, Diaz LA Jr, Schmidt-Kittler O, et al. Mutant *PIK3CA* promotes cell growth and invasion of human cancer cells. Cancer Cell. 2005; 7:561–573. [PubMed: 15950905]
27. Campbell IG, Russell SE, Choong DY, et al. Mutation of the *PIK3CA* gene in ovarian and breast cancer. Cancer Res. 2004; 64:7678–7681. [PubMed: 15520168]
28. Lee JW, Soung YH, Kim SY, et al. *PIK3CA* gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene. 2005; 24:1477–1480. [PubMed: 15608678]

29. Li VS, Wong CW, Chan TL, et al. Mutations of *PIK3CA* in gastric adenocarcinoma. BMC Cancer. 2005; 5:29. [PubMed: 15784156]
30. Carpten JD, Faber AL, Horn C, et al. A transforming mutation in the pleckstrin homology domain of *AKT1* in cancer. Nature. 2007; 448:439–444. [PubMed: 17611497]
31. Bleeker FE, Lamba S, Zanon C, et al. Absence of *AKT1* mutations in glioblastoma. PLoS One. 2009; 4:e5638. [PubMed: 19461960]
32. Qiu W, Schönleben F, Li X, et al. *PIK3CA* mutations in head and neck squamous cell carcinoma. Clin Cancer Res. 2006; 12:1441–1446. [PubMed: 16533766]
33. Ikenoue T, Kanai F, Hikiba Y, et al. Functional consequences of mutations in a putative Akt phosphorylation motif of B-raf in human cancers. Mol Carcinog. 2005; 43:59–63. [PubMed: 15791648]
34. Broderick DK, Di C, Parrett TJ, et al. Mutations of *PIK3CA* in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. Cancer Res. 2004; 64:5048–5050. [PubMed: 15289301]
35. Wang Y, Helland A, Holm R, et al. *PIK3CA* mutations in advanced ovarian carcinomas. Hum Mutat. 2005; 25:322. [PubMed: 15712344]
36. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008; 321:1801–1806. [PubMed: 18772397]
37. Kuboki Y, Shiratori K, Hatori T, et al. Association of epidermal growth factor receptor and mitogen-activated protein kinase with cystic neoplasms of the pancreas. Mod Pathol. 2010; 23:1127–1135. [PubMed: 20495538]
38. Jimenez RE, Warshaw AL, Z'graggen K, et al. Sequential accumulation of K-*ras* mutations and p53 overexpression in the progression of pancreatic mucinous cystic neoplasms to malignancy. Ann Surg. 1999; 230:501–509. discussion 509–511. [PubMed: 10522720]
39. Janku F, Lee JJ, Tsimberidou AM, et al. *PIK3CA* mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. PLoS One. 2011; 6:e22769. [PubMed: 21829508]
40. Ihle NT, Lemos R Jr, Wipf P, et al. Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. Cancer Res. 2009; 69:143–150. [PubMed: 19117997]
41. Di Nicolantonio F, Arena S, Tabernero J, et al. Deregulation of the PI3K and *KRAS* signaling pathways in human cancer cells determines their response to everolimus. J Clin Invest. 2010; 120:2858–2866. [PubMed: 20664172]
42. Schönleben F, Qiu W, Remotti HE, et al. *PIK3CA*, *KRAS*, and BRAF mutations in intraductal papillary mucinous neoplasm/carcinoma (IPMN/C) of the pancreas. Langenbecks Arch Surg. 2008; 393:289–296. [PubMed: 18343945]

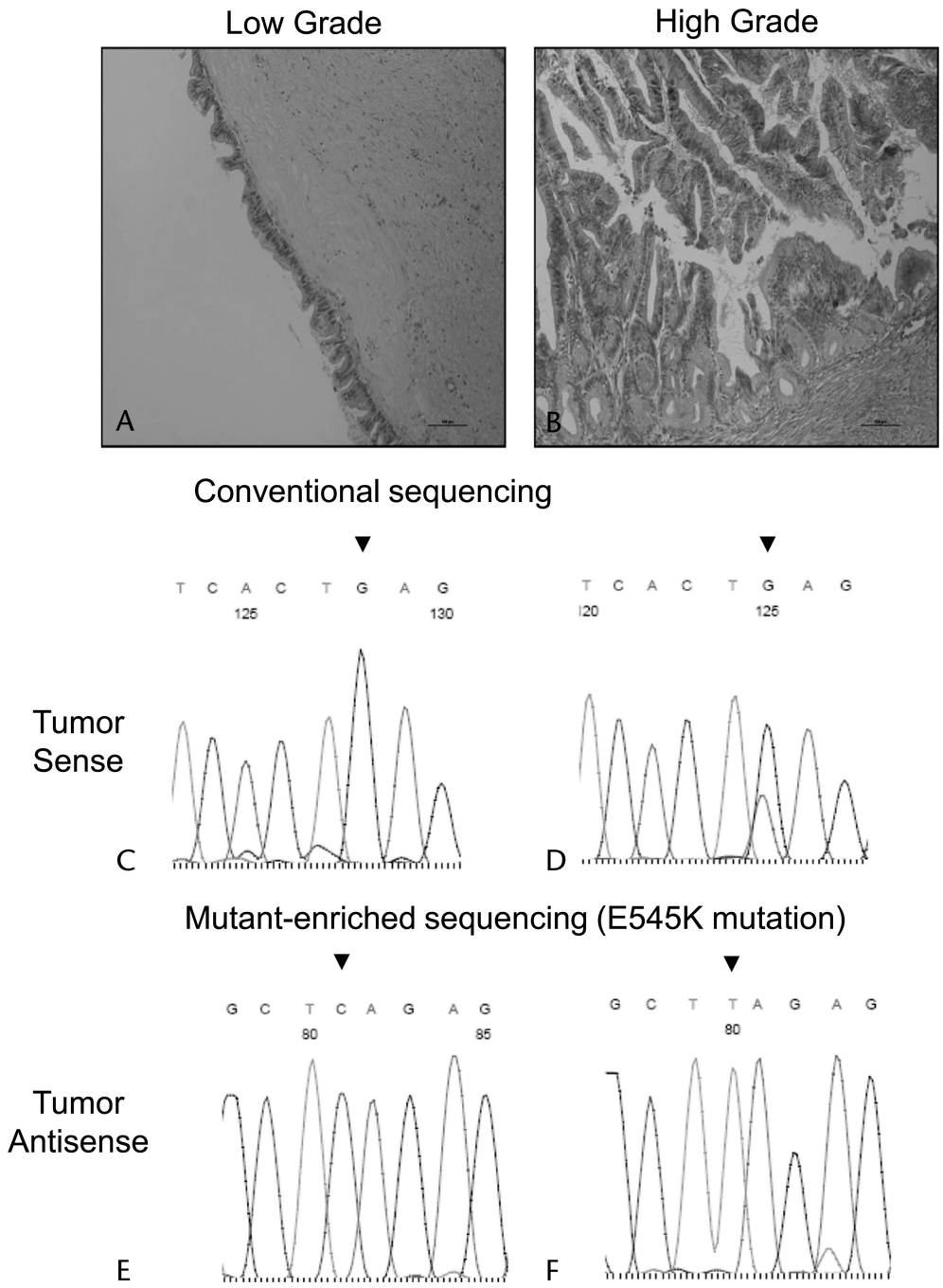

FIGURE 1.

PIK3CA E545K mutation was detected in the high-grade dysplasia of a patient with MCN. Morphology and mutational analysis of the same patient with MCN with 2 grades of dysplasia detected—low-grade (A, C, and E) and high-grade dysplasia (B, D, and F; H&E, original magnification ×400). Using the conventional sequencing method, *PIK3CA* E545K hot-spot mutation was not detected in the area with low-grade dysplasia (C), but both wild-type and mutant peaks were observed in the high-grade dysplasia in the same MCN (D; black arrows). Using a mutant-enriched sequencing method designed specifically for the

E545K mutation, the results showed the wild-type allele in the low-grade (E) areas and only the mutant allele in the high-grade (F) areas of the same patient with MCN (black arrow). As expected, the wild-type allele disappeared from the high-grade sample when the mutant-enriched sequencing method was applied.

TABLE 1

Summary Reports of the 15 Patient Samples

Case No.	Sex (Decade)	Site	Lesion Analyzed	Location	Size, cm	*PIK3CA* Mutation‡	*AKT1* Mutation
1	F (50s)	Distal pancreas	MCN with high-grade dysplasia	Tail	12	−	−
2	F (50s)	Distal pancreas	MCN with no dysplasia	Tail	5.5	−	−
3	F (40s)	Distal pancreas	MCN with no dysplasia	Tail	2	−	−
4	F (50s)	Distal pancreas	MCN with no dysplasia	Tail	13	−	−
5	F (40s)	Distal pancreas	MCN with no dysplasia	Tail	4.2	−	−
6	F (60s)	Distal pancreas	MCN with high-grade dysplasia and invasive adenocarcinoma	Tail	4.5*	+	−
7	F (60s)	Distal pancreas	MCN with no dysplasia	Tail	2.8	−	−
8	F (60s)	Distal pancreas	MCN with no dysplasia	Tail	8	−	−
9	F (70s)	Distal pancreas	MCN with high-grade dysplasia	Tail	6.6	−	−
10	F (50s)	Distal pancreas	MCN with high-grade dysplasia and microscopic invasive adenocarcinoma	Tail	9.5†	−	−
11	F (30s)	Proximal pancreas	MCN with no dysplasia	Body	6	−	−
12	F (20s)	Distal pancreas	MCN with high-grade dysplasia and invasive adenocarcinoma, multiple foci	Tail	25.0†	−	−
13	F (50s)	Distal pancreas	MCN with high-grade dysplasia and invasive adenocarcinoma, multiple foci	Tail	5.0†	−	−
14	F (50s)	Distal pancreas	MCN with high-grade dysplasia	Tail	8	−	−
15	F (40s)	Distal pancreas	MCN with high-grade dysplasia	Tail	1.2	−	−

*Mucinous cystic neoplasm with an associated invasive ductal adenocarcinoma (size, 2.5 cm).

†Mucinous cystic neoplasm with an associated microscopic focus of invasive ductal adenocarcinoma.

‡"+" means positive for mutation, and "−" means negative for mutation.