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Solid-Pseudopapillary Tumors of the Pancreas Are Genetically Distinct from Pancreatic Ductal Adenocarcinomas and Almost Always Harbor *β-catenin* Mutations

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Solid-pseudopapillary tumors (SPTs) are unusual pancreatic neoplasms of low malignant potential that most frequently affect young women. Genetic events contributing to the development of SPTs are unknown. Whereas the more common ductal adenocarcinomas of the pancreas essentially never harbor *β-catenin* or *APC* gene mutations, we have recently identified alterations of the *APC/β-catenin* pathway in other nonductal pancreatic neoplasms including pancreatoblastomas and acinar cell carcinomas. We analyzed a series of 20 SPTs for somatic alterations of the *APC/β-catenin* pathway using immunohistochemistry for β-catenin protein accumulation, direct DNA sequencing of *β-catenin* exon 3, and direct DNA sequencing of the mutation cluster region in exon 15 of the *APC* gene in those SPTs that did not harbor *β-catenin* mutations. Immunohistochemical labeling for cyclin D1 was performed to evaluate the overexpression of this cell-cycle protein as one of the putative downstream effectors of *β-catenin* dysregulation. In addition, we analyzed the SPTs for genetic alterations commonly found in pancreatic ductal adenocarcinomas, including mutations in the *K-ras* oncogene and *p53* and *DPC4* tumor suppressor genes, using direct DNA sequencing of *K-ras* and immunostaining for p53 and Dpc4. Almost all SPTs harbored alterations in the *APC/β-catenin* pathway. Nuclear accumulation of β-catenin protein was present in 95% (19 of 20), and activating *β-catenin* oncogene mutations were identified in 90% (18 of 20) of the SPTs. Seventy-four percent (14 of 19) showed overexpression of cyclin D1, ranging from 10 to 70% of tumor nuclei. In contrast, no *K-ras* mutations were present in any of the 20 SPTs, and Dpc4 expression was intact in all 16 SPTs for which immunohistochemical labeling was successful. Overexpression of p53 was limited to only 3 of 19 (15.8%) SPTs. These results emphasize the two distinct, divergent genetic pathways of neoplastic progression in pancreatic ductal and nonductal neoplasms. *(Am J Pathol 2002, 160:1361–1369)*

Solid-pseudopapillary tumors of the pancreas (SPTs) are uncommon tumors, constituting only ~1% of pancreatic neoplasms.[1] SPTs are histologically, clinically, and prognostically quite distinct from the more common pancreatic ductal adenocarcinomas. In contrast to the infiltrative growth and almost invariably lethal behavior of ductal adenocarcinomas among older individuals, SPTs are neoplasms of young women and are frequently grossly sharply circumscribed and cystic lesions (although their microscopic margins may be indistinct). Most importantly, despite their often large size (mean of >10 cm at presentation in one study),[2] the vast majority of SPTs are indolent neoplasms. They are confined to the pancreas in 85% of patients, and even the 10 to 15% of patients with liver or peritoneal metastases from SPTs commonly enjoy long-term survival.[2–6]

The histopathological appearance of SPTs is distinctive among the primary pancreatic neoplasms.[1] The neoplastic epithelial cells are uniform, polygonal, and discohesive in nature. Smaller SPTs may be primarily arranged in solid sheets with a rich microvasculature (Figure 1A). Frequent degenerative changes in larger tumors, however, lend a characteristic pseudopapillary pattern because of residual epithelial cells that form perivascular rosettes (Figure 1B). SPTs are known to consistently express vimentin,[7–9] α1-anti-trypsin,[7–11] neuron-specific enolase,[8,10,11] progesterone receptors,[10] and more recently, CD10.[9] However, attempts to discern a cell of

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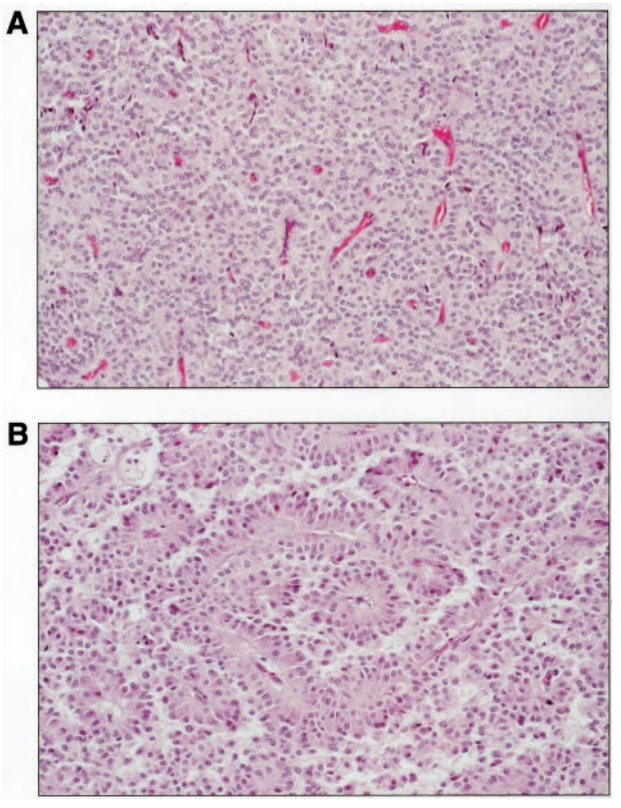

Figure 1. Histological appearance of SPTs. **A:** In solid areas, patternless sheets of uniform epithelial cells are punctuated by numerous small vascular channels. **B:** Degenerative changes and the discohesive nature of the epithelial cells give rise to characteristic pseudopapillae in which residual tumor cells appear to rosette around vascular cores.

origin or even a line of cellular differentiation for SPTs based on these results have been unrevealing.[10,12,13]

Likewise, genetic alterations that characterize SPTs have remained elusive. Numerous flow cytometric evaluations of SPTs have been reported,[7,14–20] with only occasional neoplasms demonstrating aneuploidy[7,14–16] or other structural chromosomal abnormalities.[20–22] Only a few SPTs have been evaluated for molecular alterations, and these studies have been limited to evaluation of the *K-ras* oncogene and *p53*, *p16*, and *DPC4* tumor suppressor gene. These genes are frequently involved in the pathogenesis of pancreatic ductal adenocarcinomas. Not surprisingly, given the marked clinicopathological differences between SPTs and ductal adenocarcinomas, alterations in these genes have not been detected in SPTs.[18,19,23–27]

In contrast to pancreatic ductal adenocarcinomas in which *β-catenin* or *APC* gene mutations are essentially never present, we have recently identified somatic alterations of the *APC/β-catenin* pathway in other pancreatic nonductal neoplasms including pancreatoblastomas and acinar cell carcinomas.[28,29] We therefore undertook a molecular characterization of a series of 20 SPTs for alterations in the *APC/β-catenin* pathway and for cyclin D1 protein overexpression, a putative downstream effector of *β-catenin* dysregulation.[30–33] In addition, we analyzed the SPTs for alterations in the *K-ras* oncogene and *p53* and *DPC4* tumor suppressor genes that are known to characterize pancreatic ductal adenocarcinomas.

Materials and Methods

Case Selection

The study population consisted of 20 patients with pancreatic SPTs who either underwent surgical resection or whose surgical material was seen in consultation at The Johns Hopkins Hospital (9 cases) or Memorial Sloan-Kettering Cancer Center (11 cases) between 1989 and 2000. Clinical information including patient age, gender, tumor size, tumor location in the pancreas, and presence or absence of metastatic disease was collected from patient charts or the computerized patient files.

Immunohistochemistry for β-Catenin, p53, and Dpc4

Immunohistochemical labeling using diaminobenzidine as the chromogen was performed on the Techmate 1000 automatic labeling system (BioTek Solutions, Tucson, AZ). Deparaffinized sections of formalin-fixed tissue at 5 μm thickness were labeled with β-catenin antibody (1:500 dilution, mouse monoclonal; Becton Dickinson Transduction Laboratories, Lexington, KY), cyclin D1 antibody (1:50 dilution, rabbit polyclonal; Oncogene Research Products, San Diego, CA), p53 antibody (1:100 dilution, mouse monoclonal clone D07; DAKO, Carpinteria, CA), and Dpc4 antibody (1:100 dilution, monoclonal clone B8; Santa Cruz Biotechnology, Santa Cruz, CA). Heat-induced antigen retrieval using steam for 20 minutes at 80°C was used before incubation with all four antibodies.

For β-catenin, immunohistochemical labeling was evaluated for the presence of nuclear, cytoplasmic, and membranous β-catenin accumulation in the SPTs and surrounding nonneoplastic pancreas, if present. Nuclear and cytoplasmic accumulation of β-catenin in SPTs was graded according to the percentage of neoplastic cells with strong immunolabeling. For cyclin D1, the percentage of moderately or strongly positive tumor nuclei was evaluated. For p53, the percentage of positively labeled nuclei was recorded; we considered strong nuclear labeling in ≥30% of neoplastic cells as the cutoff for positivity.[34] For Dpc4, SPTs were classified as showing intact Dpc4 protein expression if they showed the normal pattern of strong, diffuse cytoplasmic labeling and labeling of scattered nuclei. They were classified as showing loss of normal Dpc4 expression only if they showed a complete loss of cytoplasmic and nuclear Dpc4 labeling.[35]

DNA Extraction

Microdissection of SPTs for DNA extraction was performed from formalin-fixed, paraffin-embedded tissues. A 27½-guage-needle tip was used for microdissection of routinely processed 5-μm hematoxylin and eosin-stained

slides under a low-power (×4) objective. Genomic DNA was extracted as described previously.[36] Corresponding normal control DNA was available in 17 cases and was extracted from adjacent nonneoplastic pancreatic acini or stroma in 16 cases and from adjacent duodenum in 1 case.

Mutation Analysis of the β-Catenin and APC Genes

Genomic DNA from each SPT and normal tissue (if present) was amplified by polymerase chain reaction (PCR) using the primer pair: 5′-ATGGAACCAGACA-GAAAAGC-3′ (sense) and 5′-GCTACTTGTTCTGAGT-GAAG-3′ (anti-sense). These amplified a 200-bp fragment of exon 3 of the *β-catenin* gene that encompasses the region for GSK-3β phosphorylation. PCR reactions were performed under standard conditions in a 50-μl volume containing 38 μl of Platinum PCR SuperMix (Life Technologies, Rockville, MD), 5 μl of both 5′ and 3′ oligonucleotides (final concentration of 1 μmol/L), and 2 μl (~50 ng) of genomic DNA. PCR conditions consisted of an initial denaturation at 94°C for 3 minutes, 40 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 2 minutes, and a final extension at 72°C for 7 minutes. PCR products were purified with spin columns using QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA) before sequencing. Automated sequencing of purified PCR products was performed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Inc., Foster City, CA) using the internal primers: 5′-AAAGCGGCTGTTAGT-CACTGG-3′ (sense) and 5′-CCTGTTCCCACTCATA-CAGG-3′ (anti-sense), and the resulting sequence data were analyzed with the Sequencher analysis program (Gene Codes, Ann Arbor, MI). All mutations were verified in both sense and anti-sense directions on independent PCR products.

Direct sequencing for *APC* gene mutations was attempted for SPTs that did not contain identifiable *β-catenin* mutations. Four sets of oligonucleotide primers (A1: 5′-CAGACTTATTGTGTAGAAGA-3′ and A2: 5′-CTCCT-GAAGAAAATTCAACA-3′ for codons 1260–1359; B1: 5′-AGGGTTCTAGTTTATCTTCA-3′ and B2: 5′-TCTGCTTG-GTGGCATGGTTT-3′ for codons 1339–1436; C1: 5′-GGCATTATAAGCCCCAGTGA-3′ and C2: 5′-AAATGG-CTCATCGAGGCTCA-3′ for codons 1417–1516; D1: 5′-ACTCCAGATGGATTTTCTTG-3′ and D2: 5′-GGCTGGCT-TTTTTGCTTTAC-3′ for codons 1497–1596) were used to amplify the mutation cluster region of the *APC* gene.[37] PCR reactions were performed in 50-μl volumes using the reaction mixture described above. PCR conditions consisted of an initial denaturation step of 94°C for 3 minutes, 40 cycles (94°C for 1 minute, 55°C for 1 minute, and 68°C for 1.5 minutes for APC-B, APC-C, and APC-D primer pairs and 94°C for 1 minute, 52°C for 1 minute, and 68°C for 1.5 minutes for APC-A), followed by a final extension at 72°C for 7 minutes.

Mutation Analysis of the K-ras Oncogene

The oligonucleotide primers 5′-GAGAATTCATGACT-GAATATAAACTTGT-3′ (sense) and 5′-TCGAATTCCTC-TATTGTTGGATCATATTCG-3′ (anti-sense) were used to amplify a region in exon 1 of the *K-ras* gene that includes codons 12 and 13. PCR reactions were performed in 50-μl volumes using the reaction mixture described above, with an initial denaturation at 94°C for 3 minutes, 40 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute, and a final extension at 72°C for 7 minutes. PCR products were purified and sequenced as described above using the internal primer 5′-ATTCGTC-CACAAAATGAT-3′.

Results

A summary of the clinicopathological features of the 20 SPTs (designated S1 to S20) is shown in Table 1, and the corresponding molecular findings are shown in Table 2.

Clinicopathological Characteristics

Clinical information was available in 19 patients with SPTs. Sixteen (84.2%) patients were female and three (15.8%) were male. They ranged from 13 to 75 years with a mean patient age of 40.3 years. The SPTs were located throughout the pancreas without a particular site predilection. Mean size of the SPTs was 4.6 cm, although there was a wide variation in tumor size, ranging from 1 cm to 11.5 cm. Histopathologically, the 20 SPTs showed uniformly small, regular nuclei with a focal area of nuclear pleomorphism in only one (5%) of the SPTs. Five of the 19 SPTs (26.3%) for which pathological staging was available showed tumor infiltration either into vascular/perineural spaces (three SPTs) or into the adjacent bowel wall and/or peripancreatic fat (two SPTs). However, metastatic disease was present in only one patient (S10, with metastases to the liver), who subsequently died of disease 10 years after initial presentation.

Mutations in the β-Catenin Gene

Activating mutations in exon 3 of the *β-catenin* oncogene were present in nearly all (18 of 20, 90%) SPTs. All 18 were 1-bp missense mutations that either affected serine residues at GSK-3β phosphorylation sites in codon 33 (one SPT) and codon 37 (six SPTs), or immediately around serine/threonine GSK-3β phosphorylation sites in codon 32 (six SPTs) and codon 34 (five SPTs). All of these mutations were somatic in nature, as evidenced by the lack of mutations in the corresponding normal tissues (available for sequencing in 17 of 20 SPTs overall and 15 of 18 SPTs with *β-catenin* mutations). All chromatograms showing *β-catenin* mutations contained an admixture of the mutated and wild-type peaks, consistent with the known dominant-positive nature of *β-catenin* gene mutations (Figure 2).

We attempted to sequence the two SPTs without *β-catenin* mutations for mutations in the mutation cluster

Table 1. Clinicopathological Features of Pancreatic Solid-Psuedopapillary Tumors

Case	Age/sex	Tumor size	Location in pancreas	Nuclear pleomorphism	Invasion of adjacent structures	Metastases
S1	57/M	6.0 cm	Tail	–	–	–
S2	35/F	3.5 cm	Body	–	–	–
S3	42/F	2.0 cm	Head	–	–	–
S4	25/F	7.5 cm	Body/tail	–	–	–
S5	38/F	4.7 cm	Body/tail	–	–	–
S6	37/F	2.5 cm	Body	+ (Focal)	+ (Peripancreatic fat)	–
S7	51/F	3.5 cm	Uncinate	–	–	–
S8	57/F	5.2 cm	Head	–	+ (Mesocolon; duodenum; peripancreatic fat)	–
S9	75/F	5.0 cm	Head	–	–	–
S10	56/F	4.0 cm	Distal	–	–	+ (Liver)*
S11	18/F	7.5 cm	Head	–	–	–
S12	N/A	N/A	N/A	–	N/A	N/A
S13	13/M	5.5 cm	N/A	–	–	–
S14	49/F	5.5 cm	Distal	–	+ (Vascular, perineural)	–
S15	38/F	1.0 cm	Distal	–	+ (Perineural)	–
S16	38/M	>2 cm	Tail	–	+ (Capsular vessels)	–
S17	42/F	2.5 cm	Distal	–	–	–
S18	26/F	11.5 cm	Head	–	–	–
S19	42/F	3.0 cm	Distal	–	–	–
S20	27/F	4.5 cm	Head	–	–	–

*This patient died of disease 10 years after presentation.
N/A, information was not available.

region of the *APC* gene, but the PCR amplification failed in both cases.

Supposed Downstream Effects of β-Catenin Mutation

The location of the identified *β-catenin* mutations either at or around GSK-3β phosphorylation sites would be expected to interfere with normal phosphorylation by GSK-3β and subsequent ubiquitin-mediated degradation of β-catenin protein. Consistent with this effect, strong nuclear and cytoplasmic accumulation of β-catenin protein was present in nearly all SPTs. In 17 of 20 cases (85%), the immunolabeling was strong and diffuse, present throughout the tumor in >90% of neoplastic epithelial cells (Figure 3A). In an additional 2 of 20 cases (10%, both outside material with unknown tissue fixation parameters) nuclear and cytoplasmic accumulation was patchy, detected in 30% of the neoplastic epithelial cells in one SPT, and 20% of neoplastic epithelial nuclei with only faint cytoplasmic staining in the other SPT. In the one remaining SPT (also outside material), all immunolabeling

Table 2. Genetic Alterations in Pancreatic Solid-Pseudopapillary Tumors

Case	*β-catenin* mutation	Nuclear β-catenin, %	Nuclear cyclin D1, %	*K-ras* mutation	p53 accumulation	Dpc4 loss
S1	G34V	>90	50	Wild-type	–	Intact
S2	S37F	>90	30	Wild-type	–	Intact
S3	D32Y	>90	40	Wild-type	–	Intact
S4	G34V	>90	50	Wild-type	–	Intact
S5	S37F	>90	70	Wild-type	–	Intact
S6	G34V	>90	10	Wild-type	+ (Patchy)	Intact
S7	S33A	>90	20	Wild-type	–	Intact
S8	S37F	>90	10	Wild-type	–	Intact
S9	D32V	>90	10	Wild-type	–	Intact
S10	Wild-type	>90	–	Wild-type	–	Intact
S11	D32N	20	–	Wild-type	–	N/A
S12	S37F	30	–	Wild-type	–	Intact
S13	S37F	>90	15	Wild-type	–	Intact
S14	Wild-type	>90	–	Wild-type	40%	Intact
S15	G34R	>90	10	Wild-type	–	Intact
S16	S37F	>90	30	Wild-type	–	Intact
S17	D32Y	>90	30	Wild-type	–	N/A
S18	D32N	>90	–	Wild-type	–	Intact
S19	D32Y	>90	10	Wild-type	+ (Patchy)	N/A
S20	G34R	N/A	N/A	Wild-type	N/A	N/A

Locations of somatic mutations in *β-catenin* are shown by codon.
Nuclear β-catenin, cyclin D1, and p53 accumulation were evaluated based on the percentage of labeled tumor cell nuclei.
N/A, immunohistochemistry failed.

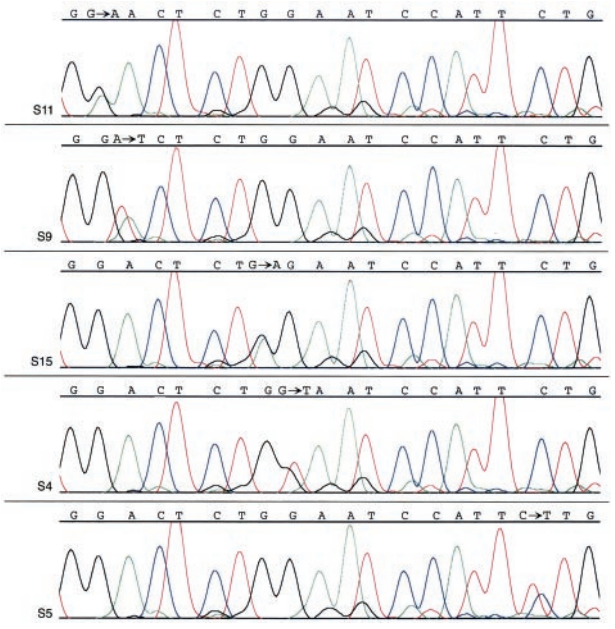

Figure 2. *β-catenin* oncogene mutations in SPTs. Representative DNA sequencing chromatograms demonstrate a GAC (aspartic acid)→AAC (asparagine) mutation in codon 32 of case S11, a GAC (aspartic acid)→GTC (valine) mutation in codon 32 of S9, a GGA (glycine)→AGA (arginine) mutation in codon 34 of S15, a GGA (glycine)→GTA (valine) mutation in codon 34 of S4, and a TCT (serine)→TTT (phenylalanine) mutation in codon 37 of S5. In each case, a mixture of the mutant and wild-type peaks is present because of the dominant-positive nature of *β-catenin* mutations.

reactions failed, with no normal membranous pattern of β-catenin staining in the adjacent nonneoplastic tissue. In all other SPTs that contained adjacent nonneoplastic tissues, only the normal membranous pattern of β-catenin labeling was observed.

There was no correlation between poor immunolabeling for β-catenin and lack of detectable *β-catenin* mutations. Each of the three SPTs that either showed only patchy or negative β-catenin protein accumulation contained *β-catenin* gene mutations on sequencing, and the two SPTs that did not contain *β-catenin* gene mutations showed strong and diffuse nuclear β-catenin protein accumulation. This finding suggests that β-catenin was dysregulated in all 20 SPTs examined in this study.

A majority of SPTs also demonstrated overexpression of cyclin D1, a downstream target of nuclear β-catenin. Seventy-four percent of SPTs (14 of 19 cases in which immunostaining was successful) contained moderately or strongly labeled nuclei that ranged from 10 to 70% of neoplastic epithelial cells (mean, 27.5%) (Figure 3B). We were better able to detect cyclin D1 overexpression among the 9 in-house SPTs with relatively uniform fixation conditions (9 of 9 cases positive, with a mean nuclear labeling of 32.2%) than among the 11 outside SPTs (5 of 11 cases positive, mean nuclear labeling of 19% in positive cases), suggesting that the true frequency of cyclin D1 activation may be higher than we were able to confirm in this study. No nuclear cyclin D1 was present in normal pancreatic acini or ductal epithelial cells; strong cytoplasmic and nuclear labeling of occasional islet cells was present, of uncertain etiology.

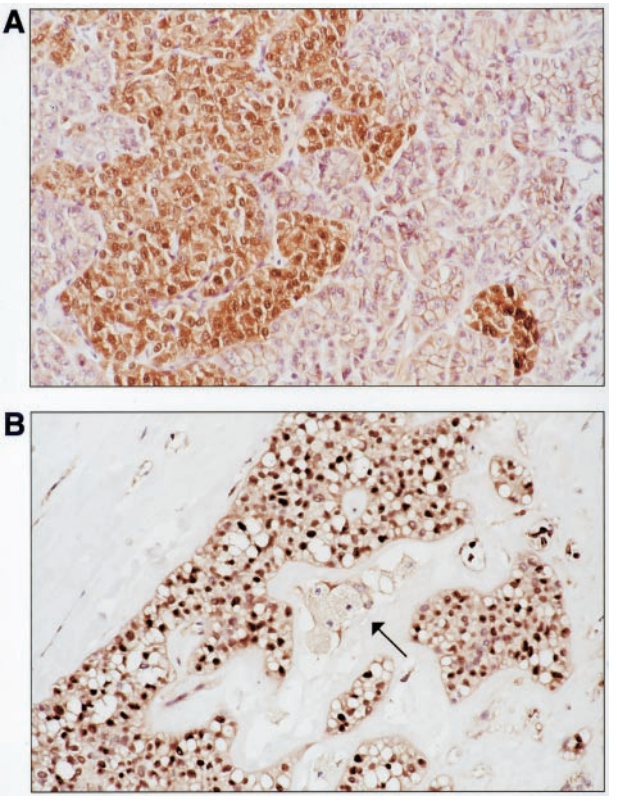

Figure 3. Downstream effects of β-catenin dysregulation in SPTs. **A:** Nuclear translocation and accumulation of β-catenin protein is seen in neoplastic epithelial cells that have infiltrated into normal pancreas at the edge of the tumor. Nonneoplastic acini show only normal membranous β-catenin labeling, but are negative for nuclear accumulation. **B:** Overexpression of cyclin D1, a downstream transcriptional target of nuclear β-catenin-Tcf/Lef complex. Cyclin D1 labeling is seen in the nuclei of neoplastic epithelial cells of this SPT, but not in the foamy histiocytes at center (**arrow**).

Alterations in K-ras, Dpc4, and p53

All 20 SPTs (100%) contained only wild-type *K-ras* gene sequences around codons 12 and 13 (Figure 4A). Normal Dpc4 protein expression was preserved in all 16 SPTs (100%) for which the immunolabeling was successful (Figure 4B). Only 3 of 19 (15.8%) SPTs demonstrated nuclear p53 overexpression. In one case, ~40% of neoplastic epithelial cell nuclei were positive for p53 in a diffuse pattern throughout the tumor. In the other two cases, intense nuclear labeling for p53 was present but was confined to distinct patchy foci that overall occupied <30% of the tumor volume (Figure 4C).

Discussion

We have shown that nearly all SPTs (18 of 20, 90%) harbor *β-catenin* gene mutations. β-catenin protein functions both as a submembranous component of the cadherin-mediated cell adhesion system and as a downstream transcriptional activator in the Wnt signaling pathway.[38,39] Normal β-catenin degradation is promoted by the APC tumor suppressor protein, which in conjunction with glycogen synthase kinase-3β (GSK-3β) and AXIN, promotes phosphorylation of β-catenin on serine/

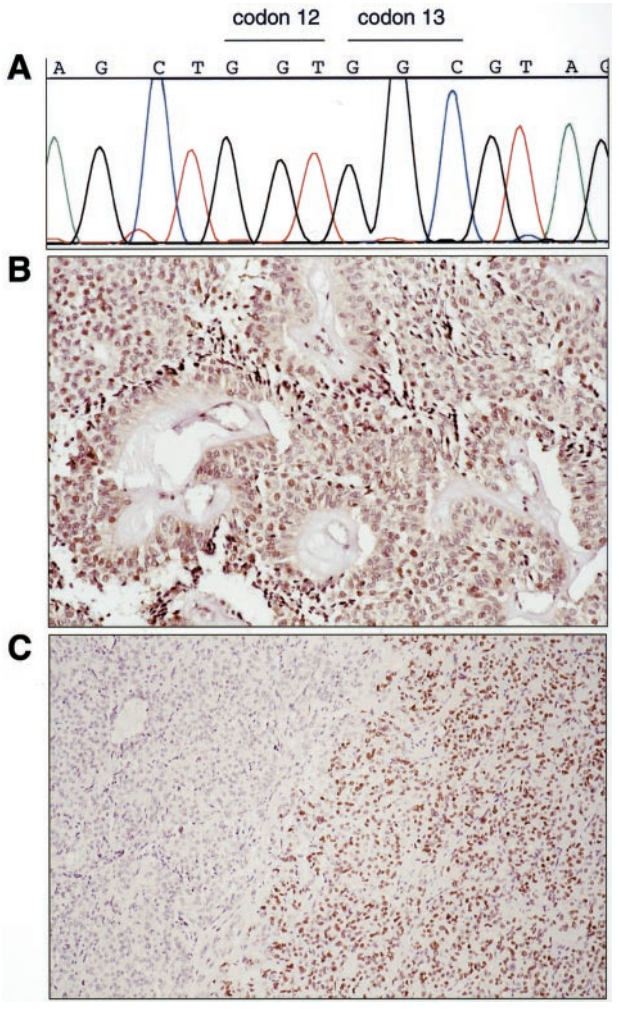

Figure 4. SPTs rarely harbor alterations in the genes commonly involved in ductal adenocarcinomas. **A:** Only wild-type *K-ras* sequences were present at codons 12 and 13 in all SPTs. **B:** Intact Dpc4 protein expression was present in all SPTs. **C:** Only 3 of 19 (15.9%) SPTs showed p53 protein overexpression, as in this case (S6) that contained a distinct patch of neoplastic epithelial cells with nuclear labeling.

threonine residues that are encoded in exon 3 of the *β-catenin* gene.[38,40,41] Normal levels of β-catenin protein are maintained by this phosphorylation, which targets β-catenin for subsequent ubiquitin-mediated degradation.[42,43] Abnormal accumulation of β-catenin and loss of β-catenin regulatory activity can result from stabilizing *β-catenin* gene mutations, truncating *APC* tumor suppressor gene mutations, or *AXIN* mutations.[41,44,45] For example, most colorectal adenomas and carcinomas can be demonstrated to contain either bi-allelic inactivation of the *APC* gene or (less commonly) activating *β-catenin* oncogene mutations.[46,47] All *β-catenin* mutations detected in SPTs in this study were 1-bp missense mutations that affected either serine phosphorylation residues in codons 33 and 37 or residues immediately surrounding phosphorylation sites in codons 32 and 34. These mutations would therefore be predicted to interfere with normal phosphorylation and degradation of β-catenin protein.

In addition to the high frequency of *β-catenin* gene mutations in SPTs, the results of this study demonstrate in part the downstream effects of β-catenin dysregulation. The consequences of cytosolic β-catenin accumulation because of mutations in the *APC/β-catenin* pathway include its interaction with T-cell transcription factor (Tcf)/lymphoid enhancer-binding factor (Lef) and translocation of the β-catenin-Tcf/Lef complex to the nucleus.[30–33] Consistent with this, we observed abnormal nuclear and cytoplasmic β-catenin accumulation in nearly all SPTs, including the two SPTs that did not contain identifiable *β-catenin* or *APC* gene mutations. In fact, all 20 SPTs in this study showed evidence for a dysregulated *APC/β-catenin* pathway in the form of nuclear β-catenin accumulation or activating *β-catenin* mutation on sequencing.

Nuclear translocation of the β-catenin-Tcf/Lef complex, in turn, has been shown in studies of colon carcinoma cells to stimulate transcription of target genes involved in cell cycle regulation, including *c-myc* and *cyclin D1*.[30,48] The β-catenin complex activates transcription of *cyclin D1* via binding to consensus sequences on the *cyclin D1* promoter; high levels of cyclin D1 mRNA and constitutive production of cyclin D1 protein have previously been demonstrated in colon carcinoma cells harboring *β-catenin* mutations.[30] Consistent with this, we found overexpression of cyclin D1 by immunohistochemical labeling in 74% of SPTs, ranging from 10 to 70% of neoplastic epithelial cell nuclei. We observed a greater frequency of cyclin D1 labeling, as well as a higher proportion of labeled nuclei, in our in-house SPTs in which tissue fixation and processing were more uniform (9 of 9 in-house cases, mean nuclear labeling of 32.2% *versus* 5 of 11 outside cases, mean nuclear labeling of 18%), suggesting that the true rate of cyclin D1 overexpression in SPTs may be higher than what was demonstrable in this study. Muller-Hocker and colleagues[25] have also recently shown overexpression of cyclin D1 (as well as the cell-cycle protein cyclin D3) in 50% and 30% of tumor nuclei in two SPTs, respectively, although analysis of β-catenin was not performed.

In contrast to the nearly universal presence of an abnormal *APC/β-catenin* pathway in SPTs, we found little evidence for involvement of genes known to be important in the stepwise molecular pathogenesis of the more common pancreatic ductal adenocarcinomas, including *K-ras*, *DPC4*, and *p53. K-ras* oncogene mutations occur early in the development of nearly all *in situ* ductal neoplasms,[49,50] *DPC4* tumor suppressor gene inactivation later in the progression of slightly more than 50% of cases,[51,52] and *p53* inactivation in up to 70% of invasive pancreatic adenocarcinomas.[27,49,53,54] In contrast, alterations of the *APC/β-catenin* pathway are essentially never observed in ductal adenocarcinomas. Only a few SPTs have previously been investigated for similar molecular genetic alterations. A total of 16 SPTs in four previous studies have been shown to harbor only wild-type *K-ras* gene sequences,[19,23,24,27] and 18 SPTs from four other studies have been shown to be negative for p53 alterations by immunohistochemistry[18,25,26] or gene sequencing.[27] In addition, four SPTs analyzed by Moore and colleagues[27] were negative for *p16* and *DPC4* gene mu-

tations. We found only wild-type *K-ras* gene sequences in codons 12 and 13 among our 20 SPTs, intact Dpc4 protein expression in all 16 stainable SPTs, and low-level and/or patchy abnormal p53 accumulation in only 3 of 19 (15.8%) SPTs. Notably, immunohistochemical labeling for Dpc4 has been shown to accurately mirror the status of *DPC4* gene inactivation.[35] Although there is imperfect correlation between immunohistochemical labeling for p53 and *p53* gene mutations (p53 may be overexpressed for reasons other than gene mutation, and conversely, truncating *p53* mutations may be present that do not result in p53 protein accumulation by immunohistochemistry), the results of this study and the previous investigations of p53 immunohistochemistry and gene sequencing in SPTs suggest that p53 alterations are at least uncommon in SPTs.

These marked molecular differences between SPTs and pancreatic ductal adenocarcinomas in this study and previous studies are not surprising given the sharp clinicopathological and prognostic distinction between the two types of neoplasms. In contrast to ductal adenocarcinomas, SPTs are characteristically tumors of young women, although cases in males,[55–58] children,[3,4,58,59] and in elderly patients[55] have been described. Furthermore, SPTs are typically indolent, with examples of long-term survival even among the 10 to 15% of patients who have liver or peritoneal metastases.[2–6] The results of this study do not explain the distinctive histopathological appearance of SPTs, nor identify the cell of origin/cellular differentiation of the neoplastic epithelial cells of SPTs, concepts that have remained elusive despite numerous studies detailing their immunophenotype. It is also conceptually difficult to establish a connection between a disrupted *APC/β-catenin* pathway and the peculiar tendency for SPTs to occur primarily in young females. However, we have shown other examples of lesions with frequent *APC/β-catenin* alterations including breast fibromatoses and juvenile nasopharyngeal angiofibromas that also show a distinct sex predilection.[60,61]

We have recently studied the genetic alterations present in two other clinicopathologically distinct nonductal pancreatic neoplasms, pancreatoblastomas and acinar cell carcinomas.[28,29] Like SPTs, pancreatoblastomas and acinar cell carcinomas contrast with ductal adenocarcinomas in their distinctive histopathological features, characteristically young age at presentation (in the case of pancreatoblastomas), and somewhat better prognosis than the usual ductal adenocarcinoma.[62,63] Also like SPTs, these neoplasms only rarely harbor the alterations in *K-ras*, Dpc4, and p53 that are common to ductal adenocarcinomas, but 67% of pancreatoblastomas and 23.5% of acinar cell carcinomas had either activating *β-catenin* mutations or inactivating *APC* mutations.[28,29,64–66] The results of this study suggest that pancreatic ductal and nonductal neoplasms progress genetically through two primarily dichotomous pathways: *K-ras*, *p16*, *DPC4*, and *p53* gene alterations in the case of ductal neoplasms, and varying rates of *APC/β-catenin* alterations in nonductal neoplasms, including virtually all SPTs.

Note added in proof

Subsequent to submission of this manuscript, similar molecular findings were reported in solid-pseudopapillary tumors in a study by Tanaka et al.[67]

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