

Genetic and Immunohistochemical Analysis of Pancreatic Acinar Cell Carcinoma

Frequent Allelic Loss on Chromosome 11p and Alterations in the APC/ β -Catenin Pathway

Susan C. Abraham,* Tsung-Teh Wu,*
Ralph H. Hruban,* Jae-Hyuk Lee,*
Charles J. Yeo,[†] Kevin Conlon,[‡] Murray Brennan,[‡]
John L. Cameron,[†] and David S. Klimstra[§]

From the Department of Pathology,* Division of GI/Liver Pathology, and the Department of Surgery,[†] The Johns Hopkins University School of Medicine, Baltimore, Maryland; and the Departments of Surgery[‡] and Pathology,[§] Memorial Sloan-Kettering Cancer Center, New York, New York

Acinar cell carcinomas (ACCs) are rare malignant tumors of the exocrine pancreas. The specific molecular alterations that characterize ACCs have not yet been elucidated. ACCs are morphologically and genetically distinct from the more common pancreatic ductal adenocarcinomas. Instead, the morphological, immunohistochemical, and clinical features of ACCs overlap with those of another rare pancreatic neoplasm, pancreatoblastoma. We have recently demonstrated a high frequency of allelic loss on chromosome arm 11p and mutations in the APC/ β -catenin pathway in pancreatoblastomas, suggesting that similar alterations might also play a role in the pathogenesis of some ACCs. We analyzed a series of 21 ACCs for somatic alterations in the APC/ β -catenin pathway and for allelic loss on chromosome 11p. In addition, we evaluated the ACCs for alterations in p53 and Dpc4 expression using immunohistochemistry, and for microsatellite instability (MSI) using polymerase chain amplification of a panel of microsatellite markers. Allelic loss on chromosome 11p was the most common genetic alteration in ACCs, present in 50% (6 of 12 informative cases). Molecular alterations in the APC/ β -catenin pathway were detected in 23.5% (4 of 17) of the carcinomas, including one ACC with an activating mutation of the β -catenin oncogene and three ACCs with truncating APC mutations. One ACC (1 of 13, 7.6%) showed allelic shifts in four of the five markers tested (MSI-high), two (15.4%) showed an allelic shift in only one of the five markers tested (MSI-low), and no shifts were detected in the remaining 10 cases. The MSI-high ACC showed medullary

histological features. In contrast, no loss of Dpc4 protein expression or p53 accumulation was detected. These results indicate that ACCs are genetically distinct from pancreatic ductal adenocarcinomas, but some cases contain genetic alterations common to histologically similar pancreatoblastomas. (Am J Pathol 2002, 160:953–962)

Acinar cell carcinomas (ACCs) are rare neoplasms of the exocrine pancreas, comprising less than 1% of primary pancreatic tumors.^{1,2} ACCs are distinct from the more common pancreatic ductal adenocarcinomas. Histologically and immunohistochemically, ACCs recapitulate the growth pattern and secretory products of nonneoplastic pancreatic acini, including frequent production of the digestive enzymes trypsin, lipase, chymotrypsin, and, less commonly, amylase.^{1,3–5} In some patients the overproduction of lipase by the neoplasm produces a distinctive syndrome of subcutaneous fat necrosis and polyarthralgia, in contrast to the more frequent jaundice in patients with pancreatic ductal adenocarcinomas.^{1,6–10} In addition, although the prognosis of ACC in adults is poor, with the majority of patients showing evidence of metastatic disease either at or subsequent to diagnosis,^{1,11} the reported mean survival of 18 months and the occasional long-term survival of patients with ACC contrasts with the significantly worse prognosis for patients with pancreatic ductal adenocarcinomas.^{1,4,12}

Several studies have examined genetic alterations in ACCs, including evaluation for mutations in the *K-ras* oncogene and *DPC4* and *p53* tumor suppressor genes that characterize the stepwise molecular and histological progression of pancreatic ductal neoplasms.^{13–19} Not surprisingly, given the clinicopathological differences between ductal adenocarcinoma and ACC, alterations of

Supported by a National Cancer Institute SPORE grant in gastrointestinal cancer (grant P50-CA62924).

Accepted for publication November 30, 2001.

Address reprint requests to Susan Abraham, M.D., Division of GI/Liver Pathology, Dept. of Pathology, Ross Bldg., Room 632, The Johns Hopkins University School of Medicine, 720 Rutland Ave., Baltimore, MD 21205-2196. E-mail: sabraham@jhmi.edu.

these genes have either been absent or only rarely present in ACCs.^{17,20–24} Indeed, aside from the presence of aneuploidy²⁵ and the recent report of ACC allelotype,²⁴ specific molecular alterations characterizing ACCs have not yet been identified.

We have recently studied genetic alterations in another rare pancreatic neoplasm, pancreatoblastoma, and found frequent involvement of the *APC/β-catenin* pathway and allelic loss on chromosome arm 11p in these neoplasms.²⁶ In pancreatoblastomas, the rationale for molecular evaluation of the *APC/β-catenin* pathway and for chromosome 11p loss lies in their occasional occurrence in patients with familial adenomatous polyposis (FAP) and Beckwith-Wiedemann syndrome, respectively.²⁶ Beckwith-Wiedemann syndrome, a maldevelopmental disorder with tissue overgrowth and increased neoplastic risk, is characterized by dysregulation of cell-cycle genes on a heavily imprinted chromosomal region on 11p15.5. FAP, caused by germline mutation of the *APC* gene on chromosome 5q, imparts a markedly increased risk for colonic and extra-colonic neoplasms through second-hit alterations of *APC* (either intragenic mutation or allelic loss of 5q). In turn, the sporadic variants of FAP-associated neoplasms also frequently involve the *APC/β-catenin* pathway, either through bi-allelic *APC* inactivation or by activating mutations in the *β-catenin* oncogene.

Several clinicopathological similarities exist between ACC and pancreatoblastoma. Both these tumors are characterized histologically by variably sheet-like, trabecular, and acinar growth patterns, both consistently show acinar differentiation as detected by immunohistochemical labeling,^{1,3,21,27,28} both may contain varying proportions of endocrine cells,^{1,27,29–33} and both may produce α -fetoprotein.^{1,4,11,27,34–38} We therefore undertook a molecular characterization of a series of ACCs. The alterations that characterize pancreatoblastomas,²⁶ including allelic loss on chromosome 11p and mutations in the *APC/β-catenin* pathway were examined, as were alterations in the *p53* and *DPC4* tumor suppressor genes that characterize the more common adult pancreatic ductal adenocarcinomas.

Materials and Methods

Case Selection

The study population consisted of 21 patients with pancreatic ACC who underwent biopsy (4 cases) or surgical resection (17 cases) between 1983 and 2001. Five cases were from The Johns Hopkins Hospital and 16 cases were from Memorial Sloan-Kettering Cancer Center. ACCs were diagnosed based on characteristic histological and immunohistochemical features that included varying proportions of sheet-like, trabecular, and acinar growth, as well as the absence of squamoid corpuscles, which distinguish pancreatoblastomas from ACCs (Figure 1).^{1,27}

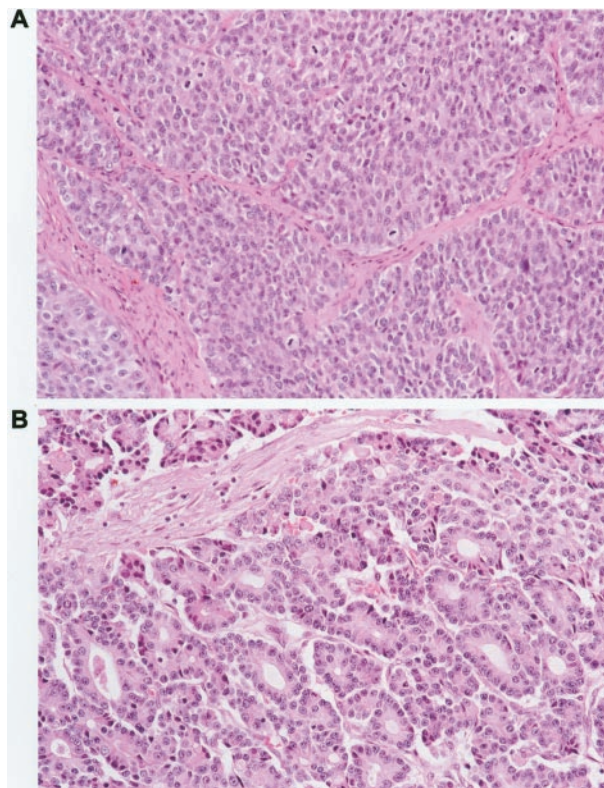


Figure 1. Histopathological appearance of pancreatic ACC. The neoplastic epithelial cells show variable growth patterns including areas of sheet-like growth divided by bands of fibrous tissue (A) and areas of acinar differentiation (B). Identical growth patterns can be present in ACCs and pancreatoblastomas, but characteristic squamoid corpuscles are not seen in the former.

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- μ m thickness were labeled with β -catenin antibody (1:500 dilution, mouse monoclonal; Becton Dickinson Transduction Laboratories, Lexington, KY), *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 three antibodies.

For β -catenin, immunohistochemical labeling was evaluated for the presence of nuclear, cytoplasmic, and membranous β -catenin accumulation in both the ACCs and any normal surrounding tissues. Nuclear and cytoplasmic accumulation of β -catenin in ACCs was graded according to the percentage of neoplastic cells with strong immunolabeling. For *p53*, the percentage of positively labeled nuclei was recorded; we considered strong nuclear labeling in $\geq 30\%$ of neoplastic cells as the cutoff for positivity.³⁹ For *Dpc4*, ACCs were classified as showing intact *Dpc4* expression if they showed the normal pattern of strong, diffuse cytoplasmic labeling and

labeling of scattered nuclei. ACCs were classified as showing loss of normal Dpc4 expression if they showed a complete loss of cytoplasmic and nuclear Dpc4 labeling.⁴⁰

DNA Extraction

Microdissection of ACCs for DNA extraction was performed from formalin-fixed, paraffin-embedded specimens. A 27 1/2-gauge-needle tip was used for microdissection of routinely processed, 5- μ m hematoxylin and eosin-stained slides under a low-power ($\times 4$) objective. Genomic DNA was extracted as described previously.⁴¹ Corresponding normal control DNA was available in 16 cases and was extracted from adjacent nonneoplastic tissue (adjacent pancreatic acini and/or stroma in 11 cases, duodenum in 3 cases, liver in 1 case, and colon in 1 case).

Mutation Analysis of the β -Catenin Gene

Genomic DNA from each sample was amplified by polymerase chain reaction (PCR) using the primer pair: 5'-ATGGAACCAGACAGAAAAGC-3' (sense) and 5'-GCTACTTGTCTGAGTGAAG-3' (anti-sense). These amplified a 200-bp fragment of exon 3 of the β -catenin gene that encompasses the region for GSK-3 β phosphorylation. PCR reactions were performed under standard conditions in a 50- μ l volume containing 38 μ l of Platinum PCR SuperMix (Life Technologies, Inc., Rockville, MD), 5 μ l of both 5' and 3' oligonucleotides (final concentration of 1 μ mol/L), and 2 μ l (~ 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'-AAAGCG-GCTGTTAGTCACTGG-3' (sense) and 5'-CCTGTTC-CCACTCATACAGG-3' (anti-sense), and the resulting sequence data were analyzed with the Sequencher analysis program (Gene Codes, Ann Arbor, MI). Mutations were verified in both sense and anti-sense directions on independent PCR products.

Mutation Analysis of the APC Gene

Four sets of oligonucleotide primers (A1: 5'-CAGACTTAT-TGTGTAGAAGA-3' and A2: 5'-CTCCTGAAGAAAATTCA-ACA-3' for codons 1260 to 1359; B1: 5'-AGGGTTCTAGTT-TATCTTCA-3' and B2: 5'-TCTGCTTGTTGGCATGGTTT-3' for codons 1339 to 1436; C1: 5'-GGCATTATAAGCCCCA-GTGA-3' and C2: 5'-AAATGGCTCATCGAGGCTCA-3' for codons 1417 to 1516; D1: 5'-ACTCCAGATGGATTT-TCTTG-3' and D2: 5'-GGCTGGCTTTTTTGTCTTAC-3' for codons 1497 to 1596) were used to amplify the mutation cluster region of the APC gene.⁴² PCR reactions were per-

formed in 50- μ l 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. PCR products were purified and sequenced as described above using the same primers as for genomic DNA amplification. All mutations were verified in both sense and anti-sense directions on independent PCR products.

Allelic Loss on Chromosome 5q

Loss of heterozygosity (LOH) on 5q was evaluated in the ACCs for which nonneoplastic control tissue was available. LOH was assessed by microsatellite assays using PCR amplification of three microsatellite markers (D5S82, D5S299, and D5S346) as previously described.⁴³ Assays were performed in 96-well plates in 10- μ l volumes, each containing 5 μ l of PCR Master (Boehringer Mannheim, Mannheim, Germany), 3.5 μ l of water, 1 μ l of genomic DNA, 0.06 μ l of 3' oligonucleotide, and 0.4 μ l of end-labeled 5' oligonucleotide. The 5' oligonucleotide was end-labeled with (γ -³²P)-ATP (NEN DuPont, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). For D5S82 and D5S299, 38 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute were performed, and for D5S346, 38 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute were performed. PCR products were separated on 6% denaturing polyacrylamide gels and the gels were subjected to autoradiography. LOH was considered to be present when there was complete or near-complete disappearance of a heterozygous band in the ACC as compared with nonneoplastic control tissue in at least one informative marker.

Allelic Loss on Chromosome 11p

LOH on 11p was evaluated in the ACCs for which nonneoplastic control tissue was available, using the microsatellite markers TH (a tetranucleotide repeat polymorphism on 11p15.5-p15) and D11S1984 (a dinucleotide repeat on 11p15.5). Assays were performed and interpreted as described above using annealing temperatures of 62°C for TH and 55°C for D11S1984.

Microsatellite Instability (MSI) Analysis

MSI was evaluated in the ACCs for which nonneoplastic control tissue was available. MSI testing was performed using the five microsatellite loci (D5S346, as described above for 5q LOH, plus D2S123, D17S250, Bat-25, and Bat-26) recommended by the 1997 National Cancer Institute (NCI)-sponsored consensus conference.⁴⁴ Assays were performed as described above for 5q LOH analysis using annealing temperatures of 55°C for D2S123, D17S250, Bat-25, and Bat-26. The resultant bands on autoradiographs were interpreted according to the crite-

Table 1. Genetic Alterations in Pancreatic Acinar Cell Carcinomas

Case	Age/sex	p53 accumulation	Dpc4 loss	MSI	Nuclear β -catenin	β -catenin mutation	APC mutation	11p LOH
A1	15/F	—	—	MSS	—	Wild-type	Wild-type	—
A2	21/F	—	—	MSS	—	Wild-type	1444X	—
A3	32/F	—	—	MSS	—	Wild-type	Wild-type	—
A4	73/F	—	—	MSI-L	—	Wild-type	Wild-type	+
A5	51/M	—	—	MSI-L	50%	Wild-type	Wild-type	—
A6	55/F	—	—	MSS	—	Wild-type	Wild-type	N/I
A7	2/F	—	—	MSS	—	Wild-type	Wild-type	+
A8	70/M	—	—	MSS	—	Wild-type	Wild-type	—
A9	76/M	—	—	MSS	—	Wild-type	Wild-type	+
A10	58/M	—	—	MSS	—	Wild-type	Wild-type	—
A11	31/M	—	—	MSS	—	Wild-type	Wild-type	+
A12	80/M	—	—	MSS	—	Wild-type	Wild-type	+
A13	31/M	—	—	N/N	20%	T41I	Wild-type	N/N
A14	61/M	N/A	N/A	N/A	N/A	N/A	N/A	N/A
A15	63/M	—	—	N/A	—	N/A	N/A	N/A
A16	76/M	—	—	N/N	60%	Wild-type	1554–1556FS	N/N
A17	54/M	—	—	N/A	—	N/A	N/A	N/A
A18	60/M	—	—	N/N	—	Wild-type	1554–1556FS	N/N
A19	75/M	—	—	MSI-H	—	Wild-type	Wild-type	+
A20	74/M	—	—	N/A	—	N/A	N/A	N/A
A21	79/M	—	—	N/N	—	Wild-type	Wild-type	N/N

Locations of somatic mutations in β -catenin and APC are shown by codon.

Nuclear β -catenin accumulation was evaluated based on the percentage of strongly staining tumor cell nuclei.

FS, frameshift mutation; LOH, loss of heterozygosity; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable; N/A, DNA did not amplify or immunohistochemistry failed; N/I, non-informative for allelic loss; N/N, no corresponding normal tissue for evaluation of allelic loss or MSI assays.

ria described in detail by Berg and colleagues.⁴⁵ MSI-high (MSI-H) was considered to be present when at least two of the five microsatellite loci showed shifting, MSI-low (MSI-L) when only one locus was shifted, and microsatellite stable (MSS) when none of the loci were shifted, as per the NCI criteria.⁴⁴

Results

A summary of the clinicopathological and molecular findings in the 21 ACCs (designated A1 to A21) is presented in Table 1.

Clinicopathological Characteristics

Nineteen of the ACCs arose in adults ranging from 21 to 80 years (mean, 59 years) and two cases were in pediatric patients aged 2 and 15 years. Six patients (29%) were female and 15 (71%) were male.

Alterations in the APC/ β -Catenin Pathway

Amplifiable DNA for mutation analysis of β -catenin and APC was obtained from 17 of the 21 ACCs, and β -catenin or APC gene mutations were detected in a total of four (23.5%) of the 17 ACCs. One ACC (5.8%, case A13) contained a β -catenin gene mutation, a 1-bp C \rightarrow T missense mutation at threonine codon 41 that would be predicted to result in β -catenin activation because of alteration of a presumptive residue for glycogen synthase kinase-3 β (GSK-3 β) phosphorylation. A mixture of both the wild-type and mutant peaks was present on DNA sequencing of this neoplasm, corresponding to the dominant nature of β -catenin gene alterations (Figure 2).

Three additional ACCs (17.6%, cases A2, A16, and A18) contained APC gene mutations, each of which would be predicted to result in APC inactivation because of premature protein truncation. In cases A16 and A18, frameshifts from insertion of a base A into a 6-base poly(A) tract spanning codons 1554 to 1556 were present. Although normal tissue was not available in either A16 or A18 for 5q LOH analysis, both cases demonstrated loss of the wild-type allele on DNA sequencing of the region of the mutated poly(A) tract, indicative of bi-allelic APC inactivation (Figure 2). In case A2, a 1-bp C \rightarrow T substitution at codon 1444 resulted in the formation of a premature stop codon (Figure 2). Normal tissue was available for analysis in this case, and the somatic nature of this APC mutation was confirmed by the presence of only wild-type APC in the nonneoplastic tissue from this patient. When DNA from this ACC was sequenced, both the mutant and wild-type peaks were present, and no 5q LOH was present on allelic loss analysis. Bi-allelic APC inactivation therefore could not be demonstrated in this case. The 13 ACCs for which normal tissue was available were analyzed for 5q LOH, and allelic loss was present in two separate ACCs (15%, cases A8 and A9) for which no corresponding intragenic APC gene mutations were detected.

Immunohistochemical labeling for β -catenin protein revealed strong nuclear and cytoplasmic accumulation in three (15%) ACCs (cases A5, A13, and A16). The labeling was patchy in nature in all three cases, ranging from 20 to 60% of the neoplastic cells (Figure 3). Nonneoplastic pancreatic acini, ducts, and gastrointestinal epithelial cells showed the expected membranous and faint cytoplasmic labeling, but no nuclear or strong cytoplasmic β -catenin. Stromal cells in fibrous tissue between lobules

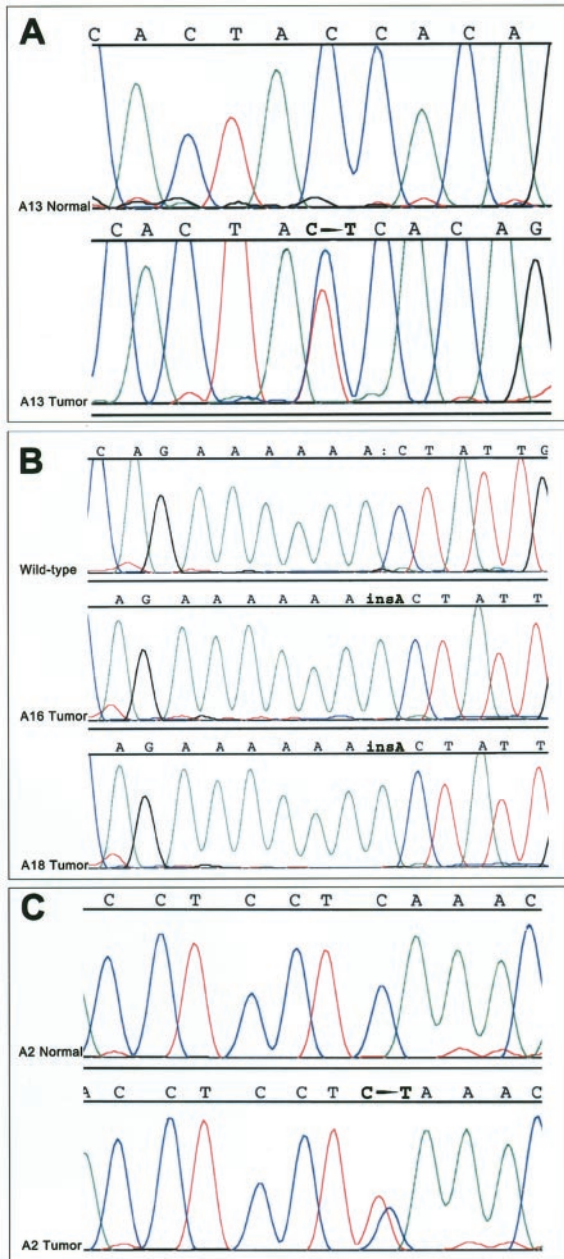


Figure 2. Alterations in the *APC*/ β -*catenin* pathway in ACCs. **A:** DNA sequencing from case A13, showing an ACC (threonine) \rightarrow ATC (isoleucine) mutation at codon 41 of the β -*catenin* gene. Sequencing of nonneoplastic tissue from this patient shows only wild-type β -*catenin*, confirming the somatic nature of the β -*catenin* mutation. **B:** Frameshift *APC* mutations in cases A16 and A18. DNA sequencing shows a 1-bp insertion A into a 6 base poly(A) tract spanning codons 1554 to 1556 in each case. The corresponding wild-type *APC* sequence from a different ACC is shown above. **C:** Nonsense *APC* mutation in case A2. DNA sequencing shows a CAA (glycine) \rightarrow TAA (stop) mutation at codon 1444 that is present in the ACC but not in the nonneoplastic tissue from this patient.

of neoplastic epithelial cells did not show β -catenin accumulation.

Only a moderate degree of correlation was present between the detection of β -catenin or *APC* gene mutation within an ACC and β -catenin protein accumulation by immunohistochemistry. Of the four ACCs with β -catenin or *APC* mutations by sequencing, two (50%, cases A13

and A16) demonstrated patchy nuclear accumulation of β -catenin in 20% and 60% of neoplastic cells, respectively, whereas the other two did not. One additional ACC (case A5) demonstrated nuclear β -catenin in 50% of neoplastic cells but did not contain detectable β -catenin or *APC* mutation.

Allelic Loss on 11p

Allelic loss on 11p15.5 was present in 6 of 12 (50%) ACCs that contained amplifiable DNA and were informative in one or both 11p microsatellite markers (Figure 4). All three cases that were informative at both TH and D11S1984 showed LOH on both markers in the ACCs.

MSI

MSI was present in 3 of 13 ACCs (23%) that contained amplifiable DNA and for which normal control tissue was available. One ACC (case A19) showed MSI-high, with allelic shifts in four of five NCI microsatellite markers (D5S346, D17S250, Bat-25, and Bat-26) as well as in D5S299 and D11S1984. Two ACCs showed MSI-low, with allelic shifts only in one marker (D17S250 in case A4 and Bat-26 in case A5). Correlation of the histopathological features in these neoplasms revealed that the ACC with MSI-high (A19) showed an interesting pattern of sharp demarcation between the neoplasm and the nonneoplastic pancreas, poor differentiation, and areas of syncytial growth of the neoplastic epithelial cells, features that have previously been described in medullary carcinomas of the pancreas.^{46,47} There was not a pronounced inflammatory infiltrate in this ACC, but this feature is also lacking in the reported pancreatic medullary carcinomas.^{46,47} However, unlike the other previously reported cases of medullary carcinoma of the pancreas, histological foci of clear-cut acini were present in this neoplasm (Figure 5), and acinar differentiation of the neoplastic epithelial cells as evidenced by diffuse immunohistochemical expression of trypsin, chymotrypsin, and lipase was also present.

Alterations in DPC and p53

Normal Dpc4 protein expression was preserved in all 20 ACCs. No significant p53 accumulation was detected by immunohistochemistry in any of the 20 ACCs (immunohistochemistry failed in one case).

Discussion

Studies of pancreatic ACCs to date have demonstrated the lack or rarity of genetic alterations commonly present in ductal adenocarcinomas, including mutations in the *K-ras* oncogene and *p53* and *DPC4* tumor suppressor genes.^{17,20–23} The specific molecular alterations that do characterize ACCs have remained obscure.²⁴

Both the histopathological and immunohistochemical features of ACCs overlap with those of pancreatoblasto-

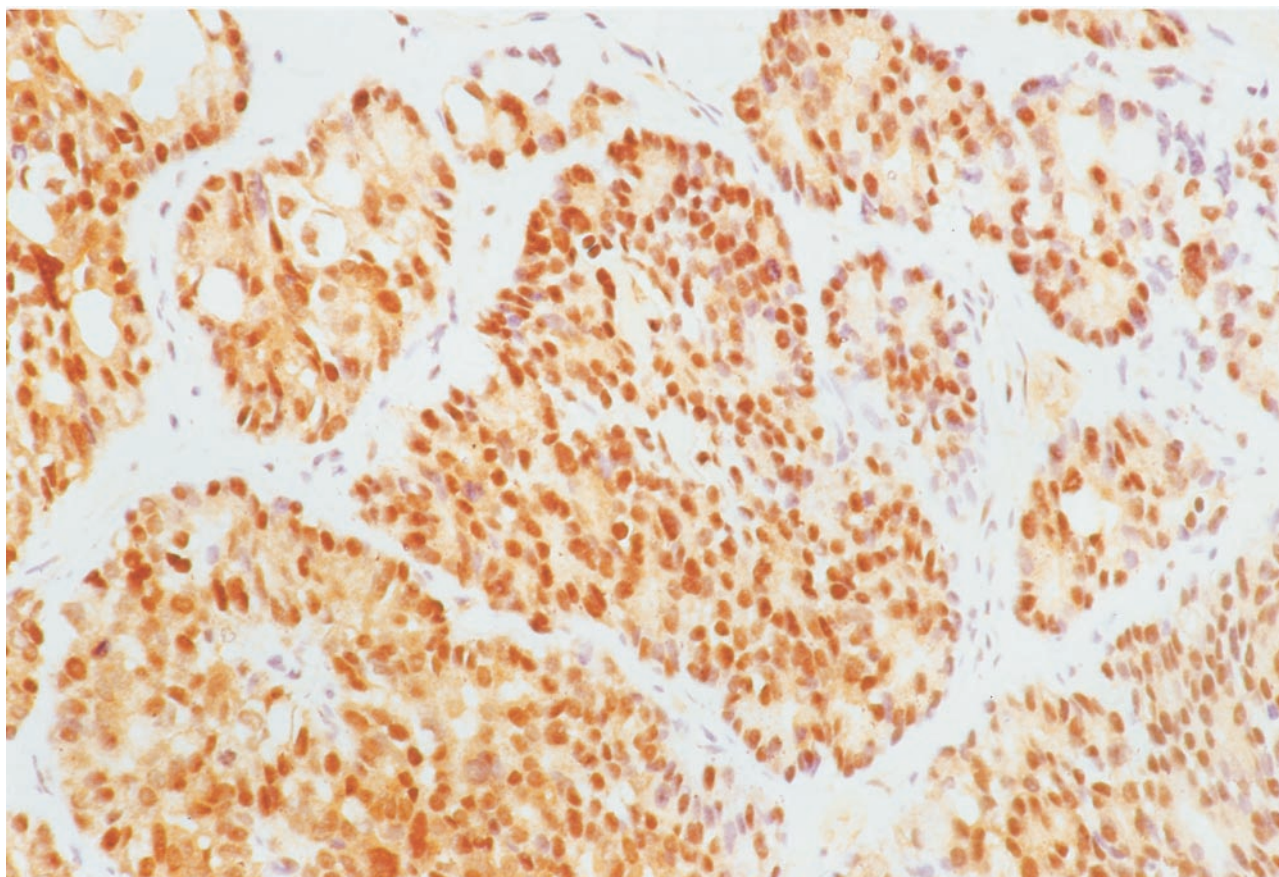


Figure 3. Immunohistochemical labeling for β -catenin in an ACC. Nuclear and cytoplasmic accumulation of β -catenin in neoplastic epithelial cells is present in this example (case A16), which also showed a truncating *APC* gene mutation. Stromal cells in the intervening fibrous bands show membranous β -catenin labeling, but are negative for nuclear and cytoplasmic accumulation.

mas, another rare but distinctive pancreatic malignancy. Although pancreatoblastomas occur predominantly in the pediatric population and ACCs predominantly in adults, occasional cases of ACCs in children (2 in this series of 21 ACCs)^{1,48,49} and pancreatoblastomas in adults^{27,50–54} are encountered, and some investigators regard pancreatoblastomas as the pediatric counterpart of ACCs.²⁷ We have recently characterized genetic alterations in pancreatoblastomas and have demonstrated high frequencies of both allelic loss on chromosome 11p and mutations in the *APC*/ β -catenin pathway, a molecular genotype that is distinct from that of pancreatic ductal adenocarcinoma.²⁶ The histological and clinicopathological overlap between ACCs and pancreatoblastomas suggests that ACCs and pancreatoblastomas might share similar genetic alterations.

The most common molecular alteration we identified in ACCs was allelic loss on chromosome 11p. Fifty percent of ACCs in this series (6 of 12 informative cases) showed LOH for TH1 and D11S1984, microsatellite markers near the WT-2 locus on 11p15.5. This frequency is somewhat higher than the 25% reported by Rigaud and colleagues²⁴ in the only previous reported allelotyping of pancreatic ACC. However, that study of eight informative ACCs used different 11p microsatellite markers (including one of two markers on 11p15.3),²⁴ making direct comparison of results difficult. The WT-2 locus on

11p15.5 is a heavily imprinted area containing growth- and cell cycle-regulatory genes.^{55,56} Congenital disruption of this locus is associated with Beckwith-Wiedemann syndrome, a maldevelopment syndrome of tissue overgrowth and organomegaly that is characterized by an increased risk for embryonal malignancies including hepatoblastoma, Wilm's tumor, and rhabdomyosarcoma.^{55,56} Several cases of pancreatoblastomas in patients with Beckwith-Wiedemann syndrome have also been reported,^{57,58} and indeed, among pancreatoblastomas studied by us, 86% also showed allelic loss on 11p15.5.²⁶ The somewhat lower rate of 11p LOH in ACCs compared with pancreatoblastomas mirrors the relationship between another typically adult tumor—hepatocellular carcinoma (HCC)—and its embryonal or pediatric counterpart—hepatoblastoma. Allelic loss on 11p (and in particular, loss of the maternal allele) is reported in up to 75% of hepatoblastomas,^{59–63} but at lower rates in HCCs.^{64–68}

The second most common genetic alteration we identified in ACCs was mutation in the *APC*/ β -catenin pathway in 23.5% (4 of 17) cases. One ACC contained a β -catenin gene mutation at threonine codon 41 that would be predicted to disrupt the *APC*/ β -catenin pathway by constitutive β -catenin protein activation, and the other three ACCs contained *APC* gene mutations predicted to result in *APC* protein truncation. In comparison, we previously

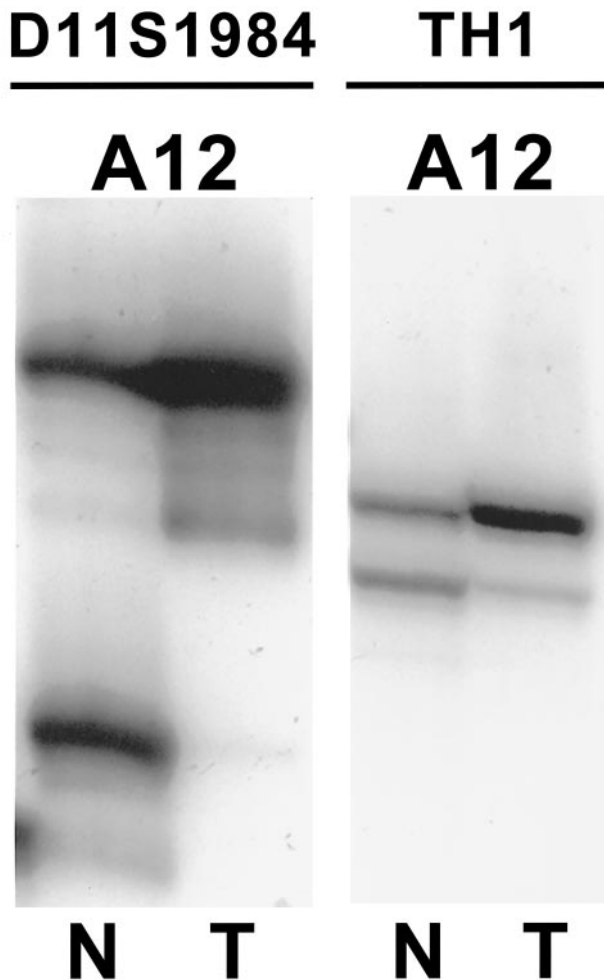


Figure 4. Allelic loss on 11p15.5 in ACC. LOH is present with both the D11S1984 and TH1 microsatellite markers for case A12, which was informative for both markers. N, normal; T, ACC.

found a much higher *APC/β-catenin* mutation frequency of 67% in pancreatoblastomas.²⁶ Interestingly, both of the adult pancreatoblastoma cases in that study demonstrated targeting of this pathway,²⁶ whereas neither of the two pediatric ACC cases in the current series contained *APC* or *β-catenin* mutations. One of the adult pancreatoblastoma cases in our previous study occurred in a patient with familial adenomatous polyposis,²⁶ although none of the ACCs in the current study did.

The lower rate of *APC/β-catenin* targeting in ACCs in comparison with pancreatoblastomas again mirrors the molecular findings in adult HCCs and childhood hepatoblastomas. Among hepatoblastomas, alterations in the *APC/β-catenin* pathway are frequently present.^{69–75} These most commonly take the form of activating *β-catenin* gene mutations, reported in one-half to two-thirds of hepatoblastomas in larger series.^{71,73} In contrast, *β-catenin* mutations are found on average only in approximately one-fifth of HCCs.^{76–82} Additionally, whereas most hepatoblastomas demonstrate strong nuclear accumulation of *β-catenin* protein on immunohistochemistry, the frequency of *β-catenin* labeling in HCCs is lower and the correlation between *β-catenin* mutation and immunola-

beling is less clear; some HCCs contain *β-catenin* mutations without the expected nuclear accumulation of the stabilized protein, and some show *β-catenin* immunolabeling without corresponding mutation.^{78,79,81,82} Similarly, we found that whereas the majority of pancreatoblastomas demonstrate strong nuclear *β-catenin* with good correlation between labeling and mutation status,²⁶ only 15% of ACCs show nuclear *β-catenin* accumulation, and only moderate correlation exists between the immunolabeling and mutation results.

The etiology of the discrepancy between *β-catenin* immunohistochemistry and *APC/β-catenin* mutation analysis in this study is not clear. However, in addition to some HCCs,^{78,79,81,82} multiple other human organ systems have also been noted to share this discrepancy between nuclear *β-catenin* labeling and *APC/β-catenin* gene mutations. In colorectal polyps, thyroid carcinomas, uterine and ovarian carcinomas, and soft tissue sarcomas, some neoplasms harbor mutations in the *APC/β-catenin* pathway without the expected nuclear accumulation of *β-catenin* protein, whereas others show nuclear *β-catenin* labeling without demonstrable mutations.^{83–88} In colonic adenomas, tumor size has been reported as a factor in nuclear *β-catenin* accumulation,⁸⁴ whereas proliferative activity is more closely correlated with nuclear *β-catenin* expression in high-grade sarcomas.⁸⁸

One ACC (7.6%) in the current series was suspected to harbor MSI based on the finding of allelic shifts in the 11p and 5q allelic loss assays, and this neoplasm subsequently demonstrated high-level MSI with the NCI microsatellite markers.⁴⁴ The MSI phenotype has recently been described in a subset of poorly differentiated carcinomas of the pancreas that show distinctive histological features common to medullary carcinomas of the colon (including a syncytial growth pattern, poor differentiation, and a pushing rather than infiltrative border, but interestingly only uncommonly a prominent lymphocytic infiltrate) as well as distinctive molecular features (frequent wild-type *K-ras* genes).^{46,47} Histopathologically, the ACC with MSI-H in this study was characterized by areas of syncytial growth, poor differentiation, and a pushing border (although this latter feature is also common to ACCs in general), but areas of well-defined acinar differentiation both histologically and by immunohistochemistry were also present. Although the medullary carcinomas reported by Goggins and colleagues⁴⁶ and Wilentz and colleagues⁴⁷ were considered to represent poorly differentiated adenocarcinomas, the finding of MSI in an ACC in the present series suggests that MSI might constitute an alternative molecular pathway of neoplastic progression in a fraction of ACCs as well.

In contrast to the above genetic alterations in ACCs, we found no evidence for involvement of *p53* or *DPC4* genes in the molecular pathogenesis of ACC. Our findings are in agreement with previous investigations that have shown no or only rare alterations of the *K-ras*, *DPC4*, and *p53* genes in pancreatic ACCs.^{17,20–24} Among conventional pancreatic ductal adenocarcinomas, early mutational activation of the *K-ras* oncogene occurs in nearly all neoplasms,^{13,14} inactivation of the *DPC4* tumor suppressor gene occurs in slightly more than one-half,^{18,19}

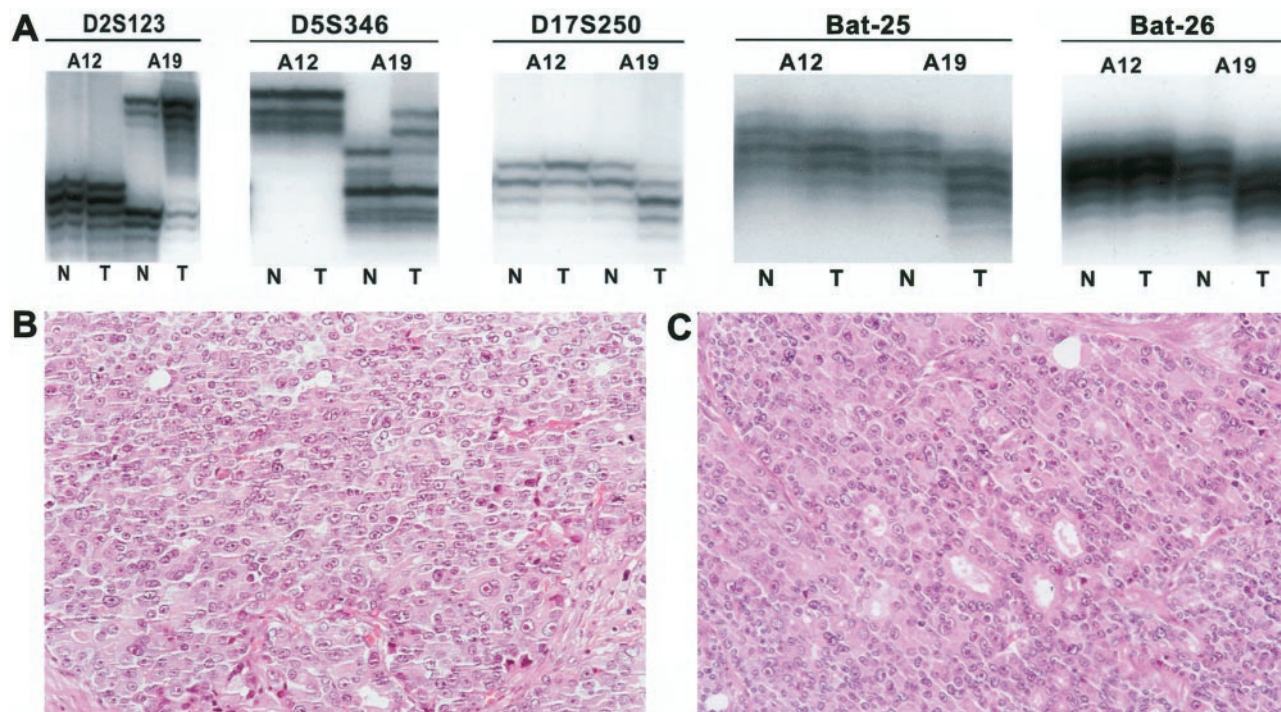


Figure 5. MSI in an ACC. **A:** Case A19 demonstrates MSI-high, with allelic shifts in the microsatellite markers D5S346, D17S250, Bat-25, and Bat-26. Only D2S123 is stable. In contrast, no allelic shifts are present in case A12, which is microsatellite stable. **B:** The histopathological features of case A19 overlapped with those of medullary carcinoma of the pancreas, with areas of poor differentiation and syncytial growth. **C:** Unlike cases of previously described medullary carcinoma, foci of clear-cut acinar differentiation were also present.

and late inactivation of *p53* occurs in up to 70%.^{13,15–17} The findings in this study underscore the sharp clinicopathological and genetic contrast between pancreatic ACCs and ductal adenocarcinomas, and suggest that at least a subset of ACCs share both pathological and molecular genetic features with pancreatoblastomas.

References

- Klimstra DS, Heffess CS, Oertel JE, Rosai J: Acinar cell carcinoma of the pancreas. A clinicopathologic study of 28 cases. *Am J Surg Pathol* 1992, 16:815–837
- Chen J, Baithun SI, Ramsay MA: Histogenesis of pancreatic carcinomas: a study based on 248 cases. *J Pathol* 1985, 146:65–76
- Morohoshi T, Kanda M, Horie A, Chott A, Dreyer T, Kloppel G, Heitz PU: Immunocytochemical markers of uncommon pancreatic tumors. Acinar cell carcinoma, pancreatoblastoma, and solid cystic (papillary-cystic) tumor. *Cancer* 1987, 59:739–747
- Shinagawa T, Tadokoro M, Maeyama S, Maeda C, Yamaguchi S, Morohoshi T, Ishikawa E: Alpha fetoprotein-producing acinar cell carcinoma of the pancreas showing multiple lines of differentiation. *Virchows Arch* 1995, 426:419–423
- Caruso RA, Inferrera A, Tuccari G, Barresi G: Acinar cell carcinoma of the pancreas. A histologic, immunocytochemical and ultrastructural study. *Histol Histopathol* 1994, 9:53–58
- Foulet A, Copin MC, Jaillard S, Wurtz A, Gosselin B: Acinar cell carcinoma of the pancreas revealed by Weber-Christian syndrome. *Ann Pathol* 1995, 15:438–442
- Kuerer H, Shim H, Pertsemilidis D, Unger P: Functioning pancreatic acinar cell carcinoma: immunohistochemical and ultrastructural analyses. *Am J Clin Oncol* 1997, 20:101–107
- MacMahon HE, Brown PA, Shen EM: Acinar cell carcinoma of the pancreas with subcutaneous fat necrosis. *Gastroenterology* 1965, 49:555–559
- Burns WA, Matthews MJ, Hamosh M, Weide GV, Blum R, Johnson FB: Lipase-secreting acinar cell carcinoma of the pancreas with polyar-
- thropathy. A light and electron microscopic, histochemical, and biochemical study. *Cancer* 1974, 33:1002–1009
- Radin DR, Colletti PM, Forrester DM, Tang WW: Pancreatic acinar cell carcinoma with subcutaneous and intraosseous fat necrosis. *Radiology* 1986, 158:67–68
- Kawamoto S, Hiraoka T, Kanemitsu K, Kimura M, Miyauchi Y, Takeya M: Alpha-fetoprotein-producing pancreatic cancer—a case report and review of 28 cases. *Hepatogastroenterology* 1992, 39:282–286
- Kuopio T, Ekfors TO, Nikkanen V, Nevalainen TJ: Acinar cell carcinoma of the pancreas. Report of three cases. *APMIS* 1995, 103:69–78
- Hruban RH, Goggins M, Parsons J, Kern SE: Progression model for pancreatic cancer. *Clin Cancer Res* 2000, 6:2969–2972
- Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M: Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell* 1988, 53:549–554
- Redston MS, Caldas C, Seymour AB, Hruban RH, da Costa L, Yeo CJ, Kern SE: p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. *Cancer Res* 1994, 54:3025–3033
- DiGiuseppe JA, Hruban RH, Goodman SN, Polak M, van den Berg FM, Allsion DC, Cameron JL, Offerhaus GJA: Overexpression of p53 protein in adenocarcinoma of the pancreas. *Am J Clin Pathol* 1994, 101:684–688
- Moore PS, Orlandini S, Zamboni G, Capelli P, Rigaud G, Falconi M, Bassi C, Lemoine NR, Scarpa A: Pancreatic tumours: molecular pathways implicated in ductal cancer are involved in ampullary but not in exocrine nonductal or endocrine tumorigenesis. *Br J Cancer* 2001, 84:253–262
- Schutte M, Hruban RH, Hedrick L, Cho KR, Nadasdy GM, Weinstein CL, Bova GS, Isaacs WB, Cairns P, Nawroz H, Sidransky D, Casero Jr RA, Meltzer PS, Hahn SA, Kern SE: DPC4 gene in various tumor types. *Cancer Res* 1996, 56:2527–2530
- Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE: DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 1996, 271:350–353
- Terhune PG, Heffess CS, Longnecker DS: Only wild-type c-Ki-ras

- codons 12, 13, and 61 in human pancreatic acinar cell carcinomas. *Mol Carcinog* 1994, 10:110-114
21. Hoorens A, Lemoine NR, McLellan E, Morohoshi T, Kamisawa T, Heitz PU, Stamm B, Ruschhoff J, Wiedenmann B, Kloppel G: Pancreatic acinar cell carcinoma. An analysis of cell lineage markers, p53 expression, and Ki-ras mutation. *Am J Pathol* 1993, 143:685-698
 22. Pellegata NS, Sessa F, Renault B, Bonato M, Leone BE, Solcia E, Ranzani GN: K-ras and p53 gene mutations in pancreatic cancer: ductal and nonductal tumors progress through different genetic lesions. *Cancer Res* 1994, 54:1556-1560
 23. Longnecker DS: Molecular pathology of invasive carcinoma. *Ann NY Acad Sci* 1999, 880:74-82
 24. Rigaud G, Moore PS, Zamboni G, Orlandini S, Taruscio D, Paradisi S, Lemoine NR, Kloppel G, Scarpa A: Allelotype of pancreatic acinar cell carcinoma. *Int J Cancer* 2000, 88:772-777
 25. Hsueh C, Kuo TT: Acinar cell carcinoma of the pancreas. Report of two cases with complex histomorphologic features causing diagnostic problems. *Int J Pancreatol* 1992, 12:305-313
 26. Abraham SC, Wu TT, Klimstra DS, Finn L, Lee JH, Yeo CJ, Cameron JL, Hruban RH: Distinctive molecular genetic alterations in sporadic and familial adenomatous polyposis-associated pancreatoblastomas: frequent alterations in the APC/ β -catenin pathway and chromosome 11p. *Am J Pathol* 2001, 159:1619-1627
 27. Klimstra DS, Wenig BM, Adair CF, Heffess CS: Pancreatoblastoma. A clinicopathologic study and review of the literature. *Am J Surg Pathol* 1995, 19:1371-1389
 28. Lonardo F, Cubilla AL, Klimstra DS: Microadenocarcinoma of the pancreas—morphologic pattern or pathologic entity? A reevaluation of the original series. *Am J Surg Pathol* 1996, 20:1385-1393
 29. Klimstra DS, Rosai J, Heffess CS: Mixed acinar-endocrine carcinomas of the pancreas. *Am J Surg Pathol* 1994, 18:765-778
 30. Kloppel G: Mixed exocrine-endocrine tumors of the pancreas. *Semin Diagn Pathol* 2000, 17:104-108
 31. Muramatsu T, Kijima H, Tsuchida T, Konagaya M, Matsubayashi H, Tada N, Nakamura M, Ueyama Y: Acinar-islet cell tumor of the pancreas: report of a malignant pancreatic composite tumor. *J Clin Gastroenterol* 2000, 31:175-178
 32. Cho KJ, Kim JY, Lee SS, Khang SK, Kim CW: Mixed acinar-endocrine carcinoma of the pancreas—a case report. *J Korean Med Sci* 1996, 11:188-192
 33. Ordonez NG, Mackay B: Acinar cell carcinoma of the pancreas. *Ultrastruct Pathol* 2000, 24:227-241
 34. Cingolani N, Shaco-Levy R, Farruggio A, Klimstra DS, Rosai J: Alpha-fetoprotein production by pancreatic tumors exhibiting acinar cell differentiation: study of five cases, one arising in a mediastinal teratoma. *Hum Pathol* 2000, 31:938-944
 35. Nojima T, Kojima T, Kato H, Sato T, Koito K, Nagashima K: Alpha-fetoprotein-producing acinar cell carcinoma of the pancreas. *Hum Pathol* 1992, 23:828-830
 36. Ono J, Sakamoto H, Sakoda K, Yagi Y, Hagio S, Sato E, Katsuki T: Acinar cell carcinoma of the pancreas with elevated serum alpha-fetoprotein. *Int Surg* 1984, 69:361-364
 37. Balian A, Hammel P, Terris B, Sauvanet A, Belghiti J, Ruszniewski P, Bernades P: Increase of serum alpha-fetoprotein in a patient with acinar cell carcinoma of the pancreas. *Gastroenterol Clin Biol* 1997, 21:231-232
 38. Ishizaki A, Koito K, Namieno T, Nagakawa T, Murashima Y, Suga T: Acinar cell carcinoma of the pancreas: a rare case of an alpha-fetoprotein-producing cystic tumor. *Eur J Radiol* 1995, 21:58-60
 39. Baas IO, Mulder JW, Offerhaus GJ, Vogelstein B, Hamilton SR: An evaluation of six antibodies for immunohistochemistry of mutant p53 gene product in archival colorectal neoplasms. *J Pathol* 1994, 172:5-12
 40. Wilentz RE, Su GH, Dai JL, Sparks AB, Argani P, Sohn TA, Yeo CJ, Kern SE, Hruban RH: Immunohistochemical labeling for Dpc4 mirrors genetic status in pancreatic adenocarcinomas: a new marker of DPC4 inactivation. *Am J Pathol* 2000, 156:37-43
 41. Moskaluk CA, Kern SE: Microdissection and polymerase chain reaction amplification of genomic DNA from histological tissue sections. *Am J Pathol* 1997, 150:1547-1552
 42. Yashima K, Nakamori S, Murakami Y, Yamaguchi A, Hayashi K, Ishikawa O, Konishi Y, Sekiya T: Mutations of the adenomatous polyposis coli gene in the mutation cluster region: comparison of human pancreatic and colorectal cancers. *Int J Cancer* 1994, 59:43-47
 43. Wu TT, Watanabe T, Heitmiller R, Zahrak M, Forastiere AA, Hamilton SR: Genetic alterations in Barrett esophagus and adenocarcinomas of the esophagus and esophagogastric junction region. *Am J Pathol* 1998, 153:287-294
 44. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S: A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998, 58:5248-5257
 45. Berg KD, Glaser CL, Thompson RE, Hamilton SR, Griffin CA, Eshleman JR: Detection of microsatellite instability by fluorescence multiplex polymerase chain reaction. *J Mol Diagn* 2000, 2:20-28
 46. Goggins M, Offerhaus GJ, Hilgers W, Griffin CA, Shekher M, Tang D, Sohn TA, Yeo CJ, Kern SE, Hruban RH: Pancreatic adenocarcinomas with DNA replication errors (RER+) are associated with wild-type K-ras and characteristic histopathology. Poor differentiation, a syncytial growth pattern, and pushing borders suggest RER+. *Am J Pathol* 1998, 152:1501-1507
 47. Wilentz RE, Goggins M, Redston M, Marcus VA, Adsay NV, Sohn TA, Kadkol SS, Yeo CJ, Choti M, Zahurak M, Johnson K, Tascilar M, Offerhaus GJ, Hruban RH, Kern SE: Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas. A newly described and characterized entity. *Am J Pathol* 2000, 156:1641-1651
 48. Osborne BM, Culbert SJ, Cangir A, MacKay B: Acinar cell carcinoma of the pancreas in a 9-year-old child: case report with electron microscopic observations. *South Med J* 1977, 70:370-372
 49. Mah PT, Loo DC, Tock EP: Pancreatic acinar cell carcinoma in childhood. *Am J Dis Child* 1974, 128:101-104
 50. Robin E, Terris B, Valverde A, Molas G, Belghiti J, Bernades P, Ruszniewski P: Pancreatoblastoma in adults. *Gastroenterol Clin Biol* 1997, 21:880-883
 51. Palosaari D, Clayton F, Seaman J: Pancreatoblastoma in an adult. *Arch Pathol Lab Med* 1986, 110:650-652
 52. Dunn JL, Longnecker DS: Pancreatoblastoma in an older adult. *Arch Pathol Lab Med* 1995, 119:547-551
 53. Hoorens A, Gebhard F, Kraft K, Lemoine NR, Kloppel G: Pancreatoblastoma in an adult: its separation from acinar cell carcinoma. *Virchows Arch* 1994, 424:485-490
 54. Levey JM, Banner BF: Adult pancreatoblastoma: a case report and review of the literature. *Am J Gastroenterol* 1996, 91:1841-1844
 55. Li M, Squire JA, Weksberg R: Molecular genetics of Beckwith-Wiedemann syndrome. *Curr Opin Pediatr* 1997, 9:623-629
 56. Li M, Squire JA, Weksberg R: Molecular genetics of Wiedemann-Beckwith syndrome. *Am J Med Genet* 1998, 79:253-259
 57. Kohda E, Iseki M, Ikawa H, Endoh M, Yokoyama J, Mukai M, Hata J, Yamazaki H, Miyauchi J, Saeki M: Pancreatoblastoma. Three original cases and review of the literature. *Acta Radiol* 2000, 41:334-337
 58. Koh TH, Cooper JE, Newman CL, Walker TM, Kiely EM, Hoffmann EB: Pancreatoblastoma in a neonate with Wiedemann-Beckwith syndrome. *Eur J Pediatr* 1986, 145:435-438
 59. Albrecht S, von Schweinitz D, Waha A, Kraus JA, von Deimling A, Pietsch T: Loss of maternal alleles on chromosome arm 11p in hepatoblastoma. *Cancer Res* 1994, 54:5041-5044
 60. Byrne JA, Simms LA, Little MH, Algar EM, Smith PJ: Three non-overlapping regions of chromosome arm 11p allele loss identified in infantile tumors of adrenal and liver. *Genes Chromosom Cancer* 1993, 8:104-111
 61. Kiechle-Schwarz M, Scherer G, Kovacs G: Cytogenetic and molecular studies on six sporadic hepatoblastomas. *Cancer Genet Cytogenet* 1989, 41:286
 62. Koufos A, Hansen MF, Copeland NG, Jenkins NA, Lampkin BC, Cavenee WK: Loss of heterozygosity in three embryonal tumours suggests a common pathogenetic mechanism. *Nature* 1985, 316:330-334
 63. Blaker H, Hofmann WJ, Reiker RJ, Penzel R, Graf M, Otto HF: Beta-catenin accumulation and mutation of the CTNNB1 gene in hepatoblastoma. *Genes Chromosom Cancer* 1999, 25:399-402
 64. Sheu JC, Lin YW, Chou HC, Huang GT, Lee HS, Lin YH, Huang SY, Chen CH, Wang JT, Lee PH, Lin JT, Lu FJ, Chen DS: Loss of heterozygosity and microsatellite instability in hepatocellular carcinoma in Taiwan. *Br J Cancer* 1999, 80:468-476
 65. Yumoto Y, Hanafusa T, Hada H, Morita T, Ooguchi S, Shinji N, Mitani

- T, Hamaya K, Koide N, Tsuji T: Loss of heterozygosity and analysis of mutations of p53 in hepatocellular carcinoma. *J Gastroenterol Hepatol* 1995, 10:179-185
66. Nishida N, Fukuda Y, Kokuryu H, Sadamoto T, Isowa G, Honda K, Yamaoka Y, Ikenaga M, Imura H, Ishizaki K: Accumulation of allelic loss on arms of chromosomes 13q, 16q and 17p in the advanced stages of human hepatocellular carcinoma. *Int J Cancer* 1992, 51: 862-868
 67. Kiechle-Schwarz M, Scherer G, Kovacs G: No evidence for loss of alleles at 11p in HBV negative hepatocellular carcinomas. *Genes Chromosom Cancer* 1990, 1:312-314
 68. Zhang WD, Hirohashi S, Tsuda H, Shimamoto Y, Yokota J, Terada M, Sugimura T: Frequent loss of heterozygosity on chromosomes 16 and 4 in human hepatocellular carcinoma. *Jpn J Cancer Res* 1990, 81: 108-111
 69. Kurahashi H, Takami K, Oue T, Kusafuka T, Okada A, Tawa A, Okada S, Nishisho I: Biallelic inactivation of the APC gene in hepatoblastoma. *Cancer Res* 1995, 55:5007-5011
 70. Oda H, Imai Y, Nakatsuru Y, Hata J, Ishikawa T: Somatic mutations of the APC gene in sporadic hepatoblastomas. *Cancer Res* 1996, 56: 3320-3323
 71. Koch A, Denkhaus D, Albrecht S, Leuschner I, von Schweinitz D, Pietsch T: Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. *Cancer Res* 1999, 59:269-273
 72. Park WS, Oh RR, Park JY, Kim PJ, Shin MS, Lee JH, Kim HS, Lee SH, Kim SY, Park YG, An WG, Kim HS, Jang JJ, Yoo NJ, Lee JY: Nuclear localization of beta-catenin is an important prognostic factor in hepatoblastoma. *J Pathol* 2001, 193:483-490
 73. Wei Y, Fabre M, Branchereau S, Gauthier F, Perilongo G, Buendia MA: Activation of beta-catenin in epithelial and mesenchymal hepatoblastomas. *Oncogene* 2000, 19:498-504
 74. Jeng YM, Wu MZ, Mao TL, Chang MH, Hsu HC: Somatic mutations of beta-catenin play a crucial role in the tumorigenesis of sporadic hepatoblastoma. *Cancer Lett* 2000, 152:45-51
 75. Takayasu H, Horie H, Hiyama E, Matsunaga T, Hayashi Y, Watanabe Y, Suita S, Kaneko M, Sasaki F, Hashizume K, Ozaki T, Furuuchi K, Tada M, Ohnuma N, Nakagawara A: Frequent deletions and mutations of the beta-catenin gene are associated with overexpression of cyclin D1 and fibronectin and poorly differentiated histology in childhood hepatoblastoma. *Clin Cancer Res* 2001, 7:901-908
 76. Legoux P, Bluteau O, Bayer J, Perret C, Balabaud C, Belghiti J, Franco D, Thomas G, Laurent-Puig P, Zucman-Rossi J: Beta-catenin mutations in hepatocellular carcinoma correlate with a low rate of loss of heterozygosity. *Oncogene* 1999, 18:4044-4046
 77. de La Coste A, Romagnolo B, Billuart P, Renard CA, Buendia MA, Soubrane O, Fabre M, Chelly J, Beldjord C, Kahn A, Perret C: Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci USA* 1998, 95:8847-8851
 78. Van Nhieu JT, Renard CA, Wei Y, Cherqui D, Zafrani ES, Buendia MA: Nuclear accumulation of mutated β -catenin in hepatocellular carcinoma is associated with increased cell proliferation. *Am J Pathol* 1999, 155:703-710
 79. Hsu HC, Jeng YM, Mao TL, Chu JS, Lai PL, Peng SY: β -catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. *Am J Pathol* 2000, 157:763-770
 80. Miyoshi Y, Iwao K, Nagasawa Y, Aihara T, Sasaki Y, Imaoka S, Murata M, Shimano T, Nakamura Y: Activation of the β -catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res* 1998, 58:2524-2527
 81. Terris B, Pineau P, Bregeaud L, Valla D, Belghiti J, Tiollais P, Degott C, Dejean A: Close correlation between β -catenin gene alterations and nuclear accumulation of the protein in human hepatocellular carcinomas. *Oncogene* 1999, 18:6583-6588
 82. Huang H, Fujii H, Sankila A, Mahler-Araujo BM, Matsuda M, Cathomas G, Ohgaki H: β -catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C infection. *Am J Pathol* 1999, 155:1795-1801
 83. Valizadeh A, Karayiannakis AJ, El-Hariry I, Kmiet W, Pignatelli M: Expression of E-cadherin-associated molecules (α -, β -, and γ -catenins and p120) in colorectal polyps. *Am J Pathol* 1997, 150: 1977-1984
 84. Brabletz T, Herrmann K, Jung A, Faller G, Kirchner T: Expression of nuclear β -catenin and c-myc is correlated with tumor size but not with proliferative activity of colorectal adenomas. *Am J Pathol* 2000, 156: 865-870
 85. Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangiu ML, Rimm DL: Frequent mutation and nuclear localization of β -catenin in anaplastic thyroid carcinoma. *Cancer Res* 1999, 59:1811-1815
 86. Fukuchi T, Sakamoto M, Tsuda H, Maruyama K, Nozawa S, Hirohashi S: β -catenin mutation in carcinoma of the uterine endometrium. *Cancer Res* 1998, 58:3526-3528
 87. Palacios J, Gamallo C: Mutations in the β -catenin gene (CTNNB1) in endometrioid ovarian carcinomas. *Cancer Res* 1998, 58:1344-1347
 88. Kuhn C, Herter P, Muller O, Muehlberger T, Krause L, Homann H, Steinau HU, Muller KM: β -catenin in soft tissue sarcomas: expression is related to proliferative activity in high-grade sarcomas. *Mod Pathol* 2000, 13:1005-1013