Pancreatic Carcinoma in Carriers of a Specific 19 Base Pair Deletion of CDKN2A/p16 (p16-Leiden)\(^1\)


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ABSTRACT

Purpose: The purpose is to document the clinical, pathological, and genetic features of pancreatic carcinoma (PC) in carriers of a specific p16-Leiden mutation (a 19-bp deletion in exon 2 of the CDKN2A gene).

Experimental Design: Clinical data and paraffin embedded tissue were obtained from 12 patients of p16-Leiden-positive families with PC. Because of the known 19-bp germ line deletion, we could specifically analyze the genotype of the wild-type allele for loss of heterozygosity.

Results: Immunostaining for p16, Tp53, Smad4, and cyclooxygenase 2 was performed. The average age of subjects that developed PC (8 males) was 58 years (range, 43–74 years). Histology was considered as conventional ductal adenocarcinoma in 11 of 12 and neuroendocrine carcinoma (1 of 12). The carcinomas were located in the head (10 of 12), corpus (1 of 12), and tail (1 of 12) of the pancreas. The specific p16-Leiden mutation was confirmed in the tissue of all subjects. Loss of heterozygosity of the wild-type allele was present in 2 of 7 tumors analyzed. Immunostaining for p16 was negative in 10 of 10. Tp53 mutations were detected in 5 of 12. Smad4 was negative in 5 of 12 and cyclooxygenase 2 was overexpressed in 11 of 12. K-ras codon 12 mutations were present in 9 of 10 and in three precursor lesions even before abrogation of p16 protein expression was seen (one of three).

Conclusions: The p16-Leiden deletion was associated with progression toward conventional ductal adenocarcinomas in all cases but one. Our observations might support the feasibility of early diagnosis of PC in p16-Leiden mutation carriers and might also indicate that chemoprevention needs consideration.

INTRODUCTION

Carcinoma of the pancreas is the fifth most common cause of death from cancer in the Western world and its incidence is still growing (1). Because of aggressive growth and early dissemination, the overall 5-year survival rate of patients with pancreatic carcinoma is only 3–5\% (2). Surgery gives the only chance of cure of the disease. Unfortunately, however, only 15–20\% of the carcinomas are detected at a resectable stage (3). The 5-year survival rate after surgery is \(\sim 20\%\) (3, 4). When the tumor is detected at an early stage, the overall 5-year survival rate increases (5–7). Sporadic pancreatic tumors are mostly mucin-producing ductal cell carcinomas that arise in the pancreatic head in 60–70\% of the cases (8). As in many types of cancer, aggregation of pancreatic cancer in certain families has been described either site specific or in the context of Peutz-Jeghers, BRCA2, or FAMMM\(^3\) (9, 10).

FAMMM syndrome is characterized by the familial occurrence of melanoma of the skin in combination with multiple atypical precursor nevi (11). Previous studies demonstrated that FAMMM families with a germ-line mutation in the CDKN2A gene are not only at risk for melanoma but also for the development of other cancers, in particular pancreatic cancer (12, 13). Recently, we demonstrated that FAMMM family members with a specific 19-bp deletion of p16 (a 19-bp deletion in exon 2 of the CDKN2A gene): p16-Leiden have a 17\% risk of developing pancreatic cancer by the age of 75 years (9). The possibility of DNA testing, in known p16-Leiden FAMMM families in the Netherlands, makes it possible to identify carriers of this gene defect.

In nonfamilial conventional pancreatic cancer, p16 is commonly altered. p16 is an inhibitor of the cyclin-dependent kinases that control passage through G1 (14). Somatic inactivation of p16 in sporadic pancreatic cancer has been found in \(\geq 95\%\) of the cases (15). This inactivation occurs by intragenic mutation in one allele associated with loss of the other allele (LOH), by

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\(^1\)The abbreviations used are: FAMMM, familial atypical multiple mole melanoma; LOH, loss of heterozygosity; TNM, tumor-node-metastasis; PanIN, pancreatic intraepithelial neoplasia nomenclature: AIF, allelic imbalance factor; IHC, immunohistochemistry; COX-2, cyclooxygenase 2; CDA, conventional ductal adenocarcinoma.

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deletion of both alleles (homozygous deletion), or by promoter hypermethylation (16–23). Much of the multistep pathway to pancreatic cancer is still unknown. For carriers of a specific 19-bp deletion of p16, there may be similarities in the progression to pancreatic cancer such as the inactivation of the wild-type allele of p16 and inactivation of other tumor suppressor genes. The aim of the present study was to document the clinical, pathological, and genetic features of pancreatic cancer in carriers of a specific 19-bp deletion of p16 to obtain insights pertinent for a possible surveillance program.

PATIENTS AND METHODS

The FAMMM Registry. Data were obtained from the Dutch population-based registry of FAMMM families. The registry was founded in 1989 as the Netherlands Foundation for the Detection of Hereditary Tumors. The aims of the FAMMM Registry are as follows: (a) to promote the identification of families with the FAMMM syndrome; (b) to implement surveillance programs within these families; and (c) to guarantee the continuity of lifelong surveillance by a computerized follow-up system. The organization and methods of the registry have previously been reported (24). By January, 1, 2001, 130 FAMMM families had been registered. Only a subset of these families was available for Cdk2 mutation detection: a founder mutation (p16-Leiden) has been identified in 20 families. Within these families, we obtained clinical data and paraffin-embedded tumor tissue of all the subjects known to have developed pancreatic cancer for whom tissue was still available. Clinical data and paraffin-embedded tumor tissue of 12 pancreatic cancer patients (8 males) of 11 FAMMM families with this p16-Leiden germ-line mutation were obtained. Relatives of the deceased subjects gave informed consent.

Clinical Data. The tumor differentiation and staging according to the TNM classification, the location as well as the type of surgery were derived from the medical reports (25). The age of the subjects was registered at the time of tissue diagnosis of pancreatic carcinoma. Survival was measured in months from date of tissue diagnosis of pancreatic cancer until the date of death.

Tumor Analysis. Two pathologists revised the histology (H. M. and G. J. A. O.). When precursor ductal lesions were identified, these were classified using the PanIN (26, 27). Briefly, PanIN-1 lesions have a flat or papillary mucinous epithelium without cellular atypia, whereas PanIN-2 lesions show increasing signs of cellular atypia and a prevalence of papilliferous architecture. Finally, PanIN-3 lesions correspond to carcinoma in situ lesions. Areas with precursor lesions and with highest tumor density were selected for DNA isolation and additional molecular analysis. Serial sections were produced for immunohistochemical analysis (see “IHC”).

DNA Isolation. Genomic DNA of normal, precursor lesions and tumor tissue was isolated from formalin-fixed, paraffin-embedded material by both microdissection (Palm Microlaser Technologies) and by punching 0.6-mm tissue of areas containing >40% tumor (Beecher Instruments). Using a Chelex extraction method, DNA was isolated from punches, resuspended in 96 μl of PK-1 lysis buffer [50 mM KCl, 10 mM Tris (pH 8.3), 2.5 mM MgCl2, 0.45% NP40, 0.45% Tween 20, and 0.1 mg/ml gelatin] containing 5% Chelex beads (Bio-Rad, Hercules, CA) and 5 μl of proteinase K (10 mg/ml) and incubated for 12 h at 56°C. The suspension was incubated 10 min at 100°C, centrifuged, and the supernatant was carefully decanted.

PCR Amplification. The p16-Leiden deletion extends 19 bp and removes nucleotides at positions 225–243 of exon 2 (28). Genomic DNA from tumor and normal tissue was subjected to PCR amplification using labeled primers containing the 225–243 region; p16-forward-TET M1 (tumor) or FAM M1 (normal), sequence 5'-ATGATGGGCAGCGCCCGAGT-3' and p16-reverse A2, sequence 5'-ACCAGCGTGTCAG-GAAG-3' (Life Technologies). The total volume/reaction was 12 μl, including 0.5 μmol of each primer, a mix of 0.25 μl of deoxyribonucleoside triphosphate (10 mM), 1.2 μl of magnesium chloride (20 mM), 1.2 μl of BSA (1 mg/ml), 1.2 μl of AmpliTaq gold buffer (without MgCl2), and 0.25 μl of AmpliTaq gold DNA polymerase, 10 ng of normal or tumor DNA and H2O. The following conditions were used: 10 min denaturation at 96°C, 33 cycles of 1 min 94°C; 2 min 60°C; 1 min 72°C and a final elongation step of 6 min 72°C in a Gene AMP 9700 thermocycler (Applied Biosystems, Foster City, CA). Mixtures of 24 μl of dionised formamide, 1 μl of 6-carboxyfluorescein-labeled nucleotide probe for the wild-type K-ras-2, 500 (Applied Biosystems), and 1.2 μl of PCR product were run on an ABI 310 Genetic analyzer (Applied Biosystems) for 20 min with run profile GS STR POP4 (1.0 ml) C and analyzed with Genescan 3.1 computer software (Perkin-Elmer Corp.).

LOH Analysis. LOH was scored when both normal tissue and tumor tissue of the pancreas or metastases were present. LOH was scored when the intensity of one allele was decreased in the tumor sample with respect to the matched wild-type allele from normal tissue. The quotient of the peak height ratios from normal and tumor DNA served as AIF. The threshold for allelic imbalance was defined as 40% reduction of one allele, corresponding with an AIF of ≤0.59 or ≥1.7. The threshold for retention was defined to range from 0.76 to 1.3 as previously empirically determined (29). AIFs of 0.60–0.75 and 1.3–1.69 were considered to belong to a so-called gray area, for which no definitive decision has been made.

K-ras-2 Gene Mutations. The microdissected foci were evaluated for activating point mutations in codon 12 of K-ras-2 as has been described previously (30). Briefly, DNA was isolated and subjected to PCR amplification using primers A (5'-ACTGAAATATAAATTTGTTGAATGCACCT-3') and D (5'-TCATGAAATTTGTTCAAGAAACC-3'). This set of primers introduces an MvaI restriction site in PCR products derived from wild-type codon 12 alleles. Mutant enrichment was performed by digestion of the PCR product using the restriction enzyme MvaI. This enzyme cleaves the wild-type but not the mutant alleles of K-ras-2. A 1-μl aliquot of the digested first PCR was then subjected to a second round of amplification using the seminested primers A and B (5'-TCAAAAGATT-GTCTCTGGACC-3'). The resulting DNA fragments were then spot-blotted onto seven different nylon membranes, and each of these membranes was hybridized with an allele-specific oligonucleotide probe for the wild-type K-ras-2 sequence or for one of the six possible mutations in codon 12 of K-ras-2. Positive controls included cloned wild-type and mutant sequences, and
no DNA was added in the negative controls. All allele-specific oligonucleotide analyses were performed in duplicate.

IIIC. Four-μm tissue sections were prepared on 3 amino propyltriethoxysilane (Sigma A3648)-coated slides and dried overnight in a 37°C stove. Sections were deparaffinized in xylene (3 × 5 min), endogenous peroxidase was blocked by incubation in methanol/H2O2 0.3% for 20 min, and rehydration took place with alcohol and distilled water. Antigen retrieval for p16, Tp53, Smad4, and COX-2 immunostaining was performed by microwaving in boiling 0.01 M sodium citrate buffer (pH 6.0) for 10 min. After cooling for 2 h and washing (2 × 5 min) in PBS, the sections were incubated overnight at room temperature with monoclonal mouse antihuman antibodies: p16 (1:500, clone JC8; Neomarkers, Fremont, CA); Tp53 (1:1000, clone DO-7; Neomarkers); Smad4 (1:100, B-8; Santa Cruz Biotechnology, Santa Cruz, CA); COX-2 (1:100, 160112; Cayman Chemical Co., Ann Arbor, MI); chromogranin (1:250; Dako, Glostrup, Denmark); and synaptophysin (1:50; Dako) in PBS/BSA 1%. Sections were subsequently washed (3 × 5 min in PBS) and incubated (30 min) with biotinylated secondary antibody in PBS/BSA 1%, washed (3 × 5 min in PBS), and incubated (30 min) with a horseradish peroxidase/streptavidin complex. This complex was prepared 30 min before the sections were incubated. Diaminobenzidine tetrahydrochloride was used as a chromogen, followed by counterstaining with hematoxylin. Slides were dehydrated in xylene. For a negative control, the primary antibody was omitted.

Light Microscopic Evaluation. Expression was evaluated by light microscopy. Loss of p16 and Smad4 expression was considered as present when there was no positive staining of the nuclei in the pancreatic tumor tissue and when an internal positive control (nuclei of normal tissue that stained positive) was observed. As a result of a short half-life, the normal Tp53 is not detectable immunohistochemically; however, the mutant form, accumulating within tumor cells, is usually easily detectable. As a result of a short half-life, the normal Tp53 is not detectable immunohistochemically; however, the mutant form, accumulating within tumor cells, is usually easily detectable (31, 32). There appears to be a good correlation between over expression of Tp53 and Tp53 gene mutations (32). The percentages of nuclei that stained positive were assessed. Expression of Tp53 was categorized into four groups: (a) no expression (<1% positive); (b) low expression (1% less than positive-stained nuclei < 25%); (c) moderate expression (25% less than positive-stained nuclei < 75%); and (d) high expression (>75% positive). This scoring was used to determine the likelihood of a Tp53 mutation. (a) No mutation cannot be ruled out, (b) probably wild-type, (c) possible mutation, (d) most likely a mutation. The immunoreactivity score for COX-2 was divided into four categories: absent; weak (1+); moderate (2+); and strong (3+). A score of ≥2+ was considered as positive (overexpression). The immunoreactivity score of chromogranin and synaptophysin was scored as absent or present.

RESULTS

Clinical data. All subjects diagnosed with pancreatic cancer were symptomatic at the time of diagnosis. Histological typing comprised CDA in 11 of 12 and neuroendocrine carcinoma (1 of 12). The average age of developing pancreatic cancer was 58 years (range, 43–74 years). Detected tumors were at TNM stages I (n = 1), II (n = 1), III (n = 2), IV (n = 7), and unknown (n = 1). Ten of 12 tumors (83%) were located in the head of the pancreas, 1 tumor (8%) was located in the transition zone between head and corpus, and 1 (8%) was located in the tail of the pancreas. Four of 12 subjects (33%) underwent a pancreateoduodenectomy according to Whipple (33). Two subjects (17%) underwent palliative surgery (both underwent a biliodesophagean anastomosis, and 1 underwent an additional gastrecterostomy). Six subjects (50%) underwent no surgery at all. The average survival of all 12 patients was 7 months (range, 0–13 months). The above data are listed in Tables 1 and 2.

PCR and LOH Analysis. In all subjects, the p16-Leiden mutation was confirmed by PCR as shown in Fig. 1. LOH could be assessed for 7 of 12 tumors. The other 6 subjects only underwent a diagnostic biopsy in which tumor tissue but not enough control tissue was present. LOH was present in 2 of 7 tumors as illustrated in Fig. 1 and Table 2. Retention of the wild-type allele was present in the 5 remaining tumors.

K-ras-2 Mutations Analysis. Mutation analysis of the K-ras-2 oncogene in codon 12 revealed an activating mutation in 9 tumors (Table 2). Wild-type codon 12 was detected in one tumor (tumor no. 8). The amount of tumor in the two remaining tumors was too little for reliable testing. A bp substitution from wild-type glycine (GGT) to aspartic acid (GAT) was detected in 5 tumors (56%), to valine (GTT) in 3 tumors (33%), and to arginine (CGT) in 1 tumor (11%). Furthermore, mutation analysis revealed activating mutations in three precursor lesions of two tumors. In the PanIN-Ia lesion of tumor no. 1 (Table 3), the same mutation was detected (i.e., GGT→GAT) as in the adenocarcinoma. In the PanIN-II lesion of tumor no. 3 (GGT→GTT), two additional mutations were detected (i.e., AGT and GCT).

Immunohistochemistry. The results of immunohistochemistry are shown in Tables 2 and 3. Fig. 2 shows an example of different stainings of the CDA no. 2 (Tables 1 and 2). When precursor lesions were present, these were also scored as shown in Table 3. Expression of the p16 gene product was absent in all tumors. However, in 2 of 12 tumors, there was no positive staining of internal control tissue (tumor nos. 8 and 12). p16 stained positive in the analyzed precursor lesions of 2 tumors as shown in Fig. 3. Tp53 stained strongly positive (category 4) in 5 of 12 tumors, perhaps indicative of a mutation. Smad4 was

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Table 1. Clinical features of pancreatic cancer in p16-Leiden mutation carriers.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>TNM stage</th>
<th>Location</th>
<th>Surgery</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>74</td>
<td>I</td>
<td>Head</td>
<td>Whipple</td>
<td>9.1</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>60</td>
<td>II</td>
<td>Head</td>
<td>Whipple</td>
<td>13.2</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>51</td>
<td>III</td>
<td>Head</td>
<td>Whipple</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>50</td>
<td>U</td>
<td>Head</td>
<td>Whipple</td>
<td>13.3</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>56</td>
<td>III</td>
<td>Head</td>
<td>Palliative</td>
<td>7.6</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>62</td>
<td>IV</td>
<td>Tail</td>
<td>No</td>
<td>8.3</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>58</td>
<td>IV</td>
<td>Head</td>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>49</td>
<td>IV</td>
<td>Head</td>
<td>No</td>
<td>†</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>72</td>
<td>IV</td>
<td>H-Corpus</td>
<td>No</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>46</td>
<td>IV</td>
<td>Head</td>
<td>No</td>
<td>1.9</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>64</td>
<td>IV</td>
<td>Head</td>
<td>Palliative</td>
<td>0.3</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>43</td>
<td>IV</td>
<td>Head</td>
<td>No</td>
<td>7.3</td>
</tr>
</tbody>
</table>

*F, female; M, male; U, unknown; †, diagnosis during autopsy.
negative in 5 of 12, and COX-2 was positive in 11 of 12. In two of the analyzed precursor lesions, COX-2 was only weakly positive (1+). Ten of 12 tumors were negative for both chromogranin and synaptophysin. One was positive, an indication of its neuroendocrine-like phenotype. The remaining 1 was not analyzed.

DISCUSSION

Recently, we demonstrated that FAMMM family members with a specific 19-bp deletion of CDKN2A (p16): p16-Leiden have an increased risk of developing pancreatic cancer on statistical grounds (9). This study demonstrates by molecular genetic methods that all subjects who developed pancreatic cancer within the p16-Leiden-positive FAMMM families were carriers of the specific deletion. All but 1 tumor were CDA.

Because of limited amount of paraffin tissue containing primarily adenocarcinoma, we were not able to estimate whether the frequency of precursor lesions is increased in p16-Leiden mutation carriers in comparison with sporadic pancreatic carcinomas. Inactivation of p16 is an early event in the genetic progression in pancreatic ducts. In sporadic pancreatic cancer, already 30–50% of the PanIN-I lesions lack expression of p16 (15, 34). In this study, the analyzed PanIN-I lesions of 2 of 4 tumors lacked expression of p16. In invasive carcinomas, inac-

Table 2 Pathological features of pancreatic cancer in p16-Leiden mutation carriers

<table>
<thead>
<tr>
<th>No.</th>
<th>Tissue available</th>
<th>Histology</th>
<th>T percentage</th>
<th>LOH</th>
<th>K-ras-2</th>
<th>p16</th>
<th>Tp53</th>
<th>Smad4</th>
<th>COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whipple</td>
<td>CDA</td>
<td>50</td>
<td>No</td>
<td>GAT</td>
<td>–</td>
<td>1</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Duodenum bp</td>
<td>CDA</td>
<td>40</td>
<td>No</td>
<td>GAT</td>
<td>–</td>
<td>1</td>
<td>+</td>
<td>Pos</td>
</tr>
<tr>
<td>3</td>
<td>Whipple</td>
<td>CDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pancreas bp</td>
<td>CDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Duodenum bp</td>
<td>CDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Liver bp</td>
<td>CDA</td>
<td>50</td>
<td>Yes</td>
<td>GAT</td>
<td>–</td>
<td>4</td>
<td>+</td>
<td>Pos</td>
</tr>
<tr>
<td>7</td>
<td>Autopsy</td>
<td>CDA</td>
<td>40</td>
<td>No</td>
<td>GTT</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>Pos</td>
</tr>
<tr>
<td>8</td>
<td>Autopsy</td>
<td>CDA</td>
<td>&gt;50</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>Autopsy</td>
<td>CDA</td>
<td>&gt;40</td>
<td>No</td>
<td>GTT</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>Pos</td>
</tr>
<tr>
<td>10</td>
<td>Autopsy</td>
<td>CDA</td>
<td>40</td>
<td>No</td>
<td>GAT</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>Pos</td>
</tr>
<tr>
<td>11</td>
<td>Pancreas bp</td>
<td>CDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Only few tumor cells.

No internal positive control.

NC: neuroendocrine carcinoma; Pos, positive.

Fig. 1 LOH result of analyzed pancreatic carcinoma of p16-Leiden mutation carrier no. 6 (Tables 1 and 2). The p16-Leiden deletion extends 19 bp and removes nucleotides at positions 225–243 of exon 2. Genomic DNA from tumor and normal tissue was subjected to PCR amplification using labeled primers (tumor tissue, green; normal control, blue) containing the 225–243 region. Because of the known 19-bp deletion, we could specifically analyze the genotype of the wild-type allele in terms of LOH; LOH of the wild-type p16 allele was present in 2 of 7 tumors analyzed.
tivation of p16 has been found in >95%. In this study, p16 seemed inactivated in all pancreatic carcinomas. Because of the specific 19-bp deletion, we could analyze the fate of the wild-type allele in terms of LOH. LOH as a second hit of inactivation was present in 2 of 7 cases analyzed. However, the quality of the isolated DNA was poor, and the LOH that was present could have been missed. Other mechanisms of inactivation of the wild-type allele remain to be determined as p16 was inactivated in all cases. Unfortunately, however, we were not able to perform methylation-specific PCR because of the poor DNA quality.

In contrast to p16, alterations in Tp53 and Smad4 seem to be late events in the genetic progression of tumor in pancreatic ducts. Overexpression of Tp53 is a frequent event in pancreatic carcinogenesis and may also be associated with a poor prognosis (35, 36). We found strong overexpression in 5 of 12 (42%) of the pancreatic tumors of p16-Leiden mutation carriers, perhaps indicative of a mutation (37). However in 6 other cases (5 with lack of protein expression and 1 with moderate expression), mutations in Tp53 could not be ruled out. Overexpression of Tp53 was not related to a poorer prognosis, but numbers are, of course, small. Smad4 was negative in 5 of 12 (42%) of the carcinomas, similar to the results in sporadic pancreatic cancer
Staining intensities of both Tp53 and Smad4 in the few analyzed precursor lesions suggested a wild-type status, thereby implying that the involvement of Tp53 and Smad4 in carcinogenesis are late events.

To date, few options exist for the treatment of pancreatic cancer. Therefore, chemoprevention would be of great interest for the increasing number of identified subjects at risk for pancreatic cancer (e.g., p16-Leiden mutation carriers). Multiple lines of evidence suggest that the enzyme COX, specifically COX-2, may be a promising chemotherapeutic target (41). Therefore, we performed immunostaining for COX-2 on the archival p16-Leiden-pancreatic carcinomas. COX-2 stained positive in all but 1 tumor. In the few PanIN lesions we analyzed, the expression of COX-2 was only weak. Whether p16-Leiden mutation carriers may benefit from COX-2 inhibitors has yet to be determined. K-ras-2 mutations occur before the development of invasive cancer and might offer an opportunity to screen for early disease (42–43). In p16-Leiden mutation carriers as well as in sporadic pancreatic cancer, the most common alteration in codon 12 of K-ras-2 is a bp substitution from wild-type glycine (GGT) to aspartic acid (GAT). The latter mutation was also found in the PanIN lesions of two tumors analyzed, even with retained protein expression of p16 in one of the PanINs that could be characterized in both ways. Whether in p16-Leiden mutation carriers, total abrogation of p16 often occurs after K-ras-2 activation remains to be further determined. Nitrosamines contained in cigarette smoke might induce G-to-A transitions at the second nucleotide of a GG pair (44). Discouraging smoking in the group of mutation carriers seems a very important preventive measure anyway.

In many tumor syndromes, surveillance of subjects at risk of developing cancer leads to detection of tumors at an early stage and improves overall survival (24, 45). Pancreatic cancer, detected in symptomatic patients, has an extremely poor prognosis (2). The prognosis improves when surgery takes place at an early stage (5–7). In view of these findings, p16-Leiden mutation carriers might benefit from regular surveillance examinations. However, when suspected lesions are detected, diagnostic accuracy is extremely important as major surgical procedures are involved.

In conclusion, in all but 1 case, the p16-Leiden deletion was associated with progression toward CDAs. As in sporadic pancreatic cancer, LOH of the wild-type p16 allele might be the mechanism of inactivation in part of these tumors. Results of immunostaining for different tumor suppressor genes and K-ras-2 mutation analysis in 12 historic pancreatic carcinomas of p16-Leiden-positive subjects were also quite similar to the observations in sporadic pancreatic cancer. Early detection by surveillance of family members at risk may be an option that needs consideration. Similarly, potential chemopreventive agents need additional study.

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Pancreatic Carcinoma in Carriers of a Specific 19 Base Pair Deletion of CDKN2A/p16 (p16-Leiden)


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