K-ras and p53 in cancer of the pancreas and extrahepatic biliary tract

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K-ras Mutations in the Duodenal Fluid of Patients with Pancreatic Carcinoma


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ABSTRACT

Many patients with carcinoma of the pancreas die because their disease is not detected until late in its course. Methods that detect these cancers earlier will improve patient outcome. Over 80% of pancreatic carcinomas contain mutations in codon 12 of the K-ras gene. Screening duodenal fluid for these mutations may lead to early detection of these cancers and assist in establishing a diagnosis of pancreatic carcinoma.

Polymerase chain reaction (PCR), with and without restriction enzyme mediated mutant enrichment, was performed on DNA isolated from duodenal fluid specimens from 61 patients who underwent pancreaticoduodenectomy (Whipple's operation) for either peripancreatic cancer or a benign condition of the pancreas. Representative sections of pancreas pathology (primary carcinoma, benign tumor, or chronic pancreatitis) from the patients with duodenal fluid specimens containing amplifiable DNA were also analyzed for K-ras mutations. Wild-type and mutant K-ras were detected by hybridization of the PCR products with K-ras codon 12 mutant and wild-type specific probes.

Seven of the 61 duodenal fluid specimens contained DNA that did not amplify. Thirteen (24% of the 54 duodenal fluid specimens with amplifiable DNA and 21% of the total of 61 specimens) contained activating point mutations at codon 12 of the K-ras gene. Mutations were detected in 13 of the 51 duodenal fluid specimens from patients with cancer (sensitivity, 25%), whereas mutations were not detected in any of the 9 amplifiable duodenal fluid specimens from patients with benign conditions of the pancreas (specificity, 100%). One duodenal fluid specimen from a patient with adenocarcinoma of the pancreas had two different K-ras mutations. DNA from three of the primary carcinomas did not amplify or was not available. Twenty-nine (69%) of the primary tumors with amplifiable DNA contained K-ras mutations, whereas 3 (30%) of the 10 pancreata with benign conditions harbored mutations. Twenty-two (65%) of 34 ductal adenocarcinomas of the pancreas with amplifiable DNA had K-ras mutations. It is noteworthy that the same mutations were present in both the duodenal fluid and the primary carcinomas in 11 (92%) of the 12 patients who had primary tumors with amplifiable DNA as well as K-ras mutations in their duodenal fluid specimens.

The identification of genetic alterations in cancer-causing genes in duodenal fluid may form the basis for the development of new approaches to the detection of carcinoma of the pancreas. Some pancreata without cancer, however, may also harbor K-ras mutations, potentially limiting the specificity of K-ras-based tests.
Introduction

Although carcinoma of the pancreas accounts for only 2% of new cancer cases in the United States, it is the fifth leading cause of cancer-related death [1]. By the time many patients with the disease are diagnosed, the carcinoma has already metastasized and is no longer curable. Although the 5-year survival for all patients with carcinoma of the pancreas is 3%, 5-year survival after successful pancreaticoduodenectomy (Whipple's operation) approaches 20% overall and maybe as high as 40% for patients with favorable prognostic factors, such as lymph node negative disease, negative margins, and diploid tumor DNA [2-4]. Therefore, methods that can detect pancreatic neoplasms earlier, when they are still resectable, may improve patient outcome.

The identification of molecular genetic changes can form the basis of such methods. For carcinomas of the pancreas, mutations in the K-ras gene, which produces a protein involved in signal transduction, are especially well suited for the task [5]. First, mutations in the K-ras gene are extremely common in pancreatic neoplasia. Between 80% and 100% of pancreatic carcinomas harbor activating point mutations in K-ras [6-10]. This suggests that K-ras is a sensitive marker for the presence of pancreatic carcinoma. Second, most of these mutations are single amino acid changes restricted to codon 12 of the K-ras gene [6-10]. This greatly reduces the number of probes that need to be employed to detect these changes, thus markedly simplifying the assays. Third, K-ras mutations are easily detectable, even when cells harboring the mutations are admixed with much larger numbers of normal cells. Mutant cells can be detected in specimens in which the cancer cells comprise only a small percentage of the cells. Indeed, K-ras mutations have already been found in pancreatic juice, fine-needle aspirations of the pancreas, endoscopic retrograde cholangio-pancreatography (ERCP) brushings, duodenal fluid, and even in the blood and stool of patients with pancreatic carcinomas [11-20]. Mutations in codon 12 of the K-ras gene are, however, not limited to invasive cancers. K-ras mutations also occur in noninvasive pancreatic ductal lesions [11,20-30].

The purpose of this study was to determine whether, in a well controlled, routine clinical setting, K-ras mutations could be detected in duodenal fluid obtained from Whipple's operation specimens from a large number of patients, and whether K-ras mutations in duodenal fluid are sensitive, specific markers for cancer. In this study, the origins of the mutations detected in the duodenal fluid specimens were confirmed by analyzing tissue obtained from the resected pancreata. This study furthers the development of relatively noninvasive molecular techniques for detecting periampullary cancer by broadening the patient base, increasing the diversity of sources in which mutations could be detected, and correlating the findings in the secondary source with those in the primary tissue.

Materials and Methods

Specimen Collection

All procedures were approved by The Johns Hopkins Medical Institutional Review Board. Sixty-one patients who underwent pancreaticoduodenectomy (Whipple's operation) at The Johns Hopkins Hospital for either periampullary cancer or a benign condition (chronic pancreatitis or serous cystadenoma) between July 6, 1994 and June 6, 1995, were randomly selected from among 108 patients without regard to diagnosis, age, gender or race. The proximal and distal enteric margins of the Whipple resection were stapled closed by the surgeon, yielding a tube of gastrointestinal tract that contained a duodenal fluid specimen. The Whipple resection was transported to the surgical pathology laboratory, dissected under sterile conditions, and duodenal fluid was collected by opening and draining the distal end of the gastrointestinal tract. The duodenal fluid speci-
men was frozen immediately and assigned a code independent of patient identifiers to assure patient confidentiality. The Whipple resection was then examined macroscopically and submitted for routine diagnostic histology [31]. All analyses were performed without knowledge of the patients' diagnoses. Preoperative duodenal fluid aspirates from ERCP were not available.

The primary neoplasms and nonneoplastic pancreatic tissue from the patients with chronic pancreatitis were analyzed by cutting 5 µm sections from formalin-fixed, paraffin-embedded tissue blocks and microdissecting areas of interest. In the case of chronic pancreatitis, sections showing papillary hyperplasia with and without atypia were selected. In the tumor cases, neoplastic tissue was harvested with as little nonneoplastic tissue as possible.

Specimen Preparation and DNA Extraction
DNA was purified from duodenal fluid using a modification of a previously described protocol [20,32]. One ml of each duodenal fluid specimen was thawed and brought to final concentrations of 1% sodium dodecyl sulfate and 100 µg/mL proteinase K. The specimens were then incubated at 56 °C for 16-18 hours, followed by a phenol-chloroform extraction. After the addition of 20 µg glycogen, 80 µL 3M Na-acetate, and 1.6 mL 96% ethanol, the specimen was cooled to -20 °C for 16-18 hours and centrifuged at 4 °C for 1 hour. The pellet was washed with 75% ethanol twice and air-dried for at least 1 hour. After resuspension in 100 µL TE buffer, the solution was stored at 4 °C for 16-18 hours and then at -20 °C until use. Negative control samples (water only) were included throughout the series. They were subjected to the identical extraction and precipitation steps as the duodenal fluid specimens and dotted to each membrane along with the duodenal fluid samples.

The primary tumors and nonneoplastic pancreatic tissue were microdissected and placed in 50-200 µL DNA isolation buffer (10 mM Tris-HCl pH = 8, 0.2% Tween 20, and 100 µg/mL proteinase K [20,33]). The mixture was incubated at 56 °C for 16-18 hours, and the proteinase was inactivated at 95 °C for 10 minutes. The mixture was stored at -20 °C until use.

Polymerase chain reaction (PCR) was performed on two independently purified sets of DNA for each duodenal fluid specimen.

Detection of K-ras Codon 12 Mutations
DNA isolated from either the duodenal fluid or the primary tissue was screened for point mutations according to a modification of a previously described protocol [8,34]. First, DNA from each specimen was amplified by PCR for 15 cycles with primers A (5' ACT GAA TAT AAA CTT GTC GTA GTT GGA CCT 3') and D (5' TCA TGA AAA TGG TCA GAG AAA CC 3'). The PCR product was then split into two equal portions. One of the two portions was digested with MvaI, an isoschizomer of BstNI (Boehringer-Mannheim, Mannheim, Germany). Then both portions were amplified again. This second PCR was performed for 35 cycles with primers A and B (5' TCA AAG AAT GGT CCT GGA CC 3'). Because MvaI cleaves wild-type but not mutant K-ras, a non-enriched sample and a sample enriched for mutant K-ras were produced. Finally, each set of PCR products was denatured at 95 °C for 10 minutes, spotted onto 7 different nylon membranes (Gene-Screen Plus, NEN Research Products, Boston, MA), and hybridized to each of the 7 32P-labeled, sequence specific oligodeoxynucleotide probes [35]. A final stringency wash at 63 °C and autoradiography were carried out. PCR products amplified from plasmid clones containing each of the 7 possible sequences at codon 12 were used as positive controls on the hybridization filters.

Statistical Analysis
All available clinical and pathologic data related to these cases were obtained from hospital records. Continuous variables, such as age and tumor size, were analyzed with one-way analysis of variance.
K-ras mutations in duodenal fluid

TABLE 1
Clinical and pathologic data on all patients who underwent pancreaticoduodenectomy (n=61)

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Periampullary cancer (n=51)</th>
<th>Benign condition (n=10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>64</td>
<td>65</td>
<td>57</td>
<td>0.07</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>54</td>
<td>55</td>
<td>50</td>
<td>0.78</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% white)</td>
<td>88</td>
<td>90</td>
<td>80</td>
<td>0.46</td>
</tr>
<tr>
<td>(% black)</td>
<td>10</td>
<td>8</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>(% other)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Tests, whereas discrete variables, such as gender, race, tumor differentiation, presence of tumor invasion, and presence of lymph node metastases, were analyzed with chi-square tests. All statistical analyses were performed with two-sided tests. P values of 0.05 and less were considered statistically significant.

Results

PCR, with and without restriction enzyme-mediated mutant enrichment, was performed on DNA isolated from duodenal fluid obtained from 61 patients who underwent pancreaticoduodenectomy (Whipple's operation) for periampullary cancer (n = 51) or a benign condition of the pancreas (n = 10, 9 cases of chronic pancreatitis and 1 case of a serous cystadenoma) (Table 1). There was no significant gender, age or race difference between patients in the two groups. Of the 51 patients with periampullary cancer, 43 had pancreatic adenocarcinomas, 3 had intraductal papillary mucinous neoplasms (one with small foci of invasive adenocarcinoma), 2 had mucinous cystadenocarcinomas, 1 had a solid and cystic papillary tumor of the pancreas (Hamoudi tumor), 1 had a bile duct adenocarcinoma, and 1 had an ampullary adenocarcinoma. One of the patients with pancreatic adenocarcinoma actually had two macroscopically distinct adenocarcinomas, one arising in the neck (4.0 cm) and one in the head (3.0 cm) of the pancreas.

Seven of the duodenal fluid specimens contained DNA that did not amplify. Six of these were from patients with periampullary cancer, and one was from a patient with a benign condition. Thirteen (24% of the 54 duodenal fluid specimens with amplifiable DNA and 21% of the total 61 specimens) contained activating point mutations at codon 12 of the K-ras gene. Mutations were detected in 13 of the 51 specimens from patients with periampullary cancer (sensitivity, 25%), whereas mutations were not detected in the amplifiable duodenal fluid of any of the 9 patients with benign conditions (specificity, 100%).

There are six known activating point mutations at codon 12 of the K-ras gene. Wild-type GGT (glycine) can be changed to TGT (cysteine), AGT (serine), CGT (arginine), GTT (valine), GAT (aspartic acid), or GCT (alanine). Five of these were found in the duodenal fluids: GTT in 7 cases, GAT in 4 cases, TGT in 1 case, GTT in 1 case, and GCT in 1 case. Fourteen mutations appeared in 13 cases because 1 specimen contained two mutations (GTT and GCT). Representative hybridizations from the duodenal fluids harboring mutations are shown in the last two columns in each of the 5 membranes in Figure 1.

The available primary pathology (primary carcinoma, serous cystadenoma, or chronic pancreatitis) from the patients from whom the duodenal fluids were obtained was also collected and analyzed. Twenty-nine (69%) of the 42 primary tumors with amplifiable DNA contained K-ras mutations. Twenty-two (65%) of the
TABLE 2
Results for duodenal fluids and tumors 1

<table>
<thead>
<tr>
<th>Diagnosis (no. of cases)</th>
<th>Fluid result</th>
<th>Tumor result</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic adenocarcinoma (43)</td>
<td>WT (26)</td>
<td>WT (11)</td>
<td>11/26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asp (7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Val (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Val (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>WT (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asp (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ala/Val (1)</td>
<td></td>
</tr>
<tr>
<td>Intraductal papillary mucinous neoplasm (3)</td>
<td>Val (2)</td>
<td>Val (2)</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asp (1)</td>
<td></td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma (2)</td>
<td>WT (2)</td>
<td>Asp (2)</td>
<td>0/2</td>
</tr>
<tr>
<td>Solid and cystic papillary tumor (1)</td>
<td>WT (1)</td>
<td>Asp (1)</td>
<td>0/1</td>
</tr>
<tr>
<td>Bile duct adenocarcinoma (1)</td>
<td>WT (1)</td>
<td>WT (1)</td>
<td>1/1</td>
</tr>
<tr>
<td>Ampullary adenocarcinoma (1)</td>
<td>Cys (1)</td>
<td>Cys (1)</td>
<td>1/1</td>
</tr>
<tr>
<td>Total 23/42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WT: wild-type; Val: valine mutation; Asp: aspartic acid; Arg: arginine; Cys: cysteine.
1 This list includes only patients for whom K-ras status in both the fluid and the primary tumor was known.

TABLE 3
Correlation between the K-ras status of duodenal fluid and primary tissue from patients with cancer 1

<table>
<thead>
<tr>
<th>Duodenal fluid</th>
<th>K-ras mutant</th>
<th>K-ras wild-type</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer K-ras mutant</td>
<td>11</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Cancer K-ras wild-type</td>
<td>1</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>30</td>
<td>42</td>
</tr>
</tbody>
</table>

1 This table includes only patients for whom K-ras status in both the fluid and the primary tumor was known.

TABLE 4
Correlation between the K-ras status of duodenal fluid and primary tissue from patients with benign conditions 1

<table>
<thead>
<tr>
<th>Duodenal fluid</th>
<th>K-ras mutant</th>
<th>K-ras wild-type</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue K-ras mutant</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tissue K-ras wild-type</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

1 This table includes only patients for whom K-ras status in both the fluid and the primary tissue was known.

34 ductal adenocarcinomas of the pancreas with amplifiable DNA harbored K-ras mutations. Multiple sections of pancreas showing papillary hyperplasia with and without atypia were analyzed from the 10 pancreata with benign processes, and 4 (15%) of these 27 sections contained K-ras mutations. These 4 sections came from 3 of the patients; therefore, 3 (30%) of the 10 pancreata without cancer harbored mutant K-ras. As discussed later, these K-ras mutations originated in the pancreatic ductal lesions.

Mutations were detected in the primary tumors of the patients with duodenal fluid containing K-ras mutations. One of the primary tumors from this group of 13 patients was not available for study. In 11 (92%) of the twelve remaining cases, the same mutation was found in the primary carcinoma and in the duodenal fluid specimen. In the single discordant case, the duodenal fluid registered a GTT mutation, whereas the tumor itself was wild-type. The specimen containing two mutations (GTT and GCT) in the duodenal fluid showed only one mutation, GTT, in the primary tumor. Twelve (40%) of the 30 patients with cancer and wild-type K-ras duodenal fluids also had wild-type K-ras primary tumors.
Therefore, the overall correlation among cancer patients between fluid and tumor results was 23 (55%) of 42. Representative results for primary tumors are shown in the first two columns of each membrane in Figure 1. The third and fourth columns in Figure 1 contain duodenal fluid samples from these same cases. The results of analyses of the duodenal fluid and primary tissue for K-ras mutations are summarized in Tables 2, 3 and 4.

The clinical and pathologic data from patients with cancer were reviewed to see if they could account for our ability to detect K-ras mutations in the duodenal fluid specimens. None of the variables, including mean age, gender, race, mean tumor size, tumor differentiation, tumor extension, or lymph node metastases, differed significantly between the mutant and wild-type fluid groups. It is noteworthy that all three intraductal papillary mucinous neoplasms produced fluids in which mutations were detected. A mutation was also identified in the fluid of the patient with the ampullary adenocarcinoma. However, mutations were not detected in the duodenal fluid specimens obtained from the patients with the solid and cystic papillary tumor, the two mucinous cystadenocarcinomas, and the bile duct adenocarcinoma.

Discussion

Point mutations in K-ras can be detected in the duodenal fluid of patients with periampullary cancer. The identification of mutations is far more specific (100%) than it is sensitive (25%). In this study, all of the specimens that produced K-ras mutations were from patients with periampullary cancers (positive predictive value = 100%). However, a duodenal fluid specimen yielding a wild-type genotype is of little value: 32 of 41 patients with wild-type duodenal fluids also had a cancer in the pancreas (negative predictive value = 22%).

Detection of mutations in codon 12 of K-ras in duodenal fluid is as specific as, but less sensitive than, detection of mutant K-ras in other secondary sources. K-ras mutations have been detected in pure pancreatic juice with reported sensitivities ranging from 55% to 100% and specificities ranging from 94% to 100% [11-15]. Similarly, K-ras mutations have been sought in stool specimens obtained from 17 patients with pancreatic adenocarcinoma, cholangiocarcinoma, or chronic pancreatitis (sensitivity = 57%, specificity = 67%) [20]. K-ras codon 12 mutations have also been reported in the blood of 2 of 6 patients with adenocarcinoma of the pancreas but not in the blood of 2 patients with insulinomas (sensitivity = 33%, specificity = 100%) [11]. Although analysis of pure pancreatic juice has generally been more sensitive than that of other sources, duodenal fluid, stool, and blood are less invasively obtained, making them more applicable as future screening tools (collecting duodenal fluid is less invasive than collecting pancreatic juice because the former uses the "secretin test", which is technically easier and less painful than obtaining pancreatic juice [16].

Iguchi et al. previously studied K-ras mutations in duodenal fluid using single-strand conformation polymorphism analysis on material obtained from 19 patients with cancer and 41 with benign conditions [16]. Our study built on this study in three ways. First, we added findings regarding more cancer patients with a variety of cancer types. Second, we used a method of mutation detection that is easier to apply clinically than is single-strand conformation polymorphism analysis (the latter technique requires confirmation with laboratory-intensive direct sequencing). Third, because we analyzed duodenal fluid from Whipple's operation specimens, we were able to correlate the presence and type of K-ras mutations in the duodenal fluid with those in the primary tissue in nearly all the cases. Importantly, Iguchi et al. showed that the detection of K-ras mutations is possible in clinically obtained specimens (in their case, duodenal fluid collected during intravenous infusion of secretin). Thus, our results obtained in the controlled environment of analyzing
Chapter 1

resected surgical specimens are indeed clinically applicable.

Of the 42 primary tumors analyzed, 29 (69%) contained K-ras mutations at codon 12. Twenty-two (65%) of 34 duct adenocarcinomas of the pancreas with amplifiable DNA showed K-ras mutations. These percentages were slightly lower than those obtained by other groups for periampullary cancers [6-10]. It is unlikely, however, that other techniques, such as standard sequencing, would have detected more mutations, as we have found our techniques to be as sensitive as sequencing in tumors we have analyzed [36]. Nonetheless, we were able to demonstrate the same mutation in the primary tumor and the duodenal fluid in 11 (92%) of 12 available cases in which mutant K-ras was detected in the duodenal fluid specimen. The duodenal fluid in the one discrepant case harbored a GTT mutation, but the patient's tumor specimen was wild-type. The histology of this lesion was reviewed, and a less differentiated second tumor morphology and extensive papillary duct hyperplasia with and without atypia were found. These additional lesions may account for the fluid-tumor mismatch, because any of the pancreatic lesions, including the duct hyperplasias, could theoretically shed cells into duodenal fluid, whereas the tumor microdissection procedure samples only one lesion. The same reasoning may account for the finding of two mutations (GTT and GCT) in the duodenal fluid of a patient whose primary tumor harbored only one mutation (GTT). Similar conclusions have been reached by others for pure pancreatic juice, stool, and tumor specimens in which mismatches or "bimutational patterns" resulted from either multifocality of the cancer or the presence of independent ductal lesions [11,20,37].

In contrast, when multiple sections of pancreas from the 10 patients with benign conditions were analyzed, 3 (30%) of these pancreata harbored mutations, whereas none of these patients' duodenal fluid specimens contained mutations. The representative tissue microdissected from these blocks contained papillary hyperplasia with and without atypia. It is possible that mutations were not detected in the duodenal fluids because too few cells containing mutations were shed from the ductal lesions into the fluid, nonetheless the identification of mutations in noninvasive pancreatic ductal lesions demonstrates that mutations in K-ras are not restricted to invasive cancers.

Noninvasive pancreatic ductal lesions are generally felt to be the precursors of adenocarcinoma of the pancreas, and these lesions may account for fluid-tumor discrepancies [11,20-30,38]. Although the prevalence of K-ras mutations in ductal hyperplasias has been debated, Caldas et al. showed that the K-ras mutations detected in the stool of 5 patients with adenocarcinoma of the pancreas, cholangiocarcinoma, or chronic pancreatitis originated in ductal hyperplasias [20,23,24,28-30].

Therefore, one may argue that although the detection of a K-ras mutation is not specific for an invasive cancer, it would at least signal the presence of ductal hyperplasia and require further evaluation of the patient. However, the frequency with which duct hyperplasia progresses to infiltrating carcinoma has not yet been established [30]. Of interest, Berthelemy et al. have recently reported two patients who had K-ras mutations detected in their pancreatic juice who developed pancreas cancer months later [39].

Detection of point mutations at codon 12 of K-ras in duodenal fluid did not have statistically significant dependence on any of the variables examined in this study, including mean age, gender, race, mean tumor size, tumor differentiation, tumor extension, or lymph node metastases. Of the 13 patients in which K-ras mutations could be detected in duodenal fluid, 9 had pancreatic adenocarcinomas, 3 had intraductal papillary neoplasms (analogous to adenoma in the adenoma-to-carcinoma paradigm of colorectal carcinoma [24,40,41]), and 1 had an ampullary adenocarcinoma. All three of the intraductal papillary neoplasms were
detectable by K-ras analysis of duodenal fluid. In contrast, neither the solid and cystic papillary neoplasm nor the two mucinous cyst adenocarcinomas were detected by mutations in duodenal fluid. This finding is reasonable because these two neoplasms do not typically communicate with the pancreatic duct system. Only 10 of 45 pancreatic, ampullary, or bile duct carcinomas contained K-ras mutations in their duodenal fluids.

One limitation of the current study that should be acknowledged is that only patients with operable pancreatic carcinomas were included, yet most patients with pancreatic carcinoma are not candidates for surgical treatment. Also, because Whipple’s operation specimens undergo extensive surgical manipulation and ischemic periods and because duodenal fluid contains bile acids, DNA degradation may have occurred. This may explain the absence of PCR amplification in some samples and may have biased the results in general.

In conclusion, mutations at codon 12 of K-ras are the most common molecular alterations in periampullary cancer. The detection of K-ras mutations in duodenal fluid and other secondary sources may form the basis for the development of new approaches to detect periampullary cancer earlier and less invasively and differentiate it from benign conditions of the pancreas.

References


