Neoplasms of the Ampulla of Vater with Concurrent Pancreatic Intraductal Neoplasia: A Histological and Molecular Study

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Adenoma and adenocarcinoma of the ampulla of Vater are uncommon neoplasms of the gastrointestinal tract. Only one report has analyzed the relationship between ampullary adenocarcinoma and pancreatic intraductal neoplasia (PanIN), the precursor lesion of pancreatic adenocarcinoma. An association between PanIN and ampullary adenoma has not been reported previously. Case reports have documented the progression of PanIN to invasive pancreatic adenocarcinoma. We reviewed five resected ampullary adenoma and 17 ampullary adenocarcinoma cases and evaluated the pancreas for PanIN. Pancreatic sections from 35 autopsies were reviewed as a control group. Immunohistochemistry for overexpression of p53 and COX-2 proteins was performed in selected cases, as was PCR analysis for *K-ras* mutations. Follow-up clinical data were obtained. All 22 ampullary neoplasms were associated with PanIN, which was high grade in two (40%) adenoma cases and seven (41%) adenocarcinoma cases. In 16 (73%) evaluable cases, PanIN extended to the pancreatic resection margin; two of which had high grade PanIN. Among the autopsy controls eight (23%) had low-grade PanIN. Seven of the 22 ampullary cases but none of the autopsy controls had coexistent pancreatitis. A smoking history was present in two of four autopsy cases in which this history was available. Overexpression of the p53 and COX-2 proteins was present in only one case of high-grade PanIN. *K-ras* mutations were present in four of four of the PanIN lesions evaluated, including one autopsy case. Clinical follow-up revealed no progression of PanIN to invasive carcinoma

in the remnant pancreas, although the follow-up period was too short to adequately assess that risk (an average of 3.8 y for adenoma cases and 2.5 y for adenocarcinoma cases). We conclude that adenomas and carcinomas of the ampulla are associated with PanIN, and often high-grade PanIN. Although its malignant potential has not been fully established, PanIN is underreported and often unrecognized. PanIN may be analogous to colorectal adenoma in that both are prevalent in the older adult population, but few progress to carcinoma.

KEY WORDS: Ampullary neoplasms, Intraductal neoplasia, Pancreas, PanIN.

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Adenoma and adenocarcinoma of the ampulla of Vater are uncommon neoplasms of the gastrointestinal tract that are found with increased frequency in familial adenomatous polyposis syndrome (1–3). Ampullary adenoma represents the precursor lesion to adenocarcinoma, and a coexistent adenoma can be identified frequently in patients with ampullary adenocarcinoma (1, 2). Because of their premalignant nature, ampullary adenomas are often resected either by polypectomy or, in the case of larger lesions, by pancreaticoduodenectomy (1, 4, 5). An association between adenomas of the ampullary region and pancreatic intraductal neoplasia has not been documented previously.

Adenocarcinoma of the pancreas is often associated with pancreatic intraductal neoplasia (PanIN), which is thought to represent the precursor lesion for this cancer (6–12). Several case reports documenting progression of high-grade (HG) PanIN to invasive pancreatic adenocarcinoma over time (17 mo to 29 y) support the role of PanIN as a precursor of carcinoma (10, 13, 14).

The relationship between ampullary adenoma and PanIN has not been investigated previously, and only one analysis of the relationship between

ampullary adenocarcinoma and PanIN has been reported (15). We reviewed five resected ampullary adenoma and 17 ampullary adenocarcinoma specimens and evaluated the accompanying pancreas for PanIN. Immunocytochemical and molecular analysis of the dysplastic epithelium was performed, and follow-up clinical data were obtained. In addition, pancreatic sections from 35 autopsies were examined histologically, and two were examined by molecular analysis for comparison.

METHODS

The files of the Departments of Pathology at the University of Washington Medical Center and Virginia Mason Medical Center were searched for cases of ampullary adenoma or adenocarcinoma over a 15-year period (1984–1999). The slides were reviewed by two pathologists (SNA, RCH) who confirmed the primary diagnosis and evaluated the accompanying pancreas for PanIN.

The PanIN was graded according to previously published criteria as low-grade (LG; flat epithelial hyperplasia and papillary hyperplasia without atypia) or HG (papillary epithelial hyperplasia with atypia and carcinoma *in situ*) PanIN (10–12). LG PanIN (Fig. 1, A–B) was defined as enlargement of the pancreatic ductal epithelial cells with abundant

apical mucin and mild nuclear enlargement with variably prominent nucleoli. The neoplastic epithelium was flat or papillary with variable nuclear stratification; however, nuclear atypia was not marked, and the nuclei maintained their basal orientation and polarity, whereby the long axis of the nuclei remained perpendicular to the epithelial basement membrane. The defining feature of HG intraductal neoplasia (Figs. 2, 3) was marked nuclear atypia with enlarged, hyperchromatic nuclei that lost their basal orientation and no longer maintained nuclear polarity. There were usually prominent nucleoli as well as mitotic figures in the neoplastic epithelium.

The pancreatic margin was assessed for the presence and grade of PanIN, and the bile duct margin was assessed for intraductal neoplasia of the biliary epithelium. The presence of a precursor adenoma in the adenocarcinoma cases was noted, as was the presence of pancreatitis. The number of sections containing pancreatic tissue was noted. Clinical follow-up was obtained from clinical charts or directly from the primary care physician. A history of stent placement was noted when available. Intraductal papillary mucinous tumors were excluded, as were duodenal adenomas occurring more than 3 cm from the ampulla of Vater. There was no history of familial adenomatous polyposis in any of the patients.

Adult autopsy cases were selected at random with the following criteria: minimal autolysis of pancreatic ductal epithelium, no history of pancreatic or ampullary adenocarcinoma, and no history of familial adenomatous polyposis. Intraductal pancreatic and biliary neoplasia, when present, were graded as above. The number of sections containing pancreatic tissue was noted. Smoking history was noted when available.

FIGURE 1. Low-grade PanIN in the pancreatic duct in (A) an autopsy control case without ampullary neoplasia and (B) in association with a tubular adenoma of the ampulla. Note the transition from the normal (arrowhead) to the neoplastic (arrow) epithelium.

FIGURE 2. High-grade pancreatic intraductal neoplasia (PanIN) of the pancreatic duct in association with a villous adenoma of the ampulla of Vater.

FIGURE 3. High-grade intraductal neoplasia of the common bile duct in association with a tubular adenoma of the ampulla of Vater.

Immunocytochemistry

Deparaffinized sections were immunostained with monoclonal antibodies to p53 (DAKO, Carpinteria, CA; 1:1000) and COX-2 (Cayman Chemical Company, Ann Arbor, MI; 1:1000). Negative controls consisted of substitutions of mouse ascites fluid for the primary antibody. Sections were subjected to heat-induced epitope retrieval using a microwave oven. Antigens were localized using the avidin-biotin method with 3,3'-diaminobenzidine as a chromogen, with nickel chloride enhancement. The staining was then assessed by two independent observers and graded as positive or negative according to previously published criteria (16–18). The COX-2 staining was reported as positive if the staining was +1 or greater. Internal positive controls for COX-2 were provided by mononuclear inflammatory cells.

K-ras Analysis

Cases in which PanIN lesions or normal ductal epithelium (in one autopsy control) could be easily manually microdissected were selected for this assay. Five-micrometer unstained sections of pancreas containing ducts with PanIN were subject to microdissection of the neoplastic epithelium. Genomic DNA was isolated from four pancreatic sections with PanIN and from one normal pancreatic duct from an autopsy case without PanIN.

K-ras exon 1 was amplified from genomic DNA using a thermal cycler (MJ Research, Watertown, MA) and primers as described by Imai *et al.* (19). Amplifications were carried out in a buffer containing 10 mM Tris-HCl; 1.5 mM MgCl₂; and 50 mM KCl, pH 8.3, with 2 U of *Taq* DNA polymerase (Boehringer Mannheim, Indianapolis, IN), 200 μ M dNTPs, and 15 pmol of each primer in a total volume of 50 μ L. Reactions were denatured for 3 minutes at 95°C, then followed by 37 cycles with the following profile: 95°C (20 s), 55°C (60 s), and 72°C (40 s). The K-ras mutational status of the 110-bp PCR product was assessed by denaturing gradient gel electrophoresis run at 56°C for 4 to 5 hours at 150 V, then visualized using Sybr Green (Molecular Probes, Eugene, OR) staining.

Statistical Analysis

Categorical variables were compared using Pearson χ^2 tests, or with Fisher's exact tests when sizes were small. Continuous variables, such as age, were compared using *t* tests or analysis of variance (ANOVA), when appropriate. Logistic regression analysis was conducted to assess the association between continuous factors, such as age, and dichotomous outcomes, such as grade of disease. In all analyses, *P* values $\leq .05$ were considered statistically significant.

RESULTS

All ampullary neoplasms, both benign and malignant, had PanIN in the pancreatic ducts, and in all but two cases, dysplasia was present in the common bile duct as well (Tables 1–3). Small, medium, and large pancreatic ducts were affected. Intraductal neoplasia was present in ducts both adjacent to the ampullary neoplasm and further away (more than 1 cm).

The pancreatic ductal epithelium in two of the five adenoma cases (Fig. 4), showed HG intraductal neoplasia (Figs. 2, 3), and three had LG PanIN (Fig. 1b). In the 17 ampullary adenocarcinoma cases, the pancreatic ductal epithelium had HG PanIN in 7 and LG in 10. All of the adenoma and adenocarci-

TABLE 1. Characteristics of Patients with Periapillary Adenomas with Concurrent Pancreatic Intraductal Neoplasia (PanIN)

Case	Age (y)/Sex	Adenoma	Grade of PanIN	Bile Duct (BD) and/or Pancreatic (PN) Margin with Ductal Neoplasia*	Pancreatitis
1	78 F	Tubulovillous	Low	N/S	No
2	39 M	Villous	High	BD with HG; PN N/S	No
3	66 F	Villous	Low	PN negative; BD N/S	No
4	45 M	Tubular	Low	BD and PN with LG	No
5	80 M	Tubular	High	N/S	Yes

* N/S indicates that the pancreatic and/or bile duct margin was not separately identified in the original surgical pathology report.

TABLE 2. Characteristics of Patients with Periampullary Adenocarcinoma with Associated Adenomas and with Concurrent Pancreatic Intraductal Neoplasia (PanIN)

Case	Age (y)/Sex	Carcinoma	Associated Adenoma	Grade of PanIN	Neoplasia Involving the Bile Duct (BD) or Pancreatic (PN) Margin*	Pancreatitis
1	49/M	Intramucosal	Villous	High (focal)	PN and BD with LG	No
2	58/F	Well differentiated	Villous	Low	BD and PN negative	No
3	75/F	Well differentiated	Villous	Low	PN with LG	Yes
4	61/M	Mod. differentiated	Tubulovillous	High (focal)	PN with HG	No
					BD with LG	
5	76/F	Mod. differentiated	Tubular	Low	PN and BD with LG	No
6	77/M	Mod. differentiated	Villous	High (focal)	PN and BD with LG	Yes
7	72/M	Mod. differentiated	Villous	High (focal)	BD and PN negative	No
8	66/F	Mod. differentiated	Tubular	Low	BD with LG	No
9	63/M	Mod. differentiated	Tubulovillous	Low	BD and PN N/S	No
10	65/F	Mod. differentiated	Tubulovillous	Low	PN and BD with LG	Yes
11	78/F	Poorly differentiated	Villous	Low	BD with LG	No
12	27/M	Poorly differentiated	Tubulovillous	Low	PN and BD with LG	No
13	69/M	Poorly differentiated	Villous	High	BD with LG	No
					PN N/S	
14	75/M	Poorly differentiated	Tubulovillous	Low	PN and BD with LG	Yes
15	61/F	Poorly differentiated	Villous	High (focal)	PN with LG	Yes
16	51/M	Poorly differentiated	Villous	High	PN with LG	Yes
17	62/M	Poorly differentiated	Tubular	Low	BD with LG	No

* N/S indicates that the pancreatic and/or bile duct margins were not separately identified in the original surgical pathology report.

noma cases with LG PanIN had papillary hyperplasia without atypia, except for one carcinoma case (Carcinoma 8, Table 2), which had only flat epithelial hyperplasia. Sixteen of the 22 cases had intraductal neoplasia extending to either the pancreatic or bile duct margins, two of which were HG (Adenoma 2, Table 1 and Carcinoma 4, Table 2). In six cases, the original surgical pathology report did not specifically identify the pancreatic and/or bile duct margins, and they therefore could not be evaluated.

The average age in the adenoma group was 62 years (median, 66 y; range, 45 to 80 y), and in the carcinoma group, 64 y (median, 65 y; range, 27 to 78 y), which was not a significant difference ($P = .8$). The average age of patients with HG PanIN was 60 years in the adenoma group (median, 60 y; range, 39 to 80 y) and 63 years in the carcinoma group (median, 61 y; range, 49 to 77 y). The male-to-female ratio was 1.6:1. Seven of the 22 cases had coexistent pancreatitis, and there was a history of stent placement in only two of the cases (one adenoma and one carcinoma case that were not associated with HG PanIN).

The pancreatic ductal epithelium in 8 (23%) of the 35 autopsy controls revealed LG PanIN: six with flat epithelial hyperplasia and two with papillary hyperplasia without atypia (Fig. 1a). None of the autopsy controls had HG PanIN. The average age for the 35 controls was 68 y (median, 69 y; range, 37 to 90 y), and the average age of those with PanIN was also 68 y (median, 66 y; range, 40 to 90 y). The male-to-female ratio was 1.7:1. Of the four patients for whom a smoking history was available, two were smokers, and two were nonsmokers. There was no evidence of pancreatitis in any of the autopsy cases.

PanIN was significantly more prevalent in the adenoma/adenocarcinoma cases compared with the case of controls ($P < .001$). However, the ampullary adenoma/adenocarcinoma cases had significantly more sections of pancreas submitted for evaluation than did the autopsy cases ($P < .05$). The adenoma cases had an average of 6.4 sections of pancreatic tissue (median of 5, range of 3–13); the adenocarcinoma cases had an average of 5.5 sections (median, 6.5; range, 3–10); and the autopsy cases, an average of 1.1 sections (median, 1; range, 1–2).

There was no significant association between the age of the patients and the grade of intraductal neoplasia ($P = .7$). However, male gender was significantly associated with the grade of PanIN ($P = .02$); only 5 of 13 cases with LG, *versus* 8 of 9 cases with HG neoplasia, were in males. In both the ampullary adenoma and adenocarcinoma cases, we observed a male predominance in cases with HG PanIN. There was no significant association between pancreatitis and the presence of intraductal

TABLE 3. Characteristics of Autopsy Controls with Pancreatic Intraductal Neoplasia (PanIN; 8 of 35 Cases)

Case	Age (y)	Sex	PanIN Grade	Smoking History*	Pancreatitis
1	90	Female	Low	N/A	No
2	57	Male	Low	Positive	No
3	87	Female	Low	N/A	No
4	86	Male	Low	N/A	No
5	74	Male	Low	Positive	No
6	40	Male	Low	N/A	No
7	55	Male	Low	Negative	No
8	53	Female	Low	Negative	No

* N/A indicates that smoking history was not available.

FIGURE 4. Villous adenoma of the ampulla of Vater.

neoplasia ($P = .09$) in the cases and controls and no significant association between pancreatitis and the grade of PanIN in the cases ($P = .3$).

Immunohistochemical analysis of p53 protein in the adenoma and adenocarcinoma cases revealed overexpression of p53 in only one focus of HG PanIN in the eight cases evaluated, whereas the associated ampullary neoplasms had overexpression in five of the eight cases (three adenomas, two adenocarcinoma; Table 4). One case (Carcinoma 17, Table 4) had overexpression of p53 in the adenoma but not in the carcinoma. COX-2 was overexpressed in only one case of HG PanIN, the associated adenocarcinoma, and in one other adenocarcinoma. All adenomas were negative for COX-2 protein overexpression. The two autopsy cases evaluated, one with LG PanIN and one with no intraductal neoplasia, had no overexpression of p53 or COX-2.

PCR analysis of *K-ras* revealed point mutations at Codon 12 in all of the PanIN lesions evaluated (four of four), including one autopsy control case (Table 4). The one autopsy pancreas control case without PanIN showed no mutation of *K-ras*.

Clinical follow-up of the adenoma cases revealed that all five patients were alive and well, with an average follow-up interval of 3.8 years (range, 0.3 to

8.8 y); the one patient with HG PanIN at the resection margin is currently disease free at 8.0 years. Of the adenocarcinoma cases, three (23%) were dead with recurrent disease, two (15%) were dead with unknown cancer status, and eight (62%) were alive and well. The average follow-up period was 2.5 years (range, 0.3 to 8.8 y). A carcinoma patient with HG PanIN at the resection margin is currently disease free at 2 years. For three patients, follow-up information was not available, and one case was too recent for follow-up.

DISCUSSION

The link between pancreatic adenocarcinoma and the precursor lesion of pancreatic intraductal neoplasia (PanIN) was first suggested by Sommers and coworkers (20). Since then, many studies have shown an association between pancreatic adenocarcinoma and intraductal neoplasia (6, 7, 9). PanIN has been estimated to occur commonly in patients without a history of pancreatic cancer in autopsy samples and in pancreatic tissue removed for nonneoplastic disease, with different studies having a prevalence of around 20 to 30%, though it is considerably more frequent in patients with carcinoma (6, 7, 8, 21). High-grade PanIN occurs only rarely in patients without a history of pancreatic neoplasia (6, 7).

Previous studies have shown some relationship between PanIN and increasing age, smoking history, chronic pancreatitis, and the number of sections of pancreatic tissue analyzed (6, 12, 21–28). We did not find a significant association between age and dysplasia, but we did find a significant association of HG PanIN with male gender, though our study is limited. Only two of the four autopsy cases with PanIN in the current study had a smoking history, indicating that it occurs in nonsmokers; more recent studies have not shown a significant association with smoking (28). (We did not assess the smoking history in ampullary neoplasia cases;

TABLE 4. p53 and COX-2 Immunohistochemistry Results for the Ampullary Neoplasm and the Intraductal Neoplastic Epithelium; K-ras PCR Results on the Microdissected Intraductal Neoplastic Epithelium (PanIN)

Case*	p53	COX-2	K-ras**
Adenoma—2	Negative in adenoma/negative in HG PanIN	Negative in adenoma/negative in HG PanIN	Positive
Adenoma—3	Positive in adenoma /negative in LG PanIN	Negative in adenoma/negative in LG PanIN	ND
Adenoma—4	Positive in adenoma /negative in LG PanIN	Negative in adenoma/negative in LG PanIN	ND
Adenoma—5	Negative in HG PanIN/negative in adenoma	Negative in HG PanIN/negative in adenoma	ND
Carcinoma—1	Negative in HG PanIN/negative in carcinoma	Negative in HG PanIN/negative in carcinoma	Positive
Carcinoma—10	Positive in carcinoma /negative in LG PanIN	Negative in carcinoma/negative in LG PanIN	ND
Carcinoma—11	Positive in carcinoma /negative in LG PanIN	Negative in carcinoma/negative in LG PanIN	ND
Carcinoma—13	Negative in carcinoma/ positive in HG PanIN	Positive in carcinoma/positive in HG PanIN	Positive
Carcinoma—17	Focally positive in adenoma /negative in LG PanIN	Positive in carcinoma /negative in LG PanIN	ND
Autopsy—1	Negative in LG PanIN	Negative in LG PanIN	Positive
Autopsy (no PanIN)	Negative in epithelium	Negative in epithelium	Negative

PanIN, pancreatic intraductal neoplasia.

* Numbers refer to case numbers in Tables 1–3.

** Positive indicates that a mutation was present in Codon 12 of the c-K-ras gene. ND indicates that k-ras PCR was not done in the specified case.

we were interested only in exploring the prevalence of PanIN in association with ampullary neoplasia and not the epidemiologic factors with which it may be associated.) As a surrogate marker for duct obstruction, all of our cases were assessed for the presence of pancreatitis, and there was no significant association in our limited study between the presence of pancreatitis and PanIN or between pancreatitis and the grade of PanIN. The adenoma/adenocarcinoma cases had significantly more sections of pancreatic tissue submitted than the autopsy controls, which may account for some of the increased frequency of PanIN detection in these cases.

Adenomas of the ampullary region have not previously been reported to be associated with PanIN. We found that ampullary adenomas not only are associated with PanIN but often with HG PanIN. In 40% of our adenoma cases, these intraductal lesions were not mentioned in the surgical pathology report, and the status of the pancreatic and biliary margins was sometimes not specified.

Only one previous report has analyzed the relationship between PanIN and ampullary adenocarcinomas (15). Liu and coworkers found intraductal neoplasia in the large pancreatic ducts in 21.9% of cases of ampullary carcinoma; however, they excluded cases of flat epithelial hyperplasia. All of the adenocarcinoma cases in the present study that had LG PanIN had papillary epithelial hyperplasia without atypia except for one case, which had flat epithelial hyperplasia. As above, these lesions were often overlooked in the original surgical pathology evaluation in 59% of the cases, although margins were more often specified than for the adenoma cases.

Surgical resection of ampullary adenomas is associated with low morbidity and mortality, and ampullary adenocarcinoma is more often resectable and has an increased survival time relative to pancreatic adenocarcinoma (2, 5, 29). Typical 5-year survival rates for ampullary adenocarcinoma after pancreaticoduodenectomy are around 60%, compared with the dismal 5-year survival rate of around 3.5% for carcinomas of pancreatic ductal origin (29, 30). The patients with ampullary adenoma in this study were all alive and well, with an average follow-up period of 3.8 years, and 62% of patients with ampullary adenocarcinoma were alive and well with a 2.5-year average postoperative follow-up period, figures that are comparable to published survival rates for ampullary adenocarcinoma.

In the past, PanIN was divided into four categories: flat epithelial hyperplasia, papillary epithelial hyperplasia, papillary hyperplasia with atypia, and carcinoma *in situ* (6, 9). Recently, because of the unknown neoplastic potential of flat and papillary

epithelial hyperplasia and the poor reproducibility among pathologists in separating papillary hyperplasia with atypia and carcinoma *in situ*, a new grading system was proposed: LG PanIN (flat and papillary epithelial hyperplasia) and HG PanIN (papillary hyperplasia with atypia and carcinoma *in situ*; 10–12). These terms are equivalent to LG and HG pancreatic ductal dysplasia and are thought to separate the lesions into those with a low probability for neoplastic progression (LG PanIN) and a high probability for progression (HG PanIN; 11, 12, 21), though this grading scheme is not yet widely accepted.

The neoplastic nature of LG and HG PanIN is supported by the discovery of multiple genetic alterations within it, most notably *K-ras* mutation, overexpression of p53 protein, and loss of p16 protein expression (31–34). *K-ras* abnormalities, most often mutations in Codon 12, have been demonstrated in neoplastic ductal epithelium isolated from patients with a history of pancreatic cancer as well as from those with no history of pancreatic neoplasia and from autopsy specimens (33–36). Overexpression of p53 is found most commonly in invasive pancreatic carcinoma; however, it may also be overexpressed in PanIN, especially HG PanIN (34, 37–39). Apple and colleagues (34) demonstrated a relationship between p53 overexpression and increasing grades of epithelial atypia. They suggested that p53 alterations may occur later in pancreatic carcinogenesis as they are found more commonly in HG lesions and invasive carcinoma (34).

To confirm that the lesions we were interpreting as PanIN were, in fact, neoplastic, we carried out *K-ras* mutational analysis and immunohistochemical assays for p53 and COX-2 protein overexpression on some of them. The intraductal neoplastic lesions we analyzed for a *K-ras* mutation, one HG and three LG PanIN, showed mutations of *K-ras* at Codon 12, including the autopsy case. The autopsy control with normal-appearing epithelium showed no mutation of *K-ras*. Overexpression of p53 was present in one case of HG PanIN, which also had a mutation in *K-ras* (Carcinoma 13, Table 4). Interestingly, this case showed no overexpression of p53 in the associated carcinoma. None of the LG intraductal lesions had overexpression of p53.

Cyclooxygenase-2 (COX-2) has been shown to play an important and early role in colorectal neoplasia, and overexpression of COX-2 has been found in approximately 72% of pancreatic adenocarcinomas (18, 40, 41). We found overexpression of COX-2 in two of the five ampullary adenocarcinomas evaluated and overexpression in one associated focus of HG PanIN. This case was the same one that showed overexpression of p53 protein in the neoplastic ductal epithelium (Carcinoma 13, Table 4). The ampullary adenoma and other cases of Pa-

nIN were negative for COX-2 overexpression. This implies that overexpression of COX-2 may play a later role in ampullary neoplasia and possibly in pancreatic ductal neoplasia than it does in colorectal neoplasia, since normal colonic epithelium near neoplastic lesions, as well as most adenomatous lesions of the colon, show COX-2 overexpression (18).

The importance of PanIN in patients with ampullary neoplasms is uncertain. Patients with ampullary neoplasms, including ampullary adenocarcinoma, have a relatively favorable prognosis with long survival times, which could increase the probability of progression of the PanIN lesions. Nordback and colleagues reported two patients with ampullary carcinoma who developed a second primary adenocarcinoma 5 and 15 years after resection of the ampullary lesions (13). The initial resection specimens in both patients had dysplasia in the hepatic duct. Other studies report the progression of HG PanIN to invasive carcinoma 17 months to 29 years after diagnosis (10, 14). This implies that progression of PanIN in patients with ampullary neoplasms is possible, especially in view of their long survival times.

The follow-up period in our study was too short to determine whether pancreatic or biliary ductal carcinomas will develop; it averaged 3.8 years (range, 0.3 to 8.8 y) for the adenoma cases and 2.5 years (range, 0.3 to 8.8 y) for the carcinoma cases. Two cases had HG PanIN extending to the resection margin, and both of those patients are currently disease free at 8 and 2 years, respectively. However, according to the previously cited reports, it may take as long as 29 years for even HG PanIN to progress to invasive carcinoma. Regular follow-up of these patients should be considered.

Although we show that ampullary adenomas and adenocarcinomas are associated with PanIN, the exact nature of this association is unknown. The one case mentioned previously that showed p53 overexpression in the HG PanIN but not in the associated carcinoma (Carcinoma 13, Table 4) suggests that these lesions may not be directly related, though drawing a conclusion from one case is a tenuous proposition at best. Whether this association could be due to a field effect similar to that seen in the urinary bladder is also speculative, and further investigation is required to determine the nature of this association.

The natural history of LG PanIN remains to be elucidated. An analogy has been drawn between the adenoma-carcinoma sequence in the colon and PanIN in the pancreas (12). Colonic adenomas are common, and the number of patients who have them increases with age, yet only a small number progress to colonic adenocarcinoma (42). Perhaps LG PanIN is analogous to tubular adenomas of the

colon in that although it is common, few progress to carcinoma. This analogy can be applied to the molecular events seen in PanIN as well, with early abnormalities of *K-ras* present in LG lesions and later, abnormalities in p53, as seen in the colonic mucosa with neoplastic progression (36, 37, 43).

In conclusion, both ampullary adenoma and adenocarcinoma are associated with PanIN, and often with HG PanIN. The explanation for the concurrence of ampullary neoplasms and intraductal neoplasia of the pancreatic and biliary epithelium is unknown, and further research is needed to establish whether this is a field effect similar to that seen in neoplasms of the urinary bladder or if it is due to other phenomena. The overexpression of p53 and COX-2 proteins in the intraductal lesions in patients with ampullary neoplasms, and the *K-ras* mutations in both the autopsy controls and the patients, provides support for the neoplastic nature of PanIN. The fact that PanIN was often not mentioned in the surgical pathology reports, even when it was present at a surgical margin, emphasizes the need for increased recognition of these intraductal lesions. Though no progression of the PanIN lesions was present in our series, our follow-up period was too short to adequately assess the risk. Intraductal neoplasia of the pancreas may be analogous to adenomas of the colon, and further investigation is needed to establish what factor(s) are responsible for progression of these lesions.

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