A Clinicopathologic and Immunohistochemical Study of 22 Intraductal Papillary Mucinous Neoplasms of the Pancreas, with a Review of the Literature

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Intraductal papillary-mucinous neoplasms (IPMNs) of the pancreas are rare lesions. We undertook this study to analyze these tumors by focusing on the diagnostic criteria and correlating the histologic features with clinical prognosis. Twenty-two cases of IPMN were retrieved from the Endocrine Tumor Registry of the Armed Forces Institute of Pathology. Blocks or unstained slides were available for histochemical and immunohistochemical studies (including proliferative markers and cell cycle regulators) and K-*ras* oncogene mutations in 15 cases. Patient follow-up was obtained in all of the cases. IPMN occurs in both genders with a slight male predominance, with a mean age at presentation of 64.4 years (range, 48–85 yr). The patients presented with abdominal pain. The neoplasms were radiologically and grossly cystic, usually (18 cases of 22) located in the head of the pancreas. Histologically, the tumors consisted of intraductal papillary proliferations protruding into and expanding the pancreatic ducts. Invasion into the surrounding pancreatic parenchyma was detected in 15 cases. Chronic pancreatitis was present in all of the cases. p27 immunoreactivity always exceeded the immunoreactivity of cyclin E. K-*ras* oncogene mutations were detected in two cases. Patients were treated with a complete surgical resection ($n = 7$) or a Whipple procedure ($n = 13$). Only 2 of 22 patients died of disease (3 died immediately postoperatively and 3 died of unrelated causes), whereas the remaining 14 patients were alive at last follow-up, without evidence of disease, an average of 58.2 months after initial presentation. IPMNs are rare, distinctive neoplasms, with complex intraductal papillae, that can be easily separated from *in situ* ductal adenocarcinoma and mucinous cystic neoplasms. The high ratio of p27 protein to cyclin E supports the excellent prognosis of these neoplasms, despite the presence of invasion and K-*ras* oncogene mutation.

KEY WORDS: Immunohistochemistry, Intraductal, Mucinous, Neoplasms, Pancreas, Papillary.

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Intraductal papillary-mucinous neoplasms (IPMNs) are a relatively new entity in pancreatic pathology, first described a decade ago by Morohoshi *et al.* (1). Because of the variety of terms used to name these lesions, a comprehensive understanding of the neoplasms is not readily available. The incidence is unknown. According to the reported cases in the literature, patients with IPMN seem to have a better prognosis than patients with conventional pancreatic ductal adenocarcinoma (2–9). Therefore, we undertook a study of 22 cases of IMPN, coupled with a review of the literature, to define the diagnostic criteria for these neoplasms, elucidate the immunophenotype of the tumor cells, identify the presence or absence of tumor proliferation and cell cycle markers, and evaluate patient outcome.

MATERIALS AND METHODS

We retreived 22 cases of IPMN from the files of the Endocrine Pathology Registry of the Armed

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Departments of the Navy, Army, or Defense.

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Forces Institute of Pathology, Washington, DC, accessioned between 1970 and 1996. Hematoxylin- and eosin-stained slides, available in all of our cases, were reviewed, with the submission of sufficient blocks (average number of section examined, 15; range, 1–56) to permit adequate evaluation of the tumor and extent of invasion (three cases had insufficient material for an adequate assessment of invasion). All of the cases met the histologic criteria of IPMN as defined by Morohoshi *et al.* (1). For inclusion in our study, adequate histologic and clinical data had to be available. Materials within our facility's files were supplemented by a review of the patient demographics, symptoms at presentation, past medical history, and laboratory data. Additionally, we reviewed the radiographic studies performed and the operative and surgical pathology reports, and we obtained information from oncology data services, either by specific mailed questionnaires or by direct communication with the patients' physicians. Patients who had pseudocysts, duct ectasia, mucin-producing adenocarcinomas, *in situ* ductal adenocarcinomas, or mucinous cystic neoplasms of low-grade malignant potential were excluded from this study, because we think that these conditions are distinct entities, easily separated by clinical and histologic review from IPMN. Specifically, "mucinous duct ectasia," as the words imply, should not be considered a neoplastic condition but rather part of ductal epithelial hyperplasia with a variable degree of atypia and mucus overproduction. It is not considered within the spectrum of IPMN.

Formalin fixed, paraffin-embedded tissue sections were stained with periodic acid–Schiff reaction with and without diastase digestion, Alcian blue (pH 2.5) with and without hyaluronidase digestion, Alcian blue (pH 0.4), Alcian blue with periodic acid–Schiff (pH 2.5), aldehyde fuchsin, Masson trichrome, and Mayer's mucicarmine. Paraffin blocks or unstained slides were available in 15 cases for immunophenotypic analysis according to the standardized avidin-biotin method of Hsu *et al.* (10). The analysis was performed on a single block in each case. A broad panel of commercially available immunohistochemical reactions was applied (Table 1). When required, proteolytic antigen retrieval was performed with predigestion for 3 minutes with 0.05% Protease VIII (Sigma, St. Louis, MO) in a 0.1 M phosphate buffer (pH 7.8) at 37°C (11). Antigen enhancement (recovery) by using formalin-fixed, paraffin-embedded tissue treated with a buffered citric acid solution and heated for 20 minutes in a calibrated microwave oven was used for c-*erb*B-2, cyclin E, Ki-67, p27, and proliferating cell nuclear antigen (12). After this, the sections were allowed to cool at room temperature in a citric acid buffer solution for 45 minutes before continuing the procedure. Standard positive controls were used throughout, with serum used as the negative control.

Point mutations in K-*ras*-2 were sought in 15 cases. Mutational analysis was performed by topographic microdissection. This type of sampling specifically selects areas of tumor from paraffin-embedded tissue sections 5 μm thick that will yield the highest concentration of tumor cells and the lowest number of "background" normal cells. Polymerase chain reaction amplification for the K-*ras*-2 exon 1 gene, using flanking intron primers, was performed as previously described (13–16). Cycle sequencing with ^{32}P was performed using dideoxy terminators and one of the amplifying primers and was subsequently analyzed on a 6% denaturing polyacrylamide gel. Suspect mutations were then reamplified and confirmed by sequencing the opposite strand.

RESULTS

Clinical Demographics, Signs, and Symptoms

There were 13 male and 9 female patients with ages at the time of diagnosis ranging from 48 to 85 years,

TABLE 1. Immunohistochemical Panel

Antigen	Primary antibody	Company	Dilution	Antigen recovery
Cytokeratin 7	Mouse monoclonal	DAKO, Carpinteria, CA	1:200	Enzyme digestion
Cytokeratin 20	Mouse monoclonal		1:50	Enzyme digestion
CAM 5.2	Mouse monoclonal	Becton Dickinson, San Jose, CA	1:100	Enzyme digestion
B72.3	Mouse monoclonal	BioMed Tech., Stoughton MA	1:20	None
CA19.9	Mouse monoclonal	Signet, Dedham, MA	Neat	Enzyme digestion
DUPAN-2	Mouse monoclonal	BioGenex Labs, San Ramon CA	Neat	Enzyme digestion
Carcinoembryonic antigen	Rabbit polyclonal	DAKO	1:800	Enzyme digestion
Proliferating cell nuclear antigen	Mouse monoclonal		1:1600	Microwave recovery
Ki-67	Mouse monoclonal	Immunotech, Westbrook, ME	1:20	Microwave recovery
p53	Mouse monoclonal	DAKO	1:50	Microwave recovery
*c-erb*B-2	Mouse monoclonal	ChemiCon Int., Temecula, CA	1:400	Microwave recovery
Collagen IV	Mouse monoclonal	DAKO	1:50	Enzyme digestion
Laminin	Mouse monoclonal	Sigma, St. Louis, MO	1:8000	Enzyme digestion
Cyclin E	Mouse monoclonal	Vector/Novocastra, Burlingame, CA	1:50	Microwave recovery
p27	Mouse monoclonal	Vector/Novocastra	1:50	Microwave recovery

TABLE 2. Radiographic Findings

Ultrasound	Radiography	Endoscopic retrograde cholangiopancreatography	Computed tomographic scanning/ magnetic resonance imaging	Angiography
Dilated ducts; solid and cystic complex mass in head, body or tail; nodule within the duct lumen	Widening of duodenal loop	Cystic mass connected to the pancreatic duct; dilatation or ectasia of the duct; abrupt filling defect usually in the head of the pancreas with partial to complete duct obstruction; occasional nodular filling defects	Dilated pancreatic duct; mass lesion in head, body or tail; inhomogeneous or low density solid to cystic lesion; small calcifications noted	Avascular mass; stenosis with neovascularization

with a mean age of 64.7 years. Pain or discomfort (usually in the epigastric or left upper quadrant region), either intermittent or continuous, was the most frequent presenting symptom (n = 11), followed by jaundice (n = 5), weight loss (n = 5), steatorrhea (n = 4), nausea or vomiting (n = 3), diarrhea (n = 3), and fever (n = 3). A palpable mass was present in three cases. Symptoms of chronic pancreatitis were present in four patients, and one patient had diabetes mellitus (the latter thought to be unrelated to the tumor). The symptoms lasted from a few days to 2 years. Two patients were asymptomatic: the tumors were discovered incidentally on imaging studies performed for unrelated reasons. Most of the patients had one or more of a variety of imaging procedures performed, including abdominal radiography, ultrasonography, computed tomographic scanning, magnetic resonance imaging, endoscopic retrograde cholangiopancreatography (ERCP), and angiography (Table 2). A rounded, well-circumscribed, retrogastric mass was identified (by ultrasonic examination, computed tomographic scanning, and magnetic resonance imaging), predominantly involving the head of the pancreas. Cystic cavities were noted that contained mucoid to hemorrhagic fluid approximating density of water (Fig. 1). ERCP showed a patulous papilla, and the sagittal images showed dilatation of the main pancreatic duct and, occasionally, filling defects within the duct (Fig. 2).

Pathologic Findings

Macroscopic Features

The majority of the tumors (n = 18) were located in the head of the pancreas. In the remaining four cases, the tumor involved the head and body (n = 2), the body only (n = 1), or the entire pancreas (n = 1). The tumors ranged from 1 to 15 cm in greatest dimension, with an average size of 6.9 cm. The majority of tumors showed cystic pancreatic ducts and were unicystic (n = 8) or multicystic (n = 13), with only one tumor described as solid. The cystic space(s) within the dilated duct(s) contained frond-like papillary proliferations (Fig. 3). Friable and necrotic debris was noted within the unilocular tumors.

Microscopic Features

In all of our cases, we found dilated duct(s) surrounded by fibrous connective tissue. The dilated ducts were lined by a proliferating epithelium forming complex papillae (Figs. 4 and 5). The papillae

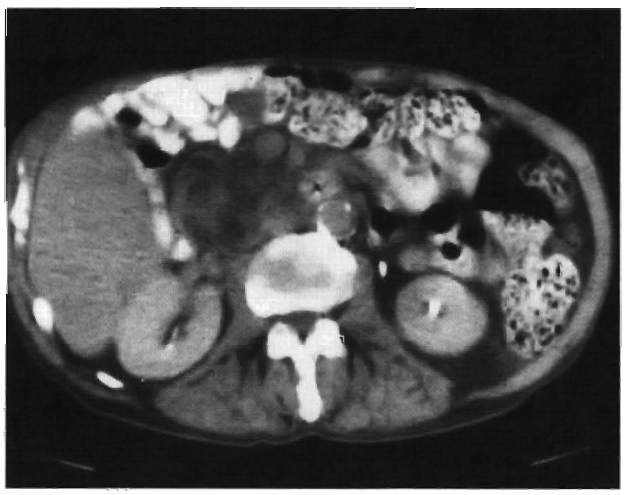

FIGURE 1. Computed tomographic scan demonstrating a multicystic mass with thin septations in the head of pancreas.

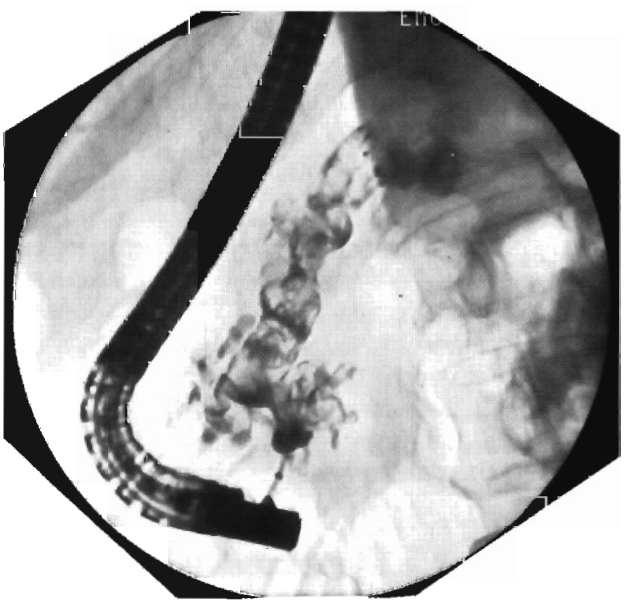

FIGURE 2. ERCP. The dilated main pancreatic duct shows filling defects, consistent with intraductal tumor.

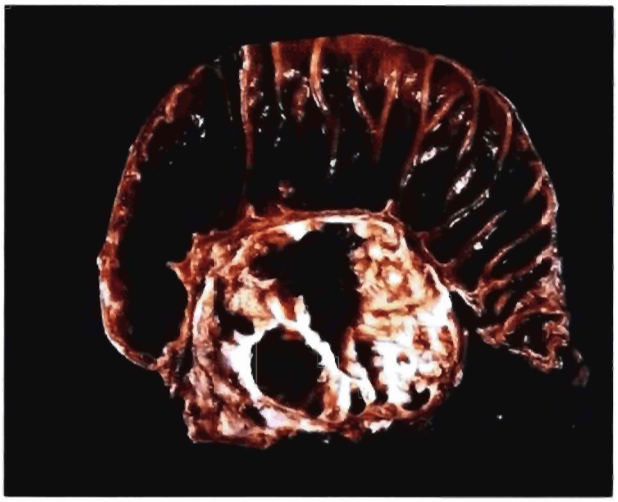

FIGURE 3. A macroscopic illustration of the tumor within cystically dilated ducts.

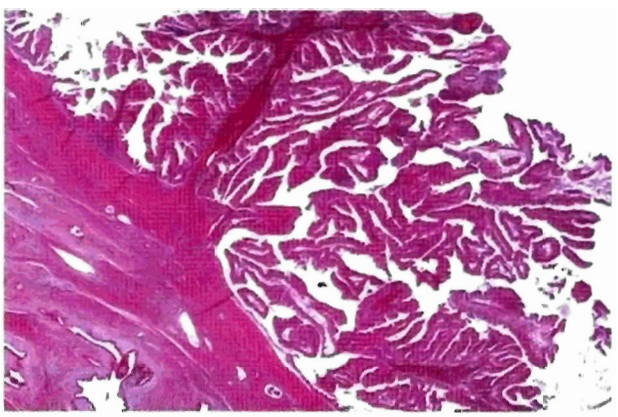

FIGURE 4. The typical appearance of the tumor, with an intraductal mass forming complex papillae, with marked fibrosis and atrophic changes in the surrounding pancreatic parenchyma (hematoxylin and eosin stain).

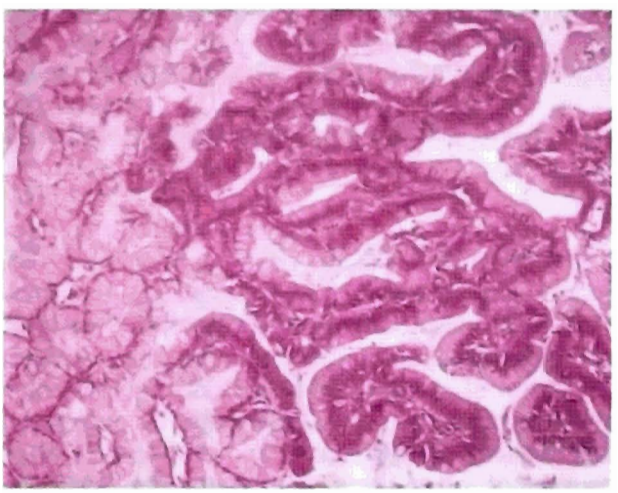

FIGURE 5. Transition of benign-appearing to atypical epithelium in a complex papillary process (hematoxylin and eosin stain).

had edematous stalks with mild-to-moderate chronic inflammation, and they were covered by tall, columnar, mucin-producing, epithelial cells (Fig. 6). Glandular profiles and a cribriform pattern

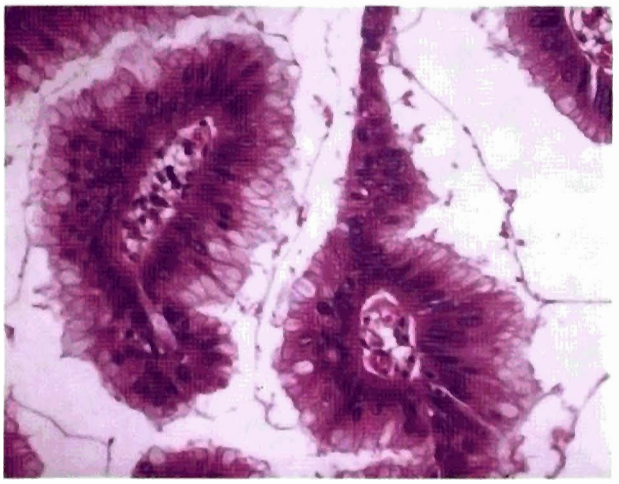

FIGURE 6. Cross section of papillae lined by atypical tall columnar epithelium (hematoxylin and eosin stain).

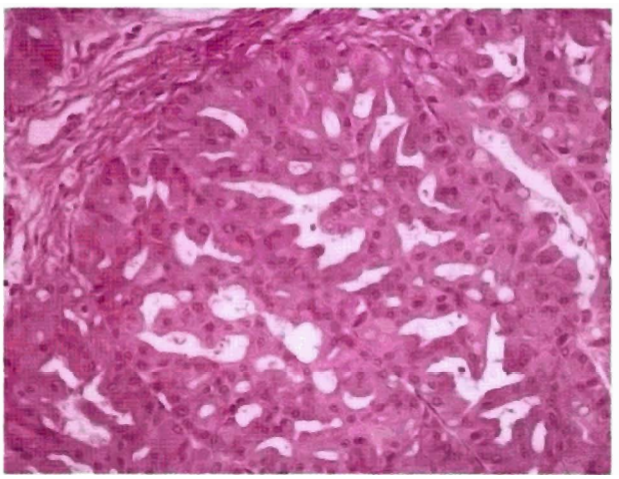

FIGURE 7. Cribriform pattern of growth of the tumor. There is a slight oxyphilia to the cytoplasm of the lining epithelial cells (hematoxylin and eosin stain).

TABLE 3. Histologic Features of Intraductal Papillary-Mucinous Neoplasms

Feature	Invasive (n = 15)	Noninvasive (n = 7)
Mild atypia	6	5
Moderate atypia	5	1
Severe atypia	4	1
Atypical mitoses	1	1
Increased mitoses	5	0

were present in five cases (Fig. 7). Psammoma bodies (laminated calcific bodies) were detected in three cases. Only two of the cases showed tumor cells with oncocytic metaplasia. Goblet cells were detected in 14 cases. Cytologic atypia was defined as an increased nuclear-to-cytoplasmic ratio, a loss of nuclear polarity, and prominent nucleoli. Cytologic atypia was of a variable degree, but present in all of our cases, even though it was focal in a few cases (Figs. 6 and 7) (Table 3). Atypia was graded as mild, moderate, or severe. Six cases with mild atypia demonstrated invasive tumor, whereas one

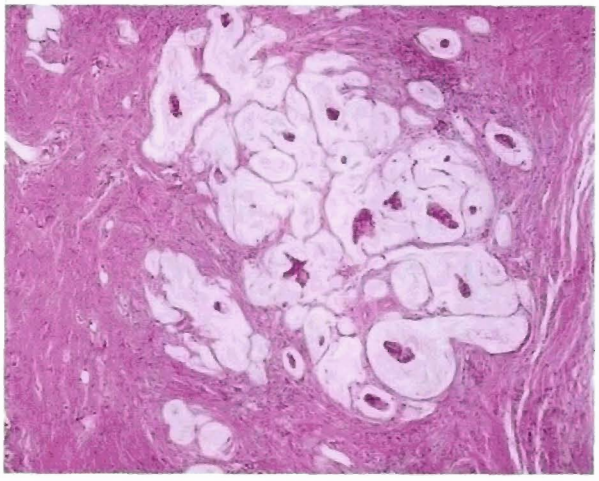

FIGURE 8. High power view of an invasive focus of the tumor (colloid carcinoma) (hematoxylin and eosin stain).

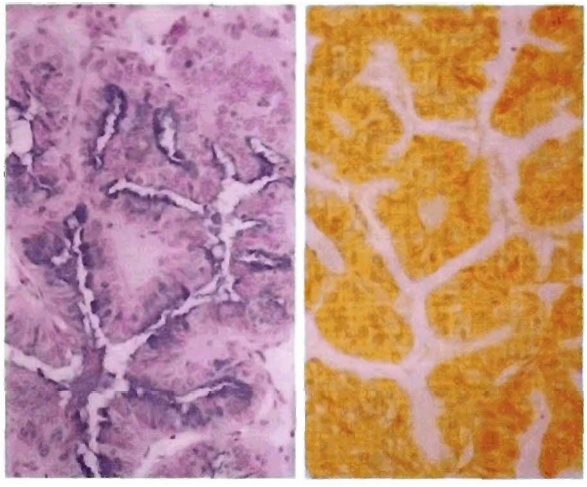

FIGURE 9. Tumor cells showing a positive reaction with Alcian blue (pH 2.5) after hyaluronidase treatment (sialated mucin) (**A**) and a negative reaction with aldehyde fuchsin (**B**).

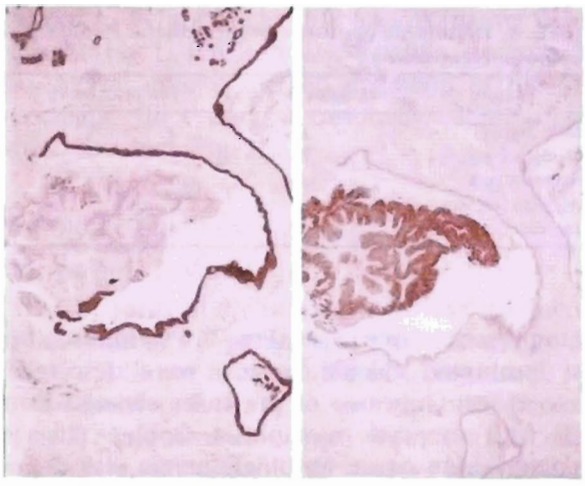

FIGURE 10. Some tumors lost their immunoreactivity with anti-CK7, although the ectatic, dilated, noninvolved ducts are reactive (**A**), whereas the tumor cells reacted with anti-CK20 (**B**).

case with moderate atypia and one case with severe atypia did not demonstrate any invasive tumor. Mitoses were present but infrequent. Neoplastic

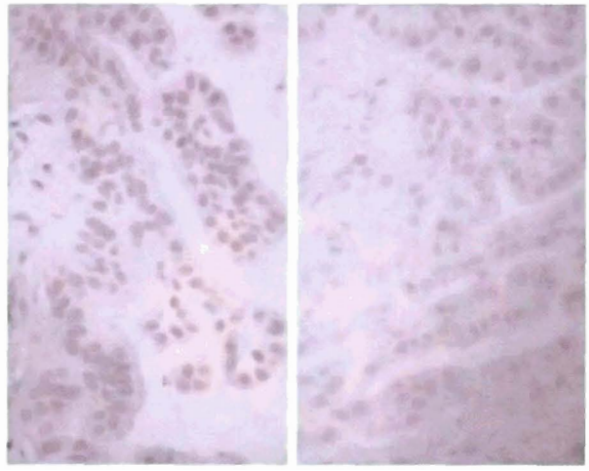

FIGURE 11. p27 (**A**) and cyclin E (**B**). This shows a more prominent and diffuse nuclear reactivity for p27 than for cyclin E.

cells invaded into the surrounding fibrous connective tissue and into the pancreatic parenchyma in 15 cases. Of these invasive cases, nine were associated with excessive mucin production and contained atypical cells and glandular profiles (noncystic mucinous or colloid carcinoma) (Fig. 8). The remaining six cases showed atypical, small, gland-like structures in the invasive areas. It was not possible to evaluate the presence or absence of invasion in three cases because of limited material. Chronic pancreatitis, with a variable degree of fibrosis, acinar cell atrophy, and ductal epithelial hyperplasia without atypia, was present in all of the cases. Metastatic adenocarcinoma was identified in the peripancreatic lymph nodes of a single case. This patient with metastatic disease had severe cytologic atypia, invasive ductal adenocarcinoma, and a *K-ras* mutation; the patient died a few days after surgery as a result of perioperative sepsis. There was no evidence of metastatic adenocarcinoma in the lymph nodes of an additional 19 cases sampled.

Histochemical Findings

The normal, uninvolved pancreatic ducts demonstrated sulfomucins by showing dark blue granules using Alcian blue (pH 0.4). After hyaluronidase digestion of Alcian blue (pH 2.5), sialomucins present within the tumor cells could be seen with a high level of specificity. Tall columnar cells made up most of the epithelium lining the papillae, demonstrating supranuclear or apical periodic acid–Schiff positive, diastase-resistant mucin, and a hyaluronidase-resistant, dark blue reaction with Alcian blue (pH 2.5), and light red to blue/purple granules with periodic acid–Schiff and Alcian blue. The reactivity decreased in intensity with increased atypia. There was weak and/or focal reactivity with

TABLE 4. Immunohistochemical Results

Case	CK7	CK20	CAM 5.2	B72.3	CA 19.9	DUPAN-2	CEA	PCNA (%)	Ki-67 (%)	p53 (%)	c-erbB-2	Cyclin E (%)	p27 (%)
1	+	+/F	n/a	n/a	n/a	n/a	n/a	n/a	n/a	50	n/a	–	10
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	–	< 1
3	+	+/F	+	–	–	–	+/ap	> 90	< 1	< 10	–	–	50
4	+/F	–	+	–	+/F	–	+/F-ap	> 90	< 10	< 10	–	–	10–30
5	–	–	+	+/F	–	–	+/F-cyt	> 90	10	10	–	–	10
6	+	–	+	–	+/F-ap	–	+/F-ap	30	–	–	–	–	< 1
7	+	–	+	+/F	–	–	+/F-cyt	70	10	70	–	–	20
8	+	+/F	+	+	+	+/F	+/cyt	> 90	–	50	–	–	> 90
9	+/F	–	+	+/F	+/F	–	+/F	> 90	–	–	–	–	< 1
10	–	–	+	+	–	–	+/ap	> 90	50	70	–	< 1	10
11	–	+	+	+/F-ap	+/ap	+/F	+/cyt	30	50	50	–	30	60
12	+/F	–	+	–	–	–	+/F/cyt	> 90	< 10	< 10	–	< 5	20
13	+	+/F	+	+	+	–	+	> 90	< 10	30	–	–	50
14	+	+	n/a	+	+	–	+/cyt	70	10	50	–	< 1	30
15	+	+	+	+	+	–	+/cyt	> 90	50	50	–	–	50

+, positive; –, negative; F, focal; ap, apical; cyt, cytoplasmic; n/a, not applied; CK, cytokeratin; CEA, carcinoembryonal antigen; PCNA, proliferating cell nuclear antigen.

Alcian blue (pH 0.4) and aldehyde fuchsin. Overall, the mucin of the neoplastic epithelial cells was of the sialated rather than the sulfated type (Fig. 9).

Immunohistochemical Reactions

Although unnecessary for the diagnosis of IPMN, a diverse panel of immunohistochemical reactions were performed on 15 cases (limited to those cases with adequate material) (Table 4) to characterize the immunophenotype of the neoplasm. In all of the cases, the neoplastic cells reacted with anti-CAM5.2, showing accentuation along the cytoplasmic membrane. In 11 of 14 cases, the neoplastic cells were positive for cytokeratin CK7. This reaction was weaker than that observed in normal ductal epithelium. Seven cases reacted with anti-CK20, whereas a focal reaction was evident in four cases (Fig. 10). The immunoreactivity of the CKs (either CK7 or CK20) did not seem to preferentially react with intestinal-type or pancreatobiliary-type areas of the intraductal or invasive portions of the tumors but instead was variably reactive throughout the lesion. DUPAN-2 (n = 2), CA19–9 (n = 8), and carcinoembryonic antigen (n = 12) had a marked heterogeneity of antigen expression, from weak and focal to more diffuse with increased intensity but variable within a single tumor.

Proliferating cell nuclear antigen, a cell cycle regulation antigen, is usually localized to sites of DNA synthesis (17). It was diffusely and strongly reactive in the nuclei of all 14 cases tested, thereby rendering this marker useless in tumor discrimination. Ki-67 monoclonal antibodies, specifically MIB-1, react with antigens expressed in all active parts of the cell cycle (17). Ki-67 was remarkably variable in expression in the 14 cases tested, from no reactivity to more than 50% of the cells reacting strongly. No definitive trend was noted. p53 protein antibody reacts with the accumulated p53 protein in the cell nucleus, usually increased in carcinomas, and consequently, it is frequently associated with a worse prognosis than that found in cases with lower levels. Our results with p53 protein showed variability similar to that of Ki-67, ranging from no reactivity to strong reactivity (> 70% of the cells' nuclei). There was no association with nuclear atypicality or a worse prognosis in the cases with increased reactivity. c-*erb*B-2 is a transmembrane protein, closely related to the epidermal growth factor receptor, and its expression is associated with a worse prognosis in breast cancer than that found in cases with lower levels of expression. None of the 14 cases we tested showed the specific cell membrane–bound type of reaction; this is in contrast to the findings of Sessa *et al.* (7). Cyclin E and p27 protein are important intranuclear proteins, with opposing roles in cell cycle regulation, *i.e.*, cyclin E enhances and p27 decreases mitotic activity. In all of the 15 cases examined, p27 reactivity always exceeded the reactivity of cyclin E (Fig. 11).

K-*ras*-2 Oncogene

Two of the 15 tumors tested for K-*ras*-2 oncogene showed a mutation, whereas the remaining 13 cases did not demonstrate a mutation and were of the wild type. Both mutations were from glycine to valine on codon 12, chromosome 12p. The two mutated cases occurred in patients with moderate-to-severe nuclear atypia, both with invasive tumors (one colloid, one ductal adenocarcinoma); one patient died perioperatively, whereas the other was alive without evidence of disease at 77.8 months. Of the remaining 13 cases, 8 were from patients with invasive disease (at 70.9 months, 5 of the 8 were alive without evidence of disease and 2 were dead, also without evidence of disease; 1 patient had died with local disease at 35.2 months) and 5 were from patients without invasion (at 73.2 months, 4 were

alive without evidence of disease, and 1 had died perioperatively).

Treatment and Follow-Up

A complete surgical excision was the treatment of choice, although it was difficult to achieve in some cases. Patients underwent a Whipple procedure (n = 13), a total pancreatectomy (n = 4), a partial pancreatectomy (n = 3) or a biopsy only (n = 2). None of the patients had adjuvant chemotherapy and/or radiation, either preoperatively or postoperatively. The overall patient survival for IPMN is good. Seventeen (77.3%) of the 22 patients in our series were alive without any evidence of disease (n = 14; mean, 58.2 mo) or had died of unrelated causes (n = 3; mean, 22.8 mo), with an overall average follow-up of 74.7 months (range, 6–172 mo). Of these 17 patients, 6 had no evidence of histologic invasive tumor (average, 75.6 mo), whereas the remaining 11 patients demonstrated invasive tumor (average, 61.9 mo). Nine patients had colloid-type or mucinous-type invasive carcinoma, and two had infiltrating ductal adenocarcinoma. Three patients (13.6%) died of complications related to the surgery in the immediate postoperative period (two patients with infiltrating ductal adenocarcinoma, one without invasive disease). One of these three patients had peripancreatic lymph node metastasis, along with severe cytologic atypia, invasive ductal adenocarcinoma, and a K-*ras* mutation. Only two (9.1%) patients died of their disease, one with widely disseminated disease (with infiltrating ductal adenocarcinoma) at 36 months, and the other patient, who had infiltrating ductal adenocarcinoma, with local recurrence at 52 months.

DISCUSSION

The first pathologic description of IPMN by Morohoshi *et al.* (1), in 1989, included lesions such as intraductal papilloma, diffuse intraductal adenocarcinoma, and *in situ* carcinoma of the pancreas. Histologically, these tumors are characterized by an intraductal epithelial proliferation of cuboidal-to-tall columnar cells, with variable degrees of atypia, a papillary or cribriform configuration, occasional comedo-type necrosis, variable sialomucin production, and few mitotic figures (1, 2, 4, 5, 7, 18–24). The original description of IMPN included a variety of tumors, each one with a different set of diagnostic criteria. Only cases with a dominant, intraluminal, protruding mass forming complex papillae and a cystic mass were included in our series. Tumors with an atypical papillary epithelium and without a large, dominant, intraluminal mass were considered to be *in situ* ductal carcinomas and were excluded. Many terms have been applied to this type of tumor, including:

- intraductal papillary-mucinous neoplasm,
- intraductal mucin-hypersecreting tumor,
- villous adenoma of the main pancreatic duct,
- papillary adenocarcinoma of the main pancreatic duct,
- mucinous ductal ectasia, and
- mucin-producing tumor (3, 6, 19, 22, 25–29).

Such a variety of terms applied to the same entity without similar histologic descriptions or findings has caused considerable diagnostic confusion. Therefore, we propose, like Sessa *et al.* (7), the term *intraductal papillary-mucinous neoplasm of the pancreas,* which seems most appropriate in reference to this distinctive tumor.

The clinical presentation included vague abdominal pain, nausea, vomiting, diarrhea, steatorrhea, and jaundice, without a specific increase in the frequency of diabetes. Specific symptoms of pancreatitis (either acute or chronic) were uncommon, except when the tumor resulted in duct obstruction. Our findings parallel results reported by other investigators (1, 2, 4, 19, 30, 31). Serum carcinoembryonic antigen and CA19–9 levels were noncontributory (2).

Computed tomographic scanning, magnetic resonance imaging, and ERCP are the most frequently used radiographic studies, with ERCP being the most sensitive and specific. Duct dilatation without proximal stricture, cysts communicating with the main pancreatic duct, and linear, round, or amorphous filling defects corresponding to mucus plugs or papillary epithelial projections are helpful as diagnostic features of these tumors (2, 4–6, 19, 20, 27, 31–35). Endoscopic brushings or biopsies usually yield a nonspecific result (31), requiring excision of the neoplasm for accurate classification. The neoplasm has been classified into four subgroups on the basis of its radiographic appearance:

- Type I: uniformly dilated main duct;
- Type II: focally dilated main duct;
- Type III: cystic sub-branches; and
- Type IV: dilated sub-branches (25).

As a result of the nature of our facility, which is a diagnostic referral center, we do not receive gross specimens with which to apply and correlate the radiographic images and descriptions with the macroscopic findings. The above classification seems to be purely of academic interest and, to our knowledge, without any prognostic significance.

In the literature, these tumors were divided into the histologic subcategories of hyperplasia, adenoma, or carcinoma (5, 27, 28, 33, 36, 37). We could not identify, however, histomorphologic criteria that can accurately and reproducibly separate these

tumors into subcategories. In addition, we do not include ductal hyperplasia *per se* as part of the spectrum of these tumors, because ductal epithelial hyperplasia, both papillary and nonpapillary, is seen in chronic pancreatitis and in a variety of other conditions (21, 38). Both hyperplastic and neoplastic cells produce neutral to sialomucin rather than sulfated mucin, as normally expected (39). There is considerable debate concerning the possibility of ductal papillary epithelial hyperplasia (with and without atypia or dysplasia) being a precursor of neoplasia (20, 21, 28, 29, 40–43). Although we cannot deny this possibility, the findings in our study do not help to resolve this controversy.

Tumor invasion might be focal (27, 44), extensive or multifocal, requiring adequate sampling of the tumor for proper examination. We examined an average of 15 sections per case, submitting additional sections when necessary to for adequate characterization of the extent of tumor invasion. In many cases, areas of extension of the tumor along the dilated or nondilated ductules might be misinterpreted as representing tumor invasion. Multiple levels and sections might be necessary to clarify this type of extension, although we did not attempt to quantitate the amount of invasion because of the nature of the specimens received. In our series, tumor invasion in IPMN seemed to be characterized by the presence of mucin pools, which frequently contained floating neoplastic epithelial cells or conventional ductal adenocarcinoma (27). The presence of invasion did not independently or adversely affect the long-term clinical outcome. Metastatic disease was reported (9), although it was not frequent. Our results confirmed those reported in the literature, in that we identified only a single case with widely disseminated disease and lymph node metastasis.

IPMN must be distinguished from atypical hyperplasia, ductal carcinoma *in situ*, and mucinous cystic neoplasms (2, 5, 7, 8, 20, 35). Ductal epithelial hyperplasia with atypia is frequently seen in association with pancreatic cancer. The atypia is of variable degree and can approach that of adenocarcinoma *in situ*. The cellular atypia in carcinoma *in situ* is remarkable, with an increased nuclear-to-cytoplasmic ratio, nuclear pleomorphism, prominent nucleoli, a loss of nuclear polarity, and a cribriform pattern. Mitotic figures are frequent. In both lesions, the papillae are small, and they lack a central fibrovascular core. These alterations of the ductal system are microscopic findings that do not show the macroscopically identifiable protruding mass lesion and dilated ducts of an IPMN.

Mucinous cystic neoplasms occur almost exclusively in middle-aged females, in the body or tail of the pancreas, are of a larger size, lack a direct connection with the pancreatic ducts, and have a characteristic ovarian-type stroma (8, 31, 45, 46). IPMN occur in an older age group of patients, affect slightly more men than women and are typically located in the head of the pancreas.

In neoplastic conditions, there is a shift to neutral and sialomucin production (incomplete glycosylation of apomucins [47, 48]), similar in character to that seen in colorectal carcinoma, as opposed to normal pancreatic ducts, which produce sulfomucin (49, 50). Sialomucins appear as hyaluronidase-resistant, dark blue granules with Alcian blue (pH 2.5) and as light red to blue/purple granules with periodic acid–Schiff and Alcian blue. The cases we studied stained as neutral to sialomucins (Fig. 9).

Tumor development and growth regulation is not well understood, but cyclin E and p27 play an important role in cell cycle regulation. Cyclins (proteins) regulate the activity of cyclin-dependent kinases (CDKs), which are active only when bound to cyclins (51, 52). The cyclin-CDK complexes propel the cell into S phase or mitosis, depending on their specificity. The major cyclin groups (G_1 cyclins [A, D, and E] and G_2 cyclins [A and B]) affect proliferation, depending on the phase of the cell cycle affected. That is, G_1 cyclins bind to CDKs in G_1 phase and are required for entering the S phase, whereas G_2 cyclins are necessary for entering the M phase (51, 52). Cyclin E acts at the G_1-S transition, with p27 counteracting the effects of cyclin E, because p27 is a stoichiometric inhibitor of the cyclin-CDK complex (along with p15) in the G_1 phase (53, 54).

High cyclin E and low p27 levels are associated with a significant increase in mortality in patients with breast cancer, although each marker is considered an independent prognostic marker (55–57). Similarly, a lack of p27 expression is associated with a worse prognosis in colorectal and gastric carcinomas, compared with cases that show higher levels of expression (58, 59). By contrast, in our cases, there were higher levels of p27 than cyclin E, suggesting a favorable prognostic outcome (a single case did not react with either marker).

K-*ras* mutations are identified in a high percentage (as high as 100%) of pancreatic cancers (60), usually increasing in frequency with greater cytologic atypia, thus perhaps affecting early pancreatic carcinogenesis and resulting in a dismal prognosis (13–16, 61–69). Reports of K-*ras* mutations in IPMN are widely disparate, from no detectable mutations (66), to intermediate levels (although not associated with cytologic atypia) (7, 61), to high levels of mutation, especially in pancreatic juice, because the tumor cells exfoliate into the fluid (65, 70). The most frequent mutation is from glycine to aspartic acid (GGT to GAT), according to Hoshi *et al.* (60). This finding contradicts the claim that this substitution is associated with a more aggressive clinical behavior (71). The two mutated cases (of 15 cases

tested) were identified from invasive tumors (one was a noncystic mucinous [colloid] carcinoma; the other was an infiltrating ductal adenocarcinoma). The IPMN portions demonstrated moderate and severe nuclear atypia, respectively. Nine, however, of the wild-type cases demonstrated mild nuclear atypia, three had moderate atypia, and one had severe atypia. There were too few cases for us to draw a statistically significant conclusion, but there was a trend toward cases with severe atypia demonstrating K-*ras* mutations. Our results, however, did not correlate with clinical outcome, because one patient died in the immediate postoperative period, and the other was alive, with no evidence of disease, at the time of last follow-up. In summary, the overall low rate of mutation, perhaps correlated with nuclear atypia (not noted in any cases with mild atypia, and only in cases with moderate to severe atypia), supports the generally good prognosis associated with this neoplasm.

Similar to our K-*ras* mutation results, there was variable detection of p53 protein mutations in IPMN (4, 7, 30, 60, 64, 69–71). It is proposed that K-*ras* mutations might exceed the frequency of p53 mutations in pancreatic cancer in general (72). In contrast to these findings, however, we identified the presence of presumed p53 mutations in nine of our cases tested, with 10 to 70% of the tumor cells demonstrating the abnormal phenotype by immunohistochemical analysis. This finding supports the proposal that p53 protein mutation occurs in an early phase of pancreatic carcinogenesis but does not match the reports in the literature that suggest that prognosis worsens with p53 protein overexpression (60, 70–74). It was reported that c-*erb*B-2 overexpression occurs in IPMN, in *in situ* ductal carcinoma, and in invasive ductal adenocarcinoma, and that it is associated with a poor clinical outcome (7, 74–77). We were unable to confirm the results of these earlier studies, because none of our cases tested with c-*erb*B-2 demonstrated overexpression. By inference, a lack of overexpression of c-*erb*B-2 would suggest a better prognosis, as seemed to be demonstrated in our patients.

The recommended therapy includes partial pancreatectomy with complete surgical excision of the neoplasm. A palliative sphincterectomy of Vater's papilla might be beneficial for high-risk surgical patients (31, 78). Only two patients died with evidence of their disease in our series, at 36 and 52 months, respectively, after the initial diagnosis. Unfortunately, the high risk of morbidity associated with pancreatic surgery must be taken into consideration (three of our patients died of surgical complications). Overall, this favorable 89% survival without disease recurrence or metastases at last follow-up (average, 74.7 mo), irrespective of clinical presentation, immunophenotype, K-*ras* mutations, or proliferation markers, supports the good clinical outcome reported in the literature (2–9, 25, 28, 34, 36, 37). Patients with invasive adenocarcinoma (ductal type) ($n = 6$) had an average survival of 50.4 months (two dead with disease, two dead of surgical complications, and two alive without disease), which is considerably better than the average survival of patients with "invasive ductal adenocarcinoma, not further specified" of 10 to 20 months, with less than 3% still alive at 36 months (79).

In summary, we think that IPMNs should be defined as neoplasms showing a protruding papillary mass within a cystically dilated pancreatic duct, either with or without invasion, lined by columnar, mucin-producing cells, frequently demonstrating high p27 reactivity and low cyclin E reactivity, a low K-*ras* oncogene mutation rate, and lack of c-*erb*B-overexpression. The conclusions proposed are on the basis of 22 cases, but inasmuch as subdivision of these tumors into benign, borderline, and malignant categories is not possible using clinical, radiographic, histomorphologic, or immunophenotypic results and seems not to be prognostically significant in our series, we suggest they should all be classified as IPMNs. A comment, where applicable, concerning evidence of invasion should be included. All of these neoplasms should be considered as tumors of low-grade malignant potential with a good prognosis. Studies with proliferative and other prognostic markers seem to support the favorable outcome of these tumors.

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