Mucinous Carcinomas of the Gallbladder

Clinicopathologic Analysis of 15 Cases Identified in 606 Carcinomas

Nevra Dursun, MD; Oscar Tapia Escalona, MD; Juan Carlos Roa, MD; Olca Basturk, MD; Pelin Bagci, MD; Asli Cakir, MD; Jeanette Cheng, MD; Juan Sarmiento, MD; Hector Losada, MD; So Yeon Kong, MPH; Leslie Ducato, BS; Michael Goodman, MD; N. Volkan Adsay, MD

• ***Context.*—There are virtually no data in the literature regarding the incidence, patterns, and clinicopathologic characteristics of mucinous carcinomas (MCs) of the gallbladder (GB).**

***Objective.*—To determine the incidence of mucinous differentiation in invasive GB carcinomas and the clinicopathologic characteristics of those that qualify as MC.**

***Design.*—Primary invasive GB carcinomas (n = 606) were reviewed for mucinous differentiation. Some degree of mucin production was identified in 40 cases (6.6%); however, only 15 (2.5%) were qualified for the World Health Organization definition of MC (stromal mucin deposition constituting >50% of the tumor).**

***Results.*—The mean age was 65 years, and the female to male ratio was 1.1 (versus 3.9 for conventional pancreatobiliary-type GB adenocarcinomas; *P* = .04). A significant proportion of the cases (8 of 12, 67%) presented with the clinical picture and intraoperative findings that were interpreted as *acute cholecystitis*. Mean and median tumor sizes were larger than those of conventional adenocarcinomas (4.8 and 3.4 cm versus 2.9 and 2.5 cm, respectively; *P* = .01). Most (13 of 15, 87%) cases presented with pT3 tumors (versus 48% for ordinary GB carcinomas; *P* = .01). Two cases had almost an exclusive colloid pattern (>90% composed of well-defined stromal mucin nodules that contained scanty carcinoma cells, most of which were floating within the mucin). Eight cases were of mixed-mucinous type, showing a mixture of colloid and noncolloid patterns. Five others had prominent signet-ring cells, both floating within the mucin (which constituted >50% of the tumor by definition) and infiltrating into the stroma as individual signet-ring cells in some areas. Immunohistochemical analysis performed on the 7 cases that had available tissue revealed CK7 in 4 of 7 (57%), CK20 in 2 of 7 (29%), MUC1 in 4 of 7 (57%), MUC2 in 6 of 7 (86%), CDX2 in 1 of 7 (14%), MUC5AC in 6 of 7 (86%), MUC6 in 0 of 7 (0%), and loss of E-cadherin in 6 of 7 (86%). The MLH1 and MSH2 were retained in 6 of 7 cases (100%). Follow-up information was available for 13 cases: 11 (85%) died of disease (1–37 months) and 2 (15%) were alive (23 months and 1 month). Overall survival of MCs was significantly worse than that of conventional adenocarcinomas (13 versus 26 months; *P* = .01); however, that did not seem to be independent of stage.**

***Conclusions.*—Mucinous carcinomas constitute 2.5% of GB carcinomas. They present with an acute cholecystitis-type picture. Most MCs are a mixed-mucinous, not pure colloid, type. They are typically large and advanced tumors at the time of diagnosis and thus exhibit more-aggressive behavior than do ordinary GB carcinomas. Immunophenotypically, they differ from conventional GB adenocarcinomas by MUC2 positivity, from intestinal carcinomas by an often inverse CK7/20 profile, from pancreatic mucinous carcinomas by CDX2 negativity, and from mammary colloid carcinomas by a lack of MUC6. Unlike gastrointestinal MCs, they appear to be microsatellite stable.**

(*Arch Pathol Lab Med.* 2012;136:1347–1358; doi: 10.5858/arpa.2011-0447-OA)

Extracellular mucin production in carcinomas has been shown to reflect activation or modification of various cellular pathways that not only impart a different morphology to the tumor but also confer distinct biologic properties to the carcinoma cells.[1–5] Carcinomas with copious mucin production are now thought to form a distinct category among malignancies of glandular organs.[1–7]

In the literature, there are virtually no data on the incidence, patterns, or biologic and clinical significance of mucinous differentiation in the gallbladder (GB). The information on ''mucinous carcinomas'' of this organ is composed of rare individual case reports[8–21] or opinions presented in textbooks.[22–25]

This study was undertaken to determine (1) the incidence of mucinous differentiation in invasive GB carcinomas, and

Accepted for publication December 13, 2011.

From the Department of Pathology, Istanbul Education and Research Hospital, Istanbul, Turkey (Dr Dursun); the Department of Pathology, University de La Frontera, Temuco, Chile (Drs Escalona Roa, and Losada); the Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York (Dr Basturk); the Department of Pathology, Rize University, Rize, Turkey (Dr Bagci); the Department of Pathology, Çorlu State Hospital, Çorlu, Turkey (Dr Cakir); the Department of Pathology, Piedmont Hospital, Atlanta, Georgia (Dr Cheng); and the Departments of General Surgery (Dr Sarmiento), Epidemiology (Ms Kong and Dr Goodman), Clinical Research (Ms Ducato), and Pathology (Dr Adsay), Winship Cancer Institute, Emory University, Atlanta.

The authors have no relevant financial interest in the products or companies described in this article.

Presented in part at the annual meeting of the United States and Canadian Academy of Pathology, Washington, DC, March 2010.

Reprints: N. Volkan Adsay, MD, Department of Pathology, Winship Cancer Institute, Emory University Hospital, Room H-180-B, 1364 Clifton Rd NE, Atlanta, GA 30322 (e-mail: volkan.adsay@emory.edu).

(2) the clinicopathologic characteristics of those tumors that qualify as mucinous carcinoma by the current definitions.

MATERIALS AND METHODS

This study was conducted in accordance with Institutional Review Board requirements.

Cases

All the pathology material available on 606 cholecystectomies with invasive GB carcinoma identified in the institutional surgical pathology files of Emory University, Atlanta, Georgia (1997–2011; n = 74 cases [12%]), Wayne State University, Detroit, Michigan (1985–2007; n = 60 cases [10%]), and the University de la Frontera, Temuco, Chile (1994–2004; n = 472 cases [78%]) were retrieved and reviewed. Cases with extracellular mucin production were identified.

Definitions

Any degree of *extracellular* mucin production was regarded as *mucinous differentiation* and was recorded. Cases in which the stromal mucin deposition constituted more than 50% of the tumor were classified as *mucinous carcinoma* (MC) according to the current World Health Organization classification,[24] and their clinicopathologic characteristics were investigated. Cases in which the mucin was confined to the lumina of the infiltrating glandular units, but not present in the stroma, were *not* qualified as MC. Ordinary adenocarcinomas of GB, composed of tubular infiltration, characteristic of pancreatobiliary-type adenocarcinomas, as seen in pancreatic ductal carcinomas or cholangiocarcinomas, were designated as *conventional GB adenocarcinomas*.

For histopathologic categorization, the cases were examined independently by the observers (N.D., A.C., P.B., and N.V.A.), and the case was placed in the group assigned by the majority of the opinions.

Evaluation of Clinicopathologic Associations

Information on the patients' demographics, clinical presentation, and follow-up were obtained through pathology databases, patients' charts, or by contacting the patients' primary physicians.

Tumor characteristics were determined by the analysis of pathology material in conjunction with clinical findings. Histologic grade, lymphovascular/perineural invasions, and lymph node and resection margin status were verified by histologic examination.

Pathologic staging was determined according to the 2010 guidelines of the American Joint of Committee of Cancer.[26] The growth pattern of a preinvasive component was classified as flat (conventional dysplasia) or polypoid/mass forming (the categories of *adenoma*, *intracystic papillary neoplasms*, and *papillary adenocarcinomas* in situ, ie, those categories that we recently proposed[27,28] be referred to as *intracholecystic papillary tubular neoplasm*), and the degree of dysplasia was graded as *low* or *high* according to the established criteria.

Immunohistochemical Analysis

Immunohistochemical analysis was performed for markers of MCs in other organs, as well as cell lineage markers known to be differentially expressed in different components of the gastrointestinal tract, which have also been used for subclassification of pancreatic and biliary neoplasms. Only 7 of the cases had blocks accessible for analysis. Those patients for whom blocks were accessible were felt to be representative of the overall MC group (3 men, 4 women; mean age, 64 years).

MUC1.—Mammary gland-type apomucin is commonly expressed in neoplasms of pancreatobiliary differentiation, including the ones arising in the GB.[29–32]

MUC2.—Intestinal goblet cell type apomucin is a fairly specific marker of intestinal differentiation as well as of mucinous colloid-type carcinomas of the exocrine organs, the pancreas, and the breast, and their precursor lesions, namely intestinal subtype of intraductal papillary mucinous neoplasms of the pancreas, and solid-papillary carcinomas of the breast, are not generally expressed in other tumor types in these organs.[2,28–33]

CDX2.—Intestinal transcription factor, an upstream regulator of MUC2, is another reliable marker of intestinal differentiation, with an expression profile closely paralleling that of MUC2. The nuclear expression of this marker is usually highly limited in pancreatobiliary adenocarcinomas compared with colonic adenocarcinomas, where it is very common and diffuse.[34–37] Along with MUC2, CDX2 is expressed uniformly in colloid carcinomas of pancreas.[2,3]

MUC5AC.—Gastric foveolar cell type apomucin is a good marker of gastric differentiation, expressed intensely in gastric foveolar epithelium. Its expression is observed in many types of pancreatobiliary neoplasms, including not only gastric foveolar type intraductal papillary mucinous neoplasms (IPMNs) but also other types of IPMNs and invasive ductal adenocarcinomas, even though MUC5AC is not expressed in normal pancreatobiliary epithelium.[29,33,38–40]

MUC6.—Gastric pyloric cell type apomucin is expressed in gastric pyloric glands as well as gastric cardiac glands and duodenal Brunner glands. Its expression is commonly observed in neoplasms or lesions with pyloric gland appearance, such as the so-called pyloric gland adenomas, a subset of IPMNs and basal glands associated with IPMNs or mucinous cystic neoplasms.[33,39,41–43]

CK7.—A subtype of high–molecular-weight cytokeratins, is expressed consistently in pancreatobiliary ductal epithelium and its neoplasms.[29,38,44–51]

CK20.—A subtype of low–molecular-weight cytokeratins, is expressed consistently in intestinal-type epithelium and its neoplasms.[38,45–51]

Biologic Markers.—*E-cadherin*.—One of the main components of the cell junction proteins, is expressed in the biliary tract epithelium. Its expression is usually decreased in biliary carcinomas.[52,53]

Microsatellite Instability Markers (MLH1 and MSH2).—The MCs of the gastrointestinal tract have some correlation with the loss of microsatellite instability markers at the immunohistochemical level.[54] In the pancreas, however, mucinous colloid carcinoma is microsatellite stable (and, therefore, stains diffusely with MLH1 and MSH2).[55] Inactivation of the mismatch repair genes occurs in early GB carcinogenesis.[56]

Methodology

Immunohistochemistry was performed using a polymer-based detection system (Envision; Dako, Carpinteria, California) with mouse monoclonal antibodies, according to the manufacturer's instructions. Sections were deparaffinized and rehydrated with deionized water. Then, they were heated in citrate buffer, pH 6.0, using an electric pressure cooker for 3 minutes at 12 to 15 pounds per square inch at approximately 120°C, and cooled for 10 minutes before immunostaining. All slides were loaded onto an automated system (Autostainer; Dako) and exposed to 3% hydrogen peroxide for 5 minutes, incubated with primary antibody for 30 minutes, incubated with labeled polymer (Envision dual link) for 30 minutes, incubated in 3′3-diaminobenzidine as a chromogen for 5 minutes, and counterstained with hematoxylin for 5 minutes. These incubations were performed at room temperature. Between incubations, sections were washed with Tris-buffered saline. Coverslips were placed with the Tissue-Tek SCA (Sakura Finetek USA, Inc, Torrance, California). Positive and negative controls were run with each batch of patient/study slides tested. The detailed specifications of the antibodies are provided in Table 1.

Evaluation of Immunohistochemical Stains

If the percentage of cells revealing cytoplasmic (MUC2, MUC5AC, MUC6, CK7, CK20), membranous (E-cadherin), apical membranous or cytoplasmic (MUC1), and nuclear (CDX2, MLH 1, MSH 2) labeling were greater than 10% of the cells, that slide was regarded as positive, as has been done in other studies.[38,49,57–59]

Table 1. Specification of the Antibodies				
Antibody	Clone	Dilution	Antibody Source	Retrieval Method
MUC1	Ma695	1:160	Leica Microsystems, Bannockburn, Illinois	Citrate
MUC2	CCp58	1:100	Leica Microsystems	Citrate
MUC5AC	CLH2	1:200	Leica Microsystems	Trilog
MUC6	CLH5	1:80	Leica Microsystems	Trilog
CK7	OB-TLI2/30	1:40	Dako, Carpinteria, California	Citrate
CK20	Ks20.8	1:40	Dako	Citrate
CDX2	CDX2-88	1:200	Biogenex, San Ramon, California	Citrate
E-cadherin	ECCD-2	1:50	Invitrogen, South San Francisco, California	Citrate
MLH1	G168-758	1:20	BD Pharmingen, San Diego, California	Citrate
MSH2	FE11	1:20	Calbiochem, San Diego	Citrate

Statistical Analysis

Differences in clinicopathologic features between the groups were analyzed by unpaired Student *t* test and analysis of variance for continuous variables; Pearson χ^2 and Fisher exact tests were used for categoric variables, and a log-rank test (Cox-Mantel) was used for survival comparisons. Overall survival was analyzed with the Kaplan-Meier method, and the differences in survival between selected groups were assessed by log-rank test. Stage-matched analyses were performed between different etiologic groups with a same stage to test whether survivals were independent of the T stage. Univariate and multivariate analyses, using the Cox proportional hazard model and R version 2.9.1 open source statistical software (R Foundation for Statistical Computing, Wien, Austria), were performed to determine the prognostic factors for survival of the patients. Statistical significance was defined as a P value less than .05.

RESULTS

At least some degree of mucinous differentiation (excessive mucin production) was identified in 40 of 606 cases (6.6%).

Fifteen of those 40 cases (38%) were qualified as MC (Figure 1) and were subjected to detailed analysis. Of the remaining 25, 17 (68%) were adenocarcinoma with focal mucinous differentiation (<50% of the tumor), and 8 (32%) were well-differentiated adenocarcinoma with intraglandular mucin, without any stromal mucin deposition. These were not regarded as MC. The clinicopathologic findings in these groups are presented and compared in Table 2.

Clinical Features

The patients ranged in age from 47 to 78 years (mean, 65 years); 8 patients (53%) were women, and 7 (47%) were men (female to male ratio, 1.1, as opposed to 3.9 in conventional GB carcinomas; $P = .04$).

Presenting symptoms information was available in 12 of 15 cases (80%) and indicated biliary obstruction in 10 of 12 cases (83%; jaundice, n = 4; abdominal colic type pain, n = 7; vomiting, n = 6; weight loss, n = 3, with many patients presenting with >1 symptom). The other 2 cases (17%) were asymptomatic and were recognized in routine image analysis with diffuse and asymmetric wall thickening.

Indication for operation was recorded as *acute cholecystitis* in most of the cases (8 of 12; 67%). Other reasons were chronic cholecystitis in 2, and asymmetric wall thickening in 1. Only one patient was suspected to have cancer preoperatively. Two patients had a history of breast carcinoma, and one had lung hydatidosis.

Main radiologic findings were thickened GB walls with pericholecystic fluid accumulation, biliary obstruction, and proximal dilatation of biliary ducts.

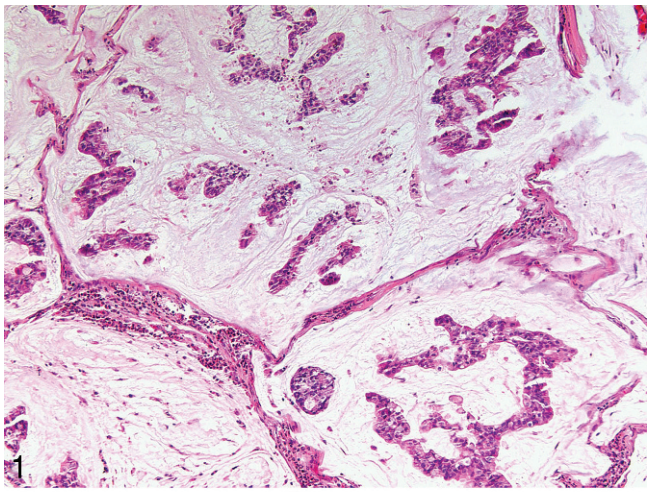
Intraoperative findings were documented in 10 cases, and 6 showed significant, acute inflammatory changes, including red, friable serosal surfaces and fibrinous adhesions. Peritoneal carcinomatosis was noted in 2 cases intraoperatively. Gallstones were present in 10 of 13 (77%).

Pathologic Findings

The overall size of the tumors ranged from 1.8 to 12 cm (mean, 4.8 cm; median, 3.4 cm; versus 2.9 and 2.5 cm, respectively, in conventional adenocarcinomas; $P = .001$). Despite the preoperative diagnosis of cholecystitis in 67% of the cases, macroscopically, tumor was apparent in 90%. Forty-five percent revealed gelatinous mass and/or gelatinous/fibrinopurulent debris (Figure 2).

Two cases of MC (13%) had an almost exclusive colloid pattern, characterized by well-defined mucin lakes in which carcinoma cells were seen floating within the mucin but not clinging to the stroma (Figure 1). Eight (54%) had ''mixed-mucinous'' features showing colloid-type areas admixed with other patterns. Of these, the nonmucinous component was conventional-type adenocarcinoma in 7 cases (47%; Figure 3), and 1 case (7%) showed a focal area (<10%) of squamoid-type differentiation in addition to the

Table 2. Comparison of Clinicopathologic and Histopathologic Characteristics of Mucinous Carcinomas, Adenocarcinomas With Focal Mucinous Differentiation, Well-Differentiated Adenocarcinomas With Intraglandular Mucin, and Conventional Adenocarcinomas of the Gallbladder				
Characteristics	Mucinous Carcinoma (n = 15)	Adenocarcinoma With Focal Mucinous Differentiation (n = 17)	Well-Differentiated Adenocarcinoma With Intraglandular Mucin (n = 8)	Conventional Gallbladder Adenocarcinoma (n = 567)
Mean age, y	65	64	72	64
Sex, F:M	1.1	7.5	7	3.9
Mean tumor size, cm (range)	4.8 (1.8–12)	3.3 (1.4–7.7)	3.9 (1.2–6.5)	2.9 (0.1–11.5)
Median tumor size, cm	3.4	3	4.7	2.5
pT3, %	87	75	50	48
Mean survival, mo (range)	13 (1–37)	30 (1–95)	51 (1–129)	26 (1–156)



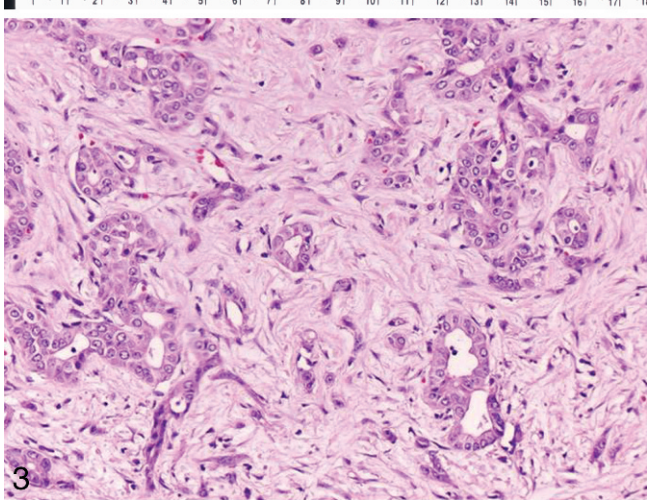


Figure 1. *Diagnostic microscopic features of mucinous carcinoma (of colloid type) characterized by pools of stromal mucin predominating the picture, with variable amount of tumor cells floating in these mucin pools (hematoxylin-eosin, original magnification ×100).*

Figure 2. *Macroscopic photograph of mucinous carcinoma. Soft, polypoid/gelatinous mass (inset) with a smooth glistening surface is located in the corpus of the gallbladder.*

Figure 3. *Mucinous carcinoma of mixed mucinous type. In addition to the mucinous component (not depicted in this figure), there are also glandular elements of conventional pancreatobiliary type ductal adenocarcinoma constituting up to 50% of the tumor (hematoxylin-eosin, original magnification ×200).*

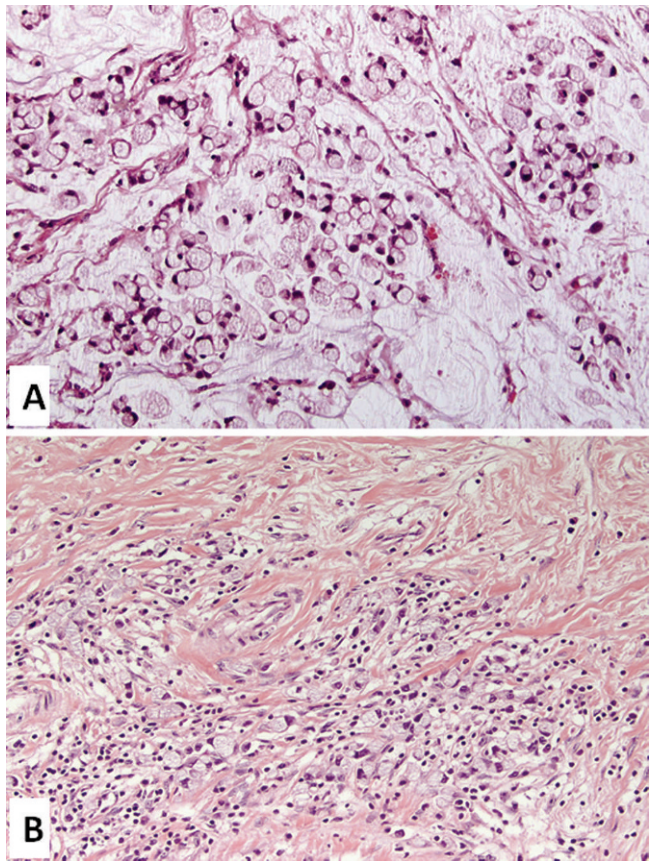


Figure 4. *Mucinous signet-ring cell carcinoma. A, Scattered signet-ring type cells predominate the picture. B, The nonmucinous component of the tumor constitutes <50% of the tumor by definition (hematoxylin-eosin, original magnifications ×200).*

conventional-type adenocarcinoma, but, by definition, greater than 50% of the tumor was mucinous. Five cases (33%) had prominent signet-ring cells with both the cells within the mucin (Figure 4, A), and those infiltrating into the stroma (individually or in cords) were mostly of signet-ring morphology (Figure 4, B).

In most cases, the tumor cells formed cribriform, stellate clusters, largely within the center of the mucin nodules (Figure 5, A). Some had foci with strips of columnar cells clinging to the stromal interface (Figure 5, B). In other areas, the cells were lying individually within the mucin (Figure 5, C). Some mucin pools were totally devoid of tumor cells (Figure 5, D). Calcifications were observed in 4 cases (27%; Figure 6).

Ten cases (67%) were graded as well or moderately differentiated and 5 (33%) as poorly differentiated with signet-ring cell morphology showing features of ''poorly cohesive carcinomas'' per World Health Organization 2010 guidelines.[60]

Lymphovascular invasion was identified in 12 cases (80%; Figure 7, A) and perineural invasion in 11 (73%; Figure 7, B). Cystic duct resection margin was involved in 4 cases (27%), and 4 cases (27%) had a tumor at the hepatic resection margin (Figure 7, C).

Seven MC cases (47%) showed high-grade, flat, intraepithelial neoplasm (dysplasia). Three cases (20%) were associated with a high-grade tumoral intramucosal papillary neoplasm (termed *adenoma* or *intracystic papillary*

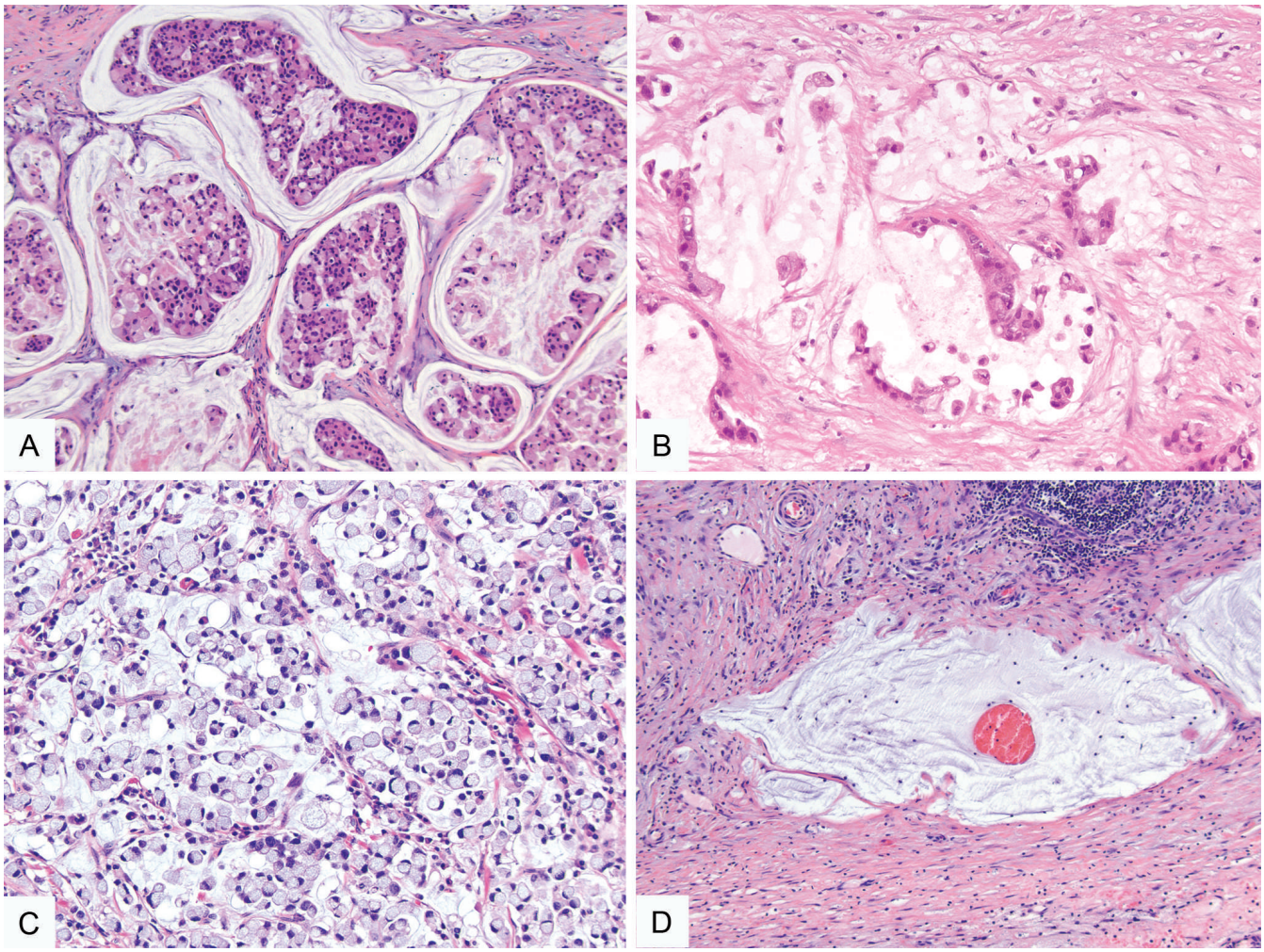


Figure 5. *Spectrum of patterns and distribution of tumor cells in mucin. A, In some cases, the tumor cells form cribriform stellate clusters floating within the mucin. B, In some cases, some cells are detached; some cling to the stroma. C, Some cases display predominantly signet-ring cell morphology with cells showing voluminous amount of intracellular mucin pushing the nuclei to the periphery. Signet-ringlike cells can be seen in clusters or lying individually within the mucin. D, Some of the mucin pools are devoid of any tumor cells (hematoxylin-eosin, original magnifications ×100 [A and D] and ×200 [B and C]).*

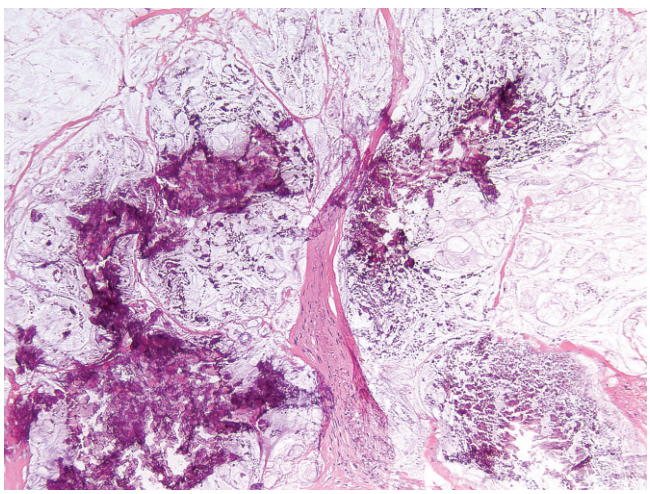


Figure 6. *Calcifications in tumoral areas were observed in 4 cases in this study (hematoxylin-eosin, original magnification ×40).*

neoplasm by the World Health Organization 2010 guidelines,[24] which we designate as *intracholecystic papillary mucinous neoplasms*[27,28]), 2 of the 3 (67%) were of intestinal subtype, and 1 (33%) was gastric. In 1 case (6%), the tumor cells in preinvasive area focally displayed the syncytial growth pattern of medullary carcinomas. In 4 cases (27%), the mucosa was denuded and was replaced by an excessive amount of acute inflammation; in these cases, the existence of a precursor lesion was difficult to determine.

In the uninvolved areas of the GBs, acute inflammation within the stroma surrounding the mucin nodules was noted in 11 cases (73%); 5 of the 11 (45%) were rich in eosinophils (Figure 8). There was marked fibrosis in 4 of 15 cases (27%). In the mucin nodules, in addition to the intact tumor cell groups, there were areas with collections of polymorphonuclear leukocytes that were destroying the tumor cell nests and leaving granular-necrotic debris in 13 of 15 cases (87%; Figure 9, A through D).

All but 2 cases (n = 13; 87%) were pT3 per American Joint Committee on Cancer 2010 guidelines[26]; 1 (7%) was pT2, and 1 (7%) was pT1 (versus 48% pT3, 40% pT2, and 12% pT1 in conventional GB adenocarcinomas; $P = .01$).

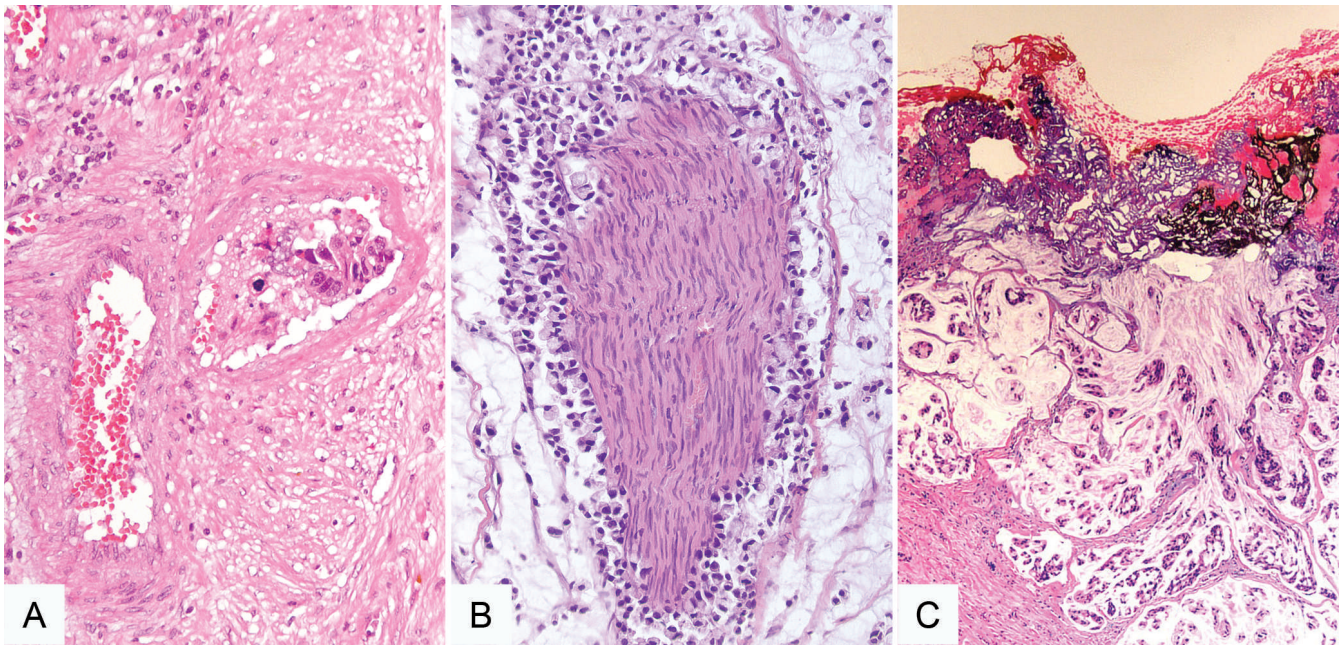


Figure 7. *A, Vascular invasion. B, Perineural invasion. C, Resection margin (inked) positivity (hematoxylin-eosin, original magnifications ×200 [A and B] and ×100 [C]).*

Regional lymph node metastasis was documented in 4 of 13 cases (31%) where lymph nodes available for microscopic examination (versus 38% in conventional GB adenocarcinomas; $P = .40$). In 2 (50%) of the involved lymph nodes, the tumor displayed characteristic features of MC, whereas in the other 2 (50%), no mucin was identified.

Immunohistochemical Features

Markers that are frequently expressed in pancreatobiliary carcinomas were also often positive in MCs: MUC5AC (foveolar marker) in 6 of 7 (86%), and CK7 in 4 of 7 (57%). In addition, however, goblet-cell/colloid marker, MUC2, which is typically negative in conventional adenocarcinomas of the GB, was positive in 6 of 7 cases (86%). In the meantime, CK20, which is commonly positive in intestinal adenocarcinomas of both ordinary and mucinous types, was positive in only 2 of 7 MCs (29%) of GB, and CDX2, which is uniformly expressed in pancreatic colloid carcinomas showed nuclear expression in only 1 of 7 (14%). Loss of membranous E-cadherin labeling was identified in 6 of 7 (86%). Microsatellite instability markers, MLH1 and MSH2, were retained in all 7 cases (100%) tested (Figure 10, A through J). The comparison of this immunoprofile to that of conventional GB carcinomas is provided in Table 3.

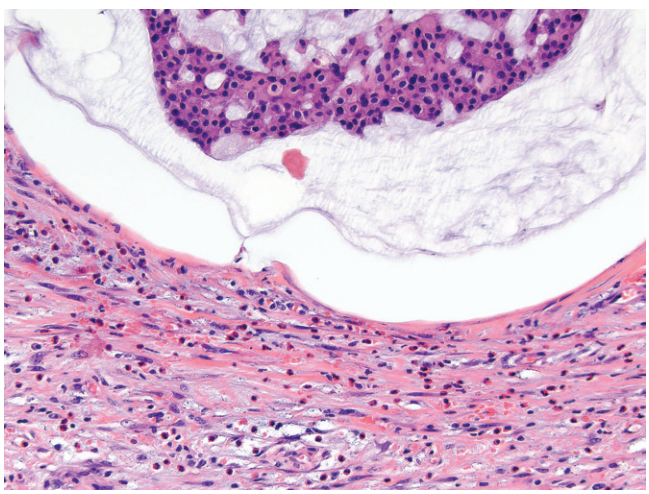


Figure 8. *Acute inflammation rich in eosinophils (hematoxylin-eosin, original magnification ×400).*

Survival Analysis

Follow-up was available in 13 MC cases. Eleven patients (85%) died of disease (1–37 months), and 2 (15%) were alive at 23 months and 1 month. Overall survival of patients with MC was significantly worse than it was for patients with conventional GB adenocarcinomas ($P = .01$) (Figure 11). The overall 3-year survival was 1%, as opposed to 39% in conventional GB adenocarcinoma. This adverse prognosis, however, appeared not to be independent of the stage. Neither the stage status nor the survival was found to be significantly dependent on sex ($P = .80$).

The survival of patients in the MC category was also compared with the survival of patients in the adenocarcinoma with focal (≤50%) mucin differentiation category, and it appeared that these tumors were not as aggressive as the full-blown MCs (P=.05; see Table 2). On the other hand, even these patients had worse prognoses than did patients with conventional GB adenocarcinomas. In contrast, patients who had adenocarcinomas with intraglandular (but not stromal) mucin appeared to have better prognoses than did those with MCs and, perhaps, even a better prognoses than that of patients with conventional GB adenocarcinomas, although that finding did not reach statistical significance (P=.20).

Additionally, the overall survival of patients with carcinomas that had signet-ring cells (6 months) appeared to be worse than that of patients with pure colloid carcinomas or mixed-mucinous ones (14 months);

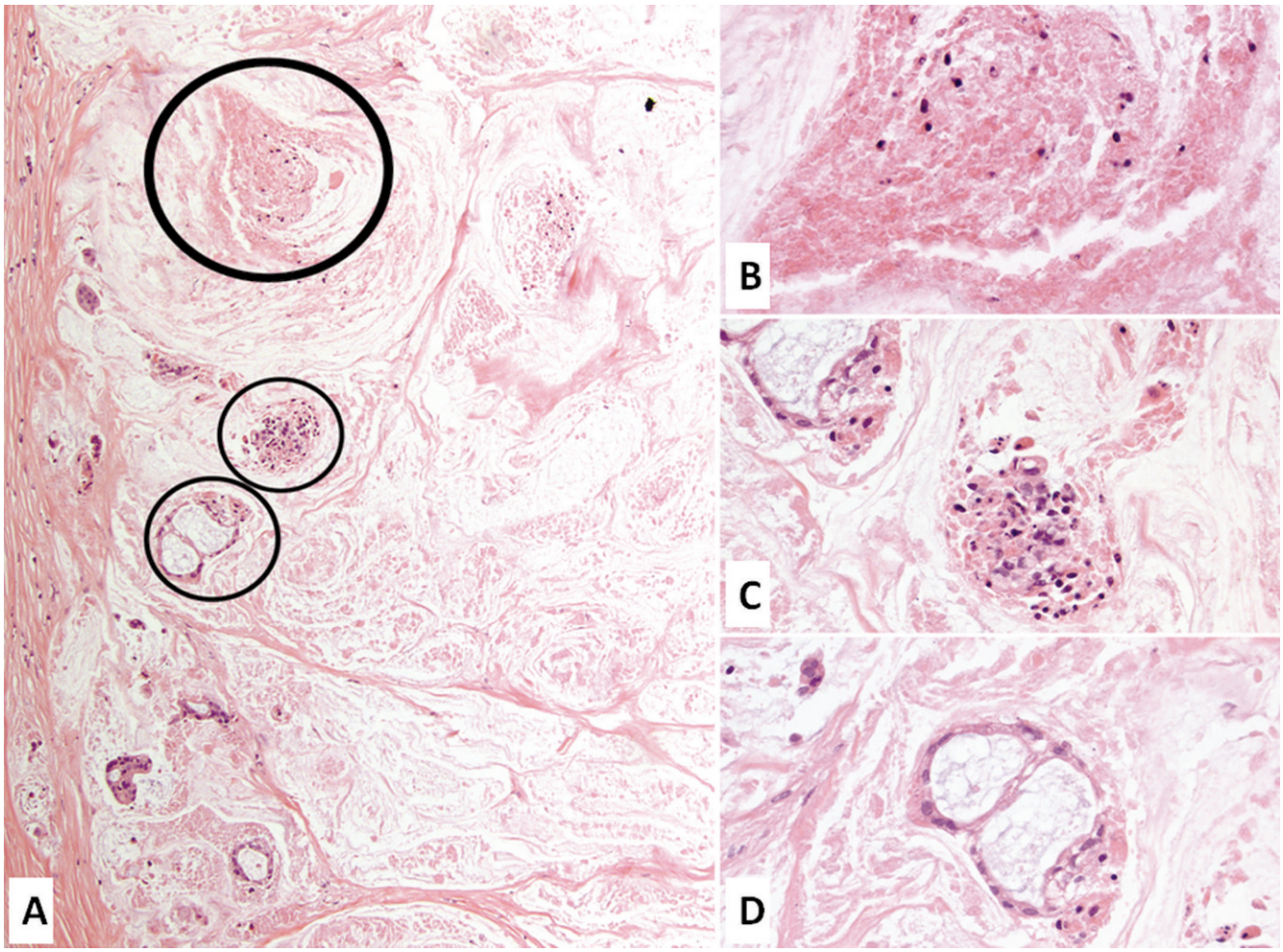


Figure 9. *There were areas (A) where polymorphous nuclear leukocytes were associated with tumor cells, as well as with eosinophilic necrotic debris in mucin (B through D) (hematoxylin-eosin, original magnifications ×40 [A] and ×200 [B through D]).*

however, the number of cases in each subgroup was too small to derive a conclusion ($P = .50$).

COMMENT

Most GB carcinomas are conventional adenocarcinomas of pancreatobiliary type. The literature on the other types of carcinoma occurring in this organ is limited. One of these other types is MC, which is rather uncommon in the GB and is noted in the literature mostly as individual case reports or small series of a handful of cases. Because of definitional variations, the reported incidence rate varied from 5% to 10.8%.[61–63]

In this clinicopathologic analysis of 606 invasive GB carcinomas, which, to our knowledge, represents the largest cohort heretofore analyzed for this purpose, *mucinous differentiation* (defined as any extracellular mucin production by carcinoma) was identified in 6.6% of the GB carcinomas (40 of 606). This mucinous differentiation, however, was seen mostly as a secondary component in otherwise conventional adenocarcinomas. It varied in amount from very minimal to extensive and was either stromal or intraglandular. In this study, in accordance with the criteria generally employed in the gastrointestinal tract,[64] we defined *MCs* as those with stromal mucin deposition constituting more than 50% of the tumor. Tumors with 50% stromal mucin or less were regarded as *adenocarcinoma with focal mucinous differentiation*, and those tumors in which the mucin was intraglandular (confined to the lumen of well-formed ductular elements), we designated *well-differentiated adenocarcinoma with intraglandular mucin*. In the GB, Albores-Saavedra and colleagues[25,65] have chosen to include this latter group into the MC category as well, which differs from what is being done in the gastrointestinal tract. Our study, however, speaks against that inclusion approach by illustrating substantial clinicopathologic differences between the 2 groups (Table 2): whereas MCs occur in men almost as commonly as in women, adenocarcinomas with intraglandular mucin production are almost exclusively in women (all but one case in this study was female; 7 of 8 [88%]). Mean tumor size was also smaller in adenocarcinomas with intraglandular mucin (3.9 cm versus 4.8 cm of MCs), and most important, although the clinical outcome for patients with MC appeared to be worse than for patients with conventional GB adenocarcinomas, those with intraglandular-only mucin appeared to have a prognosis better than that of patients with conventional GB adenocarcinomas, although the difference (mean survival, 51 months versus 26 months for conventional adenocarcinomas) did not reach statistical significance because of too few cases ($P = .20$).

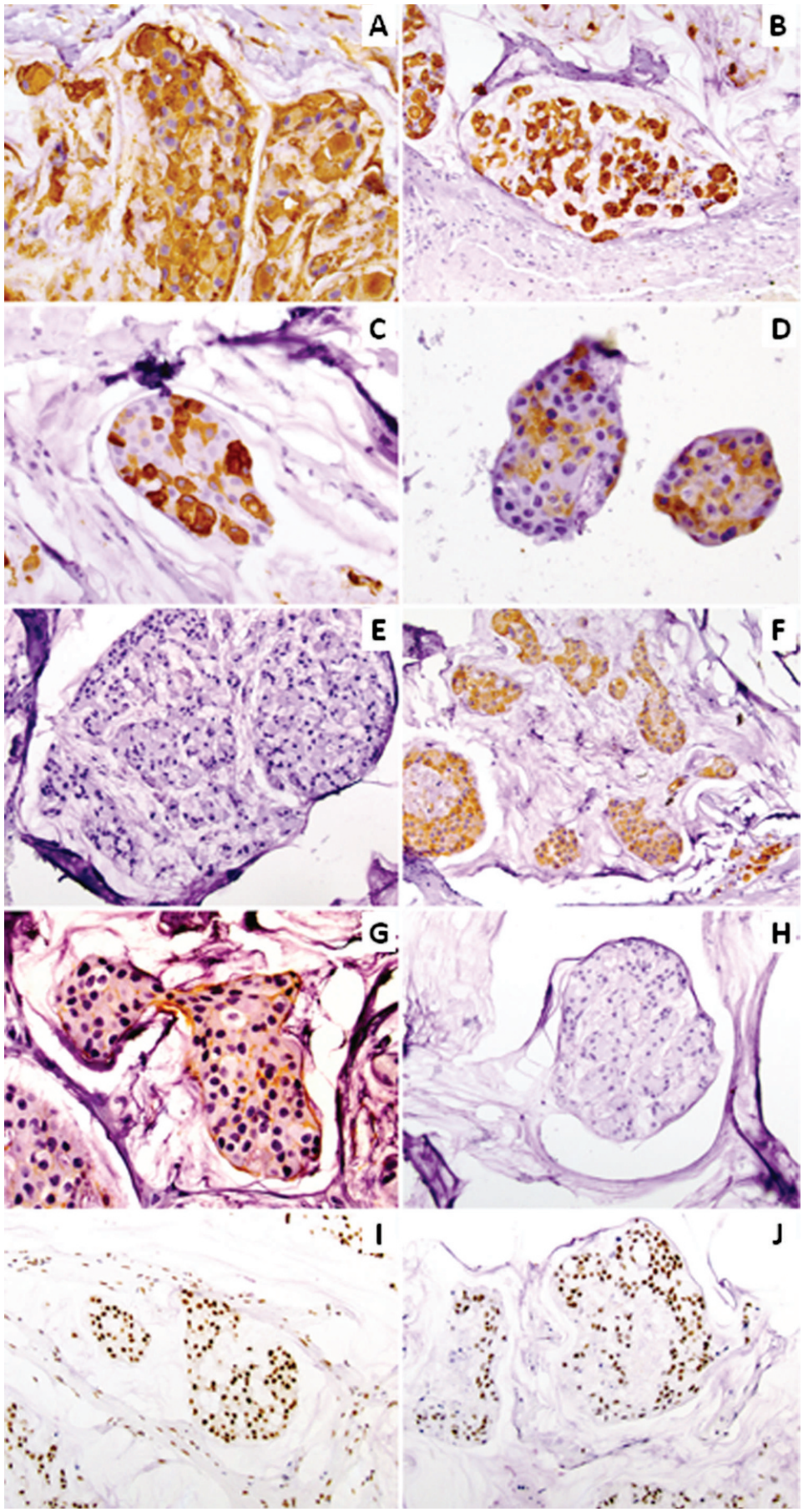


Table 3. Comparison of Immunohistochemical Characteristics of Mucinous Carcinomas and Conventional Adenocarcinomas of the Gallbladder

Type of Cancer	MUC1, %	MUC2, %	MUC5AC, %	MUC6, %	CK7, %	CK20, %	CDX2, %	MLH1 and MSH2,[a] %	E-Cadherin, %
Mucinous carcinomas (n = 7)	57	86	86	0	57	29	14	100	86
Conventional adenocarcinomas (n = 8)	75	0	71	35	88	11	0	86	N/A
P value[b]	.60	<.001	.60	.10	.30	.50	.40	>.99	N/A

Abbreviation: N/A, not applicable.

[a] Microsatellite markers, MLH1 and MSH2, were retained in all mucinous carcinomas (microsatellite stable), although 14% of the conventional adenocarcinomas tested revealed MLH1 (n = 2) or MSH2 (n = 1) lost (microsatellite instable).
[b] *P* values were based on Fisher exact test.

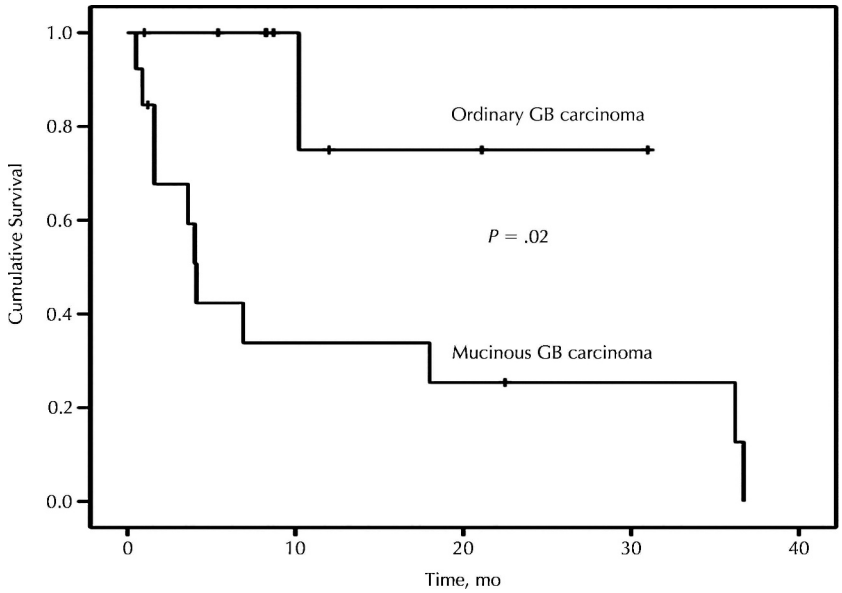


Figure 11. *Kaplan–Meier survival curves comparing patients with mucinous carcinomas and those with conventional pancreatobiliary type gallbladder (GB) adenocarcinomas.*

Defined as greater than 50% of the tumor consisting of stromal mucin deposition, MCs of the GB show the following clinicopathologic characteristic: they constitute 2.5% of the GB carcinomas. Female predominance appears not to be as striking as it is in conventional GB adenocarcinomas (1.1 versus 3.9; $P = .04$). Although conventional GB adenocarcinomas typically present with chronic cholecystitis, most patients with MC are admitted and have surgery with a working diagnosis of *acute cholecystitis*. In fact, interestingly, although the tumors are large (significantly larger than conventional GB adenocarcinomas; mean, 4.8 cm versus 2.9 cm), only 1 case (7%) had the preoperative diagnosis of carcinoma (the remainder [93%] were diagnosed with *acute cholecystitis*), presumably because the inflammatory picture overshadows the findings of the tumor. This may not be surprising, considering that mucin and mucin-related glycoproteins have been shown to have a striking ability of activating an inflammatory cascade.[66–69] That inflammation was also manifest in our cases, intraoperatively and on macroscopic examination, as adhesions and fibrinous serosal changes, and histologically, as of the many polymorphonuclear leukocytes noted in the vicinity of the tumors, in addition to the edema, granulation tissue type fibroblasts, and other signs of acute injury. As has been described in the literature, calcifications may be seen in MCs of the GB, and it was identified in 4 (27%) of our MC cases as well.[17,61,70–76]

Unlike MCs of exocrine organs (breast, pancreas, and skin), which are invariably colloid types with protracted clinical courses that are significantly better than the courses of patients with conventional carcinomas at the corresponding sites,[1,3–7] the GB MCs are seldom of colloid type (only 2 of 15 [13%] in this study). Moreover, many GB MCs (5 of 15 [33%] in this study) shows prominent signet-ring cell formation not only in the mucin lakes but also infiltrating into the stroma as individual cells or cords (''poorly cohesive cell type'' in the new World Health

←

Figure 10. *A, Common expression of MUC1. B, Nearly all cases expressed MUC2. C, Nearly all expressed MUC5AC. D, MUC6 was expressed in <10% of the cells in 1 case. E, CK20 was negative in 5 cases. F, CK7 was expressed in 4 cases. G, E-cadherin expression was retained in only one case. H, CDX2 was negative in all but one case. Nuclear expression of MLH1 (I) and MSH2 (J) were observed in all cases (original magnifications ×200 [A through D, and G] and ×100 [E, F, and H through J]).*

Organization classification).[60] The supposition is that once colloid carcinoma cells overcome the protective barrier created by the mucin and infiltrate into the stroma as individual cells, they acquire (or reflect) an aggressive behavior that practically negates the survival advantage seen in pure colloid carcinomas. The findings in this study lend further support to this hypothesis because the MCs of the GB were found to have an aggressive course, even more so than the conventional GB adenocarcinomas. Additionally, in this study, the overall survival of patients who had carcinomas with signet-ring cells appeared to be worse (6 months) than for pure colloid cases and mixed-mucinous ones (14 months), although, there were too few cases in each subgroup to derive a definitive conclusion. These findings, if confirmed, would be in accordance with what has recently been documented in the colon by Sung et al,[77] indicating that patients who have MCs with prominent signet-ring cells may have a worse prognosis than do patients with other MCs.

The GB MCs are often fairly advanced tumors at the time of diagnosis. In addition to their large size, which is in parallel with the MCs of other organs, MCs are also high stage in cholecystectomy specimens, with 87% of the cases showing T3 tumors, as opposed to 48% in conventional GB adenocarcinomas. This is probably another factor why GB MCs have a more aggressive behavior. The fact that MCs occur almost as commonly in men as in women may also be considered to account for the more dismal outcome of these tumors because GB carcinomas have been found to be more aggressive in men[78]; however, neither the stage status nor the survival was found to be sex-dependent in this study.

Immunophenotype of MCs analyzed in 7 cases in this study is also interesting. It differs from that of the conventional GB adenocarcinomas by consistent MUC2 positivity (Table 3). MUC2 expression is exceedingly uncommon in conventional GB adenocarcinomas,[28] whereas it was found in almost all cases of MC, as it is in mucinous carcinomas of other sites.[2,58] In GB MCs, however, the MUC2 expression is decoupled from the expression of CDX2 that is typical of *pancreatic* colloid carcinomas.[2,3,57,58] Instead, GB MCs show expression of MUC1, a marker of aggressive phenotype in the pancreatobiliary tract, which is not seen in pancreatic colloid carcinomas, which are known to be associated with protracted clinical course. Additionally, most pancreatic colloid carcinomas arise in association with intraductal papillary mucinous neoplasms,[2,3,57] in particular, the intestinal subtype of IPMNs, which are also uniformly positive for both MUC2 and CDX2, which has led to the conclusion that colloid carcinomas of the pancreas represent an ''intestinal pathway'' of carcinogenesis, developing from intestinal-type IPMN to colloid-type MC.[58] This intestinal pathway does not seem to be as important in the GB. Only 3 of 15 GB MCs (20%) had a mass-forming preinvasive neoplasm (what we call intracholecystic papillary tubular neoplasm) similar to IPMN.[58] However, of note, 2 of these 3 (67%) were indeed of the intestinal subtype.

Similarly, MCs of GB are different from mammary MCs by the rarity of the pure colloid pattern (most mammary MCs are colloid types) as well as by the absence of MUC6, which is very common in breast MCs.[79,80]

The GB MCs can be distinguished from intestinal-type adenocarcinomas (including those that are mucinous) by the negativity of CDX2, in addition to their often inverse CK7/CK20 profile (showing CK7 positivity and rare CK20 expression). This may be helpful in the differential diagnosis of these tumors, especially considering that GB MCs are often large tumors and may mimic a metastatic lesion in the hepatic region. Moreover, GB MCs also differ from intestinal MCs by being microsatellite stable; all 7 cases tested (100%) retained MLH1 and MSH2.

In conclusion, MCs of the GB, defined as tumors in which stromal mucin deposition constitute more than 50% of the lesion, exhibit significant clinicopathologic differences from conventional GB adenocarcinomas as well as from carcinomas with other types and patterns of mucin formation. The MCs occur almost as commonly in men as they do in women and present with an acute cholecystitis picture. Patients with MCs have large and advanced tumors at diagnosis, which are seldom of the pure colloid type, and thus, not surprisingly, they typically display aggressive clinical behavior.

This study was supported in part by Fondecyt grant 1090171, Chile, and in part by the Georgia Cancer Coalition Distinguished Cancer Clinicians and Scientists Program, Atlanta.

References

1. Adsay NV, Klimstra DS. Not all ''mucinous carcinomas'' are equal: time to redefine and reinvestigate the biologic significance of mucin types and patterns in the GI tract. *Virchows Arch*. 2005;447(1):111–112.

2. Adsay NV, Merati K, Nassar H, et al. Pathogenesis of colloid (pure mucinous) carcinoma of exocrine organs: coupling of gel-forming mucin (MUC2) production with altered cell polarity and abnormal cell-stroma interaction may be the key factor in the morphogenesis and indolent behavior of colloid carcinoma in the breast and pancreas. *Am J Surg Pathol*. 2003;27(5):571–578.

3. Adsay NV, Pierson C, Sarkar F, et al. Colloid (mucinous noncystic) carcinoma of the pancreas. *Am J Surg Pathol*. 2001;25(1):26–42.

4. Komaki K, Sakamoto G, Sugano H, Morimoto T, Monden Y. Mucinous carcinoma of the breast in Japan: a prognostic analysis based on morphologic features. *Cancer*. 1988;61(5):989–996.

5. Solcia E, Luinetti O, Tava F, et al. Identification of a lower grade muconodular subtype of gastric mucinous cancer. *Virchows Arch*. 2004; 445(6):572–579.

6. Breiting L, Christensen L, Dahlstrom K, Breiting V, Winther JF. Primary mucinous carcinoma of the skin: a population-based study. *Int J Dermatol*. 2008; 47(3):242–245.

7. Kazakov DV, Suster S, LeBoit PE, et al. Mucinous carcinoma of the skin, primary, and secondary: a clinicopathologic study of 63 cases with emphasis on the morphologic spectrum of primary cutaneous forms: homologies with mucinous lesions in the breast. *Am J Surg Pathol*. 2005;29(6):764–782.

8. Araida T, Kogure M, Takasaki K. Mucin producing gallbladder cancer [in Japanese]. *Ryoikibetsu Shokogun Shirizu*. 1996;(9):352–355.

9. Hirsch EF, Gerber L, Coffey RJ, Goldman ML. Mucoid carcinoma of the gallbladder: clinical management of excessive drainage. *Surgery*. 1964;55:759–761.

10. Huang CP, Chiou YY, Chou YH, Chiang JH, Chang CY. Imaging findings in mucin-producing carcinoma of the gallbladder. *J Formos Med Assoc*. 2006; 105(5):427–430.

11. Kuroki M, Sato K, Kashimura J, et al. A case of poorly differentiated mucinous carcinoma of the gallbladder with remarkable imaging features [in Japanese]. *Nippon Shokakibyo Gakkai Zasshi*. 2003;100(10):1224–1228.

12. Mizuno T, Eimoto T, Tada T, Tateyama H, Inagaki H, Murase T. Mucinous tumor of the gallbladder with a separate nodule of anaplastic carcinoma. *Arch Pathol Lab Med*. 1999;123(12):1280–1284.

13. Nobusawa H, Hashimoto T, Munechika H, et al. US and CT findings of mucinous carcinomas of the gallbladder [in Japanese]. *Nippon Igaku Hoshasen Gakkai Zasshi*. 1994;54(14):1359–1367.

14. Noda H, Chiba F, Toyama N, Konishi F. Mucin-producing carcinoma of the gallbladder associated with primary sclerosing cholangitis and ulcerative colitis. *J Hepatobiliary Pancreat Surg*. 2009;16(1):83–85.

15. Oshiro H, Matsuo K, Mawatari H, et al. Mucin-producing gallbladder adenocarcinoma with focal small cell and large cell neuroendocrine differentiation associated with pancreaticobiliary maljunction. *Pathol Int*. 2008;58(12): 780–786.

16. Ozan H, Ozerkan K, Aker S, Bulbul M. A case with three primary tumors of the ovary, endometrium and gallbladder. *Eur J Gynaecol Oncol*. 2008;29(5): 551–553.

17. Rogers LF, Lastra MP, Lin KT, Bennett D. Calcifying mucinous adenocarcinoma of the gallbladder. *Am J Gastroenterol*. 1973;59(5):441–445.

18. Tian H, Matsumoto S, Takaki H, et al. Mucin-producing carcinoma of the gallbladder: imaging demonstration in four cases. *J Comput Assist Tomogr*. 2003; 27(2):150–154.
19. Yamamoto A, Ozeki Y, Ito Y, et al. A case of well differentiated mucinous carcinoma of the gallbladder [in Japanese]. *Nippon Shokakibyo Gakkai Zasshi*. 2010;107(11):1821–1827.
20. Zhang S. Mucinous adenocarcinoma originating in localized type adenomyomatosis of the gallbladder. *Chin Med J (Engl)*. 2001;114(9):994–996.
21. Yoshimitsu K, Irie H, Aibe H, et al. Well-differentiated adenocarcinoma of the gallbladder with intratumoral cystic components due to abundant mucin production: a mimicker of adenomyomatosis. *Eur Radiol*. 2005;15(2):229–233.
22. Adsay NV. Gallbladder, extrahepatic biliary tree, and ampulla. In: Mills SE, Carter D, Greenson JK, Reuter VE, Stoler MH, eds. *Sternberg's Diagnostic Surgical Pathology*. Vol 2. 5th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2010:1600–1663.
23. Adsay NV, Klimstra DS. Benign and malignant tumors of the gallbladder and extrahepatic biliary tract. In: Odze RD, Goldblum JR, eds. *Surgical Pathology of the GI tract, Liver, Biliary Tract, and Pancreas*. Philadelphia, PA: Saunders Elsevier; 2009:845–875.
24. Albores-Saavedra J, Adsay N.V., Crawford JM, et al. Carcinoma of the gallbladder and extrahepatic bile ducts. In: Bosman FT, Carneiro F, Hruban R, Theise ND, eds. *WHO Classification of Tumors of Digestive System*. 4th ed. Lyon, France: IARC Press; 2010:266–274. *World Health Organization Classification of Tumours*; vol 3.
25. Albores-Saavedra J, Henson DE, Klimstra DS. Tumors of the gallbladder, extrahepatic bile ducts, and ampulla of vater. *Atlas of Tumor Pathology*. Vol 27. 3rd ed. Washington DC: Armed Forces Institute of Pathology; 2000:61–106.
26. Edge SE, Byrd DR, Comptom CC, Fritz AG, Greene FL, Trotti A. *AJCC Cancer Staging Handbook: From the AJCC Cancer Staging Manual*. 7th ed. New York, NY: Springer; 2010:255–263.
27. Jang KT, Dursun N, Basturk O, et al. Classification of tumoral intraepithelial neoplasms of the gallbladder under a unified category of intracholecystic papillary tubular neoplasms (ICPN) with 4 subsets discernible by correlation of morphology and immunophenotype [abstract]. *Mod Pathol*. 2011;24(suppl 1):363A.
28. Jang KT, Dursun N, Basturk O, et al. Immunohistochemical analysis of the progression of flat versus tumoral intraepithelial neoplasia (intracholecystic papillary-tubular neoplasm) in gallbladder carcinogenesis [abstract]. *Mod Pathol*. 2011;24(suppl 1):363A.
29. Lee MJ, Lee HS, Kim WH, Choi Y, Yang M. Expression of mucins and cytokeratins in primary carcinomas of the digestive system. *Mod Pathol*. 2003; 16(5):403–410.
30. Levi E, Klimstra DS, Andea A, Basturk O, Adsay NV. MUC1 and MUC2 in pancreatic neoplasia. *J Clin Pathol*. 2004;57(5):456–462.
31. Tamada S, Goto M, Nomoto M, et al. Expression of MUC1 and MUC2 mucins in extrahepatic bile duct carcinomas: its relationship with tumor progression and prognosis. *Pathol Int*. 2002;52(11):713–723.
32. Yonezawa S, Nakamura A, Horinouchi M, Sato E. The expression of several types of mucin is related to the biological behavior of pancreatic neoplasms. *J Hepatobiliary Pancreat Surg*. 2002;9(3):328–341.
33. Sasaki M, Yamato T, Nakanuma Y, Ho SB, Kim YS. Expression of MUC2, MUC5AC and MUC6 apomucins in carcinoma, dysplasia and non-dysplastic epithelia of the gallbladder. *Pathol Int*. 1999;49(1):38–44.
34. Li MK, Folpe AL. CDX-2, a new marker for adenocarcinoma of gastrointestinal origin. *Adv Anat Pathol*. 2004;11(2):101–105.
35. Moskaluk CA, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF Jr. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. *Mod Pathol*. 2003;16(9):913–919.
36. Tot T. Identifying colorectal metastases in liver biopsies: the novel CDX2 antibody is less specific than the cytokeratin 20+/7− phenotype. *Med Sci Monit*. 2004;10(5):BR139–143.
37. Werling RW, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol*. 2003;27(3):303–310.
38. Ji H, Isacson C, Seidman JD, Kurman RJ, Ronnett BM. Cytokeratins 7 and 20, Dpc4, and MUC5AC in the distinction of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors: Dpc4 assists in identifying metastatic pancreatic carcinomas. *Int J Gynecol Pathol*. 2002;21(4):391–400.
39. Kim GE, Bae HI, Park HU, et al. Aberrant expression of MUC5AC and MUC6 gastric mucins and sialyl Tn antigen in intraepithelial neoplasms of the pancreas. *Gastroenterology*. 2002;123(4):1052–1060.
40. Tajiri T, Tate G, Kunimura T, et al. Histologic and immunohistochemical comparison of intraductal tubular carcinoma, intraductal papillary-mucinous carcinoma, and ductal adenocarcinoma of the pancreas. *Pancreas*. 2004;29(2): 116–122.
41. Basturk O, Khayyata S, Klimstra DS, et al. Preferential expression of MUC6 in oncocytic and pancreatobiliary types of intraductal papillary neoplasms highlights a pyloropancreatic pathway, distinct from the intestinal pathway, in pancreatic carcinogenesis. *Am J Surg Pathol*. 2010;34(3):364–370.
42. Park SY, Roh SJ, Kim YN, et al. Expression of MUC1, MUC2, MUC5AC, and MUC6 in cholangiocarcinoma: prognostic impact. *Oncol Rep*. 2009;22(3): 649–657.
43. Shibahara H, Tamada S, Goto M, et al. Pathologic features of mucin-producing bile duct tumors: two histopathologic categories as counterparts of pancreatic intraductal papillary-mucinous neoplasms. *Am J Surg Pathol*. 2004; 28(3):327–338.
44. Baars JH, De Ruijter JL, Smedts F, et al. The applicability of a keratin 7 monoclonal antibody in routinely Papanicolaou-stained cytologic specimens for the differential diagnosis of carcinomas. *Am J Clin Pathol*. 1994;101(3):257–261.
45. Cathro HP, Stoler MH. Expression of cytokeratins 7 and 20 in ovarian neoplasia. *Am J Clin Pathol*. 2002;117(6):944–951.
46. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol*. 2000;13(9):962–972.
47. Duval JV, Savas L, Banner BF. Expression of cytokeratins 7 and 20 in carcinomas of the extrahepatic biliary tract, pancreas, and gallbladder. *Arch Pathol Lab Med*. 2000;124(8):1196–1200.
48. Goldstein NS, Bassi D. Cytokeratins 7, 17, and 20 reactivity in pancreatic and ampulla of vater adenocarcinomas: percentage of positivity and distribution is affected by the cut-point threshold. *Am J Clin Pathol*. 2001;115(5):695–702.
49. Rullier A, Le Bail B, Fawaz R, Blanc JF, Saric J, Bioulac-Sage P. Cytokeratin 7 and 20 expression in cholangiocarcinomas varies along the biliary tract but still differs from that in colorectal carcinoma metastasis. *Am J Surg Pathol*. 2000;24(6):870–876.
50. Tot T. Adenocarcinomas metastatic to the liver: the value of cytokeratins 20 and 7 in the search for unknown primary tumors. *Cancer*. 1999;85(1):171–177.
51. Tot T. Cytokeratins 20 and 7 as biomarkers: usefulness in discriminating primary from metastatic adenocarcinoma. *Eur J Cancer*. 2002;38(6):758–763.
52. Mukai S, Miyazaki K, Yakushiji H. The role of E-cadherin in the differentiation of gallbladder cancer cells. *Cell Tissue Res*. 2001;306(1):117–128.
53. Nemeth Z, Szasz AM, Somoracz A, et al. Zonula occludens-1, occludin, and E-cadherin protein expression in biliary tract cancers. *Pathol Oncol Res*. 2009;15(3):533–539.
54. Shia J, Ellis NA, Klimstra DS. The utility of immunohistochemical detection of DNA mismatch repair gene proteins. *Virchows Arch*. 2004;445(5):431–441.
55. Luttges J, Beyser K, Pust S, Paulus A, Ruschoff J, Kloppel G. Pancreatic mucinous noncystic (colloid) carcinomas and intraductal papillary mucinous carcinomas are usually microsatellite stable. *Mod Pathol*. 2003;16(6):537–542.
56. Roa JC, Roa I, Correa P, et al. Microsatellite instability in preneoplastic and neoplastic lesions of the gallbladder. *J Gastroenterol*. 2005;40(1):79–86.
57. Adsay NV, Merati K, Andea A, et al. The dichotomy in the preinvasive neoplasia to invasive carcinoma sequence in the pancreas: differential expression of MUC1 and MUC2 supports the existence of two separate pathways of carcinogenesis. *Mod Pathol*. 2002;15(10):1087–1095.
58. Adsay NV, Merati K, Basturk O, et al. Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms: delineation of an ''intestinal'' pathway of carcinogenesis in the pancreas. *Am J Surg Pathol*. 2004;28(7):839–848.
59. Ohike N, Kim GE, Tajiri T, et al. Intra-ampullary papillary-tubular neoplasm (IAPN): characterization of tumoral intraepithelial neoplasia occurring within the ampulla: a clinicopathologic analysis of 82 cases. *Am J Surg Pathol*. 2010;34(12):1731–1748.
60. Lauwers GY, Carniero F, Graham DY, et al. Gastric carcinoma. In: Bosman FT, Carneiro F, Hruban R, Theise ND, eds. *WHO Classification of Tumors of Digestive System*. 4th ed. Lyon, France: IARC Press.; 2010:48–63. *World Health Organization Classification of Tumours*; vol 3.
61. Joo YE, Kim HS, Choi SK, et al. Case of mucinous adenocarcinoma with porcelain gallbladder. *J Gastroenterol Hepatol*. 2003;18(8):995–998.
62. Shani M, Hart J, Modan B. Cancer of the biliary system: a study of 445 cases. *Br J Surg*. 1974;61(2):98–100.
63. Lack EL. Tumors of the gallbladder and cystic duct. In: Lack EL, ed. *Pathology of the Pancreas, Gallbladder, Extrahepatic Biliary Tract, and Ampullary Region*. New York, NY: Oxford University Press, Inc.; 2003:465–511.
64. Jass JR, Sobin LH, eds. *Histological Typing of Intestinal Tumours*. 2nd ed. New York, NY: Springer-Verlag; 1989. *World Health Organization International Histological Classification of Tumours*.
65. Henson DE, Albores-Saavedra J, Corle D. Carcinoma of the gallbladder: histologic types, stage of disease, grade, and survival rates. *Cancer*. 1992;70(6): 1493–1497.
66. Carlos CA, Dong HF, Howard OM, Oppenheim JJ, Hanisch FG, Finn OJ. Human tumor antigen MUC1 is chemotactic for immature dendritic cells and elicits maturation but does not promote Th1 type immunity. *J Immunol*. 2005; 175(3):1628–1635.
67. Kadayakkara DK, Beatty PL, Turner MS, Janjic JM, Ahrens ET, Finn OJ. Inflammation driven by overexpression of the hypoglycosylated abnormal mucin 1 (MUC1) links inflammatory bowel disease and pancreatitis. *Pancreas*. 2010; 39(4):510–515.
68. Monti P, Leone BE, Zerbi A, et al. Tumor-derived MUC1 mucins interact with differentiating monocytes and induce IL-10highIL-12low regulatory dendritic cell. *J Immunol*. 2004;172(12):7341–7349.
69. Reid MD, Basturk O, Thirabanjasak D, et al. Tumor-infiltrating neutrophils in pancreatic neoplasia [published online ahead of print August 5, 2011]. *Mod Pathol*. 2011; 24(12):1612–1619. doi:10.1038/modpathol.2011.113.
70. D'Altorio RA. Calcification in a gastric mucinous adenocarcinoma. *Am J Dig Dis*. 1973;18(5):419–492.
71. De Carvalho JC, Francischetti EA, De Barros Filho GA, Cerda JJ. Calcified mucinous adenocarcinoma of the stomach. *Am J Gastroenterol*. 1978;69(4):481–484.
72. Ghahremani GG, Meyers MA, Port RB. Calcified primary tumors of the gastrointestinal tract. *Gastrointest Radiol*. 1978;2(4):331–339.
73. Kasahara Y, Mori N, Sonobe N, Sakai K, Fuji R, Imano M. Calcification in carcinoma of the stomach: report of a case. *Nippon Geka Hokan*. 1994;63(2):76–84.

74. Kitagawa M, Kimata S. Diffuse Calcification in mucinous cell carcinoma of stomach. *Acta Pathol Jpn*. 1963;13:287–294.
75. Ohsaki A, Niimi K, Mandai K, Seto Y, Niimoto M, Fujii Y. [A calcified mucinous adenocarcinoma of the stomach—case report]. *Gan No Rinsho*. 1990; 36(2):187–192.
76. Rao P, Lyons B. Pure mucinous carcinoma of the breast with extensive psammomatous calcification. *Histopathology*. 2008;52(5):650–652.
77. Sung CO, Seo JW, Kim KM, Do IG, Kim SW, Park CK. Clinical significance of signet-ring cells in colorectal mucinous adenocarcinoma. *Mod Pathol*. 2008; 21(12):1533–1541.
78. Zaydfudim V, Feurer ID, Wright JK, Pinson CW. The impact of tumor extent (T stage) and lymph node involvement (N stage) on survival after surgical resection for gallbladder adenocarcinoma. *HPB (Oxford)*. 2008;10(6): 420–427.
79. Matsukita S, Nomoto M, Kitajima S, et al. Expression of mucins (MUC1, MUC2, MUC5AC and MUC6) in mucinous carcinoma of the breast: comparison with invasive ductal carcinoma. *Histopathology*. 2003;42(1):26–36.
80. Rakha EA, Boyce RW, Abd El-Rehim D, et al. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol*. 2005;18(10):1295–1304.