Microsatellite Instability in Intraductal Papillary Neoplasms of the Biliary Tract

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Intraductal papillary neoplasms of the biliary tree are unusual lesions characterized by solitary or diffuse growth along the intra- and/or extrahepatic biliary tract. Biliary papillary neoplasms bear some clinicopathologic similarity to intraductal papillary mucinous neoplasms of the pancreas. Like intraductal papillary mucinous neoplasms of the pancreas, biliary papillary neoplasms can be purely intraductal lesions or can give rise to invasive adenocarcinomas. We recently studied the genetic alterations present in a series of biliary papillary neoplasms and noted the presence of allelic shifts in some biliary tumors during allelic loss assays on chromosomes 5q and 18q. This suggested that microsatellite instability might play a role in the molecular pathogenesis of biliary papillary neoplasms. Genomic DNA was extracted from 17 intraductal papillary neoplasms, 6 associated invasive cholangiocarcinomas, and corresponding normal tissues, and microsatellite instability testing was performed using the 5 microsatellite loci recommended by the 1997 National Cancer Institute–sponsored consensus conference (D2S123, D5S346, D17S250, Bat-25, and Bat-26). High-level microsatellite instability was considered to be present when at least two of five microsatellite loci showed allelic shifts, and low-level microsatellite instability, when only one locus was shifted, as per the National Cancer Institute criteria. We also determined the methylation status of the DNA mismatch repair gene *hMLH1* by bisulfite treatment of genomic DNA, followed by methylation-specific PCR. High-level microsatellite instability was present in 2 of 17 (11.8%) biliary papillary neoplasms, including 1 case of purely intraductal tumor and 1 case with both intraductal and invasive cholangiocarcinoma components. In both cases there was extensive microsatellite instability, with allelic shifts in five of five and four of five microsatellite markers, respectively. Low-level microsatellite instability was present in 6 of 17 (35.3%) biliary papillary neoplasms, including 2 cases of purely intraductal tumor and 4 cases with both intraductal and invasive cholangiocarcinoma components. Interestingly, the pattern of allelic shifts was frequently not identical between the intraductal and invasive cholangiocarcinoma components; although the same microsatellite markers were shifted, alleles of differing lengths were generated in the intraductal and invasive components of the neoplasms with high-level microsatellite instability and of two neoplasms with low-level microsatellite instability. None of the biliary papillary neoplasms (0 of 10 cases with adequate DNA for evaluation) showed methylation of *hMLH1*. These results indicate that microsatellite instability is a relatively frequent event in papillary neoplasms of the biliary tree but is not associated with *hMLH1* promoter hypermethylation. The finding that alleles of differing lengths were frequently generated between the intraductal and invasive components of those tumors with microsatellite instability suggests that there is significant genetic heterogeneity within these neoplasms.

KEY WORDS: Biliary papillomatosis, Biliary tract, Cholangiocarcinoma, Microsatellite instability.

Mod Pathol 2002;15(12):1309–1317

VOL. 15, NO. 12, P. 1309, 2002 Printed in the U.S.A.
Date of acceptance: September 4, 2002.
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DOI: 10.1097/01.MP.0000038461.80167.34

Papillary tumors derived from biliary epithelium are unusual neoplasms that can arise in the intra- and/or extrahepatic bile ducts and can either remain localized or spread extensively along the biliary tree. The World Health Organization (WHO) recognizes both benign (*e.g.*, biliary papillomatosis,

biliary intraepithelial neoplasia/dysplasia) and malignant (papillary cholangiocarcinoma) forms (1). Biliary papillomatosis has been described extensively (2–16) despite its relative rarity because of its dramatic presentation: diffusely dilated bile ducts filled with delicate papillary excrescences that can result in mechanical obstruction, ascending cholangitis, and death, even when the neoplasm itself is purely intraductal (7). Other intraductal papillary neoplasms have also been described that are not classified by the WHO, including localized papillary epithelial proliferations that are termed *papillary adenomas* or *papillomas* (17) and *villous adenomas* (18) and more diffuse papillary epithelial proliferations that are sometimes termed *mucin-hypersecreting bile duct tumors* (19).

With the relatively recent description of pancreatic intraductal papillary mucinous neoplasms (pancreatic IPMNs) has come increasing recognition that there exists a distinctive subset of these biliary papillary neoplasms bearing some similarity to pancreatic IPMNs with respect to radiologic features, biologic behavior, and histologic appearance (19–22). In particular, these lesions arise within the bile ducts and in all cases demonstrate a predominant component of intraductal growth. Like pancreatic IPMNs, however, they can give rise to invasive cholangiocarcinomas (4, 6, 19, 20, 22–25) and therefore are considered to represent neoplasms of low-grade malignant potential. Architecturally, these neoplasms, like pancreatic IPMNs, are defined by their growth as papillary fronds containing fine vascular cores (22). The neoplastic epithelial cells themselves are often of biliary type but can also show gastric or intestinal differentiation, including the presence of goblet cells and Paneth cells (22). Finally, like pancreatic IPMNs, many of these distinctive biliary papillary neoplasms show overproduction of extracellular intraductal mucin. This is not a universal feature, however; in a recent large study of intraductal papillary neoplasms of the liver, Shimonishi *et al.* (22) reported that only 39 of 66 (59%) cases showed mucin overproduction.

The genetic alterations that contribute to the neoplastic progression of these distinctive biliary papillary neoplasms have only rarely been investigated. One case of biliary papillomatosis arising in association with a congenital choledochal cyst was found to carry an activating mutation in the *K-ras* oncogene (11). Shimonishi *et al.* (22) found that nuclear expression of p53 correlated with progression of intraductal papillary neoplasms of the liver. We recently studied the genetic alterations in a series of biliary papillary neoplasms and their associated invasive cholangiocarcinomas and found relatively low frequencies of *K-ras* gene mutations, nuclear β-catenin protein accumulation, and allelic loss on chromosomes 18q and 5q (20). During those investigations, we noted allelic shifts among several of the biliary papillary neoplasms using microsatellite markers for allelic loss analysis, although these were not the microsatellite markers recommended by the National Cancer Institute (NCI) for formal evaluation of microsatellite instability (MSI; 26). Nevertheless, this suggested that MSI might play a role in the molecular pathogenesis of a subset of these tumors.

The purpose of this study was therefore to formally investigate microsatellite instability in biliary papillary neoplasms. We evaluated a series of 17 biliary papillary neoplasms (selected based on their histologic similarity to pancreatic IPMNs) and 6 associated invasive cholangiocarcinomas for microsatellite instability using the NCI criteria and evaluated the methylation status of the human Mut L homologue (*hMLH1*) gene promotor, one of the key DNA mismatch repair genes.

MATERIALS AND METHODS

Case Selection and Histologic Classification

The study population consisted of 17 patients with biliary papillary neoplasms of the intra- and/or extrahepatic biliary tract, including 15 patients who underwent biopsy or resection at The Johns Hopkins Hospital between 1985 and 2001 and 2 patients who underwent resection at the University of Pennsylvania Medical Center in 1999 and 2000. All neoplasms located in the distal common bile duct or ampullary region were excluded from the study to avoid overlap with ampullary carcinomas and pancreatic IPMNs. Clinical and pathologic data, including age, gender, location of tumor, and type of surgical procedure, were obtained from the computerized patient files and from the surgical pathology files.

These cases were selected during histologic review of a larger set of biliary tumors in which the term *papillary* had been used in the diagnostic report. Criteria for inclusion included predominant intraductal growth within dilated bile ducts, an architectural pattern of fine papillary fronds with vascular cores, and an overall impression during review that the tumor histologically resembled a pancreatic IPMN. The cases were further classified as showing either localized or extensive growth (intraductal papillary growth in multiple intra- and/or extrahepatic bile ducts). Those tumors with extensive intraductal growth constituted the majority of cases ($n = 15$) in this study. However, we also included cases of localized growth ($n = 2$) that otherwise histologically resembled pancreatic IPMNs, because pancreatic IPMNs can be either solitary or extensively involve the pancreatic duct system (27). Because no established criteria for grading dysplasia

within these distinctive biliary neoplasms have been published, we graded the epithelial dysplasia within the biliary IPNs according to WHO criteria for pancreatic IPMNs (28). Briefly, these were classified based on the highest degree of dysplasia as intraductal papillary adenomas if they showed columnar epithelial cells with little to no cytologic atypia, a low nuclear to cytoplasmic ratio with abundant cytoplasm, and little nuclear pseudostratification; as intraductal papillary borderline neoplasms if they showed mild to moderate nuclear atypia and hyperchromatism as well as nuclear pseudostratification that was limited to the basal two thirds of the epithelium; and as intraductal papillary carcinomas if they showed severe cytologic atypia, loss of nuclear polarity, or architectural cribriforming or papillary fusion (29). Cases with both intraductal papillary tumor and invasive adenocarcinoma were classified as intraductal papillary neoplasms with associated invasive cholangiocarcinoma.

DNA Extraction

Microdissection of H&E-stained slides for DNA extraction was performed from formalin-fixed, paraffin-embedded specimens. For most cases, we dissected an area of ~0.5–1 cm^2 from a 5-μm section into 100 μL of digestion buffer, although the exact number of neoplastic cells varied with the cellularity of the tumor, the size of the neoplastic cells, and the abundance of extracellular mucin and/or matrix material. Genomic DNA was extracted as described previously (30). Tissue corresponding to the intraductal papillary tumor was microdissected in all 17 cases, and in 6 cases, associated invasive cholangiocarcinoma was also microdissected. Normal control DNA was extracted from non-neoplastic liver parenchyma (13 cases), nonneoplastic periductal connective tissue (2 cases), benign lymph node (1 case), or normal colonic biopsy (1 case).

Microsatellite Instability Analysis

MSI testing was performed using the five microsatellite loci (D2S123, D5S346, D17S250, Bat-25, and Bat-26) recommended by the 1997 National Cancer Institute (NCI)–sponsored consensus conference (26). Primer sequences are given in Table 1. Assays were performed by fluorescent-labeled polymerase chain reaction (PCR) amplification using fluorescent dye-labeled forward primer and unlabeled reverse primer. The forward primer was end-label with 6-FAM (Applied Biosystems, Foster City, CA). PCR amplification was performed in 15-μL reaction volumes containing 1 μL of genomic DNA, 9 μL of ABI Prism True Allele PCR Premix (Applied Biosystems), 5 pmol of 6-FAM-labeled forward primer, and 10 pmol of unlabeled reverse primer. The following cycling conditions were used for all PCR reactions: denaturation at 95° C for 6 minutes; 45 cycles of 94° C for 45 seconds, 55° C for 45 seconds, and 72° C for 1 minute; and a final extension at 72° C for 30 minutes. The PCR products were diluted with 30 μL of H_2O, and a 1.0-μL aliquot of each diluted fluorescent-labeled PCR product was combined with 12 μL of formamide and 0.5 μL of GeneScan 400HD (ROX) size standard (Applied Biosystems). The samples were then capillary electrophoresed on an ABI 3700 DNA Analyzer and analyzed using GeneScan Analysis software (Applied Biosystems).

Chromatograms were interpreted according to the criteria described in detail by Berg *et al.* (31). High-level microsatellite instability (MSI-high) was considered to be the case when at least two of five microsatellite loci showed shifting; low-level microsatellite instability (MSI-low), when only one locus was shifted; and microsatellite stable, when none of the loci were shifted, as per the NCI criteria (26).

TABLE 1. Microsatellite Loci and Primer Sequences for Microsatellite Instability Analysis

Microsatellite Locus (Label): Primer Sequence	Product Size (bp)
D2S123 (Fam)	197–227
Forward: 5′-AAACAGGATGCCTGCCTTTA-3′	
Reverse: 5′-GGACTTTCCACCTATGGGAC-3′	
D5S346 (Fam)	96–122
Forward: 5′-ACTCACTCTAGTGATAAATCGGG-3′	
Reverse: 5′-AGCAGATAAGACAGTATTACTAGTT-3′	
D17S250 (Fam)	151–169
Forward: 5′-GGAAGAATCAAATAGACAAT-3′	
Reverse: 5′-GCTGGCCATATATATATTTAAACC-3′	
Bat-25 (Fam)	120
Forward: 5′-TCGCCTCCAAGAATGTAAGT-3′	
Reverse: 5′-TCTGCATTTTAACTATGGCTC-3′	
Bat-26 (Fam)	116
Forward: 5′-TGACTACTTTTGACTTCAGCC-3′	
Reverse: 5′-AACCATTCAACATTTTTAACCC-3′	

Methylation Status of hMLH1

The methylation status of *hMLH1* was determined by bisulfite treatment of genomic DNA, followed by methylation-specific PCR, as previously described, with modification (32). Briefly, 2 μg of microdissected genomic DNA was denatured with 2 M NaOH at 37° C for 10 minutes, followed by incubation with 3 M sodium bisulfite (pH 5.0) at 50° C for 16 hours in the dark. After treatment, the DNA was purified using a DNA cleanup kit as recommended by the manufacturer (Promega, Madison, WI), incubated with 3 M NaOH at room temperature for 5 minutes, precipitated with 10 M ammonium acetate and 100% ethanol, washed with 70% ethanol, and finally resuspended in 20 μL of distilled water. *hMLH1* methylation status for each sample was determined by using 2 μL of bisulfite-treated DNA as the template

for PCR reactions using primers specific for methylated and unmethylated alleles. Primer sequences for the unmethylated reaction were as follows: 5'-AGAGTGGATAGTGATTTTTAATGT-3' (forward) and 5'- ACTCTATAAATTACTAAATCTCTTCA-3' (reverse), and for the methylated reaction they were as follows: 5'-GATAGCGATTTTTAACGC-3' (forward) and 5'-TCTATAAATTACTAAATCTCTTCG-3' (reverse). Amplification was carried out in a GeneAmp PCR System 9700 thermocycler (Perkin Elmer, USA) with 40 cycles of 95° C for 30 seconds, 53° C for 45 seconds, and 72° C for 30 seconds, followed by a final extension at 72° C for 4 minutes. Ten-microliter aliquots of the PCR products were electrophoresed on 5% acrylamide gels and visualized by ethidium bromide staining. DNA from the RKO colon cancer cell line (American Type Culture Collection, Manassas, VA) was used as a control for methylation.

RESULTS

A summary of the clinicopathologic and molecular findings in the 17 biliary papillary neoplasms (designated B1 to B17) is presented in Table 2.

Clinicopathologic Characteristics

All were tumors of adult patients ranging from 39 to 81 years (mean, 63.4 y). There was a slight predominance of women, with 10 (59%) women and 7 (41%) men. The biliary papillary neoplasms involved only the intrahepatic biliary tree in 7 (41%) patients, only the extrahepatic biliary tree (exclusive of distal common bile duct and ampullary neoplasms) in 5 (29%) patients, and both intrahepatic and extrahepatic ducts in 5 (29%) patients. In one case, histologic classification was performed on biopsy material only (Case B1), whereas in the remaining cases, complete evaluation of the tumors was possible based on surgical resection specimens (including liver explant in two patients).

Fifteen of 17 biliary papillary neoplasms showed extensive growth within the biliary tree, and two were solitary tumors. Histopathologically, the intraductal component in the majority of the tumors (16 of 17, 94%) was graded as intraductal papillary carcinoma (Fig. 1A–B), and in 7 cases (41%), the biliary intraductal papillary carcinoma was associated with an infiltrating cholangiocarcinoma (only 6 cholangiocarcinomas were microdissected and analyzed for MSI status because of marked stromal contamination in one infiltrating tumor, Case B17). Associated infiltrating cholangiocarcinomas were all of ordinary tubular type (Fig. 1C), and no example of colloid-type carcinoma was present. Only one biliary papillary neoplasm was graded as intraductal papillary adenoma, and this case (B13) was not associated with invasive cholangiocarcinoma.

Microsatellite Instability and hMLH1 Methylation

High-level microsatellite instability (MSI-high) was present in 2 (11.8%) neoplasms (Cases B15 and B16). In Case B15, a purely intraductal neoplasm, there was

TABLE 2. Clinicopathologic Characteristics and Microsatellite Instability in Biliary Papillary Neoplasms

Case	Age (y)/Sex	Location	Pattern of Growth	Histology	MSI Status	Markers Shifted	hMLH1 Methylation
B1	61/M	EHD	localized	IPC	MSI-low	D5S346	N/A
B2	79/F	IHD	extensive	IPC	MSI-low	D2S123	N/A
B3	69/F	IHD	extensive	IPC	stable	—	unmethylated
B4	76/F	IHD	extensive	IPC	stable	—	unmethylated
B5	39/F	IHD	localized	IPC	stable	—	N/A
B6	49/F	EHD+IHD	extensive	IPC	MSI-low	D5S346	N/A
				invasive	MSI-low	D5S346	N/A
B7	53/M	IHD	extensive	IPC	stable	—	unmethylated
B8	74/F	IHD	extensive	IPC	MSI-low	D17S250	unmethylated
				invasive	MSI-low	D17S250	unmethylated
B9	75/M	EHD	extensive	IPC	stable	—	N/A
B10	67/F	EHD	extensive	IPC	stable	—	unmethylated
B11[a]	65/M	EHD+IHD	extensive	IPC	MSI-low	D17S250	N/A
				invasive	MSI-low	D17S250	N/A
B12[a]	64/M	EHD	extensive	IPC	MSI-low	D2S123	N/A
				invasive	MSI-low	D2S123	N/A
B13	50/F	EHD+IHD	extensive	IPA	stable	—	unmethylated
B14	54/F	EHD+IHD	extensive	IPC	stable	—	unmethylated
				invasive	stable	—	unmethylated
B15	65/M	IHD	extensive	IPC	MSI-high	D5S346, D17S250, D2S123, Bat-25, Bat-26	unmethylated
B16[a]	57/M	EHD+IHD	extensive	IPC	MSI-high	D5S346, D17S250, D2S123, Bat-26	unmethylated
				invasive	MSI-high	D5S346, D17S250, D2S123, Bat-25, Bat-26	N/A
B17	81/F	EHD	extensive	IPC	stable	—	unmethylated

EHD, extrahepatic biliary tree; IHD, intrahepatic biliary tree; IPA, intraductal papillary adenoma; IPC, intraductal papillary carcinoma; MSI, microsatellite instability; N/A, insufficient DNA for evaluation or reaction failed (Case B16—invasive).

[a] For Cases B11, B12, and B16, the length of the newly generated alleles differed between the intraductal and invasive components.

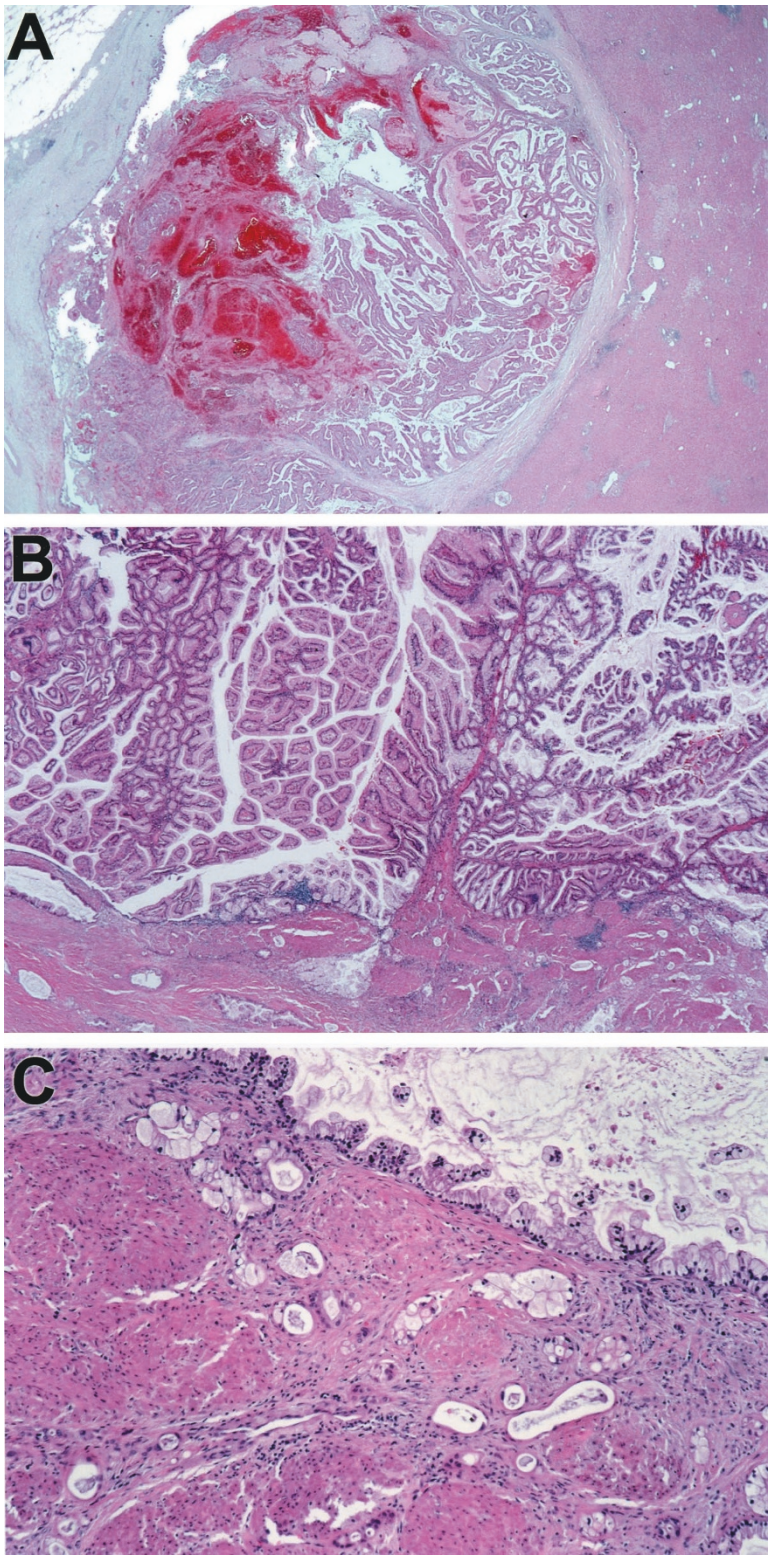

FIGURE 1. Histologic appearance of biliary papillary neoplasms. **A,** intraductal papillary carcinoma filling a dilated bile duct within the liver (hematoxylin and eosin stain; original magnification, 20×). **B,** intraductal papillary carcinoma with abundant luminal mucin, here located within a dilated cystic duct (hematoxylin and eosin stain; original magnification 60×). **C,** underlying invasive carcinoma of tubular type is present within the wall of the duct from (**B**) (hematoxylin and eosin stain; original magnification, 200×).

extensive MSI, with shifting observed in all five NCI-recommended microsatellite loci. In Case B16, both the intraductal papillary carcinoma and its associated invasive cholangiocarcinoma showed MSI-high. Interestingly, however, only four loci were shifted in this biliary intraductal papillary carcinoma (D2S123, D5S346, D17S250, and Bat-26), whereas Bat-25 was also shifted in the cholangiocarcinoma. In addition, for each common shifted locus (D2S123, D5S346, D17S250, and Bat-26) the amplicon length also differed between the intraductal and cholangiocarcinoma components (Fig. 2).

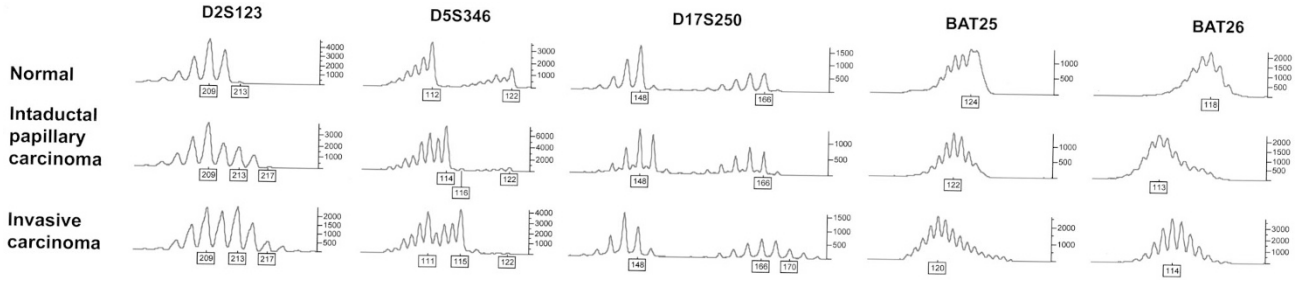

FIGURE 2. High-level microsatellite instability in Case B16. Allelic shifts in D2S123, D5S346, D17S250, and Bat-26 are present in the intraductal papillary carcinoma component, whereas the associated invasive cholangiocarcinoma also shows shifting in Bat-25. The shifting pattern for D17S250 and Bat-25 is subtle, but even if these markers are discounted, criteria for high-level microsatellite instability are met. In addition, the amplicon lengths of the shifted alleles differ between the intraductal and invasive carcinoma components of this neoplasm.

Low-level microsatellite instability (MSI-low) was present in 6 (35.3%) neoplasms (Cases B1, B2, B6, B8, B11, and B12; Fig. 3). Cases B1 and B2 contained intraductal biliary papillary carcinoma only. Cases B6 and B8 contained both intraductal and invasive cholangiocarcinoma components, and in these cases both the intraductal tumors and their associated cholangiocarcinomas demonstrated shifts in the same markers and generation of identical amplicon lengths. Cases B11 and B12 also contained both intraductal and invasive cholangiocarcinoma components with shifting of the same locus, but in these cases the intraductal biliary papillary neoplasms and their associated cholangiocarcinomas generated differing amplicon lengths.

There was no significant tendency for those biliary papillary neoplasms with MSI to be located preferentially in the intra- or extrahepatic biliary tree; of the 8 biliary tumors with MSI, 2 involved the extrahepatic ducts exclusively, 3 the intrahepatic ducts exclusively, and 3 involved both the intra- and extrahepatic ducts. The remaining 9 (53%) neoplasms were microsatellite stable. Evaluation for hypermethylation of the *hMLH1* promoter was possible in 10 cases for which adequate DNA was present, and no methylation was detected in any of these 10 biliary papillary neoplasms (including the 2 cases with MSI-high) or 2 associated cholangiocarcinomas (Fig. 4).

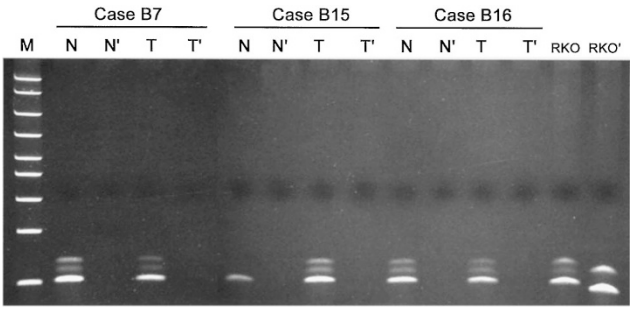

FIGURE 4. Lack of *hMLH1* promoter region hypermethylation in Cases B7 (microsatellite- stable), B15 (high-level MSI), and B16 (high-level MSI). In each case the DNA fails to amplify using methylation-specific primers (denoted by &vprime;) but amplifies with primers specific for the unmethylated allele. *Lane M,* DNA marker; *N,* normal control tissue; *T,* intraductal papillary neoplasm; *RKO,* positive control colon cancer cell line.

DISCUSSION

Intraductal papillary neoplasms of the biliary tree are uncommon neoplasms, and little is known regarding their molecular pathogenesis. Although the histologic and clinicopathologic features of those biliary papillary neoplasms that clinicopathologically resemble pancreatic IPMNs have been described in clinical case reports and several series (2–19, 22–25), their genetic alterations have only rarely been investigated (11, 20). One case of biliary papillomatosis was shown to carry an activating mutation of the *K-ras* oncogene (11), but we recently found only relatively infrequent *K-ras* mutations, relatively infrequent allelic losses on chromosomes 5q and 18q, and no evidence for p53/chromosome 17p alterations in biliary IPNs (20). During these investigations we observed several neoplasms with shifts in microsatellite markers during allelic loss analysis, a finding suggesting that

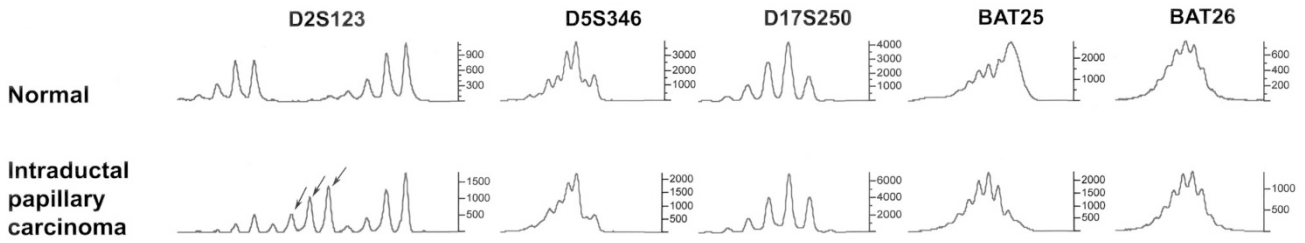

FIGURE 3. Low-level microsatellite instability in Case B2. Allelic shift in this intraductal papillary carcinoma is seen only in D2S123 (*arrows*).

microsatellite instability might play a role in the pathogenesis of a subset of these tumors.

The results of this study using the NCI-recommended investigation of microsatellite instability indicate that MSI is relatively common in biliary papillary neoplasms (47%, including high-level MSI in 11.8% and low-level MSI in 35.3%) and does not depend on the intra- or extrahepatic location of the neoplasm in the biliary tree. Interestingly, there is also evidence for clonal heterogeneity in the development of invasive cholangiocarcinomas from intraductal biliary papillary precursor lesions. Our series included 5 intraductal biliary papillary neoplasms and their associated cholangiocarcinomas that demonstrated MSI (high-level MSI in 1 case and low-level MSI in 4 cases); in 3 of these cases, the intraductal and invasive components of the neoplasms showed differing amplicon sizes in the shifted microsatellite loci, and in one case with high-level MSI, the invasive component showed shifting of one additional locus (Bat-25) that was not present in the intraductal component.

The frequency of MSI in these biliary papillary neoplasms is therefore somewhat higher than that reported for other neoplasms derived from (nonampullary) biliary epithelium. Among gallbladder carcinomas, MSI was detected in 0 of 22 (0%) carcinomas by Saetta *et al.* (33); in 3 of 15 (20%) carcinomas by Kim *et al.* (34); in 5 of 30 (17%) carcinomas by Yoshida *et al.* (35); and "widespread" MSI, in only 1 of 32 carcinomas by Chang *et al.* (36). Among cholangiocarcinomas, MSI was detected in 5 of 38 (13%) extrahepatic cholangiocarcinomas by Suto *et al.* (37), in 4 of 22 (18%) intrahepatic cholangiocarcinomas by Momoi *et al.* (38), and in 3 of 18 (17%) intrahepatic cholangiocarcinomas by Kawaki *et al.* (39). In addition, MSI was absent from multiple samples of the preneoplastic biliary epithelium of the intra- and extrahepatic biliary tree in 21 patients with primary sclerosing cholangitis (40). Only biliary tract carcinomas arising in association with pancreaticobiliary maljunction have been reported to show more frequent MSI; MSI was present in 16 of 23 (70%) such tumors (including 9 of 15 gallbladder carcinomas and 7 of 8 bile duct carcinomas) in the study by Nagai *et al.* (41). Of note, reliable comparison between studies is difficult because most investigations of MSI in biliary-derived neoplasms have not employed the NCI-recommended loci and because frequently any shifted locus is taken as evidence for MSI. For example, among all of the studies cited above, high-level MSI was present in only 1 of 32 gallbladder carcinomas (36) and in 4 of 22 intrahepatic cholangiocarcinomas (38), using a criterion of shifting in $\geq$40% of microsatellite loci examined.

The biliary papillary neoplasms studied here constitute a distinct subset of biliary tumors. It has become increasingly recognized that tumors such as these resemble pancreatic IPMNs in some of their clinicopathologic features (2, 19, 21, 22). Histologically, like pancreatic IPMNs they are comprised of a predominant intraductal growth of papillary fronds with fine vascular cores. Although the degree of extracellular mucin production may not always be as extensive as that found in most pancreatic IPMNs (22), like IPMNs they are comprised of a neoplastic proliferation of epithelial cells with varying biliary, foveolar, or intestinal differentiation and showing varying degrees of epithelial dysplasia. Both biliary and pancreatic tumors can spread extensively along a dilated duct system (as was the case for 15 biliary lesions studied here) or can constitute localized, solitary growths within a dilated duct (2 of the lesions in this study). Both the biliary and pancreatic intraductal components can serve as the precursor lesions for invasive carcinomas (invasive cholangiocarcinomas or pancreatic ductal adenocarcinomas, respectively), but even in the absence of invasion, both lesions can cause significant morbidity and mortality because of obstruction of the excretory ducts (4, 6, 7, 19, 23–25). It would therefore be of interest to compare the frequency of MSI in these two distinctive neoplasms. However, despite otherwise relatively numerous studies of the immunohistochemical and molecular genetic alterations in pancreatic IPMNs (42–50), we are not aware of any published investigation of MSI in pancreatic IPMNs.

Although a replication-error phenotype is known to occur with varying frequencies among the neoplasms arising in many different organ systems, the mechanism of MSI has been most thoroughly elucidated in colorectal, gastric, and endometrial adenocarcinomas. In sporadic colorectal carcinomas, high-level MSI is observed in approximately 15% of cases, particularly right-sided lesions, and the majority of such tumors show epigenetic hypermethylation of CpG islands in the *hMLH1* promotor, one of the key genes involved in DNA mismatch repair (51–54). In sporadic gastric adenocarcinomas, hypermethylation of the *hMLH1* promoter is present in the majority of MSI-H tumors (55–61), but neither *hMLH1* gene mutations (57) nor alterations of *hMSH2* are found (56, 57) (although several sporadic gastric carcinomas with *hMSH6* mutations have been reported) (62). Some investigators also report *hMLH1* methylation in gastric carcinomas with low-level MSI, although less frequently than in high- level MSI cases (56, 58, 59). Approximately one quarter of sporadic endometrial carcinomas are MSI-H, and in the majority of cases the replication-error phenotype is again associated with epigenetic hypermethylation of the

hMLH1 promoter rather than with *hMLH1* or *hMSH2* gene mutations (63).

In contrast, the mechanism of (less frequently observed) MSI in biliary-derived neoplasms, including invasive cholangiocarcinomas and gallbladder adenocarcinomas has only rarely been investigated. In a series of extrahepatic biliary carcinomas, Suto *et al.* (37) found loss of heterozygosity at the *hMLH1* and *hMSH2* gene loci in only 4% and 6.1% of cases, respectively. In a series of biliary carcinomas associated with pancreaticobiliary maljunction and displaying more frequent MSI, Nagai *et al.* (41) found loss of heterozygosity at the *hMLH1* and *hMSH2* loci in 12.5% and 25%. However, the methylation status of the *hMLH1* promoter in these tumors has not previously been investigated. The results of this study suggest that microsatellite instability plays a role in the pathogenesis of a subset of intraductal biliary papillary neoplasms, a clinicopathologically distinctive neoplasm among biliary tumors, but no evidence for *hMLH1* hypermethylation was found.

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