Analysis of Concurrent Intracholecystic Papillary Neoplasms and Biliary Intraepithelial Neoplasia Reveals Distinct Histologic and Molecular Profiles

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• ***Context.***—**Intracholecystic papillary neoplasms (ICPNs) and biliary intraepithelial neoplasia (BilIN) are presumed precursors to gallbladder adenocarcinomas, but their relationship is incompletely understood.**

Objective.—**To perform morphologic and molecular characterization of concurrent ICPNs, nonpolypoid mucosa, and adenocarcinomas to determine whether these lesions are related at the DNA level.**

Design.—**Background mucosa and 36 ICPNs were graded by a pathologist blinded to original diagnoses. Separate areas of ICPNs, BilIN (n = 5), nondysplastic adjacent mucosa (n = 8), and invasive adenocarcinoma (n = 3) were amplified and sequenced on a next-generation sequencer. Data were manually curated to identify pathogenic somatic variants.**

Results.—**High-grade ICPNs were associated with low-grade (n = 1) or high-grade (n = 3) BilIN or no dysplasia (n = 5). Fifteen were associated with invasive adenocarcinoma. Low-grade ICPNs were associated with low-grade BilIN (n = 3) or no dysplasia (n = 9). Pathogenic variants included *CTNNB1* (catenin beta 1) exon 3 (7); *TP53* (tumor protein p53) (6); *APC* (APC regulator of WNT signaling pathway) (2); *RB1* (RB transcriptional corepressor 1) (1); *KRAS* (KRAS proto-oncogene, GTPase) (1); and *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) (1). Pathogenic variants in ICPNs were not detected in BilIN or nondysplastic mucosa. Mutations in invasive cancers included *TP53*, *PIK3CA*, and *RB1*, concordant with the ICPN, but not with BilIN, in all 3 cases.**

Conclusions.—**BilIN in the background of ICPNs was of the same or lower grade than ICPNs. Synchronous ICPNs and BilIN lacked concordant somatic mutations. Mutations in adenocarcinomas aligned with the ICPNs. This suggests that ICPN and BilIN are independent at the DNA level and that the presence of ICPNs may not imply risk for subsequent flat dysplasia elsewhere in the biliary tree.**

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Gallbladder adenocarcinoma (GBC) is a rare malignancy accounting for only 1.2% of all cancers and 1.7% of all cancer deaths worldwide.[1] The incidence of GBC varies widely among different geographic regions and ethnic groups. Presumably, a variety of preinvasive dysplastic epithelial lesions precede the development of GBC, but their molecular genetic features and relationships to one another are incompletely understood. Widespread use of laparoscopic cholecystectomy to treat clinically benign biliary disease (ie, chronic cholecystitis and cholelithiasis) has increased the incidental detection of GBC (0.2%–2.9% of all cholecystectomies done for gallstone disease) and gallbladder dysplasia; thus, insights into their etiology and natural history can provide valuable clinical guidance.[2] Flat dysplasia of the biliary epithelium is termed *biliary intraepithelial neoplasia* (BilIN). Polypoid dysplastic lesions include pyloric gland adenomas, which rarely harbor high-grade dysplasia or carcinoma, and intracholecystic papillary neoplasms (ICPNs).[3] Up to 6% of gallbladder carcinomas arise in association with ICPN, and ICPNs are found in 0.4% of all cholecystectomies.[4–6] ICPNs are further subclassified into biliary, gastric, intestinal, and oncocytic types according to morphologic and immunohistochemical features of their epithelial lining. ICPNs are classified as low- and high-grade on the basis of the highest degree of cytoarchitectural atypia in the epithelium.[7–9]

Cholecystectomy specimens are routinely sampled for histopathologic examination. Random sections are taken from those without abnormal gross findings, whereas sections target intraluminal masses or areas of mural thickening when present. Detection of BilIN typically prompts additional representative sampling or submission of the entire gallbladder for histologic evaluation[3,10] ICPNs are usually submitted entirely, but extent of sampling of flat mucosa in these cases is highly variable and evidence-based guidelines are lacking.[3,6] Although some authorities believe that gallbladders with ICPN are at risk for concurrent or subsequent neoplasia elsewhere in the biliary tree, a circumstance that would warrant additional sampling, this possibility has not been systematically evaluated.[3,6] The purpose of this study was 2-fold: first, we assessed findings in the background mucosa of gallbladder with ICPNs to

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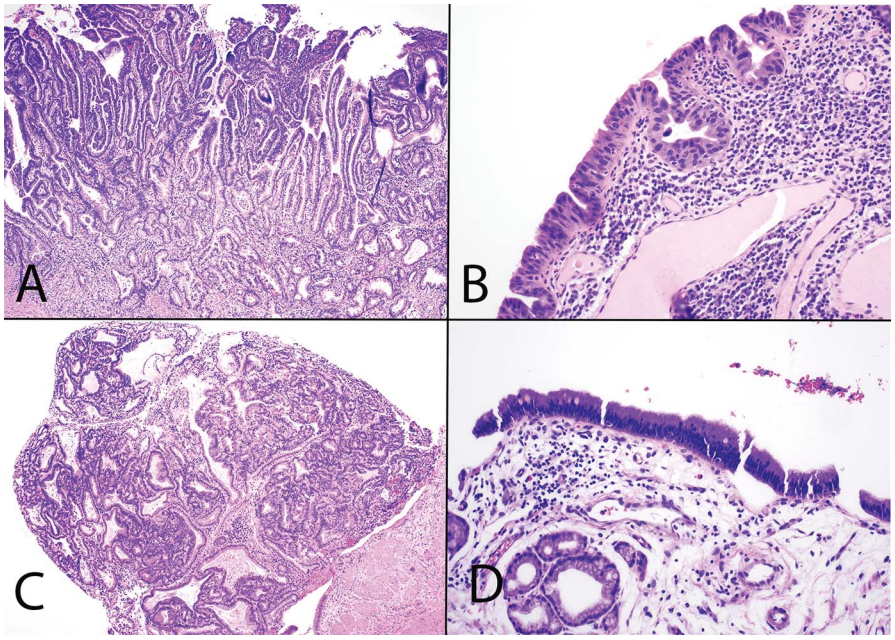

Figure 1. *High-grade intracholecystic papillary neoplasm (pancreatobiliary type) (A) with high-grade biliary intraepithelial neoplasia (BilIN) in the flat mucosa (B). High-grade intracholecystic papillary neoplasm (pancreatobiliary type) (C) with low-grade BilIN in the flat mucosa (D) (hematoxylin-eosin, original magnifications ×4 [A and C] and ×20 [B and D]).*

determine whether additional sampling revealed clinically important findings. Second, we performed segregated molecular characterization of BilIN, ICPNs, and associated GBC to determine whether carcinomas arising in association with these lesions share a common molecular landscape with identifiable drivers of malignant transformation. In so doing, we aim to guide sampling of cholecystectomy specimens with these neoplasms and guide clinical follow-up for this patient population.

METHODS

Case Selection

We retrospectively searched the pathology database for cholecystectomy specimens harboring ICPNs between the years 2000 and 2022. Owing to evolution of terminology applied to these lesions during the study period, search terms included *pyloric gland adenoma*, *intracholecystic papillary neoplasm*, *ICPN*, and *adenoma*. Thirty-six papillary neoplasms with slides available for review were identified. Data regarding clinical presentation, imaging, gross findings, and extent of sampling were obtained from the electronic medical record and pathology reports. This study was approved by the Institutional Review Board of Montefiore Medical Center (Bronx, New York).

Histologic Evaluation

All cases were routinely sampled to include the cystic duct margin (inked) and representative sections of the gallbladder wall from the neck, body, and fundus. The standard practice in our department is if any dysplasia is identified, additional sections are submitted for further evaluation and in some cases the entire gallbladder to rule out invasive GBC.[3] A pathologist blinded to the original diagnoses reviewed sections from nonpolypoid mucosa to confirm the diagnosis and classify the background mucosa as negative or positive for BilIN; in the latter case, as low-grade or high-grade BilIN. Briefly, low-grade BilIN comprised flat epithelium showing nuclear enlargement, hyperchromasia, pseudostratification, and increased mitoses without conspicuous nucleoli and loss of polarity. High-grade BilIN contained flat epithelium with pleomorphic nuclei, prominent nucleoli, and loss of polarity.[9] ICPNs were classified by papilla type and grade of dysplasia according to World Health Organization criteria.[9] Biliary-type ICPNs contained cuboidal epithelium with clear to eosinophilic cytoplasm; gastric lining resembled foveolar epithelium; intestinal morphology paralleled that of colonic epithelium; and oncocytic examples had abundant eosinophilic granular cytoplasm. Cytologic atypia is assessed similarly to BilIN (Figure 1, A through D; Figure 2, A through D).

Molecular Studies

Nineteen cases were selected for next-generation sequencing, based on adequacy of available tissue and feasibility of segregating the adenocarcinoma, ICPN, and flat mucosa. Thirteen cases of ICPN and flat mucosa, with no dysplasia, low-grade BilIN, or high-grade BilIN, were sequenced separately. In 3 additional cases, coexistent invasive adenocarcinoma was also sequenced. We also sequenced 3 cases of invasive adenocarcinoma with only high-grade BilIN in the flat/background mucosa. The regions of interest, marked and quantified (percentage of cells of interest) by a pathologist, were manually macrodissected for DNA extraction from 5 to 10 unstained, 5-μm, formalin-fixed, paraffin-embedded tissue sections. The extracted DNA was amplified by using a multiplex polymerase chain reaction primer set designed to amplify 207 amplicons covering approximately 2800 COSMIC mutations from 50 oncogenes and tumor suppressor genes (Table 1). Sequencing was performed on a next-generation sequencer and the data were manually curated to identify pathogenic somatic variants.

Statistical Analysis

Analysis was done with SPSS statistical software. Mean and standard deviations were used to describe continuous variables. Categorical variables were represented by frequency and percentages. Association of somatic mutations with ICPN and coexisting BilIN in the flat/background mucosa was analyzed by using χ^2 or Fisher exact test. A P value $<.05$ was considered statistically significant.

RESULTS

Clinical and Histologic Features

The study group included 16 male and 20 female patients with a mean age of 65 years (range, 37–108 years). Imaging and pathologic features of study cases are summarized in Table 2. All ICPNs were entirely submitted. Fifteen were associated with invasive cancers, including 14 of 15 (93%) with a mass lesion or substantial mural thickening on imaging

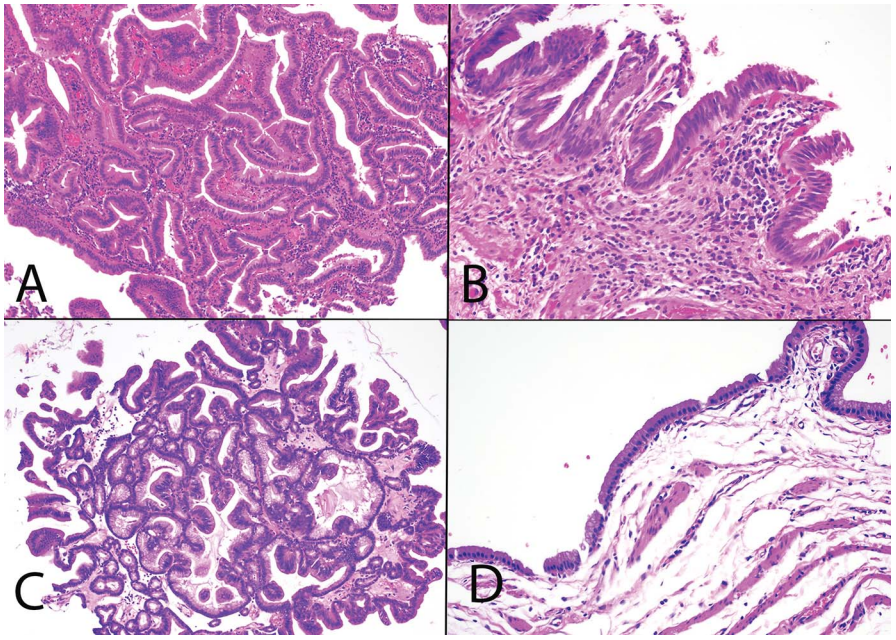

Figure 2. *Low-grade intracholecystic papillary neoplasm (gastric foveolar type) (A) with low-grade biliary intraepithelial neoplasia in the flat mucosa (B). Low-grade intracholecystic papillary neoplasm (gastric foveolar type) (C) with no dysplasia in the flat mucosa (D) (hematoxylin-eosin, original magnifications ×4 [A and C] and ×20 [B and D]).*

studies, and 1 of 15 (7%) were detected incidentally. All adenocarcinomas were present in at least 1 section underlying the corresponding ICPN, although many had lateral mural extent beyond the edges of the polypoid lesion. ICPNs with high-grade dysplasia were associated with high-grade BilIN in 3 of 9 (33%), low-grade BilIN in 1 of 9 (11%), and no BilIN in 5 of 9 (56%) cases within flat mucosa. ICPNs with low-grade dysplasia were either associated with low-grade BilIN (3 of 12; 25%) or lack of dysplasia (9 of 12; 75%) in flat mucosa, but no high-grade BilIN was detected. The flat mucosa was representatively sampled in 20 of 36 cases (56%) and was entirely submitted in 16 of 36 cases (44%). Detection of any dysplasia was significantly associated with greater extent of sampling ($P = .004$), but no higher-grade lesions were detected in any cases with low-grade ICPN, even when the gallbladder was entirely submitted. The cystic duct margin was negative for dysplasia or carcinoma in all cases.

Molecular Features

Pathogenic variants identified in the ICPN included *CTNNB1* (catenin beta 1) exon 3 (7); *TP53* (tumor protein p53) (6); *APC* (APC regulator of WNT signaling pathway) (2); *RB1* (RB transcriptional corepressor 1) (1); *KRAS* (KRAS proto-oncogene, GTPase) (1); and *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) (1) (Table 2). Eleven of 13 cases of ICPN without invasive cancer had pathogenic variants in the ICPN but not in the flat/background mucosa irrespective of the presence or grade of BilIN. This difference was statistically significant ($P < .001$). One case showed a *TP53* variant in no BilIN flat mucosa, but that same mutation was not identified in the concurrent low-grade ICPN, which showed a *CTNNB1* variant. In 1 case, no pathogenic variants were identified in the ICPN or flat/background mucosa.

The 3 cases of ICPN with concomitant invasive adenocarcinoma showed identical mutations in *TP53*, *PIK3CA*, and *RB1* in both the invasive component and the ICPN component irrespective of the grade of dysplasia in the ICPN. The background mucosa sequenced in 2 of these cases did not show these specific variants. The 3 cases of invasive carcinoma with concomitant high-grade BilIN in the background mucosa, but no ICPN, showed pathogenic variants solely in the invasive adenocarcinoma component. This difference was statistically significant with a P value of .03 (N-1 χ^2 test). Molecular features of the study cases are detailed in Table 3 and Figure 3, A through C.

Table 1. Targeted Genes in the Panel

ABL1	*EGFR*	*GNAS*	*KRAS*	*PTPN11*
AKT1	*ERBB2*	*GNAQ*	*MET*	*RB1*
ALK	*ERBB4*	*HNF1A*	*MLH1*	*RET*
APC	*EZH2*	*HRAS*	*MPL*	*SMAD4*
ATM	*FBXW7*	*IDH1*	*NOTCH1*	*SMARCB1*
BRAF	*FGFR1*	*JAK2*	*NPM1*	*SMO*
CDH1	*FGFR2*	*JAK3*	*NRAS*	*SRC*
CDKN2A	*FGFR3*	*IDH2*	*PDGFRA*	*STK11*
CSF1R	*FLT3*	*KDR*	*PIK3CA*	*TP53*
CTNNB1	*GNA11*	*KIT*	*PTEN*	*VHL*

DISCUSSION

This is the first study, to our knowledge, to systematically evaluate findings in the background mucosa of gallbladders that harbor ICPNs. Histopathologic analysis revealed that invasive carcinoma arising in ICPNs can be reliably detected by complete submission of the polypoid lesion. However, extensive additional sampling may be necessary to accurately stage invasive cancers when they are present, since the deepest extent of cancer may be in sections from beyond the gross extent of the polypoid lesion. We would recommend limited sequential sections immediately adjacent to the ICPN to determine the extent of the invasive cancer and ensure sampling of its deepest extent. In instances of high-grade ICPNs, there is a possibility of high-grade BilIN in other areas, warranting additional tissue sampling to rule out occult malignancy.

Table 2. Imaging and Pathologic Features of Study Cases			
Features	ICPN With Low-Grade Dysplasia (n = 12), No. (%)	ICPN With High-Grade Dysplasia (n = 9), No. (%)	ICPN-Associated Invasive Adenocarcinoma (n = 15), No. (%)
Imaging findings			
Intraluminal polyp/mass	8 (66)	7 (78)	11 (73)
Wall thickening	0	1 (11)	1 (7)
Cholecystitis	2 (17)	1 (11)	3 (20)
Not available	2 (17)	0	0
Histologic type			
Pyloric/gastric	10 (84)	3 (33)	1 (7)
Intestinal	1 (8)	1 (11)	1 (7)
Pancreatobiliary	1 (8)	5 (56)	13 (86)
Findings in flat mucosa			
No dysplasia	9 (75)	5 (56)	4 (26)
Low-grade dysplasia	3 (25)	1 (11)	1 (7)
High-grade dysplasia	0	3 (33)	10 (67)
Dysplasia at cystic duct margin			
Yes	0	0	0
No	12 (100)	9 (100)	15 (100)
Sampling of nonpolypoid mucosa			
Representative	7 (58)	3 (33)	10 (67)
Complete	5 (42)	6 (67)	5 (33)

Abbreviation: ICPN, intracholecystic papillary neoplasm.

While low-grade BilIN may be observed in the nonpolypoid flat mucosa associated with low-grade ICPNs, it is unlikely to impact management decisions and may not necessitate further sampling, provided that dysplasia is absent at the cystic duct margin.[3,8] Our findings are aligned with several prior investigations that evaluated BilIN. For example, Bosch et al[11] did not detect any high-grade lesions upon additional sampling of gallbladders with low-grade BilIN (n = 28), but found 2 invasive carcinomas upon additional sampling of those with high-grade BilIN (n = 20). Renshaw and Gould[12] identified high-grade BilIN upon additional sampling of 2 of 18 cases with low-grade BilIN, but no malignancies were identified. Their findings were similar to those of Akki et al[13] who also detected high-grade BilIN in the minority (4 of 18) of cases with low-grade BilIN, but no invasive lesions were seen. Finally, Rais et al[14] reported that no additional findings were present upon further sampling of 24 gallbladders with low- and high-grade BilIN. These findings suggest that the utility of complete sampling of the gallbladder is limited to cases wherein high-grade dysplasia or carcinoma are found in the mass lesion and/or flat mucosa. It has been suggested that some ICPNs may reflect a field effect that is associated with DNA alterations across multiple sites in the biliary tree. Its presence, especially at margins, may necessitate additional surveillance or surgical intervention. However, molecular investigations confirming this effect are currently lacking. Hence, we performed molecular analysis and found *CTNNB1*, *APC*, *KRAS*, and *TP53* to be the most common mutations identified in ICPN. However, there were no instances of concordant somatic mutations identified in concurrent ICPNs and flat lesion/background mucosa (BilIN or no dysplasia). In 3 cases with adenocarcinoma, mutations (*TP53*, *PIK3CA*, and *RB1*) aligned with ICPN. Three separate cases of adenocarcinoma without ICPN had mutations (*TP53*, *CDKN2A* [cyclin-dependent kinase inhibitor 2A], and *GNAS* [GNAS complex locus]), but none of these mutations were present in the concurrent high-grade BilIN. The study results support that these lesions may arise from separate clones, either of which may give rise to gallbladder adenocarcinoma. Nonpolypoid gallbladder mucosa also harbored driver mutations that differed from morphologically appreciable adenocarcinoma and ICPN. The lack of common driver mutations implies that these events may be independent at the DNA level. Surveillance of the biliary tree may not be warranted in patients with fully resected gallbladder neoplasia.

Numerous investigations have delved into the genomic landscape of GBC. For example, a recent comprehensive molecular characterization of GBC identified *TP53*, *CDK2NA*, and RTK-RAS pathway alterations as the most common via the MSK-IMPACT assay.[15] On the other hand, there are relatively few studies characterizing molecular features of ICPN. A study by Akita et al[16] identified *STK11* (serine/threonine kinase 11), *CTNNB1*, and *APC* as major driver genes for ICPNs, aligning with our study results. According to Akita et al,[16] mutations in *STK11* and *CTNNB1* were observed exclusively in ICPNs but not in polypoid/exophytic or nonpolypoid carcinomas of the gallbladder. By contrast, mutations in *TP53* and *ERBB2*/*ERBB3* (erb-b2 receptor tyrosine kinase 2/3) were more frequent in polypoid cancers than in ICPNs.[16]

Our findings are also supported by those of Lin et al,[17] whose whole-genome sequencing study identified mutations in *CTNNB1*, *ARID2* (AT-rich interaction domain 2), *TP53*, and *ERBB3* in GBC, low-grade BilIN, or high-grade BilIN. Additionally, studies have reported stepwise development of GBC through BilIN, including *TP53* and *KRAS* mutations in BilIN, and dysregulation of p16/cyclin-D1/CDK4 cell cycle pathway in both BilIN and GBC.[18,19] These authors also suggested that some gallbladder cancers arise via a BilIN independent

Table 3. Variants Identified by Next-Generation Sequencing in the Cases Sequenced

	ICPN				Flat Lesion/Background Mucosa				Invasive Adenocarcinoma		
Case	Grade of Dysplasia	Gene	Nucleotide Change	Protein Effect	Grade of Dysplasia	Gene	Nucleotide Change	Protein Effect	Gene	Nucleotide Change	Protein Effect
1	HG	*TP53*	c.124del	p.R42Gfs	HG	None	None	NA	NA	NA	NA
2	HG	*TP53*	c.747G>T	p.R249S	HG	None	None	NA	NA	NA	NA
3	HG	*TP53*	c.880G>T	p.E294*	LG	None	None	NA	NA	NA	NA
		APC	c.42insT	p.I15Yfs*							
4	HG	None	None	None	LG	None	None	NA	NA	NA	NA
5	HG	*CTNNB1*	c.122C>T	p.T41I	No dysplasia	None	None	NA	NA	NA	NA
6	HG	*CTNNB1*	c.110C>T	p.S37F	No dysplasia	None	None	NA	NA	NA	NA
7	LG	*CTNNB1*	c.121A>G	p.T41A	LG	None	None	NA	NA	NA	NA
8	LG	*CTNNB1*	c.122C>T	p.T41I	No dysplasia	*TP53*	c.902_903insG	p.G302fs*4	NA	NA	NA
9	LG	*CTNNB1*	c.122C>T	p.T41I	No dysplasia	None	None	NA	NA	NA	NA
10	LG	*CTNNB1*	c.98C>G	p.S33C	No dysplasia	None	None	NA	NA	NA	NA
11	LG	*APC*	c.4630G>T	p.E1544*	No dysplasia	None	None	NA	NA	NA	NA
12	LG	*CTNNB1*	c.122C>T	p.T41I	No dysplasia	None	None	NA	NA	NA	NA
13	LG	*KRAS*	c.35G>A	p.G12D	No dysplasia	None	None	NA	NA	NA	NA
14	HG	*TP53*	c.473G>A	p.R158H	No dysplasia	None	None	NA	*TP53*	c.473G>A	p.R158H
15	LG	*TP53*	c.796G>T	p.G266*	LG	*TP53*	c.646G>A	p.V216M	*TP53*	c.796G>T	p.G266*
		PIK3CA	c.1633G>A	p.E545K					*PIK3CA*	c.1633G>A	p.E545K
16	LG	*TP53*	c.388C>T	p.L130F	NA	NA	NA	NA	*TP53*	c.388C>T	p.L130F
		RB1	c.1981C>T	p.R661W	HG	None	None	NA	*RB1*	c.1981C>T	p.R661W
17	NA	NA	NA	NA					*TP53*	c.523C>T	p.R175C
									CDKN2A	c.225delC	p.A76fs*70
									GNAS	c.2531G>A	p.R844H
18	NA	NA	NA	NA		None	None	NA	*TP53*	c.1024C>T	p.R342*
19	NA	NA	NA	NA		None	None	NA	*TP53*	c.535C>T	p.H179Y

Abbreviations: *APC*, APC regulator of WNT signaling pathway; *CDKN2A*, cyclin-dependent kinase inhibitor 2; *CTNNB1*, catenin beta 1; *GNAS*, GNAS complex locus; HG, high-grade dysplasia; ICPN, intracholecystic papillary neoplasm; *KRAS*, KRAS proto-oncogene; LG, low-grade dysplasia; NA, not applicable; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *RB1*, RB transcriptional corepressor 1; *TP53*, tumor protein p53.

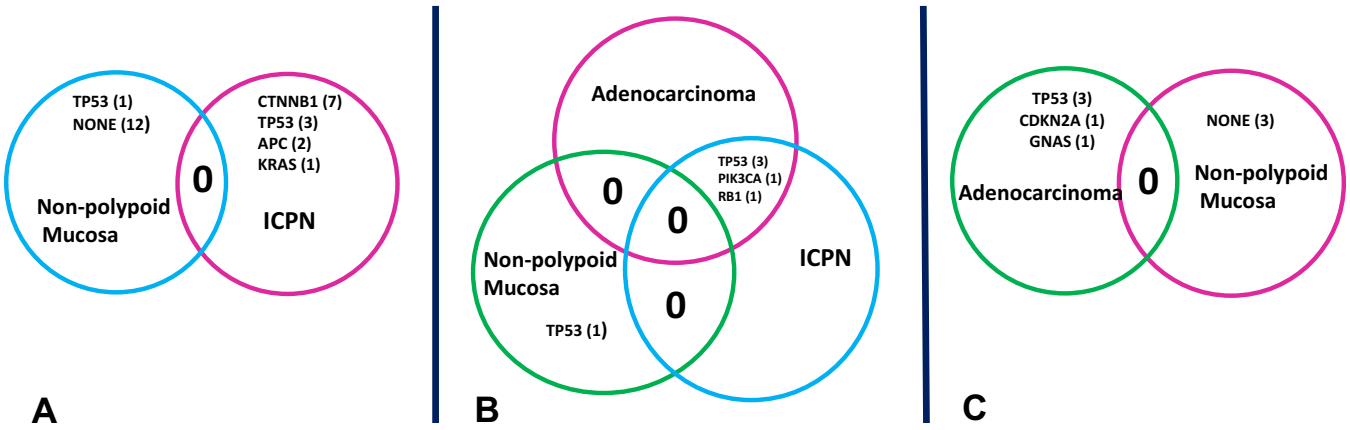

Figure 3. *Venn diagram showing somatic variants among adenocarcinoma, intracholecystic papillary neoplasm, and nonpolypoid mucosa. A, Somatic variants in agreement for cases with ICPN and nonpolypoid mucosa (n = 13). B, Somatic variants in agreement for cases with adenocarcinoma, ICPN, and nonpolypoid mucosa (n = 3). C, Somatic variants in agreement for cases with adenocarcinoma and nonpolypoid mucosa (n = 3). Abbreviations: APC, APC regulator of WNT signaling pathway; CDKN2A, cyclin-dependent kinase inhibitor 2; CTNNB1, catenin beta 1; GNAS, GNAS complex locus; ICPN, intracholecystic papillary neoplasm; KRAS, KRAS proto-oncogene, GTPase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; RB1, RB transcriptional corepressor 1; TP53, tumor protein p53.*

pathway, which may account for our findings of driver mutations in rare cases of nondysplastic mucosa as well as cases with lack of concordance between BilIN and adenocarcinoma. Some mutations in the foregoing studies were not detected in our cohort and these discrepancies likely reflect sampling error in both our study and theirs. Although our study is limited by small sample size, this is the largest series of ICPNs to undergo molecular analysis, to our knowledge. These results suggest that ICPNs and BilINs exhibit genetic heterogeneity, with different lesions harboring different combinations of genetic alterations. This heterogeneity likely reflects the complex molecular landscape of these tumors and may have implications for tumor behavior and response to therapy. Further research is needed to elucidate the full spectrum of genetic alterations associated with ICPNs and to understand their functional significance in tumor development and progression. Such insights may lead to the identification of novel therapeutic targets and the development of personalized treatment approaches for patients with ICPNs.

CONCLUSIONS

Our study results emphasize the importance of comprehensive but judicious histopathologic evaluation and tailored tissue sampling strategies in the management of cholecystectomy specimens with ICPNs. Furthermore, by conducting analysis of somatic alterations in gallbladder cancer, ICPN, and low- and high-grade BilIN, we reveal that these lesions may arise independently or may diverge after developing from a common precursor. These findings have implications for pathologic sampling of cholecystectomy specimens and clinical surveillance of the biliary tree in patients with these lesions.

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