Gallbladder Cancer with Biliary Intraepithelial Neoplasia Complicated by Pancreaticobiliary Maljunction: A Case Report

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ABSTRACT

We report herein a case of gallbladder cancer with biliary intraepithelial neoplasia (BilIN) complicated by pancreaticobiliary maljunction (PBM). A 60-year-old woman was referred to our hospital for thickening of the gallbladder wall diagnosed via ultrasonography at the referring clinic. The Radiographic images showed thickening of the gallbladder wall and a high confluence of pancreaticobiliary ducts outside the duodenal wall without dilatation of the bile duct. The amylase level in the bile duct was highly elevated. The patient was initially diagnosed with PBM without biliary dilatation, and laparoscopic cholecystectomy was performed. Histopathology of the resected specimen revealed gallbladder cancer localized in the mucosa propria with widespread BilIN. Immunohistochemical analyses showed positive results for S100P,IMP3 and $p16^{ink4a}$ in tumor cells, but a positive result for only IMP3 in adenocarcinoma. Expression of p53 was negative. Oncogenic *KRAS* mutations were not detected in tumor cells. The patient was diagnosed with gallbladder cancer with BilIN complicated by PBM. This case report may be useful in clarifying the carcinogenic process and genetic mutations for gallbladder cancer associated with PBM.

Key words biliary intraepithelial neoplasia; gallbladder cancer; immunohistochemistry; pancreaticobiliary maljunction

Pancreaticobiliary maljunction (PBM) is a congenital malformation defined as a junction of the pancreatic and bile ducts outside the duodenal wall.[1] PBM regurgitates the pancreatic juice to the bile duct, causing persistent inflammation of the biliary tract, which in turn leads to biliary tract cancer (BTC). The carcinogenesis of BTC associated with PBM is typically considered to involve a hyperplasia-dysplasia-carcinoma sequence induced by chronic inflammation with the reflux of pancreatic juice into the biliary tract, rather than via the adenoma-carcinoma sequence or de novo carcinogenesis that is carcinogenesis form of gallbladder cancer without PBM.[1]

Biliary intraepithelial neoplasia (BilIN) is a microscopic flat or micropapillary lesion of the dysplastic epithelium, the grades of which represent the most frequent dysplasia-carcinoma sequence through which BTC develops.[2] Therefore, BilIN is recognized as a precursor lesion of BTC. BilIN is frequently found in resected specimens of patients with gallbladder cancer, and PBM accounts for about 20% of the causes of gallbladder cancer.[3] However, the etiology and genetic mutations of gallbladder cancer in patients with PBM has not been fully clarified.

Here, we report a case of gallbladder cancer originating in BilIN with PBM. This case may be useful in clarifying the carcinogenic process for gallbladder cancer associated with PBM.

PATIENT REPORT

A 60-year-old woman without both hepatitis B virus and hepatitis C virus was referred to our hospital for thickening of the gallbladder wall diagnosed via ultrasonography at the referring clinic. Table 1 shows blood biochemical examination in the patient. The serum concentration of carbohydrate antigen and carcinoembryonic antigen was within the reference range. Computed tomography revealed enhanced thickening of the gallbladder wall (Fig. 1a); endoscopic ultrasonography showed similar results (Fig. 1b). Magnetic resonance cholangiopancreatography showed a long common channel of the pancreatic duct and the bile duct (Fig. 2a), and endoscopic retrograde cholangiopancreatography(ERCP) confirmed the high confluence of pancreaticobiliary ducts outside the duodenal wall without dilatation of the bile duct (Fig. 2b). Furthermore, the amylase level of the bile juice in the bile ducts sampled during ERCP was highly elevated at 2123 IU/L (30-120IU/L). Based on images, we diagnosed preoperatively the gallbladder hyperplasia

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Abbreviations: BilIN, biliary intraepithelial neoplasia; BTC, biliary tract cancer; IMP3, insulin-like growth factor II mRNA binding protein 3; PBM, pancreaticobiliary maljunction; S100P, S 100 calcium binding protein

Table 1. Laboratory data

Parameter	Value	Unit	Parameter	Value	Unit
Blood cell count			Biochemistry		
WBC	3400	/μL	TP	6.83	g/dL
RBC	421×10^4	/μL	Alb	4.09	g/dL
Hb	12.4	g/dL	AST	25	IU/L
Ht	38	%	ALT	27	IU/L
Plt	35.2×10^4	/μL	ALP	331	IU/L
			γ-GTP	36	IU/L
Immunology			T.Bil	0.6	mg/dL
HBsAg	Negative		Amy	53	IU/L
HCVAb	Negative		BUN	11.5	mg/dL
			Cre	0.53	mg/dL
Tumor marker			Na	140	mEq/L
CEA	2.9	ng/mL	K	4.4	mEq/L
CA19-9	20.4	U/mL	Cl	106	mEq/L
			CRP	0.04	mg/dL
			Glu	102	mg/dL

Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Amy, amylase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; Cl, chlorine; Cre, Creatinine; CRP, C-reactive protein; Glu, glucose; Hb, hemoglobin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; Ht, hematocrit; K, kalium; Na, Natrium; Plt, platelet; RBC, red blood cell; T.Bil, Total Bilirubin; TP, total protein; WBC, white blood cell; γ-GTP, γ-Glutamyl TransPeptidase.

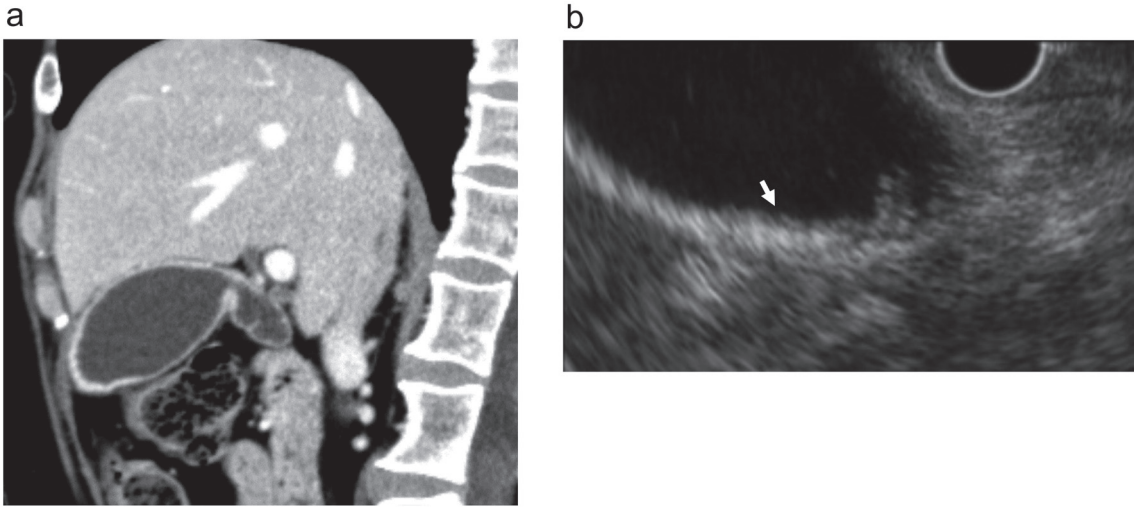

Fig. 1. Thickening of the gallbladder wall was observed by computed tomography (**a**) and endoscopic ultrasonography (arrow) (**b**).

complicated by PBM without biliary dilatation, because there are no nodules or irregular raised lesions in the gallbladder. We performed a laparoscopic cholecystectomy. The amylase level of bile juice in the removed gallbladder was high at 56,833 IU/L. Figure 3 shows the resected gallbladder specimen, displaying extensive granular mucosa. Although there were no findings of gallbladder cancer preoperatively, the histopathological findings confirmed well-differentiated adenocarcinoma located in the mucosa propria with widespread BilIN2/3 (Figs. 4a and b). Immunohistochemical examinations showed positive results for both S100P, IMP3 and

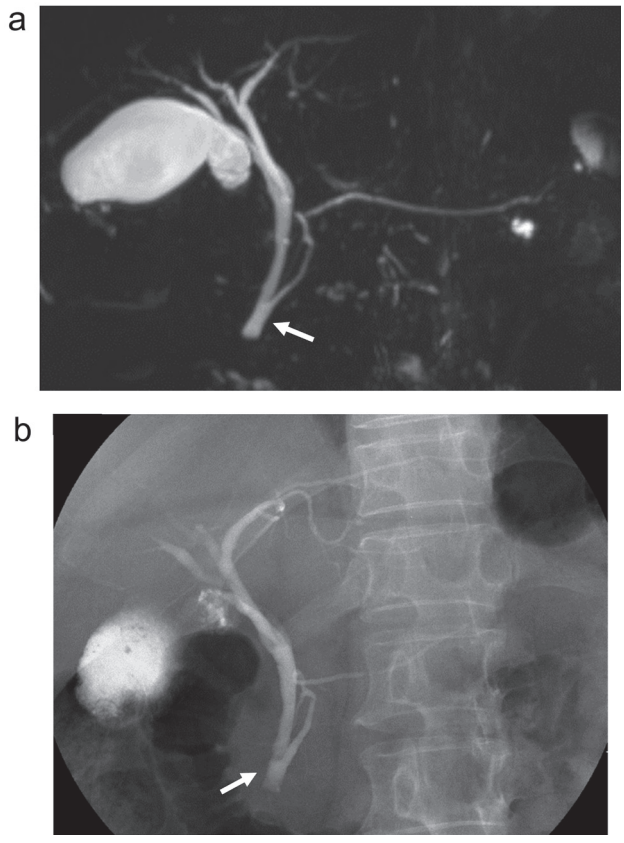

Fig. 2. (**a**) Magnetic resonance cholangiopancreatography showed the long common channel of the pancreatic duct and the bile duct (arrow). (**b**) Endoscopic retrograde cholangiopancreatography described the high confluence of pancreaticobiliary ducts located outside the duodenal wall without dilatation of the bile duct (arrow).

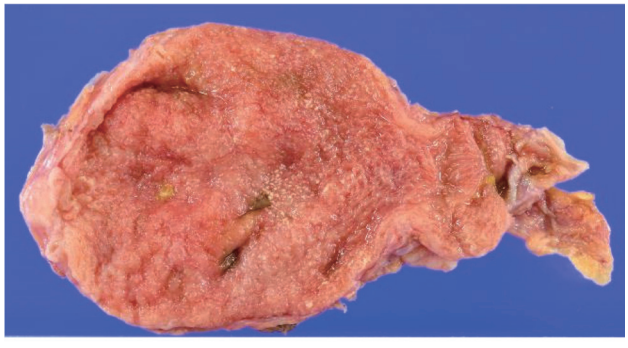

Fig. 3. Resected gallbladder specimen showing extensive granular mucosa.

p16^{ink4a} in tumor cells, but a positive result for only IMP3 in adenocarcinoma (Figs. 5a–d). Expression of p53 was negative. Oncogenic *KRAS* mutations were not detected in tumor cells. Finally, the patient was diagnosed with gallbladder cancer with BilIN complicated by PBM.

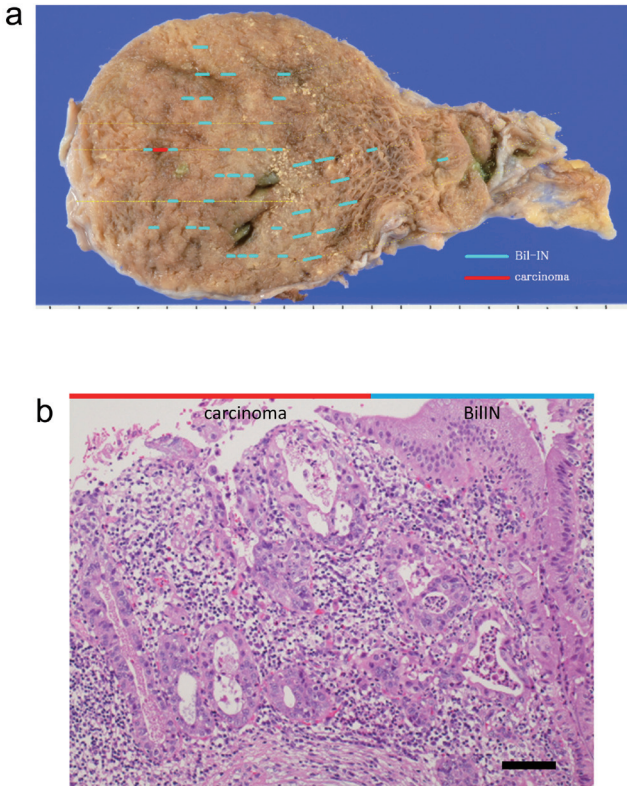

Fig. 4. Histopathological findings confirmed well-differentiated adenocarcinoma located in the mucosa propria with widespread BilINs. (**a**) Distribution of carcinoma and BilINs. (**b**) Well-differentiated adenocarcinoma located in the mucosa propria. Bar = 100 μm.

DISCUSSION

This is the report of a case of gallbladder cancer complicated by PBM with pathological findings of well-differentiated adenocarcinoma localized in the mucosa propria with extensive BilIN, which may help elucidate the contributions of PBM to the carcinogenic process. PBM patients have a high incidence of BTC; in one report, gallbladder cancer was found in 67.0% of PBM patients without biliary dilatation.[4] The mechanism of carcinogenesis in PBM is thought to be related to the persistent inflammation caused by regurgitation of pancreatic juice into the biliary tract. However, the etiology of gallbladder cancer in patients with PBM has not been fully clarified.

BilIN is a microscopic flat or micropapillary lesion of the dysplastic epithelium classed into 3 types (BilIN-1, low grade; BilIN-2, intermediate grade; or BilIN-3, high grade/carcinoma in situ) and is considered to reflect multistage carcinogenesis consisting of a dysplasia-to-carcinoma sequence for BTC.[5] In PBM patients

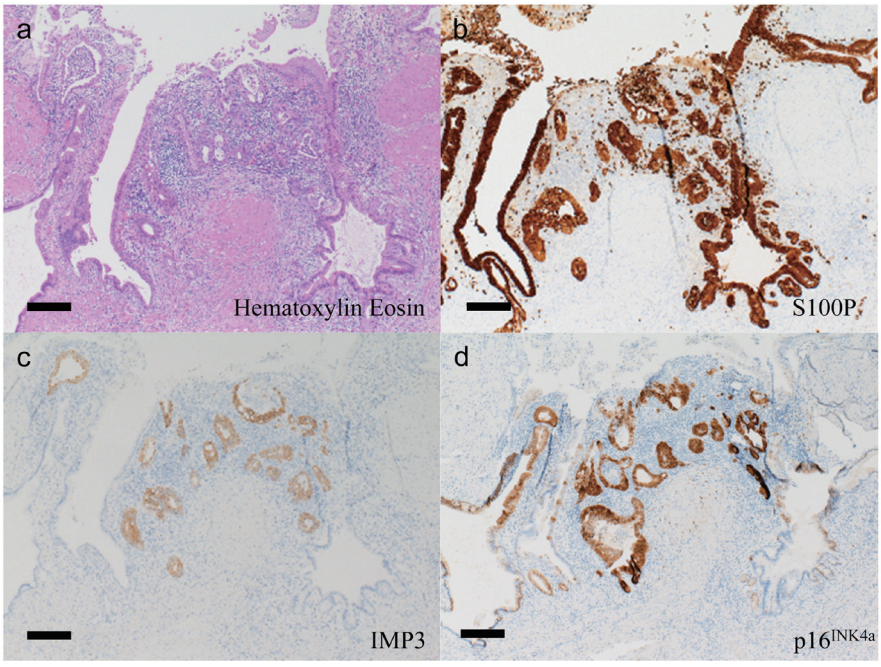

Fig. 5. Histopathological examination of gallbladder via: (**a**) hematoxylin and eosin; (**b**) S100P; (**c**) IMP3; (**d**) $p16^{INK4a}$. Bar = 250 μm.

without biliary dilatation, various pathological epithelial changes such as hyperplasia, dysplasia or carcinoma are frequently found in the gallbladder mucosa,[6] such that immunohistochemical examinations, in addition to hematoxylin and eosin staining, are desirable to accurately diagnose the pathological changes in the gallbladder mucosa. Therefore, we performed these immunohistochemical examinations using S100P and IMP3. S100P is generally expressed in several kinds of malignant tumors; furthermore, in the bile duct, S100P expression increases in a stepwise fashion from reactive epithelium to low-grade BilIN to high-grade BilIN and invasive carcinoma.[7] Bile duct carcinomas or high-grade dysplasias in the bile duct are typically strongly positive for IMP3, in contrast to the weak or lacking expression found in normal- and low-grade dysplastic bile duct epithelium.[8] Gallbladder adenocarcinoma typically demonstrates an immunostaining profile of IMP3+/S100P+. Therefore, the expression of S100P and IMP3 are useful in the distinction of adenocarcinoma or not in the gallbladder mucosa.[9] IMP3, an oncofetal protein involved in embryogenesis, is reported to promote tumor cell proliferation, adhesion, and invasion. The expression of IMP3 is detected in malignant tumors but is not found in adjacent benign tissues.[10] In this case, overexpression of S100P was detected in both the tumor cells and BilINs in the gallbladder mucosa. However, for IMP3, only the tumor cells were positive and the BilINs were negative, indicating that gallbladder carcinomas localized in the mucosa propria existed in the widespread BilIN lesions. The result of expression of these two makers in this case is similar to that of expression in the bile duct, which might indicate that carcinogenic prossess for gallbladder cancer associated with PBM. In addition to these, we performed immunohistochemical examinations for p53 and $p16^{INK4a}$, referring to known literature.[11, 12] Expression of p53 was negative, and $p16^{INK4a}$ was positive in both the carcinoma and BilIN lesions.

In oncogenic examinations, *KRAS* mutations were detected in the gallbladder epithelium of 60% of patients with PBM with biliary carcinoma, and in the gallbladder epithelium of 33.3% of patients with PBM without biliary carcinoma.[11] In this case, no *KRAS* mutations were detected in either tumor cells or BilINs. Although multiple gene mutations are reported to accumulate in various noncancerous lesions in the background biliary epithelium of patients with PBM, the mechanisms for carcinogenesis have not yet been fully elucidated.[6] Therefore, further research is required to clarify the mechanisms involved in gallbladder cancer through BilIN in patients with PBM.

We searched PubMed.gov for all cases up to July 2021 using the keywords “pancreaticobiliary maljunction”, “biliary intraepithelial neoplasia” and “gallbladder cancer or gallbladder carcinoma”. However, no case reports were found. Therefore, This case may help clarify the carcinogenesis and genetic mutations of early

gallbladder cancer with PBM.

In conclusion, we report herein a case of gallbladder cancer with BilIN, which was a precancerous lesion for BTC, complicated by PBM. This case may be useful in clarifying the carcinogenic process and genetic mutations for gallbladder cancer associated with PBM.

Ethics approval and consent to participate: Consent for publication was obtained from the patient.

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The authors declare no conflict of interest.

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