Introduction

Pancreaticobiliary maljunction (PBMj) is a congenital dysplasia in which the pancreatic duct and the bile duct join outside the duodenal wall\(^1\). In PBMj cases, the sphincteric action of the ampulla of Vater cannot reach the junction of the pancreatic duct and the bile duct, thereby causing a reflux of the pancreatic juice into the bile duct. The phenomenon persists, resulting in chronic inflammation in the bile duct epithelium, followed by an atypical change, which is known to lead to the development of biliary tract cancer at a high rate\(^2,3\). PBMj is characteristically associated with biliary tract cancer in a relatively large number cases; there have been previous reports in Japan as well on cases of synchronous or metachronous overlap of the two diseases\(^4-6\).

We recently encountered an extremely rare case in which a patient developed pre-malignant biliary neoplasms simultaneously in three sites: the gallbladder, extrahepatic bile duct, and ampulla of Vater, associated with PBMj. Herein, we report this case, as it will help understand the mechanism of the malignant transformation in PBMj and the multistep carcinogenesis of biliary tract cancer.

Case Report

The patient was a 60-year-old man who developed fever and intense right upper abdominal pain and was then urgently admitted to our hospital in 2011. Laboratory data indicated a severe inflammatory response and obstructive jaundice. The hepatitis B and C virus markers were negative; however, the carbohydrate antigen 19-9 (CA19-9) level was abnormally high at 159.9 U/mL. Abdominal computed tomography showed swelling of the gallbladder and thickening of its wall, with an extensive papillary mass in its lumen enhanced by a contrast medium (Fig. 1a). On the basis of these findings, the patient was diagnosed with a tumor obstructing the gallbladder, which led to concurrent acute cholecystitis; thus, percutaneous transhepatic gallbladder drainage (PTGBD) was performed. Endoscopic retrograde cholangiopancreatography (ERCP) showed multiple papillary tumors in the gallbladder lumen were noted, with a similar form of tumors in the proximal extrahepatic bile duct (Fig. 1b). Additionally, the extrahepatic bile duct and the pancreatic duct joined outside the wall of the duodenum; thus, PBMj was diagnosed (Fig. 1c). Although the ERCP findings had shown no abnormality in the ampulla of Vater, the bile amylase level was abnormally high at 1,333 IU/L, suggesting reflux of the pancreatic juice into the bile duct. Based on the abovementioned imaging findings, double neoplasms of gallbladder carcinoma and cholangiocarcinoma were diagnosed, and operation was performed in beginning of 2012 after obtaining a written informed consent from the patient. In laparotomy findings, no distant metastasis or peritoneal metastasis was found, the patient underwent cholecystectomy, including partial resection of segment 5 of the liver, subtotal stomach-preserving pancreaticoduodenectomy, and regional lymph node dissection. Macroscopically the resected specimen showed aggregation of multiple papillary tumors, with a size of 72 × 48 mm, extending from the neck to the base of the gallbladder (Fig. 2a). In the extrahepatic bile duct, papillary tumors sized 30 × 16 mm were noted (Fig. 2b);
however, the tumors were separated from those in the
gallbladder. The microscopic pathological findings indi-
cated that most of the papillary tumors in the gallblad-
der were adenomas but were partly carcinomas-in-situ;
further, the tumors with a similar atypia to that of car-
cinomas-in-situ had invaded the subserosal layer of the
gallbladder. Meanwhile, the bile duct lesions were mainly
adenomas with a low grade and no invasion, and were
diagnosed as carcinomas-in-situ. On the basis of these
histopathological findings, the tumors in the gallbladder
were staged as pT2 pN0 pM0 pStage II and those in the
bile duct, pTis pN0 pM0 pStage 0, according to the TNM
classification of the International Union Against Cancer,
with each tumor determined as having a curative degree
of R0. Using the World Health Organization (WHO)
classification, the gallbladder tumors were diagnosed as
intracystic (gallbladder) papillary neoplasms (ICPNs)
with an associated invasive carcinoma, as the tumors
showed invasion. The bile duct tumors were diagnosed as
intraductal (bile duct) papillary neoplasms (IPNBs) with
high-grade intraepithelial neoplasia, as the tumors con-
tained components with a slightly high grade, although
no findings of invasion were noted. In addition, further
histopathological exploration revealed carcinomas-in-
situ associated with an adenomatous component in the
ampulla of Vater. This tumor lesion was staged as pTis
pN0 pM0 pStage 0 using the TNM classification and as
biliary intraepithelial neoplasia, grade 3 (BilIN-3) using
the WHO classification. Therefore, as the three lesions
were separated from each other and were not associated
with vascular invasion, triple synchronous neoplasms
that occurred in the bile duct were diagnosed. On the

Fig. 1 Abdominal CT and Endoscopic Retrograde Cholangiopancreatography
Abdominal CT showed multiple papillary tumors in the gallbladder enhanced
by a contrast medium (arrow) (1a). Cholangiography indicated multiple papil-
lar tumors throughout the gallbladder (1b, white arrow). Additionally, elevat-
ed tumors are seen in the proximal extrahepatic bile duct (1b, black arrow).
Pancreatography revealed pancreaticobiliary maljunction where the extrahe-
opathy bile duct joined together outside the duodenal wall (1c, arrow head).
bosis of the immunopathological findings, the tumors in the gallbladder were positive for each of the following: mucin core protein (MUC)1, MUC5AC, and MUC6 (Figs. 3a-d). The tumors in the bile duct showed almost similar results to those in the gallbladder, except for the result for MUC1 (Figs. 4a-d). The tumors in the ampulla of Vater had negative findings for MUC1 and MUC6 and partially positive findings for MUC5AC (Figs. 5a-d). The immunopathological findings of the other factors are indicated in Table 1; the Ki-67 proliferation index (MIB-1) was 45% for the gallbladder, 40% for the bile duct, and 25% for the ampulla of Vater.

The postoperative course of the patient was uneventful, and he was discharged with no postoperative complications. It has been 6 years since the operation as of the time of this report; the patient has been placed under observation with no signs of recurrence. In addition, the high CA19-9 level before the operation rapidly decreased postoperatively, remaining within the normal range to date.

Discussion

Most biliary tract cancer cases develop without preceding occurrence of lesions; however, PBMj is known as a risk factor for biliary tract cancer\[^{7}\]. In the current study, the lesions were triple synchronous neoplasms that developed in association with PBMj, comprising ICPN of the gallbladder, IPNB of the extrahepatic bile duct, and BiIN of the ampulla of Vater. PBMj is already known to potentially cause multiple neoplasms in the bile duct[^4-6]. To our knowledge, this study is the first to report a case of pre-malignant biliary neoplasms developing simultaneously at three sites. ICPN/IPNB and BiIN are newly listed disease presentations in the 2010 revision of the WHO classification of tumors of the digestive system, as pre-malignant neoplasms in the multistep carcinogenesis of biliary tract cancer. Each of them corresponds to counterparts of intraductal papillary mucinous neoplasms (IPMN) of the pancreas and pancreatic intraepithelial neoplasia[^8,9]. In particular, BiIN was previously referred to as bile duct epithelial dysplasia, which was extremely difficult to diagnose preoperatively because the lesion is a pre-malignant or pre-invasive neoplasm that can be identified only via microscopic examination. In our study as well, the BiIN in the ampulla of Vater was accidentally found in the resected specimen of the bile duct tumors and was revealed to be present as a result of detailed histopathological exploration of the resected specimen. BiIN is categorized as BiIN-1 (low grade)/BiIN-2 (intermediate grade) and BiIN-3 (high grade) according to the degree of atypia[^8,9]. The tumors in the ampulla of Vater in our study were diagnosed as BiIN-3, as they were carcinomas-in-situ.
Fig. 3 Histopathological Findings of the Gallbladder
The tumors in the gallbladder were well-differentiated papillary adenocarcinomas based on the adenoma with a size of 72 × 48 mm (3a, H.E. × 100). Immunopathological findings showed that the tumors were all positive for MUC1, MUC5AC, and MUC6 (3b-d, × 40).

Fig. 4 Histopathological Findings of the Extrahepatic Bile Duct
The tumors in the bile duct were well-differentiated papillary adenocarcinomas sized 30 × 16 mm and were carcinomas-in-situ (4a, H.E.x 100). Immunopathological findings revealed that the tumors were negative for MUC1 and positive for MUC5AC and MUC6 (4b-d, × 40).
ICPN/IPNB and BilIN can be classified into four phenotypes (pancreatobiliary type, intestinal type, oncocytic type, and gastric type) according to the form of expression\(^{10, 11}\) as per the histological subtype classification based on immunohistological findings related to the prognosis or on the basis of mucin core protein expression\(^{13, 14}\). The characteristics of mucin core protein expression include a high rate of MUC5AC and low rates of MUC1 and MUC2\(^{13, 14}\). The characteristics of the ICPN and IPNB in our study are shown in Table 1, and they were definitively diagnosed as gastric types of ICPN/IPNB associated with a good prognosis based concurrently on the histopathological findings. The expressions shown in BilIN were MUC1 (-), MUC5AC (+), and MUC6 (-), which is almost in accordance with normal mucin expression. Immunopathological findings revealed that the tumors in our study were triple synchronous neoplasms; however, the other results suggest a good prognosis as compared with that in regular biliary tract cancers, i.e., early biliary tract cancer.

To determine the cause of multiple tumors developing in the bile duct, it is necessary to consider two backgrounds of the malignant potential of PBMj and the multicentric occurrence of ICPN/IPNB. In cases of PBMj, the pancreatic juice that flows backward into the bile duct is mixed with the bile, generating lysolecithin, which has...

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**Table 1 Immunostaining microscopic pathological findings**

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<th>Gallbladder</th>
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<td>CK20</td>
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<td>MIB-1</td>
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<td>β-catenin</td>
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**Fig. 5** Histopathological Findings of the Ampulla of Vater

The tumors in the ampulla of Vater were well-differentiated tubular adenocarcinomas sized 19 × 8 mm (5a, H.E. × 100). The tumors were positive for MUC1, partially positive for MUC5AC, and negative for MUC6 (5b-d, × 40).
a strong cytotoxicity. As a result, chronic inflammation associated with repeated impairment and repair of the mucosal epithelium of the bile duct occurs. This is followed by the unique process of multistep carcinogenesis through changes mainly due to hyperplasia as well as DNA mutation, i.e., hyperplasia-dysplasia-carcinoma sequence. In IPNB, as in its counterpart IPMN, it has been pointed out that each pre-malignant neoplasm associated with multicentricity could independently follow the process of the adenoma-carcinoma sequence and result in multiple occurrences. In the current study, the immunopathological findings showed no overexpression of p53, a tumor suppressor gene, in the gallbladder and bile duct tumors. However, the expression of the oncogene K-ras was found, suggesting that the hyperplasia-dysplasia-carcinoma sequence of PBMJ could have mainly been involved in the malignant transformation. Meanwhile, β-catenin was expressed in the gallbladder and bile duct tumors; it could not be ruled out that β-catenin might have excessively induced the expression of cell growth-related genes, such as cyclin D1 or human epidermal growth factor receptor 2 and cell-cycle Regulatory proteins, and further enhanced tumor formation. Given that the three tumors that were separated from each other showed different pathological findings, it seemed unlikely that the tumor in the bile duct or the ampulla of Vater metastasized from that in the gallbladder as a causative process. Triple synchronous neoplasms were eventually diagnosed.

As cases with PBMJ have diffuse hyperplastic changes in the gallbladder or bile duct epithelium already during childhood, it seems necessary to perform strict long-term follow-up observation taking the possibility of subsequent metachronous occurrences into consideration. Given that biliary tract cancer is still one of the intractable cancers, it is important how the pre-malignant biliary neoplasms of IPNB or BiIN are positioned. Accumulated knowledge on the early diagnosis and treatment of similar lesions can largely contribute to the treatment outcomes of biliary tract cancer.

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References