Clinicopathologic study on metachronous double cholangiocarcinomas of perihilar and subsequent distal bile duct origin

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Background. *Despite an increasing number of long-term survivors after the resection of perihilar cholangiocarcinoma, metachronous carcinoma in the remnant distal bile duct has not been well documented because of its rarity. The aim of this study was to clarify the feasibility of operative resection and the pathologic features for metachronous double cholangiocarcinomas.*
Methods. *Between 2003 and 2013, 6 patients underwent resections for both a primary perihilar cholangiocarcinoma and a metachronous distal cholangiocarcinoma. Their medical records were retrospectively reviewed.*
Results. *At a median of 42 months (range, 19–138 months) after the hepatectomy, a metachronous distal cholangiocarcinoma was detected by follow-up computed tomography and, interestingly, no symptoms were observed. Despite severe adhesions, a pancreatoduodenectomy was undertaken in all patients; there were no serious complications, and the procedure resulted in an R0 resection. Although 2 patients died of the disease after the second operation, the remaining 4 patients are now alive with* (n = *1) or without recurrence. A pathologic survey showed that 4 patients had changes of biliary intraepithelial neoplasia–2/3 around their primary and metachronous lesions. The primary and metachronous cholangiocarcinomas showed histologic similarity in 4 of the 6 patients and immunohistochemical concordance in 3 of the 6 patients.*
Conclusion. *Pancreatoduodenectomy for metachronous distal cholangiocarcinoma can lead to a favorable prognosis. Careful observation after the resection of perihilar cholangiocarcinoma is mandatory to detect this potentially curable disease. Pathologically, some of the multicentric cholangiocarcinomas present histologic and immunohistochemical similarities. (Surgery 2017;162:84-93.)*

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During hepatectomy for perihilar cholangiocarcinoma, the common bile duct is usually divided at the superior border of the head of the pancreas with preservation of the distal intrapancreatic bile duct. With an increasing number of long-term survivors after resection,[1] the risk for developing metachronous carcinoma in the remnant distal bile duct has been noted recently.[2] Resected primary perihilar and subsequent distal cholangiocarcinomas are rare; to our knowledge, only 8 such cases have been reported.[3-5] A few studies have mentioned the histologic similarity of multicentric lesions in the biliary tract,[2,5] but more detailed immunohistochemical and molecular analyses have not been conducted.

Multistep carcinogenesis of cholangiocarcinoma has been well highlighted by Nakanuma

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et al.[6] Two types of precancerous lesions, biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the bile duct, may precede the development of many invasive cholangiocarcinomas. Meanwhile, Slaughter et al[7] first proposed the concept of "field cancerization" to explain the multicentric development of head and neck cancer. They proposed that exposure to carcinogens causes precancerous field changes which can progress to invasive cancers with multicentricity.[8-11] This process of field cancerization appears to be consistent with the multistep hypothesis of carcinogenesis of cholangiocarcinoma.[12] This issue of field cancerization of the bile duct has been discussed only rarely because of the rare occurrence of metachronous carcinoma.

The aim of the present study was to review our experiences with metachronous distal cholangiocarcinoma after resection of the primary perihilar cholangiocarcinoma, to evaluate the feasibility of operative treatment of the metachronous cholangiocarcinoma, and to clarify the pathologic features of primary and metachronous lesions. This study is the first report to present a detailed pathologic evaluation of this issue.

PATIENTS AND METHODS

Patients. Between January 2003 and December 2013, 480 patients with perihilar cholangiocarcinoma underwent resection with curative intent at the First Department of Surgery, Nagoya University Hospital, of whom 62 underwent combined hepatectomy and pancreatoduodenectomy (hepatopancreatoduodenectomy)[13]; these patients were excluded because of concomitant resection of the distal bile duct. Of the remaining 418 patients, 6 (1.4%) developed metachronous cholangiocarcinoma in the remnant distal bile duct. Five patients underwent resection for the metachronous disease at Nagoya University Hospital, and the remaining patient (Case 4) did so at Kyoto University Hospital. The medical records of these 6 patients were reviewed retrospectively, and a detailed pathologic evaluation was conducted, including immunohistochemical and genetic analyses of the resected specimen.

During the same time period, no patients who had undergone resections of a primary distal cholangiocarcinoma developed perihilar or intrahepatic cholangiocarcinoma. Of the 480 resected patients with perihilar cholangiocarcinoma, only 1 patient had a synchronous distal cholangiocarcinoma and underwent hepatopancreatoduodenectomy. This study was approved by the Human Research Review Committee of Nagoya University Hospital.

Pathologic assessment. The extrahepatic bile duct of the resected specimen was opened longitudinally, fixed in 10% formalin for several days, and sectioned serially at 5-mm intervals. The specimens were prepared for microscopic examination using hematoxylin and eosin (HE) staining. Pathologic findings of both the primary and metachronous tumors were described according to the TNM classification of malignant tumors by the International Union Against Cancer (7th edition, 2009).[14] All histologic slides were re-evaluated by 2 pathologists (K. S. and Y. S.). In the rare instances of discrepancy, the cases were discussed at a multiheaded microscope to achieve consensus.

The extents of the invasive tumor and the superficial spreading lesion (carcinoma in situ)[15,16] were determined by examining multiple sections of the entire lesion. The distance of the distal margin of the primary resection was measured and defined as the distance from the lower border of the primary lesion to the distal cut stump of the extrahepatic bile duct. Similarly, the distance of the proximal edge of the resection of the metachronous distal cholangiocarcinoma was measured and defined as the distance from the superior border of the metachronous lesion to the proximal edge of the remnant distal bile duct.

The status of the distal ductal margin in the primary operation and the proximal ductal margin status in the second operation were examined and classified as nondysplastic epithelium, BilIN-1, BilIN-2, BilIN-3, or invasive carcinoma based on the criteria of an international interobserver agreement study.[17] The proximal biliary ductal margins in the second operations were totally resected, because the margin was intrapancreatic; thus, an R0 resection was performed even when there was a positive proximal edge.

To evaluate the distribution of high-grade dysplasia in the surrounding bile duct epithelia, the number of histologic slides containing BilIN-2 or BilIN-3 was counted. If one slide contained different degrees of BilINs, the highest-grade dysplastic lesion was used. The total number of histologic slides in each case was also counted, excluding slides that had no epithelium of the bile duct or those that had only an invasive lesion.

Immunohistochemistry. A representative block, including the main tumor in each case, was subjected to immunohistochemical staining to compare the molecular status at the protein level between paired primary and metachronous cancers. Formalin-fixed paraffin sections were analyzed using immunoperoxidase staining via an

Table I. Clinical features of 6 study patients

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*
Age (y)*/Sex	74/male	47/male	49/male	63/male	68/male	57/male
Primary hilar cholangiocarcinoma						
CA19-9 (U/mL)	65	129	80	115	956	117
CEA (ng/mL)	1.9	1.5	2.5	2.8	1.4	2.0
Bismuth type	IV	IV	III	II	IV	IV
Type of operation†	S1,2,3,4	S1,2,3,4+HA+PV	S1,2,3,4	S1,5,6,7,8	S1,2,3,4,5,8+HA	S1,2,3,4+PV
Operative time (min)	635	645	679	488	584	680
Blood loss (mL)	1,384	1,754	1,375	933	1,476	1,073
Adjuvant therapy	No	Gem + Radiation	No	Gem	Gem	No
Interval (months)‡	138	55	48	19	28	36
Second distal bile duct neoplasm						
CA19-9 (U/mL)	33	10	6	85	20	14
CEA (ng/mL)	1.4	1.1	2.0	3.9	2.6	1.7
Type of resection	SSPPD	SSPPD	SSPPD	PPPD	SSPPD	SSPPD
Operative time (min)	343	588	686	504	295	471
Blood loss (mL)	668	2,055	1,340	660	204	856
Follow-up (mo)§	17, alive (without recurrence)	37, dead (liver + local recurrence)	38, alive (without recurrence)	16, dead (bone and kidney metastasis)	20, alive (lymph node recurrence)	4, alive (without recurrence)

*At operation for primary lesion.
†Expressed as Couinaud's hepatic segments resected.
‡Time to metachronous lesion.
§After resection for metachronous lesion.
CA 19-9, Carbohydrate antigen; *CEA*, carcinoembryonic antigen; *Gem*, gemcitabine; *HA*, hepatic artery resection; *PPPD*, pylorus preserving pancreatoduodenectomy; *PV*, portal vein resection; *SSPPD*, subtotal stomach preserving pancreatoduodenectomy.

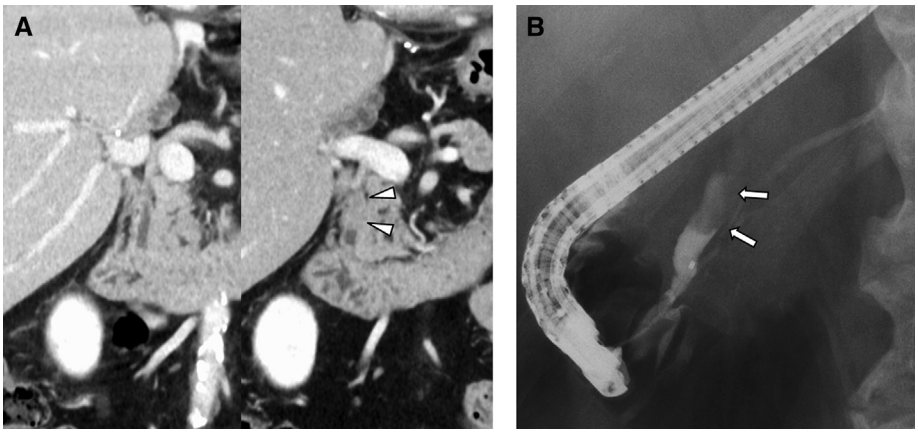

Fig 1. Preoperative images of the metachronous cholangiocarcinoma in case 6. (*A*) Coronal computed tomography in the portal phase (*right side*) indicates a slightly hypoattenuated mass (*arrowhead*) newly emerging in the remnant distal bile duct compared with the image obtained 25 months earlier (*left side*). (*B*) Endoscopic retrograde cholangiography shows a complete obstruction of the upper part of the distal bile duct (*arrow*).

avidin-biotin-peroxidase complex method. The following monoclonal antibodies were used: MUC1, MUC2, MUC5AC, MUC6, HGM (Novocastra Laboratories, Newcastle, UK), CDX2 (Bio Genex, San Romon, CA), and p53 (DAKO, Glostrup, Denmark). The immunostaining was scored semiquantitatively according to the percentage of positive cells in each lesion: score 0, 0%; score 1, 1% to 10%; score 2, 11% to 50%; and score 3, >51%.

Analysis of mutation in KRAS and NRAS. The mutational analysis of the KRAS and NRAS genes was performed by extracting genomic DNA from formalin-fixed and paraffin-embedded slides of the tumor tissue in each case. For the reverse sequence-specific oligonucleotide method with polymerase chain reaction, KRAS and NRAS mutations in codons 12, 13, 59, 61, 117, and 146 were detected using a RASKET KIT (Medical and Biological Laboratories, Nagoya, Japan) according to the manufacturer's instructions. This assay detects the G12S, G12C, G12R, G12D, G12V, G12A, G13S, G13C, G13R, G13D, G13V, G13A, and A59T, A59G, Q61K, Q61E, Q61L, Q61P, Q61R, Q61Ht, Q61Hc, K117Nc, K117Nt, A146T, A146P, and A146V mutations in KRAS and NRAS. UniMAG (MBL, Nagoya, Japan) was used to analyze raw data from Luminex 100/200 (Luminex, Austin, TX).

RESULTS

Clinical course. The clinical features of the 6 study patients are shown in Table I. All were men, and their age at the time of resection of the primary perihilar cholangiocarcinoma ranged from 47 to 74 years. No patients had known risk factors for cholangiocarcinoma, such as primary sclerosing cholangitis, hepatolithiasis, a pancreaticobiliary maljunction, or liver fluke infection.

The six patients had undergone a major hepatectomy with en bloc resection of the caudate lobe and extrahepatic bile duct for perihilar cholangiocarcinoma, with (n = 3) or without vascular resection. Bilio-enteric continuity was established by Roux-en Y hepaticojejunostomy. The jejunal limb was brought up to the hepatic ducts via retrocolic-retrogastric route.[18] All patients were discharged from the hospital in good health. Three patients underwent adjuvant therapy after resection of the hilar cholangiocarcinoma: case 2 received adjuvant chemoradiation therapy due to positive proximal ductal and radial margins, and cases 4 and 5 received adjuvant chemotherapy due to lymph node metastases.

At a median of 42 months (range, 19 to 138 months) after resection of the hilar cholangiocarcinoma, the metachronous distal cholangiocarcinoma was detected by follow-up computed tomography (Fig 1, *A*); none of these patients had any recognizable symptoms. Endoscopic retrograde cholangiography was performed (Fig 1, *B*), and histologic confirmation was made by transpapillary forceps biopsy, except in one patient (case 1).

Because of the diagnosis of metachronous distal cholangiocarcinoma, all patients underwent subtotal stomach- or pylorus-preserving pancreatoduodenectomy (PD). Despite severe adhesions, the PDs were performed without serious problems. Although a postoperative grade B pancreatic fistula occurred in 3 patients, all patients were discharged from the hospital in good health. Two patients died of the disease 37 and 16 months after

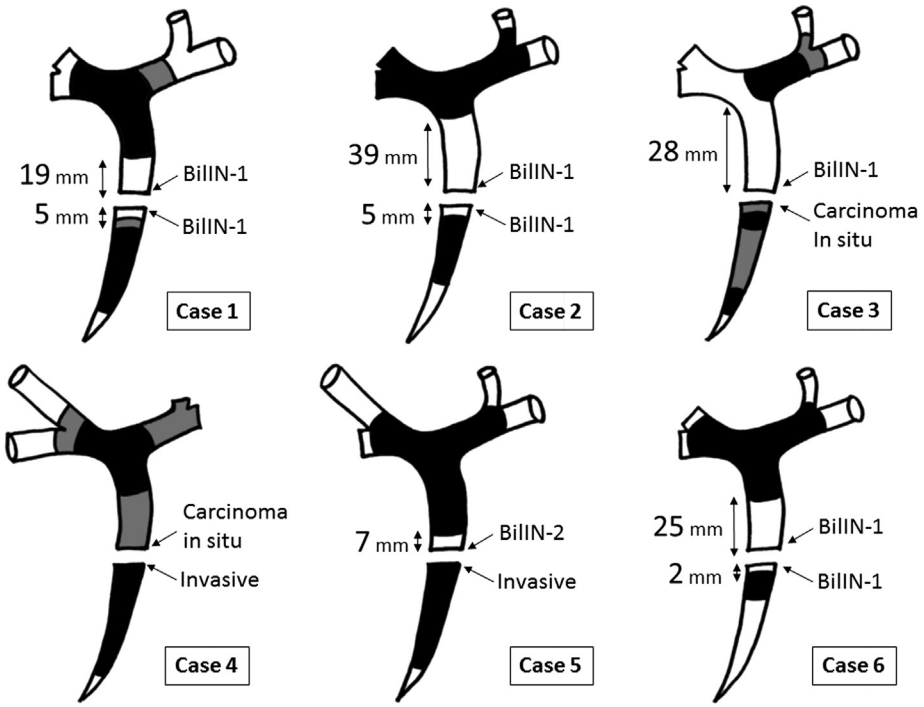

Fig 2. Anatomic distributions of paired primary and metachronous cholangiocarcinomas in 6 study patients. Black areas represent invasive carcinoma, and gray areas indicate superficial spreading, potentially premalignant lesions. Distal margin distance and status in the primary operation are recorded in each case. Similarly, the proximal edge distance and status in the second operation are recorded.

Table II. Pathologic features of 6 study cases

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*
Primary hilar cholangiocarcinoma						
Gross type	infiltrating	infiltrating	papillary	infiltrating	papillary	nodular
Histologic type	wel	mod	pap	mod	pap	mod
Mucin secretion	+	-	+	+	-	-
Stage (pTN)*	II (T2aN0)	IVA (T4N0)	I (T1N0)	IIIB (T2aN1)	IVA (T4N1)	IVA (T4N0)
R status	0	1	0	1	0	0
Total number of slides†	10	11	9	4	7	14
Slides with BilIN2/3 (%)	7 (70%)	5 (45%)	9 (100%)	4 (100%)	5 (71%)	6 (43%)
Second distal bile duct neoplasm						
Gross type	nodular	infiltrating	papillary	nodular	papillary	papillary
Histologic type	wel	wel	pap	wel	pap	pap
Mucin secretion	+	-	+	-	-	-
Stage (pTN)*	IIA (T3N0)	IVA (T4N0)	0 (T0N0)	IA (T1N0)	IIA (T3N0)	IA (T1N0)
R status	0	0	0	0	0	0
Total number of slides†	4	5	14	2	7	5
Slides with BilIN2/3 (%)	2 (50%)	0 (0%)	14 (100%)	2 (100%)	4 (57%)	0 (0%)

*According to the American Joint Committee on Cancer seventh edition.
†Excluding slides with no biliary epithelium or only invasive lesions.
mod, Moderately differentiated tubular adenocarcinoma; *pap*, papillary adenocarcinoma; *wel*, well-differentiated tubular adenocarcinoma.

the PD. The remaining 4 patients are now alive with (n = 1) or without recurrence.

Anatomic distribution of the paired carcinomas. The anatomic distributions of the paired primary and metachronous carcinomas are depicted in Fig 2. The paired lesions in 5 patients (cases 1–3, 5, and 6) were confirmed to be located separately with a distance of 7 to 44 mm. In the remaining

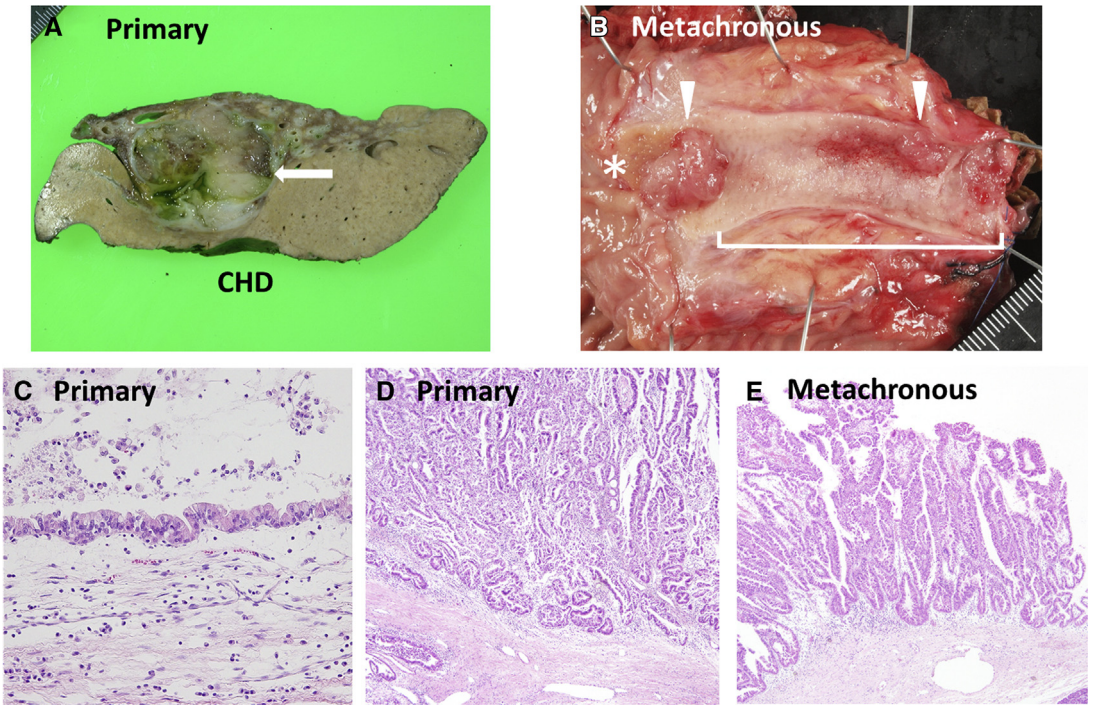

Fig 3. Resected specimens of case 3. (*A*) Macroscopically, the primary tumor proliferates in the intrahepatic duct with an intraductal growth pattern (*arrow*). (*B*) The metachronous tumor forms papillary nodules (*arrow head*) around the proximal edge of the distal bile duct and the ampulla of Vater (*asterisk*) with superficial spreading lesion (*solid line*). (*C*) Biliary epithelia surrounding the primary tumor have dysplastic changes corresponding to BilIN-2 (HE stain, ×200). (*D*) (*E*) Histologically, both the primary and metachronous lesions reveal gastric-type papillary adenocarcinomas (HE stain, ×40). *CBD*, Common bile duct. (Color version of this figure is available online.)

patient (case 4), the distal ductal margin at the time of resection of the hilar cholangiocarcinoma was positive with carcinoma in situ, and the proximal ductal edge at the time of PD 19 months later was involved in invasive carcinoma.

BilIN distribution in the surrounding bile duct. All HE sections of 12 total lesions (6 primary and 6 metachronous lesions) were reviewed to evaluate whether they contained changes of BilIN-2 or BilIN-3 around the main tumors (Table II). Ten had dysplastic areas, but the metachronous lesions of cases 2 and 6 had no detectable dysplasia. For the semiquantitative analyses of high-grade BilIN distribution, the proportions of the number of slides having BilIN-2/3 were calculated. Four patients had more than 50% of their HE slides showing BilIN-2/3 in both their primary and metachronous lesions (Fig 3, *C*).

Histologic types. The paired lesions in 4 patients showed quite similar histologic characteristics (Table II): mucin-producing, well-differentiated tubular adenocarcinomas in case 1, tubular adenocarcinomas with predominantly scirrhous components in case 2, gastric-type papillary adenocarcinomas in case 3 (Fig 3), and intestinal-type papillary adenocarcinomas in case 5. In contrast, the paired lesions of the remaining 2 patients revealed different histologic appearances. Case 4 had tubular adenocarcinoma with mucin secretion in the primary lesion but no obvious mucin secretion in the metachronous one. The primary lesion in case 6 was a tubular adenocarcinoma, whereas the metachronous lesion showed a pancreatobiliary-type papillary adenocarcinoma.

Immunophenotypes. The immunohistochemical expression patterns of mucin core proteins and p53 in the paired primary and metachronous lesions are summarized in Table III. The paired lesions in cases 1–3 showed similar immunophenotypes; those in cases 1 and 3 had a concordant scoring pattern in 6 of the 7 immunohistochemical markers, and those in case 2 did in 5 of the 7 markers. Meanwhile, the paired lesions in the remaining 3 patients (cases 4–6) represented predominantly distinct immunophenotypes: those in case 4 had a concordant scoring pattern in only 3 of the 7 markers compared to 1 of the 7 markers in cases 5 and 6.

Table III. Immunohistochemical scoring patterns and molecular analyses of 6 study patients

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*
MUC1	1/1*	1/1	0/0	3/3	1/0↓	3/1↓
MUC2	0/0	0/0	0/0	0/0	2/1↓	1/1
MUC5AC	3/3	1/0↓	3/3	3/1↓	2/1↓	2/1↓
MUC6	1/1	0/0	3/3	1/3↑	1/0↓	0/0
HGM	3/3	1/0↓	3/3	3/1↓	2/1↓	3/0↓
CDX2	0/0	1/1	1/3↑	1/0↓	1/3↑	0/3↑
P53	0/1↑	0/0	1/1	1/1	3/3	0/1↑
KRAS	wt/wt	wt/G12A	wt/wt	wt/wt	wt/wt	G12A/G12A
NRAS	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt

*Primary hilar cholangiocarcinoma/second distal bile duct neoplasm.
"↑" means an increase in the score of the metachronous lesion compared with the matched primary lesion, and "↓" means a decrease.
wt, Wild type.

Molecular studies. KRAS mutation was observed in 3 of the 12 lesions. In case 2, the metachronous lesion had a G12A KRAS mutation, whereas the primary lesion had no KRAS mutation. In case 6, both of the paired lesions had G12A KRAS mutations. No NRAS mutations were found in the paired lesions of all 6 patients.

DISCUSSION

The current study has demonstrated that PD for metachronous distal cholangiocarcinoma after resection of a hilar cholangiocarcinoma is feasible and can offer a better chance of long-term survival, although this procedure is technically demanding due to the anticipated adhesions. In our series, 2 patients survived for more than 3 years after PD. Importantly, the preoperative diagnosis of all of the distal tumors was metachronous cancer (second primary cancer), not recurrence. In one patient, the distal margin was positive with carcinoma in situ at the time of resection of the hilar cholangiocarcinoma; however, preoperative computed tomography before the second operation showed that the center of the metachronous distal lesion was separate from the distal margin of the previous resection, indicating a second primary cancer. That is why we aggressively performed a curative PD. Kim et al[5] similarly reported 6 patients who underwent PD for metachronous distal cholangiocarcinoma after the resection of hilar cholangiocarcinoma. In their series, PD was performed without mortality, and one patient survived for more than 3 years after PD. Considering the ineffectiveness of chemotherapy for cholangiocarcinoma,[19] repeat PD for metachronous distal cholangiocarcinoma appears to be a rational therapeutic option, although this is still less evident due to a paucity of literature on this topic.

Recurrence was observed in 3 patients (case 2, 4, and 5). Given the facts that resection of the hilar cholangiocarcinoma with a hepatectomy in cases 2 and 4 resulted in an R1 resection and that lymph node metastases were observed in the primary perihilar cholangiocarcinomas in cases 4 and 5, these recurrences were likely to derive from the primary lesions. Although R1 resection and N1 status are known as negative prognostic factors in perihilar cholangiocarcinoma, patients with these factors should not be precluded from a potentially curative PD for metachronous disease, because long-term survival is possible even in these patients.[1]

Meanwhile, the metachronous lesion of case 3 was histologically carcinoma in situ. It was difficult to diagnose the obvious nodule preoperatively as being noninvasive (Fig 3, *B*). In addition, a recent study has revealed that residual carcinoma in situ had a negative survival effect in early stage cholangiocarcinoma[20]; therefore, resection for noninvasive metachronous disease certainly appears to be justified.

Of note, all of our patients were asymptomatic at the time of diagnosis of the metachronous distal cholangiocarcinomas. An increased serum level of CA19-9 was observed only in one patient (case 4), and this parameter did not appear to be useful for detecting the metachronous disease. Jaundice is the most common symptom of cholangiocarcinoma, but it is not caused by metachronous disease in the remnant distal bile duct because there is no longer any bile flow due to the previous hepatectomy with biliary reconstruction.

In this respect, routine surveillance using computed tomography is most useful for detecting this potentially curable disease. Magnetic resonance cholangiopancreatography (MRCP) may be useful; however, we cannot comment on its

clinical value, because we did not use MRCP in this series. We used endoscopic retrograde cholangiography for histologic confirmation, but this invasive procedure is unsuitable for surveillance.

Although the incidence of metachronous distal disease is rare (1.4%), attention should be paid to the remnant intrapancreatic bile duct in follow-up surveillance using computed tomography. Meanwhile, metachronous lesions developed within 5 years after hepatectomy, except in one patient (case 1). Intervals to metachronous disease in the previously reported cases ranged from 3.4 to 37 months.[3-5] Although metachronous lesion may occur any time after hepatectomy, these results suggest that surveillance over 5 years may not necessarily be mandatory.

The criteria for multiple primary malignant neoplasms, first proposed in 1932 by Warren and Gates,[21] are still generally accepted: (1) each neoplasm must present a definite picture of malignancy; (2) each must be distinct; and (3) the probability that one is a metastasis of the other must be excluded. Although the paired lesions of cases 1–3 showed histologic and immunohistochemical similarities, the obvious distances between them indicate that the neoplasms in the remnant distal bile ducts were second primary lesions. Meanwhile, the paired lesions of case 4 were anatomically adjacent to one another; however, the discrepancy between the histologic types and immunohistochemical expression patterns suggests that the second distal lesion is a new primary neoplasm rather than a local recurrence.

The present study provided additional support for the assertion that multicentric neoplastic lesions of the bile duct are highly associated with a varying degree of BilIN in their carcinogenesis. Indeed, the background epithelia in 4 of our 6 patients were accompanied by changes of BilIN-2/3. Kobayashi et al[2] indicated a similar predisposition, because 9 of their 10 patients developed multicentric (metachronous or synchronous) biliary tract carcinoma probably related to superficial spreading lesions with extensive dysplasia in their biliary tract. The development of an expanding precancerous field, that is, BilIN, is speculated to be a critical step in the multicentric carcinogenesis of the biliary tract.

There were no patients with metachronous intrahepatic cholangiocarcinoma after resection of these perihilar cholangiocarcinomas. Although this may have been simply by chance, one possible reason is that we could not differentiate between intrahepatic multicentric lesions, if any, and metastases. Another potential reason is presence or absence of bile flow. After resection of a perihilar cholangiocarcinoma, bile flow is maintained in the intrahepatic biliary trees, while it is deprived in the intrapancreatic bile duct. In addition, pancreatic or duodenal juice may have promoted carcinogenesis in the absence of biliary flow, but due to the limited number of patients, further study is needed to reach any definitive conclusion.

In our series, 2 of the 6 primary perihilar lesions and 3 of the metachronous distal ones were papillary adenocarcinoma. Our previous studies reported that papillary neoplasms accounted for 11% of perihilar cholangiocarcinomas[22] and 13% of distal cholangiocarcinomas.[23] Although the number of our cases was small, papillary cholangiocarcinoma could develop more frequently in a multicentric setting, as described in other studies.[2,5] This predisposition may be explained by the fact that papillary cholangiocarcinoma is often accompanied by superficial spreading premalignant lesions (ie, BilIN-3).[15] Careful follow-up is, therefore, justified after the resection of papillary perihilar cholangiocarcinoma.

Another notable finding was that our study patients were all men. The sex difference, however, was not statistically significant compared to our overall 418 patients (data not shown), and the previously reported 8 cases included 3 women.[3-5] Although the male predominance in multicentricity of biliary tract carcinoma is not proven at present and might have happened by chance, the current results should be noted for further studies on multicentric cholangiocarcinoma.

Three paired carcinomas (cases 1–3) showed histologic similarity and major concordance in the immunohistochemical expression pattern. Their paired lesions, however, were anatomically separate from each other with a distance ranging from 24 to 44 mm, which is considered only one piece of evidence for our preferred diagnosis of a second primary cancer and not a local recurrence. Two other studies documented histologic similarities in paired multicentric carcinomas of the biliary tract.[2,5]

In our series, the paired carcinomas of the biliary tract were characterized consistently by histologic and immunohistochemical similarities, which suggests that multicentric cholangiocarcinomas do not arise as an isolated cellular phenomenon but instead tend to develop from a genetically related, single precancerous field defect. Indeed, this phenotypic similarity has been reported in other organ systems, such as lung cancers or intraductal papillary mucinous

neoplasms of the pancreas, the carcinogenesis of which is considered to be associated with field cancerization.[11,24,25] Our present data may lead to the hypothesis that field cancerization plays an important role in the pathogenesis of multicentric cholangiocarcinomas.

In contrast, the paired lesions of cases 4–6 showed discordance in the immunohistochemical expression pattern. Although epigenetic changes are not being excluded as a plausible explanation for the phenotypic variations in those paired lesions, these lesions were more likely to arise from independent fields representing distinct local genetic alterations. Further genetic and epigenetic analyses are needed to clarify this issue.

Our current molecular analysis showed that the paired carcinomas in case 2 expressed distinct KRAS mutation patterns, whereas the paired lesions of the remaining 5 patients showed identical mutation patterns in KRAS and NRAS. Although there is still considerable debate concerning the clonality of the multiple primary tumors in field cancerization (ie, polyclonality and monoclonality theory),[9] concordance in only 2 types of genetic mutation patterns would not lead to evidence of monoclonality. Further, it is noteworthy that one patient (case 2) in our study presented with clonally independent, multicentric lesions based on the discrepancy of KRAS mutation beyond the histologic and immunohistochemical similarity between the lesions. It may be valuable to further explore a rare case such as this one.

In conclusion, PD for metachronous distal cholangiocarcinoma can lead to long-term survival in selected patients. Careful surveillance after resection of perihilar cholangiocarcinoma is mandatory to detect this potentially curable disease of the remnant distal bile duct. As noted for other organ systems in which field cancerization has been described, the present study shows that some of the multicentric cholangiocarcinomas present histologic and immunohistochemical similarities.

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