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Original

Clinicopathological Study of Intracholecystic Papillary-Tubular Neoplasms (ICPNs) of the Gallbladder

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Abstract: Intracholecystic papillary-tubular neoplasm (ICPN) has recently been proposed as a new disease concept in the classification of gallbladder tumors. ICPN is defined as a papillary or polypoid glandular neoplasm forming a localized, non-invasive mass (≥ 1 cm) in the gallbladder. We analyzed the clinicopathological characteristics of ICPN. Resected gallbladder cancer specimens from 57 patients were classified as ICPN or non-ICPN and clinicopathological characteristics were compared. ICPN cell characteristics were also analyzed using immunostaining and genetic analysis. Twenty-three cases were classified as ICPN and 34 as non-ICPN. In the ICPN and non-ICPN groups, mean ages were 69 and 74 years, male:female ratios were 14:9 and 15:19, mean tumor diameters were 2.8 and 2.6 cm, invasion depths were Tis + T1/T2 + T3 in 14/9 cases and 13/21 cases, lymph node metastases were present in 6% and 43%, distant metastases in 0% and 6% and 3-year survival rates were 91% and 52%, respectively. Significant intergroup differences were seen in lymph node metastases and the 3-year survival rate. ICPN cell lineage was biliary-type in 13 cases, gastric-type in 8 and intestinal-type in 2. This proportion differs from that of pancreatic intraductal papillary mucinous neoplasm (IPMN), in which gastric- and intestinal-type are more common. *KRAS* gene mutations were only seen in 1 of 13 ICPN cases. ICPN is frequently seen in gallbladder cancer, showing similar pathology to pancreatic IPMN, which is considered to have a relatively good prognosis among pancreatic cancers. However, ICPN cell characteristics are not necessarily identical to those of pancreatic IPMN.

Key words: intracholecystic papillary-tubular neoplasm (ICPN), gallbladder, clinicopathological characteristics, cell characteristics, pancreatic intraductal papillary mucinous neoplasm (IPMN)

Introduction

Advances in clinical imaging have led to an increased detection of early-stage gallbladder cancer, but advanced cancer still accounts for many cases. Although tumor depth and staging are important factors influencing treatment approach and prognosis, the relationship between malignancy and the diverse morphologic features of gallbladder cancer is also worth

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investigating[1,2].

Adsay *et al*[1] recently proposed intracholecystic papillary-tubular neoplasm (ICPN) as a new disease concept in the classification of gallbladder tumors. ICPN is termed as an intracystic papillary neoplasm in the 4th edition of the World Health Organization Classification of Tumors of the Digestive System published in 2010[3]. ICPN resembles pancreatic intraductal papillary mucinous neoplasm (IPMN), which commonly develops in the duct of pancreatobiliary system[4,5], as well as in intraductal papillary neoplasms of the bile duct[6] and intra-ampullary papillary-tubular neoplasms[7]. ICPN is primarily defined as "a papillary or polypoid glandular neoplasm forming a localized, non-invasive mass ($\geq$ 1 cm) in the gallbladder"[1,3]. ICPN could provide a useful concept for decisions on treatment strategies for gallbladder cancer if the pathological features resemble those of pancreatic IPMN, which is considered a relatively indolent form of pancreatic cancer.

We compared the clinicopathological features of ICPN and non-ICPN cases using specimens from resected gallbladder cancers to determine whether ICPN can be considered the gallbladder counterpart of pancreatic IPMN. We also analyzed ICPN cell characteristics and compared these with pancreatic IPMN.

Subjects and Methods

The study included 57 patients with gallbladder cancers which were surgically resected at the authors' hospitals (Showa University Hospital and Showa University Fujigaoka Hospital) between 1998 and 2012. All patients gave their informed consent and personal information was hidden during the investigation of the medical records.

In accordance with the proposal by Adsay *et al*[1], tumors were considered to be ICPN if all three of the following components were present: (1) a non-invasive glandular neoplasm growing in the gallbladder mucosa; (2) formation of localized papillary or polypoid protruded lesions clearly distinguishable from the surrounding mucosa (Fig. 1); and (3) diameter of at least 1 cm. Cases showing all three components were included in the ICPN group, and all other cases were included in the non-ICPN group. As with the criteria for intraductal papillary neoplasms of the bile duct and intra-ampullary papillary-tubular neoplasms, the presence of excess mucus production was not considered relevant.

Clinicopathological analysis

We compared and analyzed the mean age, male:female ratio, mean tumor diameter, invasion depth, lymph node metastasis-positive rate, distant metastasis-positive rate and postoperative prognosis of the ICPN and non-ICPN groups. During the postoperative observation period, which ranged from 45 days to 4079 days, 6 patients died (ICPN group, n = 5; non-ICPN group, n = 1). All 6 deaths were clearly attributable to another disease (myocardial infarction, pneumonia, etc.), and were therefore excluded from the analysis of prognosis. The statistical tests used were the t-test, the chi-square test and the log-rank test for the Kaplan-Meier survival curve. Values of $P < 0.05$ were considered significant.

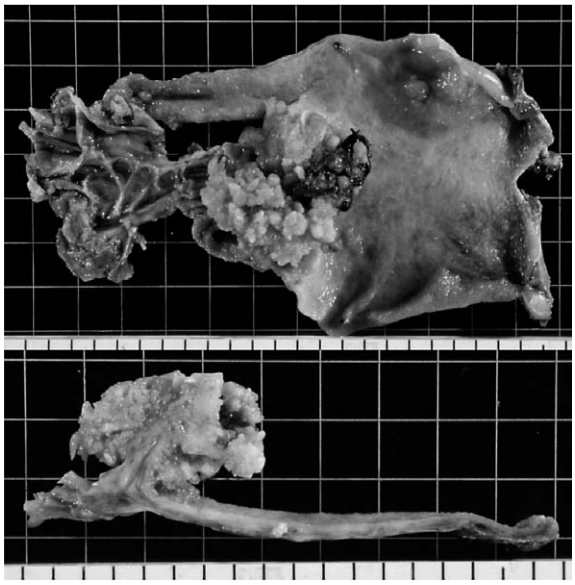

Fig. 1. Macroscopic features of intracholecystic papillary-tubular neoplasm (ICPN)
A papillary protruded mass larger than 1 cm are visible in the gallbladder. The tumor meets the ICPN concept when histologically confirmed as being formed by non-invasive proliferation of glandular neoplasm.

Analysis of ICPN cell characteristics

Cell types in the ICPN group were identified using histopathological and immunohistochemical methods.

1. Histomorphological analysis

All ICPN cases were histomorphologically classified as biliary, intestinal or gastric through observation of hematoxylin and eosin-stained specimens using light microscopy. Biliary-type cells are cuboidal or short-columnar epithelial cells with oval nuclei and poor mucinous cytoplasm, resembling proper biliary tract epithelium (Fig. 2a), while intestinal-type cells resemble those of colon adenoma or colon carcinoma, and are tall-columnar epithelial cells with spindle, pseudostratified nuclei and eosinophilic or dark cytoplasm (Fig. 2b). The presence of goblet cells and Paneth cells is also considered indicative of intestinal lineage. Gastric-type cells consist of tall-columnar epithelium with mucinous cytoplasm resembling foveolar epithelium, and/or small-tubular epithelium resembling pyloric glands (Fig. 2c). If these characteristics were seen to overlap, cell characteristics were determined by agreement between the observers (MI, NO, and TT), taking into account the dominant features and degree of atypism.

2. Immunohistochemical analysis

All specimens from the ICPN group underwent immunohistochemical staining. Thin sections (3 μm) were prepared for immunostaining using one or two formalin-fixed, paraffin-embedded tissue sections including the center of the tumor from each patient. Staining was performed using the avidin-biotin complex detection method with a BenchMark automated immunostainer

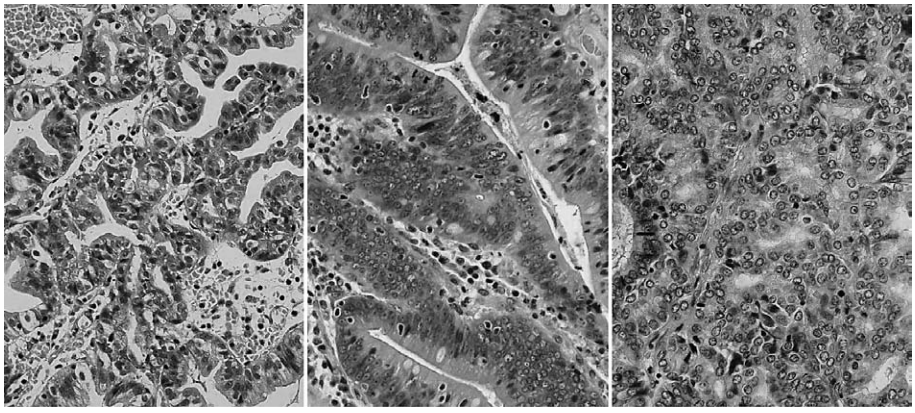

Fig. 2. ICPN cell morphology

a : Biliary-type : Characterized by cuboidal or short-columnar epithelium resembling proper biliary tract epithelium.
The patient of the figure is a 74-year-old female with subserosal invasive, 6 cm-papillary tumor.

b : Intestinal-type : Characterized by tall-columnar epithelium with spindle and pseudostratified nuclei.
The patient of the figure is a 87-year-old female with subserosal invasive, 5 cm-tubulo-papillary tumor.

c : Gastric-type : Characterized by tall-columnar epithelium resembling foveolar epithelium, and acinar or small-tubular epithelium resembling pyloric glands. The photograph mainly shows the latter component. The patient of the figure is a 68-year-old female with non-invasive, 2 cm-polypoid tumor.

(Ventana Medical Systems, Inc, Tucson, AZ). The primary antibodies used were MUC1 (Ma695, 100-fold dilution ; Leica Biosystems Newcastle, Newcastle, UK), MUC2 (Ccp58, 200-fold dilution ; Leica Biosystems Newcastle), MUC5AC (CLH2, 200-fold dilution ; Leica Biosystems Newcastle) and MUC6 (CLH5, 50-fold dilution ; Leica Biosystems Newcastle). MUC1 was treated as a marker of biliary cell lineage, MUC2 as a marker of intestinal cell lineage and MUC5AC and MUC6 as markers of gastric cell lineage. For each stain, the proportion of positive tumor cells relative to all tumor cells was calculated as a percentage.

KRAS Testing

The presence of mutations in codons 12 and 13 of the *KRAS* gene was analyzed using the ARMS-Scorpion method. Thirteen cases from Showa University Hospital were selected from among the ICPN cases. A 10-μm thick section was prepared from a formalin-fixed, paraffin-embedded tissue section including the center of the tumor from each selected case. Testing was performed by a clinical testing company (SRL, Tokyo, Japan)[8]. Briefly, the assay was designed to detect a wild-type control and the seven most common *KRAS* exon 2 mutations, ie, Gly12Ala, Gly12Asp, Gly12Arg, Gly12Cys, Gly12Ser, Gly12Val, and Gly13Asp. Real-time polymerase chain reaction (7900HT, Life Technologies, Tokyo, Japan) was performed using 15 ～20 ng of genomic DNA for each of the eight mixtures. Data regarding each mutation were interpreted according to the kit manual after curve analysis and calculation of Δ cycle threshold

(Ct) values (sample mutation assay Ct minus sample control assay Ct).

Results

Of the 57 cases of gallbladder cancer, 23 (40%) were categorized as ICPN and the remaining 34 (60%) were categorized as non-ICPN (Table 1). Excluding the 13 intraepithelial cancer (Tis) cases, 14 (32%) of the remaining 44 invasive cases were categorized as ICPN and 30 (68%) as non-ICPN.

Clinicopathological analysis (Table 1)

Comparison of the ICPN and non-ICPN groups revealed no significant differences in mean age (69 and 74 years, respectively) or male:female ratio (14:9 and 15:19, respectively). Mean tumor diameters were also similar, at 2.8 cm and 2.6 cm, for ICPN and non-ICPN, respectively. When tumor depth was classified as non-invasive tumor (Tis), tumor invading mucosa (T1a),

Table 1. Comparison of clinicopathological features between the ICPN and non-ICPN groups

	ICPN	Non-ICPN	*P*-value
Number	23	34	–
Mean age (years)	69	74	NS
Male:female	14 :9	15 :19	NS
Surgical procedure			NS
Cholecystectomy (only)	16	23	
with partial resection of liver	7	9	
with segmentectomy resection of liver	0	2	
Lymph node dissection	15	20	
Mean tumor diameter (cm)	2.8	2.6	NS
Invasion depth (T)			NS
Tis	9	4	
T1	5	9	
T2	7	14	
T3	2	7	
Lymph node metastasis-positive rate (%)	6	43	0.01
Distant metastasis-positive rate (%)	0	6	NS
3-year survival rate (%)[a]	91	52	0.03
5-year survival rate (%)[b]	67	31	NS

[a, b] Excluding death by other illness (ICPN, n = 18 ; non-ICPN, n = 33).

Abbreviations : ICPN, intracholecystic papillary-tubular neoplasm ; NS, not significant.

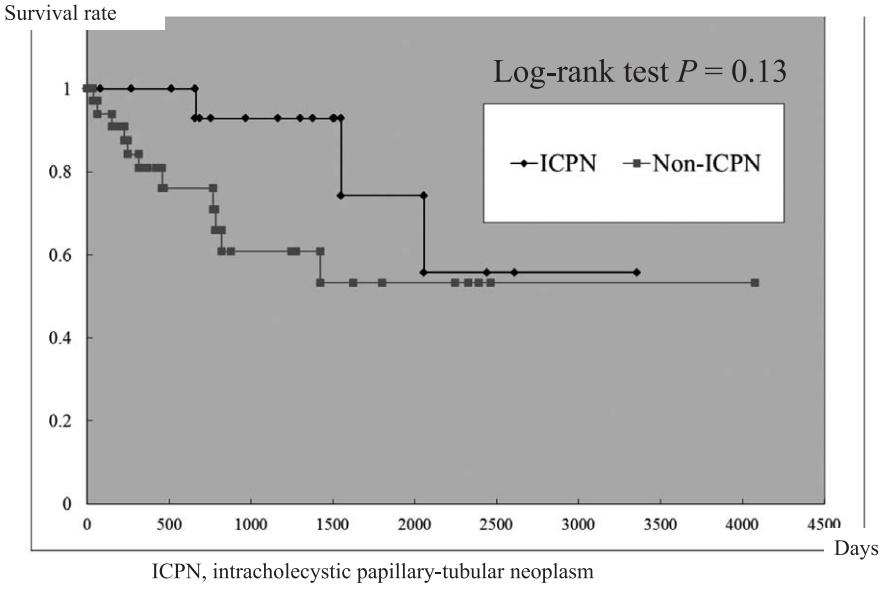

Fig. 3. Kaplan-Meier survival curve
ICPN group shows higher survival rate than non-ICPN group, but no significant difference is detected between two groups in the log-rank test.

solid tumor invading the muscle layer (T1b), tumor invading the subserosa (T2), or tumor invading the serosal surface or adjacent organs (T3), the breakdown of the ICPN group was 14 cases (61%) in the Tis + T1 group, 7 cases in the T2 group and 2 cases in the T3 group. In comparison, in the non-ICPN group, the figures were 13 (38%), 14 and 7 cases, respectively. No significant differences were apparent, but more cases in the ICPN group tended to show less-invasive tumors. The lymph node metastasis-positive rate was 6% in the ICPN group, which was significantly lower than the 43% in the non-ICPN group. Distant metastasis was not seen in the ICPN group. The postoperative 3-year survival rate was 91% in the ICPN group which was significantly higher than the non-ICPN group (52%; $P = 0.03$). The 5-year survival rate and Kaplan-Meier curve (Fig. 3) also showed better trends for the ICPN group, although the differences were not significant. When looking at the 3-year survival rate of cases with the same invasion depth, the ICPN group tended to show a higher 3-year survival rate for T2 + T3, although the number of cases was small and the difference was not significant (Table 2).

Analysis of ICPN cell characteristics

Histomorphological analysis of the 23 ICPN cases showed that 13 cases had biliary-type cells, 8 had gastric-type cells and 2 had intestinal-type cells. Immunostaining revealed that the mean proportion of positive-stained tumor cells for the biliary-type was 57% for MUC1, 0.6% for MUC2, 16% for MUC5AC and 17% for MUC6; in the gastric-type cells, values were 40% for MUC1, 0.3% for MUC2, 30% for MUC5AC and 88% for MUC6; and in the intestinal-type cells, values were 7.5% for MUC1, 37.5% for MUC2, 22.5% for MUC5AC and 35% for MUC6 (Fig. 4). These results were consistent with the histomorphological features. The predominant

Table 2. Three-year survival rate by T-category.

	(All)	ICPN	Non-ICPN	*P*-value
Tis + T1 tumor (n)	(14)	7	7	–
3-year survival rate (%)	(93)	100	86	0.3
	(All)	ICPN	Non-ICPN	*P*-value
T2 + T3 tumor (n)	(21)	4	17	–
3-year survival rate (%)	(55)	75	38	0.2

Abbreviation : ICPN, intracholecystic papillary-tubular neoplasm.

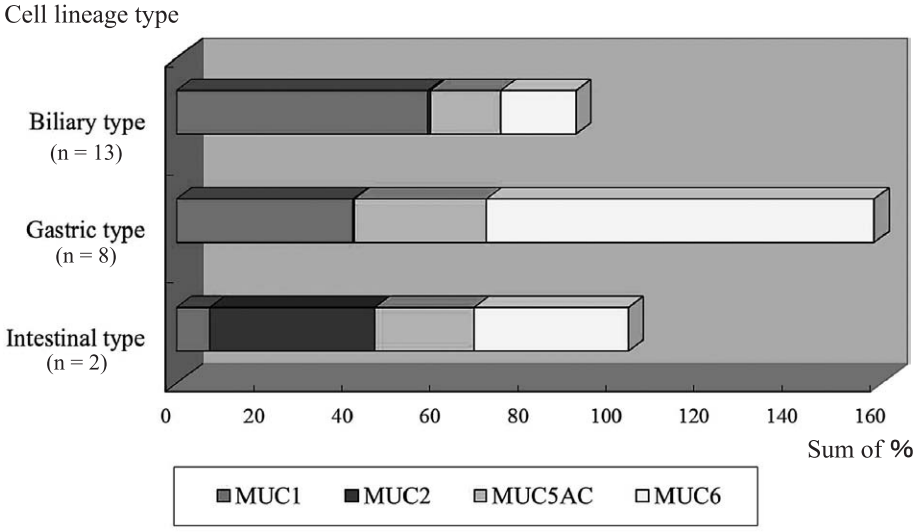

Fig. 4. Expression of MUCs by cell lineage type
Heterogeneous expression was seen in all cell types, but MUC1 was dominant in biliary type, MUC6 was dominant in gastric type and MUC2 was dominant in intestinal type. Marker expression was thus consistent with cell lineage.

growth patterns were papillary in 11 cases and tubular in 2 cases with biliary-type cells, papillary in 3 cases and tubular in 5 cases with gastric-type cells, and papillary in both cases with intestinal-type cell characteristics.

KRAS Testing

A *KRAS* gene mutation was only seen in one of the 13 ICPN specimens tested (an intestinal-type ICPN). The amino acid mutation type was G12V.

Discussion

ICPN was found in 40% (23 of 57) of the resected gallbladder cancer specimens examined, and of the 44 cases considered to be invasive cancer, ICPN was seen in 14 (32%). In Japanese papers on this subject, Akashi *et al*[9] studied material from 30 resected gallbladder

cancer cases and found 11 instances (37%) of papillary and invasive papillary tumors, which would be expected to overlap with ICPN in many cases. Kanda *et al*[10] reported 23 similar papillary tumors out of 51 gallbladder cancer cases (45%). These reports seem to support our data showing a 40% occurrence of ICPN. In contrast, Adsay *et al*[1] reported only 39 cases (6.4%) with ICPN components out of 606 cases of invasive gallbladder cancer, and the Survey Epidemiology and End Results (SEER) Program by the National Cancer Institute (1973 ～ 2001) reported papillary carcinoma in 394 of 8773 cases (4.5%) of gallbladder cancer, which included intraepithelial cancer[2]. This difference between the incidences in Japan and the United States may be due to differences in early detection rates, indications for surgery or ethnic factors. The possibility of ethnic differences is interesting, particularly when considering the incidence of gallbladder cancer, which has a high incidence in Eastern Europe, Chile and Japan[3].

We found several significant results when comparing clinicopathological factors in the ICPN and non-ICPN groups. Although the number of cases was small and no significant differences were detected, the ICPN group tended to include many cases of low invasive depth, including 14 of 23 cases (61%) of Tis + T1, compared to 13 of 34 cases (38%) in the non-ICPN group. This trend was complemented by the significantly lower lymph node metastasis rate and higher 3-year survival rate in the ICPN group compared to the non-ICPN group. Adsay *et al*[1] also reported a large number of cases with low tumor depth in the ICPN group, with 32% of cases at T1 in that group, compared to 9% in the non-ICPN group. One of the reasons for this difference could be that protruding ICPN lesions are highly likely to be detected by clinical imaging and consequently resected at a relatively early stage. However, another possibility is that invasiveness is an unlikely complication with ICPN, and invasion may progress slowly even when it occurs. The finding that ICPN had a high 3-year survival rate in this study at each category of tumor depth was noteworthy, although unfortunately the number of cases was small and no significant difference was detected. In cancers which had advanced to at least T2 (invasion of subserosa), Kanda *et al*[10] reported that the 5-year survival rate was 50% for papillary tumors, compared to 34% for invasive papillary tumors. This result may suggest that ICPN could be considered a biliary counterpart of pancreatic IPMN, which is considered a relatively indolent form of pancreatic cancer[4,5]. This should be clarified by the accumulation of further cases.

The possibility that ICPN is the biliary counterpart of pancreatic IPMN is also supported by the fact that ICPN components include a diverse histomorphological growth pattern ranging from papillary to tubular, together with diverse cell characteristics, resembling those of pancreatic IPMN. Nevertheless, there are several differences between ICPN and pancreatic IPMN. We found ICPN cell characteristics to be biliary-type in 13 cases, gastric-type in 8, and intestinal-type in 2, while Adsay *et al*[1] reported a similar proportion among ICPN cases (biliary-type, 69; gastric-type, 44; intestinal-type, 10). In contrast, pancreatic IPMNs mainly consist of cells of the gastric-type or villous intestinal-type[4,5]. This difference may be due to anatomical differences or differences in the nature of the preneoplastic lesions, but another factor could be that the presence of excess mucus production is not essential in the definition of ICPN. This is similar

for biliary intraductal papillary neoplasms and papillary-tubular neoplasms of the ampulla, where biliary-type and tubular intestinal-type cells are more frequent[6, 7], and consequently the patterns of cell characteristics in these tumors also differ from pancreatic IPMN. Additionally, the differences in cell characteristics might also be reflected in the fact that only 1 of 13 ICPN cases in our study had a *KRAS* mutation, whereas pancreatic IPMN has a high *KRAS* mutation rate of 30%～80%[3, 11, 12].

Conclusions

ICPN is frequently seen in gallbladder cancer, and could be regarded, clinicopathologically, as the gallbladder equivalent of pancreatic IPMN. However, the cell characteristics of these two diseases are not necessarily identical.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest.

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