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**Original**

# Clinicopathological Study of Mass-forming Gallbladder Cancer Focusing on the Grade of Cellular Dysplasia

Nobukazu SHIMA*[1)], Nobuyuki OHIKE[2)], Reika SUZUKI[2)], Tomoko NOROSE[2)], Tomohide ISOBE[2)], Akira SHIOKAWA[2)], Nobuyuki TAKEYAMA[3)], Junichi TANAKA[4)], Masahiko MURAKAMI[5)] and Masafumi TAKIMOTO[1)]

**Abstract**: The relationship between the clinicopathological features and the grade of cellular dysplasia of the neoplastic glands in mass-forming gallbladder cancer was investigated. In this retrospective study, 41 mass-forming (≧1 cm) gallbladder cancer specimens from 83 resected cases were examined. Tumors were classified into three groups: Group A had intraluminal masses consisting of neoplastic glands with only low-grade dysplasia; Group B had mixed low- and high-grade dysplasia, and Group C had only high-grade dysplasia. Of the 41 tumors, 13 were classified as Group A, 11 as Group B, and 17 as Group C. For Group A, B, and C, respectively, the mean tumor diameter was 1.6, 3.7 and 3.4 cm; macroscopic type (pedunculated / semi-pedunculated / sessile) was 7 / 5 / 1, 4 / 6 / 1 and 0 / 10 / 7; frequency of an invasive component inside the mass was 0%, 9% and 82%; and cell lineage (biliary / metaplastic / mixed) was 2 / 1 / 10, 8 / 1 / 2 and 14 / 1 / 2. In addition, invasion depth (Tis + T1 / T2 / T3) was 13 / 0 / 0, 7 / 4 / 0 and 3 / 10 / 4; lymph node metastases were present in 0 %, 9 % and 24 % of patients; 3-year survival rate was 100%, 100% and 82%; and 5-year survival rate was 100%, 100% and 69%, for A, B and C, respectively. Significant intergroup differences were seen for positive lymph node metastasis rate and 5-year survival rate. The present study indicates that the clinicopathological features of mass-forming gallbladder cancer are different depending on the grade of cellular dysplasia of the mass lesion. The tumors in Groups A and B were of lower malignancy than those in Group C and the prognosis of patients in the former groups was excellent. Group A and B tumors may be intracholecystic papillary-tubular neoplasms, a recently proposed new disease concept.

**Key words**: mass-forming gallbladder cancer, cellular dysplasia, clinicopathological study, intracholecystic papillary-tubular neoplasm (ICPN)

[1)] *Department of Pathology, Showa University School of Medicine,* 1-5-8, Hatanodai, Shinagawa-Ku, 142-8555, Tokyo, Japan.
[2)] *Department of Pathology, Showa University Fujigaoka Hospital.*
[3)] *Department of Radiology, Showa University Fujigaoka Hospital.*
[4)] *Department of Gastroenterological and General Surgery, Showa University Fujigaoka Hospital.*
[5)] *Department of Surgery, Division of General and Gastroenterological Surgery, Showa University School of Medicine.*
* To whom corresponding should be addressed.

## Introduction

Opportunities for the detection of elevated lesions in the lumen of the gallbladder are increasing with the widespread use of, and advances in, clinical imaging. These elevated lesions form a diverse group that includes not only cholesterol polyps, hyperplastic polyps, and adenomyomatosis, but also adenomas and adenocarcinomas. Adenocarcinomas are further subdivided into low-grade tumors, which may be confused with adenomas and hyperplastic polyps, and obviously high-grade tumors[1–3]. The present study focused on the characteristics of the neoplastic cells in intraluminal elevated lesions in patients diagnosed with mass-forming gallbladder cancer, as well as the influence of the cellular dysplastic grade on the clinicopathological features, including staging and prognosis.

## Subjects and methods

From 83 patients who underwent surgical resection at our institutions (Showa University Hospital and Showa University Fujigaoka Hospital) and were histologically diagnosed with primary adenocarcinoma of the gallbladder between 1998 and 2015, 46 patients with macro-sized (approximately $\geqq 1$ cm) polypoid or papillary mass lesions in the gallbladder lumen were initially selected. This retrospective study was approved by the institutional review board and was conducted in accordance with the Health Insurance Portability Accountability Act. The institutional review board waived the requirement for informed patient consent.

### *Clinicopathological study*

Tumors were classified into the following three groups, based on the dysplastic grade of the tumor cells that composed the elevated lesions protruding into the lumen of the gallbladder, and their clinicopathological features were compared. Group A tumors were intraluminal masses consisting solely of low-grade dysplastic glands (Fig. 1a), Group B were intraluminal masses with a mixture of low-grade and high-grade dysplastic glands (arbitrarily with each component accounting for at least 25% of the whole tumor), and Group C were intraluminal masses consisting extensively of high-grade dysplastic glands (usually showing complex papillo-tubular structures and cribriform structures; Fig. 1b). Low-grade dysplastic glands were characterized by the regular arrangement of uniform and relatively simple glands with mild nuclear enlargement, while high-grade dysplastic glands were characterized by the irregular arrangement of complex glands with prominent nuclear enlargement and more significant hyperchromasia. The mean age, sex ratio, macroscopic type, mean tumor diameter, presence of an invasive carcinoma component inside the mass lesion, presence of tumors exhibiting p53-overexpression, invasion depth, rate of lymph node metastasis, rate of distant metastasis, and postoperative prognosis were investigated to make comparisons among these three groups. The duration of postoperative follow-up ranged from 45 to 4,137 days, and 5 patients who died of other causes (such as myocardial infarction and pneumonia) were excluded from the analysis of prognosis. Statistical analysis was carried out using the Kruskal-Wallis test, with the log-rank test for Kaplan-Meier curves; $P < 0.05$ was regarded as significant.

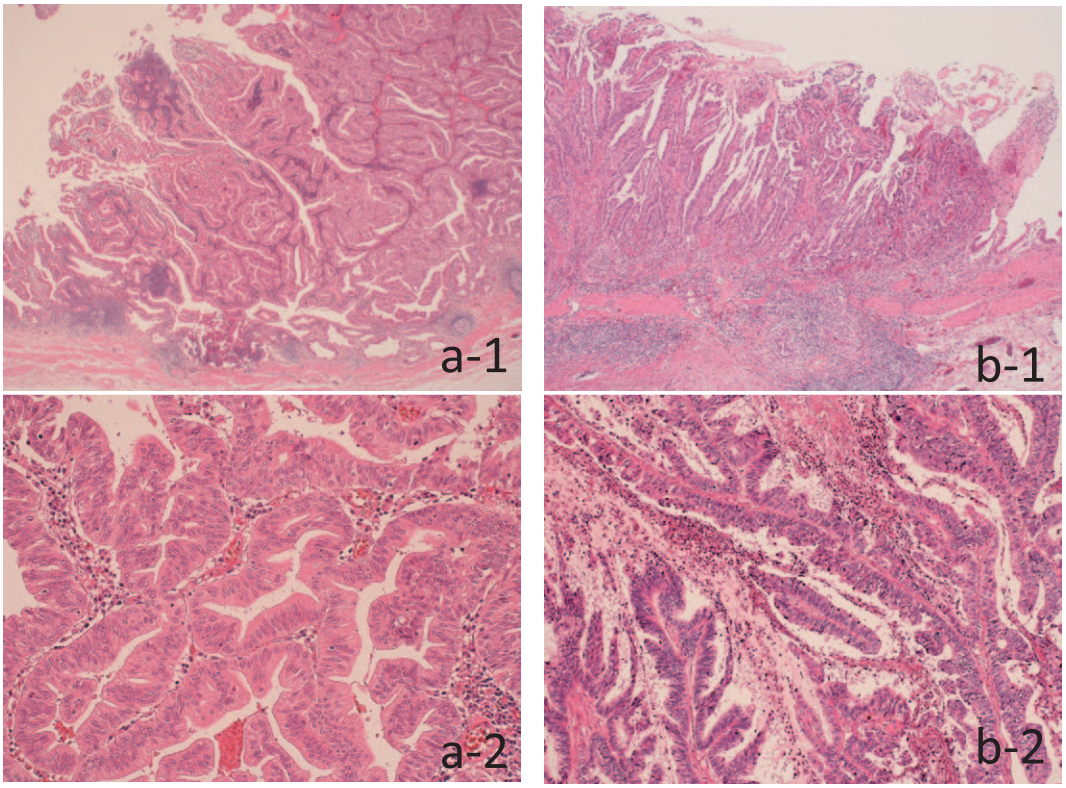


Fig. 1. Microscopic features of mass-forming gallbladder cancer. (a-1) Intraluminal mass consists extensively of low-grade dysplastic glands. Hematoxylin and eosin (H & E) stain, ×50 magnification. (a-2) Intraluminal mass consists extensively of low-grade dysplastic glands. H & E stain, ×200 magnification. (b-1) Intraluminal mass consists extensively of high-grade dysplastic glands. H & E stain, ×50 magnification. (b-2) Intraluminal mass consists extensively of high-grade dysplastic glands. H & E stain, ×200 magnification.

*Investigation of cell lineage*

The cell lineage of the neoplastic glands composing the mass lesion was investigated histomorphologically and immunohistochemically for all cases.

(a) Histomorphological observation :

Tumor cells were categorized as biliary type, intestinal (INT) type, or gastric (GAS) type through observation of hematoxylin and eosin-stained specimens under light microscopy, and each tumor was classified as either biliary, metaplastic (GAS or INT), or mixed (biliary + GAS and / or INT)[1, 2, 4]. Briefly, the histological characteristics of these different cell lineages were as follows : biliary type was characterized by cuboidal or low-columnar epithelial cells with oval nuclei and scant mucinous cytoplasm resembling proper biliary tract epithelium. INT type consisted of tall columnar epithelium with spindle-shaped, pseudostratified nuclei and strongly-stained cytoplasm resembling colorectal adenoma. The presence of goblet cells and Paneth cells was also considered indicative of INT lineage. GAS type consisted of tall columnar epithelium with mucinous cytoplasm resembling gastric foveolar epithelium or small tubular units similar to gastric pyloric glands. If more than one of these characteristics were present simultaneously, the

most prominent cell type was used, taking into account the predominant features and degree of cellular dysplasia.

(b) Immunohistochemical analysis :

For each patient, thin sections (3 μm) were prepared for immunostaining using one or two formalin-fixed, paraffin-embedded tissue sections including the maximum area of the tumor. Staining was performed using the avidin-biotin complex detection method with a BenchMark automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). The primary antibodies used were MUC1 (Ma695, Leica Biosystems Newcastle Ltd, Newcastle, UK ; 100-fold dilution), MUC2 (Ccp58, Leica ; 200-fold dilution), MUC5AC (CLH2, Leica ; 200-fold dilution), MUC6 (CLH5, Leica ; 50-fold dilution), and CDX-2 (AMT28, Leica ; 50-fold dilution). MUC1 was used as a marker for biliary type, MUC2 and CDX-2 for INT type, and MUC5AC and MUC6 for GAS type[1, 2, 4–6]. The proportion of cells staining positive for each marker as a percentage of the total number of neoplastic cells was calculated. Immunostaining for p53 (DO-7, Leica ; 800-fold dilution) was also carried out following the same staining procedure[1–3].

## Results

Of the 41 patients, 13 were classified with tumors belonging to Group A, 11 to Group B, and 17 to Group C.

*Clinicopathological study* (Table 1)

The mean age was 65 years in Group A, 71 years in Group B, and 77 years in Group C, and the male : female ratio was 10 : 3 in Group A, 4 : 7 in Group B, and 7 : 10 in Group C. Groups B and C tended to be older and to include more women than Group A, but these differences were not significant. The mean tumor diameter was 1.6 cm in Group A, but 3.7 cm in Group B and 3.4 cm in Group C, with the latter groups being significantly larger than Group A. When the tumors were classified as pedunculated, semipedunculated, or sessile on the basis of their macroscopic morphology, Group A included 7 pedunculated, 5 semipedunculated, and 1 sessile tumor, Group B included 4 pedunculated, 6 semipedunculated, and 1 sessile tumor, and Group C included only 10 semipedunculated, and 7 sessile tumors, with Group C containing significantly more sessile tumors than the other groups. The frequency of the presence of invasive carcinoma inside the mass was 0% in Group A and 9% in Group B, while it was significantly higher, at 82%, in Group C.

Invasion depth was classified as no invasion (Tis), invasion into the mucosa (T1), invasion into the muscularis propria (T2), or invasion into the subserosa (T3), and expressed as Tis + T1 / T2 / T3. The number of tumors of each invasion depth was 13 / 0 / 0 in Group A, 7 / 4 / 0 in Group B, and 3 / 10 / 4 in Group C, and these differences were significant. The rate of lymph node metastasis was 0% in Group A, 9.1% in Group B, and 23.5% in Group C, and these differences were also significant. The 3-year survival rate was 100% in Groups A and B, but only 82.4% in Group C. The 5-year survival was 100% in Groups A and B, but significantly lower in Group C at 68.8%. The significance of this difference was also confirmed by the log-rank test ($P = 0.02$).

Table 1. Clinicopathological features of mass-forming gallbladder cancer

| Number | Grp A (n = 13) | Grp B (n = 11) | Grp C (n = 17) | *P*-value |
|---|---|---|---|---|
| Mean age (years) | 65 | 71 | 77 | 0.226 |
| Sex (n) | | | | 0.078 |
| Male | 10 | 4 | 7 | |
| Female | 3 | 7 | 10 | |
| Mean tumor diameter (cm) | 1.6 | 3.7 | 3.4 | 0.0003 |
| Morphology (n) | | | | 0.0007 |
| Pedunculated | 7 | 4 | 0 | |
| Semipedunculated | 5 | 6 | 10 | |
| Sessile | 1 | 1 | 7 | |
| Frequency of invasive carcinoma inside the mass lesion (%) | 0 | 9.1 | 82.3 | < 0.0001 |
| Cell lineage (n) | | | | 0.0007 |
| Biliary | 2 | 8 | 14 | |
| Mixed | 10 | 2 | 2 | |
| Metaplastic | 1 | 1 | 1 | |
| p53-overexpression rate (%) | 7.7 | 36.3 | 41.2 | 0.113 |
| Invasion depth (n) | | | | 0.0002 |
| Tis + T1 | 13 | 7 | 3 | |
| T2 | 0 | 4 | 10 | |
| T3 | 0 | 0 | 4 | |
| Lymph node metastasis rate (%) | 0 | 9.1 | 23.5 | 0.044 |
| Distant metastasis rate (%) | 0 | 0 | 0 | — |
| 3-year survival rate (%) | 100 | 100 | 82.4 | 0.122 |
| 5-year survival rate (%) | 100 | 100 | 68.8 | 0.023 |

Grp, Group ; Tis + T1, no invasion or invasion into mucosa ; T2, invasion into muscularis propria ; T3, invasion into subserosa.

*Investigation of cell lineage and immunohistochemical studies*

When cell lineages were histomorphologically classified as biliary, metaplastic, or mixed, 2 of the tumors in Group A were classified as biliary, 1 as metaplastic, and 10 as mixed ; in Group B, 8 were classified as biliary, 1 as metaplastic, and 2 as mixed ; and in Group C, 14 were classified as biliary, 1 as metaplastic, and 2 as mixed. Group A contained significantly more mixed-type tumors, while Groups B and C contained significantly more biliary-type tumors.

In Group A, 33.6% of tumors were immunohistochemically positive for MUC1, 0.8% for MUC2, 26% for MUC5AC, 73.8% for MUC6, and 32.5% for CDX2. Similarly, the percentages in Group B were 20%, 7%, 23%, 48%, and 36%, and those in Group C were 37%, 2.6%, 15.7%, 27%, and 29.8%, respectively. Group A contained a conspicuously high proportion of

MUC6-positive tumor cells. The frequency of tumors exhibiting p53-overexpression was 7.7% in Group A, but significantly higher at 36.3% in Group B and 41.2% in Group C.

## Discussion

This study showed that the clinicopathological features of mass-forming gallbladder cancer were different depending on the grade of cellular dysplasia of the neoplastic glands within the intraluminal elevated lesions. In Group A, with mass lesions consisting of low-grade dysplastic glands, there was a high proportion of men, more lesions were pedunculated masses of mean diameter $< 2$ cm, they did not contain an invasive carcinoma component, the rate of p53-overexpression was low, tumors invaded no deeper than the mucosa, there were no lymph node metastases, and the 5-year survival rate was 100%. The cell type was most commonly mixed, consisting of biliary + GAS, and a high proportion stained positive for MUC6. In contrast, in Group C with mass lesions consisting of high-grade dysplastic glands, there was a high proportion of women, more tumors were semipedunculated or sessile masses with mean diameters greater than 3 cm, most elevated lesions already contained an invasive carcinoma component, the p53-overexpression rate exceeded 40%, many of the lesions had invaded the muscularis or deeper, lymph node metastases were present in almost a quarter of patients, and the 5-year survival rate was 68%. The cell type was most commonly biliary. Group B was intermediate between Groups A and C in most respects, but the 5-year survival rate was 100%, the same positive outcome as in Group A. Group C had tumors with a deeper invasion and poorer prognosis than Group A or B. The difference between these groups may be due to the difference in stage. In the future, legitimate evaluation of these tumors should be performed to accumulate a larger number of cases for analysis.

Histological classification focusing on the grade of cellular dysplasia has not been well established for gallbladder carcinoma. Recently, however, a new disease concept of intracholecystic papillary-tubular neoplasm (ICPN) has been proposed[1, 2, 4, 7–12]. ICPNs are defined as intraluminal mass-forming preinvasive neoplasms occurring within the gallbladder which are highly analogous to pancreatic or biliary intraductal papillary and tubular neoplasms, as evidenced by their papillary and / or tubular growth, variable cell lineage, and spectrum of dysplastic change (adenoma-carcinoma sequence)[9, 13]. ICPNs are biologically indolent ; noninvasive examples show an excellent prognosis, whereas those with invasion exhibit a malignant, but nevertheless significantly better prognosis, compared to ordinary invasive carcinomas unaccompanied by ICPN. The majority of tumors in Groups A and B in the present study would appear to be classified as ICPNs, while the majority of tumors in Group C would be excluded from ICPN and should be classified as ordinary high-grade "invasive papillary adenocarcinoma" or "tubular adenocarcinoma" because many of them contained an invasive carcinoma component within the intraluminal mass lesion.

The present results may be useful for several issues in the diagnosis and treatment of mass-forming gallbladder cancer. For instance, low-grade adenocarcinoma, as shown in tumors in Group A, might be regarded as hyperplasia or adenoma depending on the pathologist[13].

However, the present study shows that a different diagnosis would not be a crucial problem because such tumors usually show an almost "benign" course. Mass-forming gallbladder cancer may be associated with intraepithelial spread along a Rokitansky-Aschoff sinus (RAS), which often is confused with non-neoplastic RAS or invasive tubular adenocarcinoma. The present study indicates that in tumors of Group A which are rarely associated with an invasive component, it is appropriate to firstly consider non-neoplastic RAS or intraepithelial spread, even if glandular ducts are present in the deep gallbladder wall. Conversely, in tumors in Group C, the possibility of invasive tubular adenocarcinoma should be aggressively pursued. Finally, as for treatment strategies for mass-forming gallbladder cancer, particularly surgical procedures, simple cholecystectomy seems to be sufficient for tumors in Group A. In Groups B or C with lymph node metastasis, however, a surgical procedure including lymph node dissection must be considered. Therefore, if preoperative classification of the tumor into one of the Groups A–C is feasible, it would be a highly worthwhile process.

## Conclusion

The present study indicated that the clinicopathological features of mass-forming gallbladder cancer were different depending on the cellular dysplastic grade of the mass lesion. Tumors with an intraluminal mass consisting of low-grade dysplastic glands were of lower malignancy and the prognosis of patients with such tumors was excellent. These results may support the significance of the disease concept of ICPN.

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**Conflict of interest disclosure**

None of the authors have any conflicts of interest or financial ties to disclose.

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