| Journal | MSP No. | Dispatch: November 13, 2017 | CE: |
|---|---|---|---|
| JSO | 24946 | No. of Pages: 10 | PE: Michele Heal |

Received: 13 May 2017 | Accepted: 6 November 2017

DOI: 10.1002/jso.24946

RESEARCH ARTICLE

WILEY Journal of SURGICAL ONCOLOGY

# The prognostic impact of differentiation at the invasive front of biliary tract cancer

Satoshi Okubo MD[1,2] | Shuichi Mitsunaga MD, PhD[3,4] | Yuichiro Kato MD[5] | Motohiro Kojima MD, PhD[3,6] | Motokazu Sugimoto MD, PhD[1] | Naoto Gotohda MD, PhD[1] | Shinichiro Takahashi MD, PhD[1] | Ryuichi Hayashi MD, PhD[1] | Masaru Konishi MD[1]

[1] Department of Hepatobiliary and Pancreatic Surgery, National Cancer Center Hospital East, Chiba, Japan

[2] Course of Advanced Clinical Research of Cancer, Juntendo University Graduate School of Medicine, Tokyo, Japan

[3] Division of Biomarker Discovery, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Chiba, Japan

[4] Department of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Chiba, Japan

[5] Department of Surgery, Nagoya Ekisaikai Hospital, Aichi, Japan

[6] Division of Pathology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Chiba, Japan

**Correspondence**
Shuichi Mitsunaga, MD, PhD, Division of Biomarker Discovery, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan.
Email: smitsuna@east.ncc.go.jp

**Funding information**
Grants-in-aid for Cancer Research and for the Third-term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan,
Grant number: JSPS KAKENHI Grant Number 22790624; The National C



**Background:** The invasive front of tumor can provide prognostic information in many cancers. We investigated the prognostic morphological factors at the invasive front including tumor differentiation ($Dif^{inv}$) and tumor budding (Bud) in biliary tract cancer (BTC).

**Methods:** The resected specimen from the 299 BTC patients were examined. Intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and ampulla of Vater cancer were found in 16%, 48%, 17%, and 19%, respectively. $Dif^{inv}$grade (G) 3 and Bud foci ≥5 were found in 47% and 10%. Tumor with $Dif^{inv}$G3 showed the high frequencies of Bud, vascular invasion (Ve) and nodal metastasis (LN) compared to tumor with $Dif^{inv}$G1/2 (Bud: 21% vs 0%, Ve: 71% vs 50%, LN: 52% vs 36%). Multivariate analysis revealed that the independent predictors were $Dif^{inv}$G3 (HR: 1.71), Bud foci ≥5 (HR: 2.14), Ve (HR: 1.56) and LN (HR: 2.59) in overall survival and were positive resection margin (HR: 1.71), $Dif^{inv}$G3 (HR: 1.75), Ve (HR: 1.50), and LN (HR: 2.19) in relapse free survival.

**Conclusion:** Poor differentiation at the invasive front of tumor was associated with poor prognosis and early relapse in BTC patients.

KEYWORDS
biliary tract cancer, invasive front of tumor, prognostic factor, surgical pathology, tumor differentiation

## 1 | INTRODUCTION

Biliary tract cancer (BTC) arises from the ductal epithelium of the bile duct tree and is classified into intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC), gallbladder cancer (GB), and ampulla of Vater cancer (Va) according to the anatomical site of the tumor. Surgical resection for curative intent is the only approach to cure, but more than half of patients who undergo curative resection develop recurrences. The 5-year survival rate of BTC patients remains less than 40%.[1–5] The development of lymph node metastases is an important prognostic factor in all types of BTC, including ICC, ECC, GB, and Va.[6–10]

  

Various invasive behaviors of primary tumors have been reported as important prognostic factors in BTC. Vascular invasion, intrahepatic metastasis, and tumor size have been considered prognostic factors in ICC patients.[1,11] In ECC patients, differentiation, liver invasion, and pancreas invasion are candidate prognostic factors.[8,12] Differentiation and liver invasion in GB patients and perineural invasion in Va patients have also been reported to be prognostic factors.[10,13,14] The prognostic values of these candidate factors remain unclear for the entire BTC spectrum. Identification of common invasive behaviors to predict prognosis could be useful for the management of all cases of BTC and be necessary for patient allocation in clinical trials for the development of new treatment strategies.

The invasive front of tumor has been highlighted in recent histological research. Evaluation of tumor budding, which is characterized by isolated or small clusters of tumor cells at the invasive front, is considered to be a useful prognostic marker in colorectal adenocarcinomas and esophageal and squamous carcinomas.[15–17] The growth pattern is a morphological classification according to the density of desmoplasia and the invasive behavior to liver parenchyma at the invasive front of liver metastases.[18,19] The growth pattern includes a desmoplastic growth pattern, a pushing growth pattern, and a replacement growth pattern, and it is related to prognosis in patients with colorectal liver metastases. The budding component and replacement growth pattern appear to be part of poorly differentiated adenocarcinoma, but cellularity differs between the budding area and the replacement growth pattern. Cellularity is lower in the budding area and is higher in the replacement growth pattern. Thus, tumor differentiation and cellularity at the invasive front are the important elements of tumor budding and the replacement growth pattern and may have prognostic impacts in BTC.

The present study was conducted to evaluate the prognostic impact of invasive behavior, including tumor differentiation and cellularity, at the invasive front of BTC.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Between 2000 and 2012, 380 patients underwent surgical resection with curative intent for BTC at National Cancer Center Hospital East. The exclusion criteria were: (1) treatment-related death ($n = 6$); (2) histological types other than adenocarcinoma, including adenosquamous carcinoma ($n = 9$), undifferentiated carcinoma ($n = 3$), carcinosarcoma ($n = 2$), neuroendocrine carcinoma ($n = 1$), and mucinous cyst adenocarcinoma ($n = 1$); (3) carcinoma in situ ($n = 20$); (4) intraoperative radiation therapy ($n = 4$); (5) concomitant malignancies, including multifocal malignancy in the biliary tract ($n = 5$), pancreatic cancer ($n = 3$), colorectal cancer ($n = 1$), and hepatocellular carcinoma ($n = 1$); (6) preoperative treatment including cholecystectomy for incidental gallbladder carcinoma in a previous hospital ($n = 8$) and preoperative chemotherapy ($n = 2$); and (7) lost to follow-up ($n = 13$). The remaining 299 patients were analyzed in this study (Figure 1).

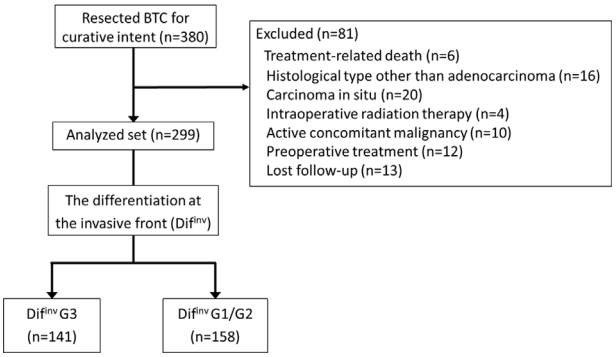


**FIGURE 1** Consort diagram. BTC: Biliary tract cancer

Clinical data were collected retrospectively from patients' medical records. Pathological data were assessed according to the tumor-node-metastasis (TNM) classification criteria outlined in the 7th edition of the Union for International Cancer Control (UICC).[20]

Overall survival was calculated from the date of surgery to death from any cause. Relapse-free survival was defined as the period from surgery to tumor relapse or death from any cause, whichever came

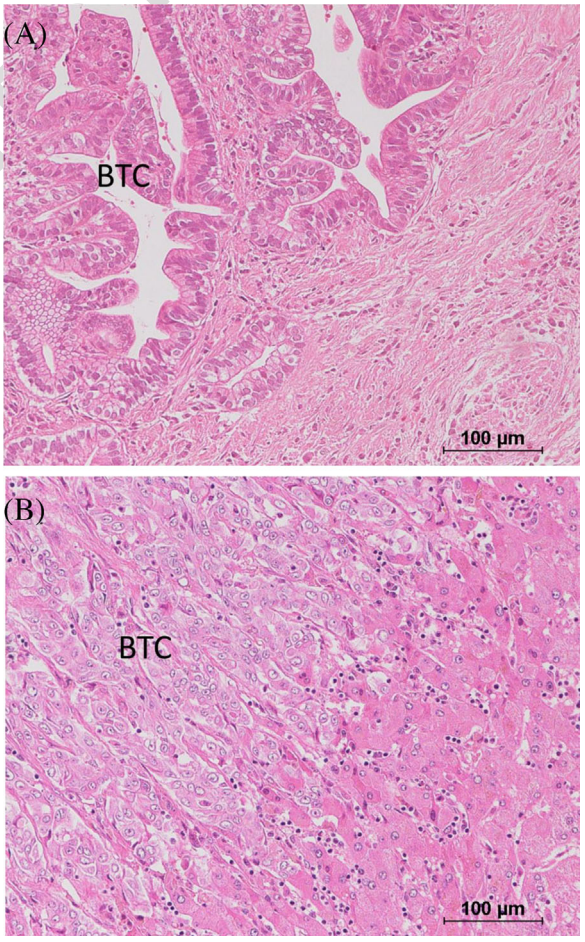


**FIGURE 2** Photographs of BTC tumors at the invasive front (HE, ×200), (A) Grade 1/2, (B) Grade 3. BTC: Biliary tract cancer



COLOR

**TABLE 1** Characteristics of BTC patients [Q3] classified by differentiation at the invasive front

| Characteristic | Total $N = 299$ (100%) | $Dif^{inv}$ G1/G2 $N = 158$ (100%) | $Dif^{inv}$ G3 $N = 141$ (100%) | *P*-value |
|---|---|---|---|---|
| Male | 200 (67) | 103 (65) | 97 (69) | 0.30 |
| Age (y) | | | | 0.17 |
| Median (range) | 68 (31-88) | 68 (31-88) | 68 (43-86) | |
| ≥70 | 135 (45) | 76 (48) | 59 (42) | |
| Period | | | | 0.26 |
| 2000-2006 | 133 (44) | 67 (42) | 66 (47) | |
| 2007-2012 | 166 (56) | 91 (58) | 75 (53) | |
| Tumor location | | | | <0.01 |
| ICC | 47 (16) | 12 (8) | 35 (25) | |
| ECC | 144 (48) | 88 (56) | 56 (40) | |
| GB | 50 (17) | 29 (18) | 21 (15) | |
| Va | 58 (19) | 29 (18) | 29 (21) | |
| Biliary drainage | 162 (54) | 83 (53) | 79 (56) | 0.31 |
| CEA ≥UNL (5 ng/mL) | 63 (21) | 28 (18) | 35 (25) | 0.08 |
| CA19-9 ≥UNL (37 U/mL) | 173 (58) | 86 (54) | 87 (62) | 0.08 |
| Lymphadenectomy | 268 (90) | 141 (89) | 127 (90) | 0.48 |
| $Dif^{whole}$ | | | | <0.01 |
| G1/G2 | 259 (87) | 156 (99) | 103 (73) | |
| G3 | 40 (13) | 2 (1) | 38 (27) | |
| $Cell^{inv}$ high | 131 (44) | 36 (23) | 95 (67) | <0.01 |
| Tumor budding positive | 29 (10) | 0 (0) | 29 (21) | <0.01 |
| Lymphatic invasion | 145 (48) | 76 (48) | 69 (49) | 0.49 |
| Vascular invasion | 179 (60) | 79 (50) | 100 (71) | <0.01 |
| Perineural invasion | 185 (62) | 92 (58) | 93 (66) | 0.11 |
| UICC T factor 3-4 | 132 (44) | 59 (37) | 73 (52) | <0.01 |
| Nodal metastasis | 131 (44) | 57 (36) | 74 (52) | <0.01 |
| UICC M factor | 11 (4) | 2 (1) | 9 (6) | 0.02 |
| Resection margin-negative | 38 (13) | 23 (15) | 15 (11) | 0.20 |
| Adjuvant chemotherapy | 22 (7) | 16 (10) | 6 (4) | 0.04 |
| Complications | 151 (51) | 81 (51) | 70 (50) | 0.44 |

BTC, biliary tract cancer; UNL, upper normal limit; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; $Dif^{inv}$, differentiation at the invasive front of the tumor; $Dif^{whole}$, predominant differentiation of the whole primary tumor; $Cell^{inv}$, cellularity at the invasive front of the tumor; UICC, Union for International Cancer Control; ICC, Intrahepatic cholangiocarcinoma; ECC, extrahepatic cholangiocarcinoma; GB, gallbladder cancer; Va, ampulla of Vater cancer.

first. The date of tumor relapse was determined as the day when the examination contributing to the diagnosis of the relapse was performed. The protocol of the present study was approved by the institutional review board of the National Cancer Center.

## 2.2 | Surgical procedure and follow-up

Patients with intrahepatic cholangiocarcinoma typically underwent extended hemihepatectomy with lymphadenectomy and extrahepatic bile duct resection or hemihepatectomy with/without lymphadenectomy or segmental or partial hepatectomy without lymphadenectomy. Patients with perihilar cholangiocarcinoma also typically underwent hemihepatectomy or extended hemihepatectomy with extrahepatic bile duct resection and regional lymphadenectomy, while patients with distal cholangiocarcinoma and ampulla of Vater cancer typically underwent subtotal stomach-preserving pancreaticoduodenectomy with regional lymphadenectomy. For gallbladder cancer, curative surgery was performed depending on tumor extension.

For surveillance after resection, patients underwent physical examinations, laboratory tests including tumor markers

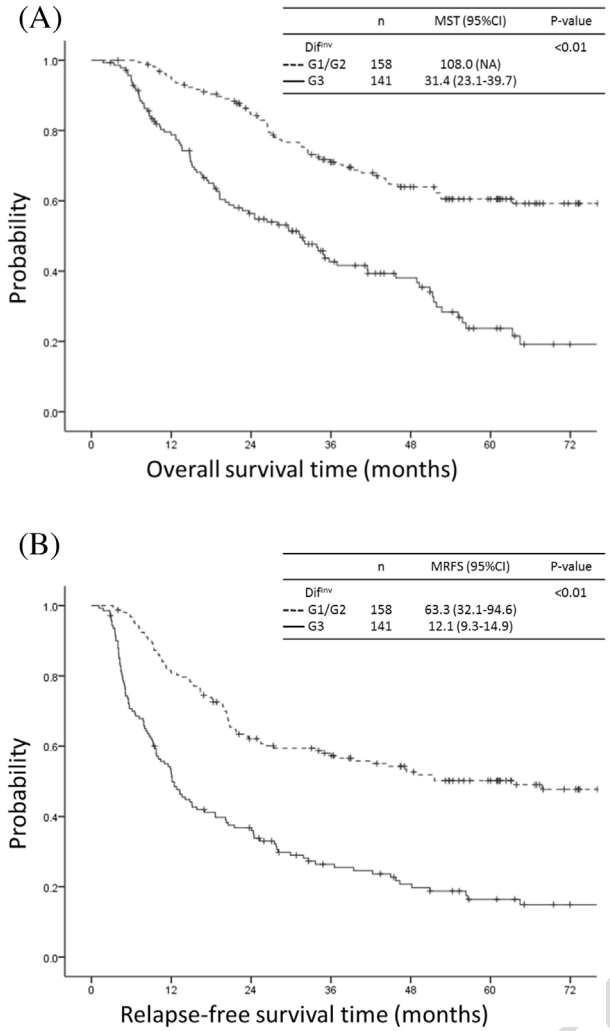


**FIGURE 3** Survival curves of BTC patients classified by the degree of differentiation at the invasive front of the primary tumor. A, Overall survival time, (B) Relapse-free survival time. BTC: Biliary tract cancer; Dif$^{inv}$: differentiation at the invasive front; MST: median overall survival time; MRFS: Median relapse-free survival time; CI: Confidence interval; NA: Not available

(carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9)) and imaging tests, including enhanced multidetector computed tomography and/or ultrasonography every 3 to 6 months for the first 2 years, every 6 months for the following 3 years, and annually thereafter. The median follow-up period was 61.6 months (95% confidence interval, 56.9-65.3 months).

## 2.3 | Morphological definitions for evaluating the invasive front

The histological findings on the hematoxylin and eosin-stained tissue sections were evaluated using a ×20 magnification objective lens by two of the authors (S.O. and S.M.). The invasive front was defined as the peripheral to whole primary tumor and in the most severe extended area of tumor to the surrounding tissue. The most severe extended area was determined on the basis of the severity on 7th edition of UICC T classification.[20] For example, in the case of Va that invaded the duodenum and pancreas, the invasive front was regarded as the site of pancreas invasion. The histological differentiation at the invasive front of the tumor (Dif$^{inv}$) was classified into: G1/2, well/moderately differentiated adenocarcinoma; and G3, poorly differentiated adenocarcinoma (Figure 2).[21] In addition, the predominant differentiation of the whole primary tumor was assessed as Dif$^{whole}$. Cellularity of tumor tissue at the invasive front (Cell$^{inv}$) was also evaluated; Cell$^{inv}$ High was defined as tumor cell-occupied area higher than the no tumor cell-occupied area at the invasive front. Tumor budding was defined as an isolated single cell or cluster of fewer than five cancer cells at the invasive front. The count of tumor budding was made in the field using a ×20 magnification objective lens. The extent of the budding was then classified as positive if there were ≥5 budding foci and negative if there were <5 budding foci or no budding focus, according to Kai's study.[22]

## 2.4 | Statistical analysis

Differences were compared among two or more groups using Fisher's exact test or the chi-squared test. Cumulative survival curves were prepared using the Kaplan-Meier method and compared using the log-rank test on univariate analysis. Survival-related factors on univariate analysis were entered into the multivariate Cox proportional hazards model with adjustment for age and sex. The level of significance was set at $P < 0.05$. All statistical evaluations were performed using the SPSS 22.0 software package (SPSS Japan, Tokyo, Japan) for Windows.

# 3 | RESULTS

## 3.1 | Patient demographics

The study patients included 200 men and 99 women, with a median age of 68 years (range, 31-88 years) (Table 1). All 299 patients underwent surgical resection with curative intent, and ICC, ECC, GB, and Va were found in 47 patients (16%), 144 patients (48%), 50 patients (17%), and 58 patients (19%), respectively; 45% of ICC patients ($n = 21$) did not undergo lymphadenectomy (Supplemental Table S1).

## 3.2 | Histological factors

Pathological examination was performed in all 299 patients (Table 1). Dif$^{inv}$G3 was found in 47%, Cell$^{inv}$ High was seen in 44% and tumor budding-positive was seen in 10% of all cases of BTC. Half of the ICC cases showed Dif$^{inv}$G3 (48%). The frequencies of Dif$^{inv}$G3 in ECC, GB, and Va were 16%, 17%, and 19%, respectively. The frequencies of vascular invasion, Dif$^{whole}$G3, and nodal metastasis were 60%, 13%, and 44% for all BTC patients. Dif$^{inv}$G3 patients showed significantly higher rates of Dif$^{whole}$G3, Cell$^{inv}$ High, tumor budding-positive, vascular invasion, UICC T3-4 factor, nodal metastasis, and UICC M factor than Dif$^{inv}$G1/2 patients.

**TABLE 2** Analyses of overall survival in BTC patients

| | | | Univariate | Multivariate | | |
|---|---|---|---|---|---|---|
| **Characteristics** | ***n*** | **MST (months)** | ***P*-value** | ***P*-value** | **HR** | **(95%CI)** |
| Sex | | | | | | |
| Male | 200 | 51.3 | 0.70 | 0.55 | | |
| Female | 99 | 52.5 | | | | |
| Age (y) | | | | | | |
| <70 | 164 | 51.9 | 0.86 | 0.99 | | |
| ≥70 | 135 | 48.9 | | | | |
| Period | | | | | | |
| 2000-2006 | 133 | 40.6 | 0.04 | 0.07 | | |
| 2007-2012 | 166 | 55.1 | | | | |
| Lymphadenectomy | | | | | | |
| Yes | 268 | 50.9 | 0.50 | | | |
| No | 31 | NR | | | | |
| Resection margin | | | | | | |
| R0 | 261 | 51.6 | 0.19 | | | |
| R1 | 38 | 40.6 | | | | |
| $Dif^{inv}$ | | | | | | |
| G1/G2 | 158 | 108.0 | <0.01 | 0.01 | ref | |
| G3 | 141 | 31.4 | | | 1.71 | (1.11-2.63) |
| $Dif^{whole}$ | | | | | | |
| G1/G2 | 259 | 52.7 | <0.01 | <0.01 | ref | |
| G3 | 40 | 16.7 | | | 1.93 | (1.21-3.08) |
| $Cell^{inv}$ | | | | | | |
| Low | 168 | 52.7 | 0.04 | 0.58 | | |
| High | 131 | 41.5 | | | | |
| Tumor budding | | | | | | |
| Negative | 270 | 55.1 | <0.01 | <0.01 | ref | |
| Positive | 29 | 18.5 | | | 2.14 | (1.25-3.68) |
| Lymphatic invasion | | | | | | |
| Absent | 154 | 63.3 | 0.06 | | | |
| Present | 145 | 41.6 | | | | |
| Vascular invasion | | | | | | |
| Absent | 120 | 108.0 | <0.01 | 0.03 | ref | |
| Present | 179 | 34.2 | | | 1.56 | (1.05-2.31) |
| Perineural invasion | | | | | | |
| Absent | 114 | 79.2 | 0.03 | 0.69 | | |
| Present | 185 | 42.5 | | | | |
| UICC T factor | | | | | | |
| T1-2 | 167 | 63.3 | <0.01 | 0.83 | | |
| T3-4 | 132 | 35.8 | | | | |
| Nodal metastasis | | | | | | |
| Negative | 168 | NR | <0.01 | <0.01 | ref | |
| Positive | 131 | 28.3 | | | 2.59 | (1.78-3.78) |
| UICC M factor | | | | | | |

(Continues)

**TABLE 2** (Continued)

| | | | Univariate | Multivariate | | |
|---|---|---|---|---|---|---|
| **Characteristics** | ***n*** | **MST (months)** | ***P*-value** | ***P*-value** | **HR** | **(95%CI)** |
| Negative | 288 | 51.6 | <0.01 | 0.35 | | |
| Positive | 11 | 18.5 | | | | |

MST, median overall survival time; Dif$^{whole}$, predominant differentiation of whole primary tumor; Dif$^{inv}$, differentiation at the invasive front; Cell$^{inv}$, cellularity at the invasive front; CI, confidence interval; UICC, Union for International Cancer Control; NR, not reached; ref, reference arm. The level of significance was set at $P < 0.05$.

### 3.3 | Overall survival analysis

For all 299 patients, 1-, 3-, and 5-year overall survival rates were 87%, 58%, and 45%, respectively. Median overall survival time (MST) was 51.5 months for all 299 patients, 31.4 months for Dif$^{inv}$G3 patients, and 108.0 months for Dif$^{inv}$G1/2 patients. The MST of patients with Dif$^{inv}$G3 was significantly shorter than that of patients with Dif$^{inv}$G1/2 (Figure 3A) On multivariate analysis, the independent predictors of a poor prognosis were Dif$^{inv}$G3, Dif$^{whole}$G3, tumor-budding positive, presence of vascular invasion, and nodal metastasis-positive (Table 2).

### 3.4 | Relapse-free survival analysis

One-, 3-, and 5-year relapse-free survival rates were 67%, 43%, and 35%, respectively. The median relapse-free survival time (MRFS) was 24.1 months for all 299 patients, 12.1 months for Dif$^{inv}$G3 patients, and 63.3 months for Dif$^{inv}$G1/2 patients. The MRFS was significantly shorter for patients with Dif$^{inv}$G3 than for patients with Dif$^{inv}$G1/2 (Figure 3B). On multivariate analysis, the independent predictors of early relapse were microscopically positive resection margin, Dif$^{inv}$G3, Dif$^{whole}$G3, presence of vascular invasion, and nodal metastasis-positive (Table 3).

### 3.5 | Subgroup analysis

The prognostic impact of Dif$^{inv}$G3 was re-evaluated by stratification using Dif$^{inv}$G3-related factors, including nodal metastasis, vascular invasion, tumor location, and Dif$^{whole}$G3. Survival curve analysis showed that the prognostic values of Dif$^{inv}$G3 were maintained in nodal metastasis-positive patients (Figure 4), patients with vascular invasion, and those with Dif$^{whole}$G1/2. In each location of primary tumor, Dif$^{inv}$G3 was also associated with shortened overall survival and relapse-free survival compared to Dif$^{inv}$G1/2 in patients with ECC (Supplemental Figure S1B and S2B), in patients with GB (Supplemental Figure S1C and S2C), and in patients with Va (Supplemental Figure S1D and Figure S2D). There were tendencies for differences in OS and RFS between Dif$^{inv}$G3 and Dif$^{inv}$G1/2 patients with ICC (Supplemental Figure S1A and S2A).

## 4 | DISCUSSION

This study showed that the existence of poorly differentiated adenocarcinoma at the invasive front is an independent prognostic factor for nodal metastasis in all BTC patients who underwent curative resection. In previous studies and the NCCN guideline, nodal metastasis was reported to be an important prognostic factor for all BTC patients.[6–10,23] On the other hand, there are no common invasive behaviors that predict prognosis in all BTC patients. In recent studies with 200 or more BTC patients, the independent prognostic factors for nodal metastasis were tumor size, vascular invasion, and intrahepatic metastasis in ICC, differentiation, liver invasion, and pancreas invasion in ECC, TNM stage in GB, and perineural invasion in Va.[6,8,10–12,23–25] There were no common independent prognostic factors for nodal metastasis in all BTC patients. This study showed that assessment of Dif$^{inv}$ was useful in the detailed evaluation of prognosis. It is possible that Dif$^{inv}$ classification could be useful for managing resectable BTC patients and to allocate patients in clinical trials.

The UICC T factor should represent prognostic invasive behavior as a primary factor. However, the UICC T factor was not an independent prognostic factor in the present study and in many previous studies.[8,10–12,25] For the precise assessment of prognosis in surgical pathology, it is necessary to identify a prognostic factor related to locoregional invasive behavior of the primary tumor. According to the results of the present study, Dif$^{inv}$ should become a part of the T classification because it is an invasive behavior of primary tumor and an independent prognostic factor for nodal metastasis.

The present study showed that Dif$^{inv}$G3 was an independent predictor of early relapse with nodal metastasis. Dif$^{inv}$G3 was associated with metastasis-related factors such as high frequency of vascular invasion, nodal metastasis, and distant metastasis. The frequency of relapse was significantly higher in Dif$^{inv}$G3 patients than in Dif$^{inv}$G1/2 patients (data not shown). Dif$^{inv}$G3 was a predictor of poor prognosis because it was highly correlated with recurrence. This study is the first to show that Dif$^{inv}$G3 is a prognostic factor and an early relapse factor.

The reason why Dif$^{inv}$G3 promotes tumor metastasis has not been reported. In the present study, about 40% of Dif$^{whole}$G1/2 cases had Dif$^{inv}$G3. In Dif$^{whole}$G1/2 patients, Dif$^{inv}$G3 was also associated with a significantly higher frequency of relapse (data not shown), as well as worse relapse-free survival and overall survival. These results showed that dedifferentiation at the invasive front and ability to metastasize are correlated. To metastasize, tumors pass

**TABLE 3** Analyses of relapse-free survival in BTC patients

| | | | Univariate | Multivariate | | |
|---|---|---|---|---|---|---|
| **Characteristics** | ***n*** | **MRFS (months)** | ***P*-value** | ***P*-value** | **HR** | **(95%CI)** |
| Sex | | | | | | |
| Male | 200 | 24.4 | 0.81 | 0.27 | | |
| Female | 99 | 21.6 | | | | |
| Age (y) | | | | | | |
| <70 | 164 | 24.3 | 0.99 | 0.47 | | |
| ≥70 | 135 | 21.5 | | | | |
| Period | | | | | | |
| 2000-2006 | 133 | 24.4 | 0.68 | | | |
| 2007-2012 | 166 | 23.4 | | | | |
| Lymphadenectomy | | | | | | |
| Yes | 268 | 22.2 | 0.63 | | | |
| No | 31 | 33.7 | | | | |
| Resection margin | | | | | | |
| R0 | 261 | 24.4 | 0.01 | 0.01 | ref ~~1.71~~ | |
| R1 | 38 | 15.8 | | | 1.71 | (1.13-2.59) |
| $Dif^{inv}$ | | | | | | |
| G1/G2 | 158 | 63.3 | <0.01 | <0.01 | ref | |
| G3 | 141 | 12.1 | | | 1.75 | (1.21-2.54) |
| $Dif^{whole}$ | | | | | | |
| G1/G2 | 259 | 27.5 | <0.01 | 0.03 | ref | |
| G3 | 40 | 8.9 | | | 1.61 | (1.05-2.47) |
| $Cell^{inv}$ | | | | | | |
| Low | 168 | 31.9 | 0.01 | 0.65 | | |
| High | 131 | 15.8 | | | | |
| Tumor budding | | | | | | |
| Negative | 270 | 25.5 | <0.01 | 0.15 | | |
| Positive | 29 | 9.8 | | | | |
| Lymphatic invasion | | | | | | |
| Absent | 154 | 31.9 | 0.17 | | | |
| Present | 145 | 20.5 | | | | |
| Vascular invasion | | | | | | |
| Absent | 120 | 64.4 | <0.01 | 0.02 | ref | |
| Present | 179 | 15.8 | | | 1.50 | (1.07-2.10) |
| Perineural invasion | | | | | | |
| Absent | 114 | 42.3 | 0.01 | 0.88 | | |
| Present | 185 | 20.2 | | | | |
| UICC T factor | | | | | | |
| T1-2 | 167 | 33.8 | 0.01 | 0.61 | | |
| T3-4 | 132 | 18.1 | | | | |
| Nodal metastasis | | | | | | |
| Negative | 168 | 56.3 | <0.01 | <0.01 | ref | |
| Positive | 131 | 14.1 | | | 2.19 | (1.58-3.05) |
| UICC M factor | | | | | | |

(Continues)

**TABLE 3** (Continued)

| Characteristics | *n* | MRFS (months) | Univariate *P*-value | Multivariate *P*-value | HR | (95%CI) |
|---|---|---|---|---|---|---|
| Negative | 288 | 24.4 | <0.01 | 0.11 | | |
| Positive | 11 | 9.8 | | | | |

MRFS, median relapse-free survival time; $Dif^{whole}$, predominant differentiation of whole primary tumor; $Dif^{inv}$, differentiation at the invasive front; $Cell^{inv}$, cellularity at the invasive front; CI, confidence interval; UICC,Union for International Cancer Control; ref, reference. The level of significance was set at $P < 0.05$.

through some steps: proliferation of primary tumor, detachment and invasion to the vasculature, and migration and invasion to metastatic sites.[27] Some studies reported a correlation between detachment of tumor and dedifferentiation.[28,29] In general, epithelial cells normally have apico-basal polarity and basolateral membrane domains attached to extracellular matrix.[30,31] If a cell becomes detached from the extracellular matrix, apoptosis triggers so-called anoikis.[32] Anoikis ensures that cells that have migrated or are in inappropriate locations are eliminated, and it prevents dysplastic growth.[33] Previous studies have reported that tropomyosin-related receptor kinase B (TrkB) was overexpressed in aggressive tumor cells, and TrkB activated the PI3K-AKT pathway, which inhibits caspase-9, causing anoikis.[34,35] Anoikis resistance allows cancer cells to survive when detached from the extracellular matrix and to be able to invade into the vascular system.[36] Tumor cells that acquire anoikis-resistance form an epithelial-mesenchymal transition-like phenotype, in which cells partially de-differentiate and show loss of polarity.[29] TrkB has also been reported to cause tumor morphology to be round in a mouse model.[37] Tanaka et al reported that the poorly differentiated component or dedifferentiated tumor cells may be regarded as anoikis-resistant.[38] $Dif^{inv}$G3 may acquire anoikis resistance and metastasize in BTC patients.

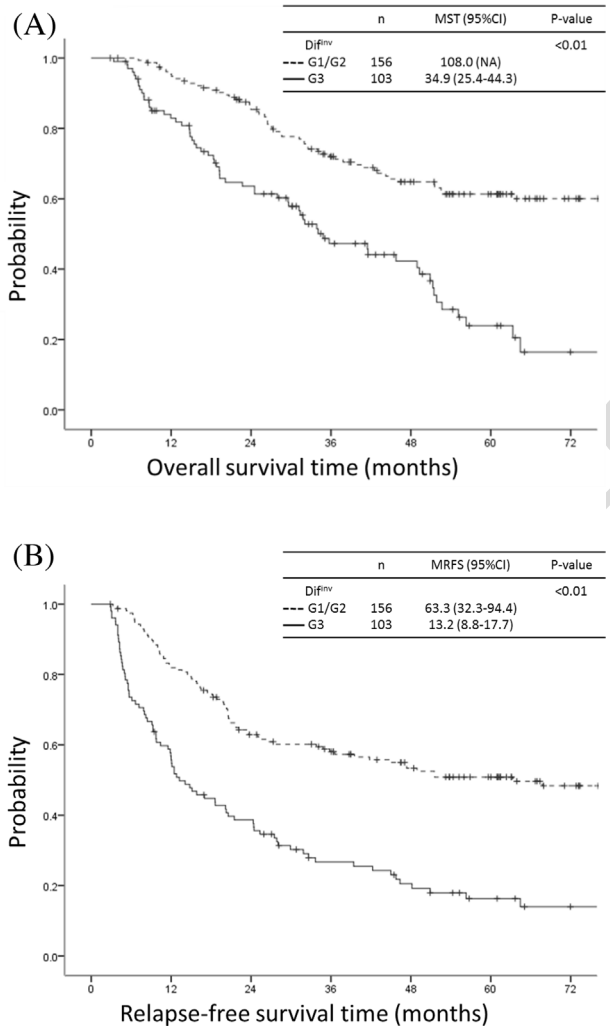


**FIGURE 4** Survival curves of BTC patients with G1/2 differentiation of the whole tumor ($n = 159$) classified by the differentiation at the invasive front of the primary tumor. A, Overall survival time, (B) Relapse-free survival time. BTC: biliary tract cancer; $Dif^{inv}$: differentiation at the invasive front; MST: median overall survival time; MRFS: Median relapse-free survival time; CI: Confidence interval; NA: Not available

There are a few limitations associated with this study. First, it was a retrospective study at a single institution, with no validation analysis. There is a possibility that the time required to diagnose tumor relapse was different for each attending doctor because the surveillance method at the time of suspected tumor relapse may differ by doctor. A validation analysis using common surveillance in a multi-center prospective study is needed in the future. Second, in ICC patients, $Dif^{inv}$G3 was not significantly associated with OS and RFS. However, OS and RFS showed substantial differences between $Dif^{inv}$G3 and $Dif^{inv}$G1/2 patients with ICC (MST 34.8 months vs NA; MRFS 8.0 months vs 10.2 months). $Dif^{inv}$G3 is considered to be useful even in ICC patients.

In conclusion, the present study demonstrated that $Dif^{inv}$G3 is an important prognostic factor for nodal metastasis in all BTC patients and has prognostic value for each primary site. Evaluation of $Dif^{inv}$ is convenient and provides useful information for predicting poor outcomes and can help in the management of patients after surgical resection.

## ACKNOWLEDGMENTS

This work was supported by Grants-in-aid for Cancer Research and for the Third-term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan;

JSPS KAKENHI Grant Number 22790624; the National Cancer Research and Develop Fund (23-A-2b)

## CONFLICTS OF INTEREST

None.

## ORCID

*Satoshi Okubo* http://orcid.org/0000-0003-3010-7776

## REFERENCES

1. de Jong MC, Nathan H, Sotiropoulos GC, et al. Intrahepatic cholangiocarcinoma: an international multi-institutional analysis of prognostic factors and lymph node assessment. *J Clin Oncol*. 2011;29:3140–3145.
2. Jarnagin WR, Fong Y, DeMatteo RP, et al. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg*. 2001;234:507–517. discussion 517–19.
3. Hong SM, Pawlik TM, Cho H, et al. Depth of tumor invasion better predicts prognosis than the current American Joint Committee on Cancer T classification for distal bile duct carcinoma. *Surgery*. 2009;146:250–257.
4. Lim H, Seo DW, Park DH, et al. Prognostic factors in patients with gallbladder cancer after surgical resection: analysis of 279 operated patients. *J Clin Gastroenterol*. 2013;47:443–448.
5. Talamini MA, Moesinger RC, Pitt HA, et al. Adenocarcinoma of the ampulla of Vater. A 28-year experience. *Ann Surg*. 1997;225:590–599. discussion 599-600.
6. Li T, Qin LX, Zhou J, et al. Staging, prognostic factors and adjuvant therapy of intrahepatic cholangiocarcinoma after curative resection. *Liver Int*. 2014;34:953–960.
7. Aoba T, Ebata T, Yokoyama Y, et al. Assessment of nodal status for perihilar cholangiocarcinoma: location, number, or ratio of involved nodes. *Ann Surg*. 2013;257:718–725.
8. Kiriyama M, Ebata T, Aoba T, et al. Prognostic impact of lymph node metastasis in distal cholangiocarcinoma. *Br J Surg*. 2015;102:399–406.
9. Pilgrim CH, Groeschl RT, Turaga KK, et al. Key factors influencing prognosis in relation to gallbladder cancer. *Dig Dis Sci*. 2013;58:2455–2462.
10. Hsu CH, Chen TD, Tsai CY, et al. Prognostic value of the metastatic lymph node ratio in patients with resectable carcinoma of ampulla of Vater. *Medicine (Baltimore)*. 2015;94:e1859.
11. Tang L, Tan YX, Jiang BG, et al. The prognostic significance and therapeutic potential of hedgehog signaling in intrahepatic cholangiocellular carcinoma. *Clin Cancer Res*. 2013;19:2014–2024.
12. Lee HJ, Chung JY, Hewitt SM, et al. HER3 overexpression is a prognostic indicator of extrahepatic cholangiocarcinoma. *Virchows Arch*. 2012;461:521–530.
13. Liu B, Zhang Y, Liao M, et al. Clinicopathologic and prognostic significance of CD24 in gallbladder carcinoma. *Pathol Oncol Res*. 2011;17:45–50.
14. Ito H, Ito K, D'Angelica M, et al. Accurate staging for gallbladder cancer: implications for surgical therapy and pathological assessment. *Ann Surg*. 2011;254:320–325.
15. Koelzer VH, Zlobec I, Lugli A. Tumor budding in colorectal cancer-ready for diagnostic practice?. *Hum Pathol*. 2016;47:4–19.
16. Nilsson PJ, Rubio C, Lenander C, et al. Tumour budding detected by laminin-5 {gamma}2-chain immunohistochemistry is of prognostic value in epidermoid anal cancer. *Ann Oncol*. 2005;16:893–898.
17. Koike M, Kodera Y, Itoh Y, et al. Multivariate analysis of the pathologic features of esophageal squamous cell cancer: tumor budding is a significant independent prognostic factor. *Ann Surg Oncol*. 2008;15:1977–1982.
18. Eefsen RL, Van den Eynden GG, Hoyer-Hansen G, et al. Histopathological growth pattern, proteolysis and angiogenesis in chemonaive patients resected for multiple colorectal liver metastases. *J Oncol*. 2012;2012:907971.
19. Van den Eynden GG, Bird NC, Majeed AW, et al. The histological growth pattern of colorectal cancer liver metastases has prognostic value. *Clin Exp Metastasis*. 2012;29:541–549.
20. International Union Against Cancer (UICC) *TNM classification of malignant tumors*, 7th edn. New York: Wiley-Liss; 2009.
21. Hamilton RS, Altonen AL. eds. *World Health Organization Classification of Tumours. Pathology and Genetics of Tumors of the Digestive System*. Lyon: IARC Press; 2000.
22. Kai K, Kohya N, Kitahara K, et al. Tumor budding and dedifferentiation in gallbladder carcinoma: potential for the prognostic factors in T2 lesions. *Virchows Arch*. 2011;459:449–456.
23. NCCN Clinical Practice Guidelines in Oncology Hepatobiliary Cancers v2. 2016. June 27, 2016. Available at: http://www.nccn.org/professionals/physician_gls/pdf/hepatobiliarypdf. Accessed August 1, 2016.
24. Chen Q, Dai Z, Yin D, et al. Negative impact of preoperative platelet-lymphocyte ratio on outcome after hepatic resection for intrahepatic cholangiocarcinoma. *Medicine (Baltimore)*. 2015;94:574.
25. Ito K, Ito H, Allen PJ, et al. Adequate lymph node assessment for extrahepatic bile duct adenocarcinoma. *Ann Surg*. 2010;251:675–681.
26. Wang RT, Zhang LQ, Mu YP, et al. Prognostic Q5significance of preoperative platelet count in patients with gallbladder cancer. *World J Gastroenterol*. 2015;21:5303–5310.
27. Guan X. Cancer metastases: challenges and opportunities. *Acta Pharm Sin B*. 2015;5:402–418.
28. Korita PV, Wakai T, Ajioka Y, et al. Aberrant expression of vimentin correlates with dedifferentiation and poor prognosis in patients with intrahepatic cholangiocarcinoma. *Anticancer Res*. 2010;30:2279–2285.
29. Klymkowsky MW, Savagner P. Epithelial-mesenchymal transition: a cancer researcher's conceptual friend and foe. *Am J Pathol*. 2009;174:1588–1593.
30. Lee M, Vasioukhin V. Cell polarity and cancer-cell and tissue polarity as a non-canonical tumor suppressor. *J Cell Sci*. 2008;121:1141–1150.
31. Khursheed M, Bashyam MD. Apico-basal polarity complex and cancer. *J Biosci*. 2014;39:145–155.
32. Frisch SM, Ruoslahti E. Integrins and anoikis. *Curr Opin Cell Biol*. 1997;9:701–706.
33. Guadamillas MC, Cerezo A, Del Pozo MA. Overcoming anoikis-pathways to anchorage-independent growth in cancer. *J Cell Sci*. 2011;124:3189–3197.
34. Thiele CJ, Li Z, McKee AE. On Trk-the TrkB signal transduction pathway is an increasingly important target in cancer biology. *Clin Cancer Res*. 2009;15:5962–5967.
35. Yu X, Liu L, Cai B, et al. Suppression of anoikis by the neurotrophic receptor TrkB in human ovarian cancer. *Cancer Sci*. 2008;99:543–552.
36. Paoli P, Giannoni E, Chiarugi P. Anoikis molecular pathways and its role in cancer progression. *Biochim Biophys Acta*. 2013;1833:3481–3498.
37. Douma S, Van Laar T, Zevenhoven J, et al. Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. *Nature*. 2004;430:1034–1039.
38. Tanaka K, Shimura T, Kitajima T, et al. Tropomyosin-related receptor kinase B at the invasive front and tumour cell

dedifferentiation in gastric cancer. *Br J Cancer*. 2014;110: 2923–2934.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Okubo S, Mitsunaga S, Kato Y, et al. The prognostic impact of differentiation at the invasive front of biliary tract cancer. *J Surg Oncol*. 2017;1–10. https://doi.org/10.1002/jso.24946

## SYNOPSIS

We investigated the Q6prognostic morphological factors at the invasive front including tumor differentiation in biliary tract cancer (BTC). Poor differentiation at the invasive front of tumor was associated with poor prognosis and early relapse in BTC patients. Evaluation of tumor differentiation at the invasive front can help manage BTC patients after surgical resection.