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Tumor invasion front morphology: a novel prognostic factor for intrahepatic cholangiocarcinoma

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Abstract. – OBJECTIVE: To explore the prognostic value of the morphology of tumor invasion front (TIF) in Intrahepatic Cholangiocarcinoma (ICC).

PATIENTS AND METHODS: Seventy-four ICC patients with complete clinicopathological data and follow-up information were enrolled in our study. The most typical morphology of TIF of each case will be classified as low-grade group or high-grade group after evaluation by two pathologists. The clinicopathological characteristics, disease-free survival (DFS), and overall survival (OS) were compared between the two groups.

RESULTS: A total of 26 (35.3%) patients were assigned to low-grade group, while 48 (64.7%) patients were assigned to high-grade group. High-grade group was associated with higher CA19-9 (p=0.032), poor differentiation (p=0.050), larger tumor diameter (p=0.016), advanced T staging (p=0.048), and higher incidence of lymph node (LN) metastasis (0.014). No significant associations were found in demographic and clinical characteristics between the two groups. On multivariable analysis, high-grade group was a significant independent predictor of worse DFS (HR=0.433, 95% CI=0.235-0.800, p=0.002) and OS (HR=0.363, 95% CI=0.187-0.704, p=0.003).

CONCLUSIONS: High-grade morphology of TIF was an independent prognostic factor of ICC.

Key Words:
Tumor invasion front (TIF), Morphology, Tumor budding (TB), Intrahepatic Cholangiocarcinoma (ICC), Prognosis.

Introduction

As one of the most common malignant primary liver tumor after hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) has shown a striking increase in the incidence of morbidity over the last 20 years[1,2]. However, most patients are diagnosed in the terminal stage due to untypical symptoms and no more than 35% of them can receive effective treatment[3]. Surgical resection is still believed to be the most effective management for ICC patients[4]. But even with radical resection, the reported outcome of 5-year survival rate is still not encouraging. Given the suboptimal management of ICC and the limited progress in adjuvant treatment, an additional and reliable prognostic predicator is urgently needed to provide a guide towards a more active and effective treatment.

Recently, the American Joint Committee on Cancer and International Union against Cancer (AJCC/UICC)[5] listed tumor border configuration and tumor budding (TB) as "additional" prognostic factors for colorectal cancer[6]. Tumor invasion front (TIF) is the region where tumor tissue meets non-tumor tissue, and it is the most active region for tumor cells infiltration and invasion. Compton[6] suggests that tumor cells at the invasive front accelerate the process of tumor invasion. Therefore, the biological behavior of tumor cells at TIF have attracted more and more concerns, because it is able to indicate the invasiveness of the primary tumor to some extent[7-10].

TIF has diverse morphology. It can be smooth, regular, tortuous, and even accompanied by the growth of TB. We speculated that the changes in the morphology of TIF may lead to different prognosis based on the poor prognosis of ICC. With that in mind, this retrospective study was aimed to evaluate the morphology of TIF in ICC and to explore its prognostic value in correlation with other established prognostic factors.

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Patients and Methods

Patients and Specimen Characteristics

Seventy-four non-consecutive ICC patients were enrolled in this study between January 2011 and December 2016. They underwent a primary attempt of a radical resection for ICC in our department, including 39 males and 35 females. Among the 74 ICC patients, 55 (74.3%) were from Henan, 8 (10.8%) were from Shandong, 4 (5.4%) were from Shanxi, 3 (4.1%) were from Hebei, and the remaining 4 were from other cities. All histomorphological and clinical data were retrospectively analyzed from the corresponding hematoxylin-eosin (HE) stained slides and medical reports, respectively. Histological analysis of all resected specimens was performed to assess tumor diameter, number, morphology, differentiation, margin, vascular and biliary invasion, lymph node status, and adjacent organ invasion. Pathologic stage was assigned based on the 8th edition of the American Joint Committee on Cancer (AJCC) guidelines[11]. Our study was approved by the Clinical Ethics Committee of the Henan Provincial People's Hospital. Since this study is a retrospective study, patient data were analyzed anonymously without the written consent of all participants.

Assay Methods

Assessment of morphology of TIF and TB

The smoothness of TIF and statistics of TB were included in the evaluation of the morphology of TIF by two experienced pathologists, and the evaluation of TB was performed as previously described[12]. First, all HE staining whole tissue slides were screened through morphological observation to the deepest infiltration of tumor under low magnification (50×). Slides with the most typical morphology of TIF or containing the highest density of buds were selected for further observation (including the evaluation of morphology and the statistics of TB) under high magnification (200×). TB was defined as a single or a cluster of < 5 tumor cells at the invasion front. The morphology of TIF, which is regular and has no tumor bud, was classified as A. The front without tumor bud but irregular was classified as B. C was defined as an average of < 5 buds, no matter whether the tumor invasion front was regular or not. The number of buds ≥ 5 or a large number of cancer cells growing into normal liver tissue were classified as D. A and B were classified as low-grade group, C and D were classified as high-grade group.

Follow-up

After discharge, all patients received routine appointment out-patient service, returning visit or telephone consultation. During the postoperative follow-up, all patients were regularly followed with laboratory examinations like serum carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), and imaging studies, including ultrasound, CT and/or magnetic resonance imaging (MRI). The primary endpoints of follow-up were disease-free survival (DFS) and overall survival (OS). DFS was defined as the time from initial resection to tumor recurrence. OS was defined as the time from initial resection to patient death or the end of the study. Recurrence was defined as the appearance of new lesions identified by CT scan or ultrasound with no definite evidence of metastasis from other cancers or recurrence elsewhere. Rational treatment options for recurrent patients were ensured according to their specific conditions, which mainly included surgical re-resection, ablation, or combined resection plus ablation.

Statistical Analysis

Statistical analyses were conducted using SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA). $p<0.05$ was considered statistically significant. The Mann-Whitney U-test was used to describe the normality of each continuous parameter's distribution. χ^2-test was used for the analysis of the categorical variables in the low-grade and high-grade groups. Quantitative results were expressed as the mean ± standard deviation. The associations between clinical and histopathological parameters with DFS and OS were analyzed by the Kaplan-Meier curves and compared by the log-rank test. Multivariable Cox proportional hazard models (backward conditional stepwise) were used to adjust for different risk factor distributions between the groups. The variables with p-value less than 0.05 were included in multivariate logistic regression analysis to determine independent variables on the basis of univariate analysis. Hazard ratios (HRs) of each independent variable were estimated by the Cox analysis and shown as relative risks with corresponding 95% confidence intervals (CIs).

Results

Baseline Characteristics

All ICC patients were treated with radical resection and the diagnoses were ultimately confirmed both clinically and pathologically. From the 99 ICC patients initially selected, 9 cases were excluded for the lack of the clinical data, and 7 cases were excluded for the preoperative treatment of transcatheter arterial chemoembolization (TACE) or ultrasound-guided radiofrequency ablation. We followed up the remaining 83 patients and obtained complete follow-up data from 74 of the patients. Of the enrolled 74 ICC cases, 59 (79.7%) were mass-forming type, 5 (6.7%) were periductal-infiltrating type, 7 (9.5%) were intraductal-growth type, and 3 (4.1%) were mixed type. At the end of the follow-up, 60 patients died, and 49 (82%) of them had a specific cause of death, including 38 patients with recurrence and/or metastasis. According to the scoring system (Figure 1), 26 patients were assigned to low-grade group, and the remaining 48 patients were determined to be high-grade group. The quantitative clinical characteristics are shown in Table I, and the categorical clinical factors are summarized in Table II.

Clinical Parameters Associated with Morphological of Tumor Invasion Front in Low-Grade and High-Grade ICCs

As shown in Table I, the levels of tumor marker CA19-9 was significantly higher in high-grade group than low-grade group ($p<0.001$). Although not statistically significant, patients in the high-grade group tend to develop larger tumors ($p=0.053$). In addition, no significant differences were observed in terms of the other quantitative clinical parameters, including age, albumin, CEA, AFP, platelets, lymphocytes, and neutrophils. Comparisons of the categorical

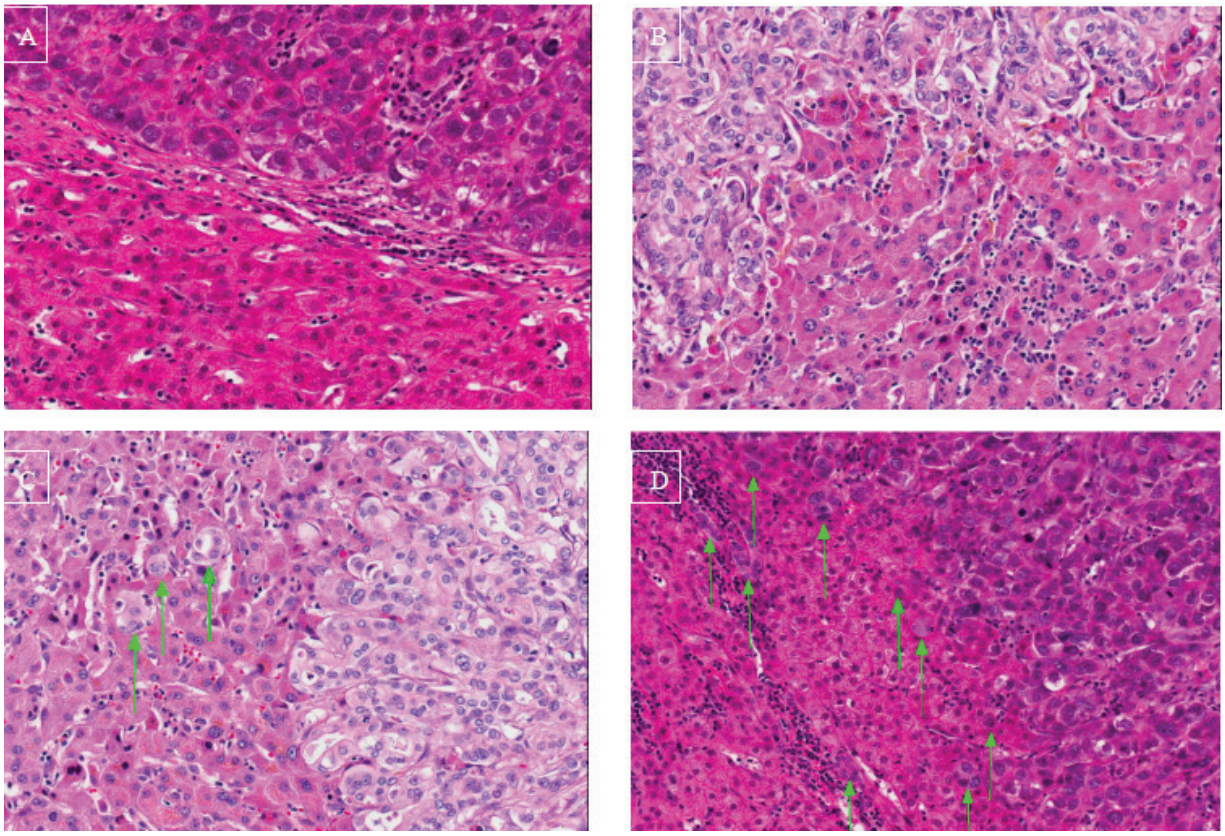

Figure 1. A, Low-grade group: the tumor invasion front, which is regular and has no tumor bud is classified as A. **B,** Low-grade group: the invasion front without TB but irregular is classified as B. **C,** High-grade group: C is defined as an average of < 5 buds (arrows), no matter whether the tumor invasion front is smooth or not. **D,** High-grade group: the number of buds ≥5 (arrows) or a large number of cancer cells growing into normal liver tissue are classified as D. [200× magnification, HE-stained sections. **(A-D)**]. (arrows).

Table I. Comparison of quantitative clinical factors between high-grade group and low-grade group.

Factors	Low-grade group		High-grade group		*p*-value
	Mean	Standard deviation	Mean	Standard deviation	
Age (years)	65.62	10.83	61.55	8.96	0.881
Albumin (g/dL)	38.46	5.44	38.12	4.91	0.787
CEA (ng/mL)	5.65	7.28	3.22	3.65	0.057
AFP (U/mL)	5.83	4.88	14.09	28.93	0.064
CA125 (U/mL)	25.21	26.34	19.72	17.68	0.293
CA19-9 (U/mL)	468.25	654.31	6678.45	8542.11	<0.001
Platelet ($\times 10^3$/mL)	237.28	59.60	228.71	61.71	0.573
Lymphocyte ($\times 10^3$/mL)	1.22	0.47	1.46	0.53	0.058
Neutrophil ($\times 10^3$/mL)	3.65	1.58	3.84	1.66	0.631
Diameter (cm)	4.38	2.24	3.65	1.25	0.053

variables in the two groups indicated that high-grade group was more likely to develop tumors with higher CA19-9 (p=0.032), larger tumor diameter (p=0.016), advanced stage (p=0.048) and higher risks of LN metastasis (0.014) (Table II). Moreover, the differentiation appeared to be higher in the low-grade group, almost reaching statistical significance (p=0.050). No significant differences were found between the two groups with respect to age, gender, CEA, AFP, tumor location, T Stage, LN metastasis or AJCC staging (Table II).

Comparison of Clinical Variables Related to DFS After Operation

On univariate analysis, CA19-9, differentiation, diameter, T stage, and classification of morphology of TIF were all independent risk factors for DFS after curative resection (all p<0.05, Table III). The above variables were selected for multivariate analysis. The results showed that T stage (HR=0.379, 95% CI 0.208-0.694, p=0.002) and high-grade group (HR=0.433, 95% CI 0.235-0.800, p=0.008) were all significant independent predictors of

Table II. Univariate analysis of clinical characteristics according to the morphology of TIF.

Characteristics		Low-grade group (N = 26)	(%)	High-grade group (N = 48)	(%)	*p*-value
Gender	Male	12	46	27	56	0.406
	Female	14	54	21	44	
Age (years)	<55	8	31	13	27	0.737
	≥55	18	69	35	73	
CEA (ng/mL)	<5	16	62	25	60	0.435
	≥5	10	38	23	40	
CA19-9 (U/mL)	<39	4	15	19	40	0.032
	≥39	22	85	29	60	
AFP (ng/mL)	<25	24	92	43	90	0.702
	≥25	2	8	5	10	
Location	Left liver	14	54	18	37	0.175
	Right liver	12	46	30	63	
Differentiation	Well to moderate	17	65	20	42	0.050
	Poor	9	35	28	58	
Diameter (cm)	≤5	15	58	14	79	0.016
	>5	11	42	34	21	
T stage	1	16	62	18	38	0.048
	2-3	10	38	30	62	
LN metastasis	Negative	17	65	17	35	0.014
	Positive	9	35	31	65	

DFS (Table III). The predictive value of high-grade group was also determined in the DFS curve (Figure 2).

Comparison of the Clinical Variables Related to OS After Liver Resection

On univariate analysis, CEA, CA19-9, differentiation, diameter, T stage, LN metastasis, and classification of morphology of TIF were all significant prognostic factors for OS (all $p<0.05$, Table III). The multivariate analysis results showed that a higher T stage (HR=0.261, 95% CI 0.134-0.508, $p=0.000$), lymph node metastasis (HR=0.458, 95% CI 0.244-0.861, $p=0.015$), and high-grade group (HR=0.365, 95% CI 0.179-0.711, $p=0.003$) were all significant independent predictors of a worse OS (Table III). The poor prognosis of high-grade group was also shown in the OS curve (Figure 3).

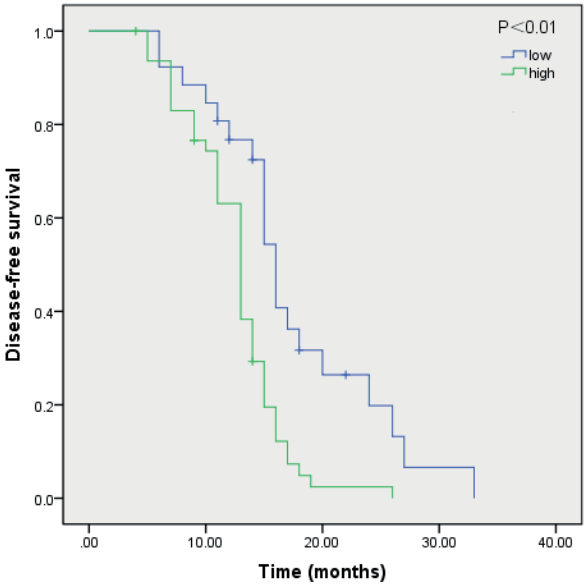

Figure 2. Kaplan-Meier curve showing that the high-grade group had a major adverse effect on the DFS (p = 0.008) of the patients.

Discussion

Tumor morphology study has always been an important way for the exploration of tumor characteristics. For example, the study of the general morphology of tumors conducive to the better classification of pathomorphology, and the morphological study of tumor cells favorable for the recognition of differentiation of tumor cells, which is of great significance for the treatment of tumors and the judgment of prognosis. However, there's limited research so far conducted on the morphology of TIF in ICC regardless of the desperate need for additional effective prognostic predicators. The novelty of this study lies in the classification of morphology of TIF combined with TB and our results underlined that the high-grade group was closely correlated with highly unfavorable prognosis.

Even after radical resection, the outcome of ICC patients is still not optimistic. Numerous histological studies[13-16] have suggested that, several prognostic factors could affect the outcomes of ICC patients who undergo surgical resection, and similar factors were also mentioned in our study. The primary tumor diameter, differentiation degree, and presence of lymph node metastases appear to be important risk factors of ICC even after radical resection. Although not mentioned in the 7th edition of AJCC guidelines, tumor diameter is still believed to be an important prognostic factor of tumor behavior, which is in accordance with our results. Accordingly, tumor diameter has been embedded in the TNM staging system again by the 8th edition of AJCC guidelines for ICC. Our findings suggest that ICC patients in high-grade group were more likely to develop tumors with poor differentiation, larger diameter, advanced T stage, and higher risks of LN metastasis. Since TB is restricted to the high-grade group, these aggressive clinicopathological features of ICC may be closely related to TB.

TB was first reported in colorectal carcinoma, and it was significantly related with poor differentiation, lymphatic and microvascular invasion, local recurrence and distant metastasis of patients with colorectal carcinoma[17-22]. Due to the nature of infiltrative growth, TB was thought to be with several dynamic and reversible characteristics of cancer stem cells[23]. In addition, TB is a mostly accepted favorable prognostic factor in other digestive system malignant tumors, including oesophageal, gastric ampullary, and pancreatic cancer[24-28]. It is noteworthy that our findings are supportive of the unfavorable prognostic value of TB reported in other malignant tumors

The dissemination of tumor cells *via* lymphatic system is a typical feature of ICC, which is rarely observed in primary HCC. TB should not be simply understood as a deviation of tu-

Table III. Univariate and multivariate analysis of clinicopathologic variables in relation to DFS and OS after curative operation.

			Disease Free Survival			Overall Survival		
				Multivariate analysis			Multivariate analysis	
Parameter		N	Univariate analysis *p*-value	HR (95% CI)	*p*-value	Univariate analysis *p*-value	HR (95% CI)	*p*-value
Gender	Male	39	0.967		0.641			
	Female	35						
Age (years)	<55	21	0.827		0.398			
	≥55	53						
CEA (ng/mL)	<5	41	0.182		0.024	1		0.671
	≥5	33					1.146 (0.610-2.153)	
CA19-9 (U/mL)	<39	23	0.010	1	0.764	0.006	1	0.715
	≥39	51		0.903 (0.465-1.756)			1.036 (0.416-1.825)	
AFP (ng/mL)	<400	67	0.146			0.176		
	≥400	7						
Location	Left liver	32	0.364			0.205		
	Right liver	42						
Differentiation	Well to moderate	40	0.041	1	0.600	0.039	1	0.908
	Poor	34		0.861 (0.492-1.506)			1.036 (0.569-1.866)	
Diameter (cm)	≤5	29	0.020	1	0.214	0.004	1	0.017
	>5	45		0.694 (0.390-1.235)			0.462 (0.244-0.873)	
T stage	1	42 (34)	<0.001	1	0.002	0.001	1	<0.001
	2-3	32 (40)		0.379 (0.208-0.694)			0.261 (0.134-0.508)	
LN metastasis	Negative	34	0.295			0.016	1	0.015
	Positive	40					0.458 (0.244-0.861)	
Morphology classification	Low-grade group	26	0.002	1	0.008	0.019	1	0.003
	High-grade group	48		0.433 (0.35-0.800)		0.356 (0.179-0.711)		

Abbreviations: LN = lymph node; HR = hazard ratio; CI = confidence interval.

mor cells but the first step of tumor progression from local to distal metastasis through its association with the presence of lymphatic invasion. Moreover, our study found that the prognostic value of TIF morphology is noteworthy in ICC patients, almost showing a comparable ability to T staging. Additionally, TB seemed to have an independent negative influence on survivals, since patients in the high-grade group had both shorter DFS and OS.

However, the mechanism of TB has not been fully illuminated. Relevant studies indicate that the development from highly differentiated epithelial mucosal cells to invasive phenotypes has been proposed to represent the epithelial-mesenchymal transition (EMT)[29]. In the EMT process, a series of changes in molecular mechanism resulting in the loss of cell-to-cell adhesion may lead to the occurrence of tumor development, invasion, and metastasis. As a de-differentiation feature occurring at TIF, TB was considered as the initial status of tumor infiltration.

The retrospective nature and the small sample size from a single center are potential limitations of our study. In spite of this, our report benefits from complete clinicopathological data and follow-up information. Besides, the use of

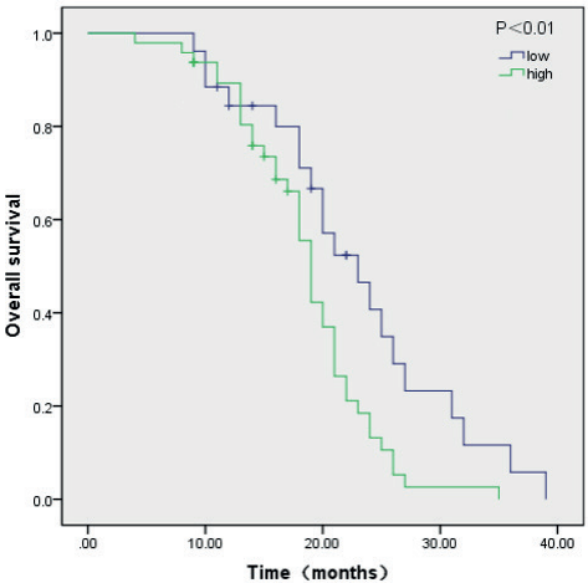

Figure 3. Kaplan-Meier curve showing that the high-grade group had a major adverse effect on the OS (p = 0.003) of the patients.

the whole tissue sections to find the most typical TIF and the utilize of morphology of TIF combined with TB are all critical factors for classification. However, in order to get better application in daily clinical diagnosis and treatment, the larger samples from multicenter studies are needed to further validate the prognostic value of morphology of TIF. In fact, we are planning to gain further insight into the morphology of TIF and TB through further refinement of the classification methods based on more patients, like comparing the prognostic effects of smooth and tortuous morphology of TIF on group A and B and discussing the effect of different grades of TB on prognosis in a comparison of group C to D.

Conclusions

The present study provides evidence that the morphology of TIF can be taken as a parameter of tumor invasiveness and as an indicator of adverse outcomes. Most importantly, TB almost showing the same prognostic value compared with other classical prognostic factors, such as TNM staging. The morphology of TIF should be recorded in medical reports after operation as these data can provide important reference for judging the prognosis and establishing individualized treatment for ICC patients.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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