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Prognostic Impact of Desmoplastic Reaction Evaluation for Intrahepatic Cholangiocarcinoma

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Abstract. *Background/Aim: The purpose of this study was to clarify the relationship between the desmoplastic reaction (DR) and clinicopathological features, and the prognosis using cases of resected intrahepatic cholangiocarcinoma (ICC). Patients and Methods: Out of 54 cases that were preoperatively diagnosed with ICC and underwent resection at our department, 47 patients were included in this study. All sections were prepared from resected specimens and were microscopically observed following H&E staining. Stroma were evaluated at the advancing edge of the cancer and stratified into three DR types: mature (DR1), intermediate (DR2), and immature (DR3). Results: DR was correlated to the serum levels of CA19-9, but not to the other tumor factors. In multivariate analysis, only DR and tumor size were determined as independent prognostic factors. Conclusion: Evaluation of DR for ICC may be useful for prognostic assessments.*

Intrahepatic cholangiocarcinoma (ICC) has poor prognosis, despite the incidence of 3-7% among primary malignant liver tumors. Surgery remains the most effective treatment. There is no clear evidence showing improvement of prognosis from chemotherapy or radiotherapy (1-3).To improve the prognosis of ICC, it is necessary to clarify the factors involved in biological malignancy and the mechanism of involvement of these.

Changes in the local microenvironment transform epithelial cells into high migratory mesenchymal cells, causing tissue remodeling. This process is called epithelial-mesenchymal transition (EMT). When EMT occurs in cancerous epithelial cells, they acquire various malignant traits such as migratory properties, invasive capacity, and resistance to apoptosis. EMT has been related to the metastatic potential of cancer cells and the acquisition of resistance to anticancer drugs (4-7). Desmoplastic reaction (DR) refers to a state in which fibroblasts have proliferated in the stroma. It has been reported that during cancer cell EMT, DR is a result of remodeling of the extracellular matrix in the cancer microenvironment (8).

Recently, DR has been reported to be useful in determining the presence or absence of submucosal invasion in early colorectal cancer. In addition, the relationship between DR and prognosis has been reported for various cancers such as gastric, colorectal, breast, and esophageal (8-10). However, there are few reports on the existence or significance of DR in ICC (11, 12). Clarification of the relationship between stroma and malignancy in ICC is important when considering the indications of surgical treatment, prognosis prediction, and planning postoperative adjuvant therapy for ICC with poor prognosis.

In this study, we examined the relationship between the prognosis and pathological features of DR at the tumor margin.

Patients and Methods

Of the 54 cases that were preoperatively diagnosed with ICC and underwent resection between April 2005 and March 2019 at our hospital, 47 patients with pathologically confirmed ICC after resection, were included in this study.

All sections were prepared from the resected specimens, and microscopic observations were performed following H&E staining. The diagnosis of DR was obtained by two pathologists. When there was a difference in diagnosis, another pathologist joined to reach a final decision. The diagnosis was performed by concealing the clinical features of each case.

This is a retrospective study and was approved by the Ethical committee of the Kurume University School of Medicine (approval no.19266). This was conducted in accordance with the Declaration

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of Helsinki. An opt-out approach was used to obtain informed consent from the patients, and personal information was protected during date collection.

DR evaluation method. Observations were made at the boundary between the cancerous tissue and normal liver tissue. Classification was conducted according to the criteria reported by Ueno et al. (8). DR1 (mature) was defined as that consisted of thin collagen fibers in multiple layers and cannot be identified as keloid-like fibers or mucus-like stroma. DR2 (intermediate) was defined as that consisted of keloid-like fibers (thick acidophilic collagen fibers) in the stroma of the cancer border. DR3 (immature) as that comprised irregular keloid-like fibers surrounded by a slightly basophilic mucus-like stroma (Figure 1).

Statistical methods. The association between DR and clinicopathological features was analyzed using Fisher's exact test. For clinicopathological features, including DR, the overall survival rate was calculated using the Kaplan-Meier method, and the survival curves were compared using the log-rank test. A p-value of <0.05 was considered statistically significant. Multivariate analysis was conducted on the relationship between survival and clinicopathological factors, including DR, using Cox's proportional hazards model. Statistical analysis was performed using JMP® Pro 11.0.0 (SAS Institute Inc, Cary, NC, USA).

Results

Subject background. The median age was 69 years (range=33-88 years) and the male-to-female ratio was 29:18. The mass-forming type was the most common morphologic subtype, which was observed in 34 cases. The UICC 8th edition (13) was used for staging; 17 cases were T1 and 30 were T2/3. Lymph node metastasis was noted in 11 cases. No arterial invasion was observed, and venous and portal vein invasion was noted in 7 and 27 cases, respectively. The breakdown of DR categorization for DR1, DR2, and DR3 were 15, 12, and 20 cases, respectively. The median serum CA19-9 levels were 41.8 U/ml and the interquartile range was 10.7-528.7 U/ml. The median observation period was 4.3 years (95%CI=1.649-10.151), and the 5-year survival rate was 48% (Table I, Figure 2).

DR and clinical pathological features. The relationship between DR and each clinical pathological feature was analyzed. The clinicopathological feature that had a significant relationship with DR was serum CA19-9. No significant relationship was found with tumor size, histology, lymph node metastasis, vascular invasion, or nerve invasion (Table II).

DR and overall survival rate. Univariate analysis was performed on clinical and pathological factors and survival rate in patients who underwent surgery. A significant relationship was noted with tumor size (p=0.0091), presence or absence of lymph node dissection (p=0.0122), CA19-9 levels (p=0.0007), and DR (p=0.0032). Multivariate analysis,

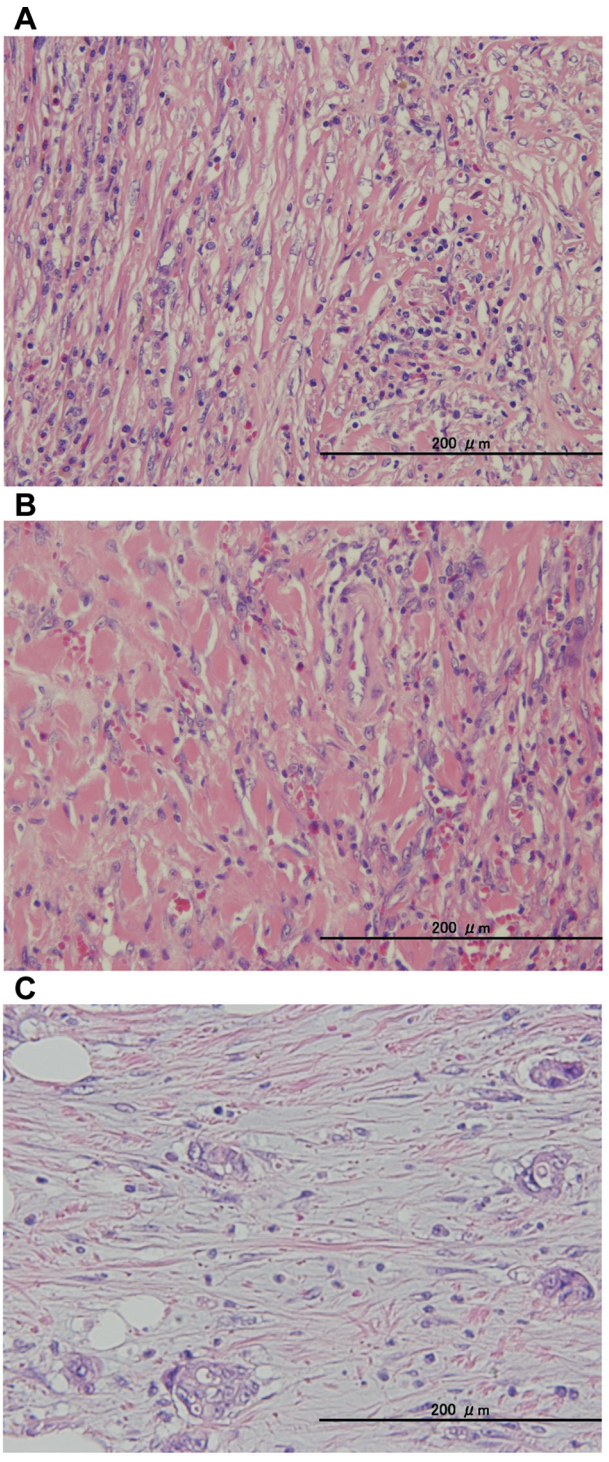

Figure 1. *Categorization of desmoplastic reaction in intrahepatic cholangiocarcinoma. (A) DR1 (mature) denotes from the presence of thin collagen fibers in multiple layers that cannot be identified as keloid-like fibers or mucus-like stroma. (B) DR2 (intermediate) denotes keloid-like fibers (thick acidophilic collagen fibers) in the stroma of the cancer border. (C) DR3 (immature) denotes irregular keloid-like fibers surrounded by a slightly basophilic mucus-like stroma. (40× magnification, hematoxylin & eosin stain).*

Table I. *Clinicopathological characteristics of patients with ICC undergoing operation.*

Age (years)	Median (range)	69 (33-88)
Gender	Male:Female	29:18
Morphologic subtype	MF: MF+PI:PI:IG	34:2:7:4
Tumor size (mm)	Average (range)	38.4 (11-92)
T category	pT1a/1b:pT2/3	17:30
Lymph node invasion	Present:Absent	11:36
Artery invasion	Present:Absent	0:47
Vein invasion	Present:Absent	7:40
Portal vein invasion	Present:Absent	27:20
Nerve invasion	Present:Absent	27:20
Bile duct invasion	Present:Absent	29:18
Underlying liver	F0:F1/2/3	29:18
CA19-9	Median (IQR)	41.8 (10.7~528.9)
DR	DR1:DR2:DR3	15:12:20

MF: Mass-forming type; PI: periductal invasion type; IG: intraductal growth type; IQR: interquartile range; DR: desmoplastic reaction.

Table II. *Correlation between clinicopathological characteristics and DR in ICC.*

	DR1 (n=15)	DR2/3 (n=32)	*p*-Value*
Tumor size			
≥32 mm	7	17	0.6797
<32 mm	8	15	
Tumor number			
Solitary	2	3	0.6816
Multiple	13	29	
T category			
T1a/T1b	6	11	0.6461
T2/T3	9	21	
Pathological			
Well/Mode	12	23	0.5515
Por	3	9	
Vascular invasion			
Present	7	21	0.2170
Absent	8	11	
Bile duct invasion			
Present	9	20	0.8695
Absent	6	12	
Lymph node metastasis			
Present	2	9	0.2642
Absent	13	23	
Nerve invasion			
Present	7	20	0.3553
Absent	8	12	
Underlying liver			
F0	9	20	0.8695
F1234	6	12	
CA19-9			
<41.1 U/ml	13	11	0.0006
≥41.1 U/ml	2	21	

Well: Well differentiated tubular adenocarcinoma; mode: moderately differentiated tubular carcinoma; por: poorly differentiated adenocarcinoma. *Using the Fisher's exact test.

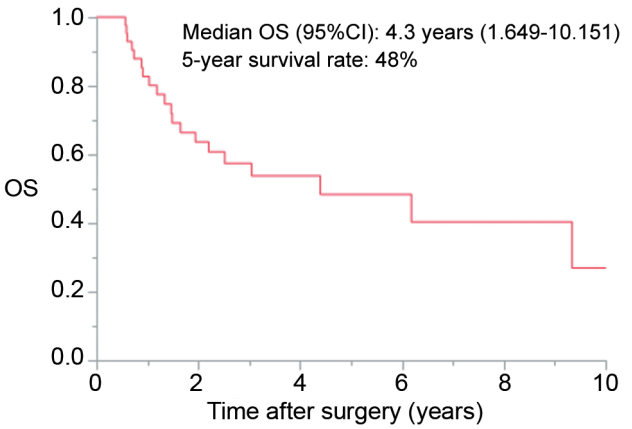

Figure 2. *Overall survival (OS) curves of patients for intrahepatic cholangiocarcinoma after the operation. The median observation period was 4.3 years (95%CI=1.649-10.151), and the 5-year survival rate was 48%.*

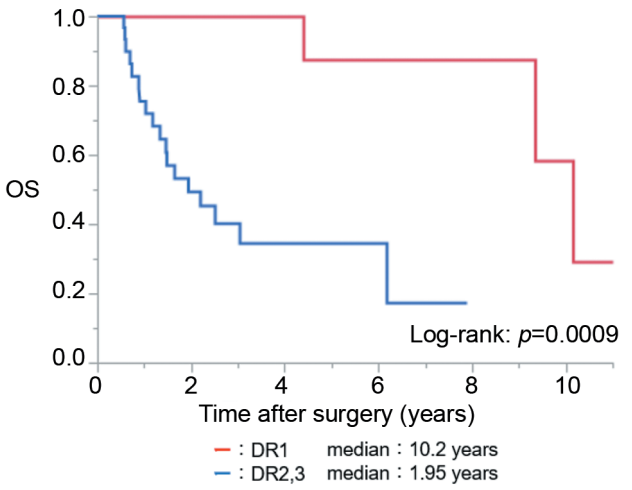

Figure 3. *Overall survival (OS) curves of patients with intrahepatic cholangiocarcinoma according to DR. The median OS of patients with DR1 was 10.2 years (95%CI=4.39-12.93 years), and with DR2,3 was 1.95 years (95%CI=1.18-6.18 years). The 5-year survival rate of patients with DR1 was 87.5%, and those with DR2,3 was 34.5%.*

which used these factors as covariates, indicated tumor size and DR as independent prognostic factors (Table III). In the present study, the overall survival (OS) curves of patients with ICC according to DR categorization showed that the median OS of patients with DR1 was 10.2 years (95%CI=4.39-12.93 years), with DR2 was 1.94 (95%CI=0.61-6.03), and with D3 was 2.20 (95%CI=0.91-6.18). The 5-year survival rate of the DR1 group was 87.5%, the DR2 was 32.5%, and DR3 was 35.4%. The difference in OS between the mature type (DR1) and the other types (DR2 and DR3; hereinafter, referred to as

Table III. *Univariate and multivariate analysis of the prognosis of patients after surgery.*

	Univariate analysis*		Multivariate analysis*	
	5-year survival (%)	*p*-Value	HR (95%CI)	*p*-Value
Tumor size				
<32 mm	50.6	0.0091	1	0.0071
≥32 mm	48.5		4.191 (1.439-15.680)	
Tumor number				
Solitary	49.4	0.2533		
Multiple	0			
T category				
T1a/T1b	41.4	0.965		
T2/T3	48.4			
Pathology				
Well/Mode	45.9	0.9246		
Por	64.9			
Vascular invasion				
Present	47.9	0.8372		
Absent	41.9			
Bile duct invasion				
Present	60.2	0.739		
Absent	0			
Lymph node metastasis				
Present	0	0.0925		
Absent	61.7			
Lymph node dissection				
Present	16.1	0.0027		0.4698
Absent	80.1			
Nerve invasion				
Present	40.8	0.1759		
Absent	61.8			
CA19-9				
<41.1 U/ml	75.0	0.0007		0.0963
≥41.1 U/ml	27.8			
DR				
DR1	87.5	0.0032	1	0.0394
DR2 / DR3	34.9		10.058 (1.546-199.01)	

Well: Well differentiated tubular adenocarcinoma; mode: moderately differentiated tubular carcinoma; por: poorly differentiated adenocarcinoma; HR: hazard ratio; CI: confidence interval. *Using the Cox's proportional hazards model.

"DR2,3") was significant. The median OS of DR2,3 was 1.95 years (95%CI=1.18-6.18 years), and the 5-year survival rate of that was 34.5% (Figure 3).

Discussion

Many clinical studies have been reported concerning the poor outcome of patients with ICC after curative-intent surgical resection. In this study, the clinicopathological features of the enrolled patients were similar to those previously reported, and the 5-year survival rate was also similar (1, 3, 14, 15).

Conventionally, the poor prognostic factors for ICC include multiple lesions, tumor size, vascular invasion, lymph node metastasis, cirrhosis, and high levels of CA19-9 (3, 16-18). However, in this study only tumor size and DR were identified as poor prognostic factors in multivariate analysis. Lymph node metastasis tended to be a prognostic factor, but was not statistically significant in our univariate analysis. However, lymph node dissection was a significant prognostic factor in univariate analysis. The prognosis was worse in patients who underwent lymph node dissection than in those who did not. In our surgical strategy, lymph node dissection was performed only in cases of a positive intraoperative lymph node sample biopsy, which may have biased our analysis results of no relationship between lymph node metastasis and the prognosis.

Regarding DR categorization, there were cases where it was difficult to distinguish between DR2 and DR3. However, DR1, which indicates mature stroma, and DR2,3 tend to be

clearly distinguishable histologically, with significant relationship in the overall survival rate between the DR1 and DR2,3 groups. The mature type (DR1) had significantly better prognosis than the non-mature types (intermediate type: DR2, immature type: DR3). There were 15 cases diagnosed as DR1. Some cases had a more than 10 years postoperative follow up period, but only 4 deaths were noted and long-term survival was obtained. Due to this, the hazard ratio may have become extremely high.

Regarding DR in colorectal cancer, there are reports on the correlation between lymphatic invasion, lymph node metastasis, and DR (8). However, in contrast to expectations, no correlation was found between lymph node metastasis and DR in this study. Further, several factors which have been reported to be the prognostic factors were not correlated to DR except CA19-9. Typically, previous clinicopathological prognostic factors were based on the evaluation of the cancer cells themselves. Recently, the evaluation of the cancer microenvironment has been gaining attention as it is consider to be related to malignant potential, prognosis and recurrence (9, 19). It is known that the EMT mechanism is greatly affected by the microenvironment around cancer cells. This study suggested that the more immature, the poor prognosis, but also the DR may be related to the malignant aggressiveness of ICC, such as the levels of tumor markers (serum CA19-9). In addition, Ueno et al. have reported (9) that although carcinomas differ, there is a relationship between the budding and DR in the advanced part of the cancer. The tumor and stromal microenvironment in ICC are likely to affect prognosis. Moving forward, the clarification of relationship between the EMT phenotypes of tumors, such as budding and spindle-shaped cells, and stroma may be useful in prognosis prediction and determining the pros and cons of postoperative adjuvant chemotherapy in ICC with poor prognosis. Furthermore, the clarification of the involvement of genes related to EMT (Snail family, ZEB family, twist, Slug, CDH2, CAFs, etc.) may contribute to the establishment of new therapeutic methods.

This study has limitations. It was a retrospective study, with a limited number of cases, performed at a single institution. Additionally, the reproducibility of DR evaluation and its association with the tumors should be validated by further research. The advantage, which we emphasize, is that the evaluation of tumor type and DR can be performed with the same H&E staining, which is an inexpensive and simple method.

In conclusion, we report that an immature desmoplastic reaction in ICC affects survival rate. In the future, if the relationship between tumor and DR will be validated and clarified, it might contribute to the identification of genes related to the establishment of the cancer microenvironment in ICC.

Conflicts of Interest

No conflicts of interest exist regarding this study.

Authors' Contributions

Satoki Kojima designed the study and wrote the initial draft of the manuscript. Satoki Kojima and Toru Hisaka contributed to analysis and interpretation of data and assisted in the preparation of the manuscript. All the other Authors contributed to data collection and interpretation and critically reviewed the manuscript. All Authors approved the final version of the manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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