Original Article
Y-box-binding protein-1 and E-cadherin are potential prognostic factors in human bladder carcinoma

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Abstract: Many studies have reported various results regarding the relationship of Y-box binding protein 1 (YB-1) and E-cadherin with many cancers, including bladder carcinoma (BC). However, there is still an incomplete understanding about the role of YB-1 and E-cadherin in BC prognosis. This study aimed to evaluate the value of YB-1 and E-cadherin in BC. In this study, the expression of YB-1 and E-cadherin was examined using immunohistochemical staining in a study cohort including 117 BC patients. The results showed that there were significant correlations of YB-1 and E-cadherin with clinical T stage, pathologic T stage, and lymph node metastasis (LNM) in BC patients. Univariate analysis showed that the patients with high YB-1 expression levels (HR = 2.732; P = 0.001), low E-cadherin expression levels (HR = 2.899; P = 0.003), high clinical T stage (HR = 3.905; P < 0.001), high pathologic stage (HR = 4.199; P < 0.001), or LNM (HR = 5.267; P < 0.001) had a significantly higher risk of worse overall survival (OS). In the multivariate analysis, YB-1 (HR = 2.031; P = 0.022) and LNM (HR = 3.546; P < 0.001) predicted worse OS. Therefore, YB-1 and E-cadherin expression is associated with BC incidence; tumor stage, grade and metastasis; and survival and may be reliable prognostic markers for patients undergoing radical cystectomy for BC.

Keywords: Bladder cancer, prognosis, YB-1, E-cadherin

Introduction

Bladder carcinoma (BC) is a rare neoplasm with a poor prognostic rate in 2012 worldwide [1]. Even though effective diagnostic strategies have led to great progress in the treatment of BC, the 5-year relative survival rates of metastatic BC is 33% [2, 3]. It has previously been demonstrated that epithelial-mesenchymal transition (EMT) is important in BC. However, how EMT regulates BC invasion remains unclear.

Y-Box-binding protein 1 (YB-1) is a transcription/translation regulatory factor that belongs to the cold-shock domain superfamily [4]. Upregulation of YB-1 expression triggers EMT, an effect that is characterized by the loss of the epithelial marker E-cadherin and the induction of mesenchymal markers [5]. In addition, clinical studies have shown elevated nuclear YB-1 expression in a wide range of human cancers, including lung, colon, breast, bladder, prostate, and renal cancers [6-10]. Moreover, overexpression of YB-1 is closely related to tumor progression and an aggressive cancer phenotype, contributing to unfavorable survival outcomes among cancer patients [11]. However, the molecular events underlying the association of increased YB-1 protein expression with cancer metastasis and poor prognosis remain elusive.

Therefore, in our study, we investigated whether YB-1 and E-cadherin could help predict long-term survival outcome in BC patients. We hypothesized that YB-1 and E-cadherin protein would be associated with unfavorable outcomes comparable to those of other urological cancers.

Patients and methods

Patients

The study included 117 consecutive patients with pathologically confirmed BC who underwent radical cystectomy (RC) from May 2011 to May 2016 in the Institute of Urology at Lanzhou University Second Hospital.

Table 1. Clinicopathological features of 117 bladder cancer patients and stratified by YB-1 and E-cadherin

Characteristic	All patience n (%)	YB-1 expression			E-cadherin expression		
		High	Low	P	High	Low	P
Age (years, n (%))				0.439			0.180
< 65	50	34	16		23	27	
≥ 65	67	40	27		22	45	
Gender, n (%)				0.399			0.059
Female	34	24	10		16	18	
Male	83	50	33		27	56	
Clinical T stage, n (%)				0.002			0.001
≤ T1	64	38	26		30	34	
≥ T2	53	36	17		13	40	
Pathologic T stage, n (%)				0.044			0.014
≤ T1	89	56	33		49	40	
≥ T2	28	18	10		23	5	
Tumor size, n (%)				0.180			0.257
< 3 cm	62	43	19		27	35	
≥ 3 cm	55	31	24		18	37	
Tumor number, n (%)				0.806			0.458
Single	21	14	7		10	11	
Multiple	96	60	36		35	61	
LVI, n (%)				0.343			0.883
Negative	64	39	25		25	39	
Positive	53	35	18		20	33	
LNM, n (%)				0.007			< 0.001
No	88	50	38		42	46	
Yes	29	24	5		3	26	
Histological subtype, n (%)				0.967			0.474
Non-UBC	22	8	14		10	12	
UBC	95	35	60		35	60	
Tissue location				0.040			< 0.001
Tumor	72	41	31		25	47	
Para-tumor	45	33	12		20	25	

LNM = lymph node metastasis; LVI = lymphovascular invasion; pT = pathological tumor stage; UBC = Urothelial Bladder Cancer.

The inclusion criteria: all patients were pathologically diagnosed as bladder cancer; each case was diagnosed with adequate imaging and clinical examination who received at least one follow-up or examination after discharge. The exclusion criteria: patients were treated with chemotherapy radiotherapy or biotherapy; patients suffered with other tumor diseases; patients with distant metastasis before treatment.

The study was approved by our institutional review board with a waiver of informed consent and therefore was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki and its later amendments.

Clinicopathological information of the patients, including age, gender, tumor grade and stage, and follow-up time, was obtained from medical records. Tumor stage and grade were based on the 2004 TNM staging system and the 1997 WHO classification.

Immunohistochemical staining

Immunohistochemistry (IHC) was performed as previously described and incubated with anti-YB-1 and E-cadherin primary antibodies (dilu-

tions 1:100 and 1:100, respectively) overnight at 4°C. The E-cadherin, YB-1 and secondary antibodies were purchased from Cell Signaling Technology (CA, USA).

Evaluation of immunohistochemistry

The staining intensity of immunostained sections was reviewed and scored independently by two pathologists who were blinded to the clinical features of patients and sample information. The score was based on the proportion of positive tumor cells multiplied by the staining intensity. The proportion of positive tumor cells was scored as 1 (< 25%), 2 (25-50%), 3 (50-75%), or 4 (> 75%). The staining intensity was scored as follows: 0 = negative staining, 1 = weak, 2 = moderate, and 3 = strong. The final immunostaining score for each protein was the product of the two abovementioned scores, ranging from 0 to 12. The protein expression levels were defined as follows: low expression, with a final staining index of 0-3; or high expression, with a final staining index of 4-12.

Statistical analysis

The endpoint of the study was overall survival (OS), which was calculated from the date of surgery to the time of death or the last follow-up date. We used *Chi*-square (χ^2) tests to compare categorical variables that were expressed as frequencies and percentages. OS curves were drawn using the Kaplan-Meier method. For all statistical tests, *P* values were two sided, and P < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 22.0 software (IBM Corporation, Somers, NY, USA).

Results

Patient characteristics and the correlation of preoperative YB-1 and E-cadherin levels with clinicopathological parameters

The clinicopathological variables of the patients are presented in **Table 1**. We enrolled 117 BC patients (median age 58.5 years; range 28-87 years), including 83 men and 34 women, in this study. The median follow-up period was 26.5 months (interquartile range 1-96 months).

Expression of YB-1 and E-cadherin expression in human BC tissue

YB-1 proteins expression were assessed in the nuclei of primary cervical cancer cells. E-cad-

herin proteins expression in cancer cells was characterized by variable degrees of cytoplasmic and membrane staining (**Figure 1A**).

We investigated the expressions of both YB-1 and EZH2 in 72 primary BC samples and 45 para-carcinoma normal tissues. YB-1 nuclear expression was detected in 52.8% (38/72) of the BC cases, while only 11.1% (5/45) in normal subjects; E-cadherin was only expressed in 30.6% (22/72) of the BC, but 51.1% (23/45) of normal samples showed E-cadherin-positive staining.

Potential relationships between YB-1 or E-cadherin and other clinicopathological factors were explored (**Table 1**). Our results revealed that elevated YB-1 or E-cadherin expression was significantly correlated with high clinical T stage and pathological T stage (all P < 0.05). Moreover, Spearman rank correlation coefficient analysis revealed a significant correlation between high YB-1 and low E-cadherin levels (P < 0.001).

Correlation of YB-1 and E-cadherin with OS in BC

For all 117 patients in our study, the median survival time was 26 months. The low-YB-1 group exhibited significantly longer OS (median OS = 36 months; mean OS = 31.43 ± 2.961 months) than the high-YB-1 group (median OS = 23.67 months; mean OS = 26.23 ± 2.272 months) (P = 0.001). The low E-cadherin group exhibited significantly shorter OS (median OS = 23 months; mean = 25.87 ± 2.446 months) than the high E-cadherin group (median OS = 35.33; mean = 31.05 ± 2.628 months) (P = 0.003; **Figure 1B**). We found that the percentage of YB-1-positive BCs increased from stage T1 to T3 and T4; we found that the percentage of E-cadherin-positive BCs decreased from stage T1 to T3 and T4 (**Figure 1C**).

We assessed the effects of YB-1 and E-cadherin on OS using the Cox proportional hazard regression model (**Table 2**), and we performed a univariate analysis to assess the risk of death after RC. YB-1 expression (HR = 2.737, P = 0.001), E-cadherin expression (HR = 2.899, P = 0.003), more advanced Clinical T stage (HR = 3.905, P < 0.001), Pathologic N stage (HR = 4.199, P < 0.001) and the presence of lymph node metastasis (LNM) (HR = 5.267, P < 0.001)
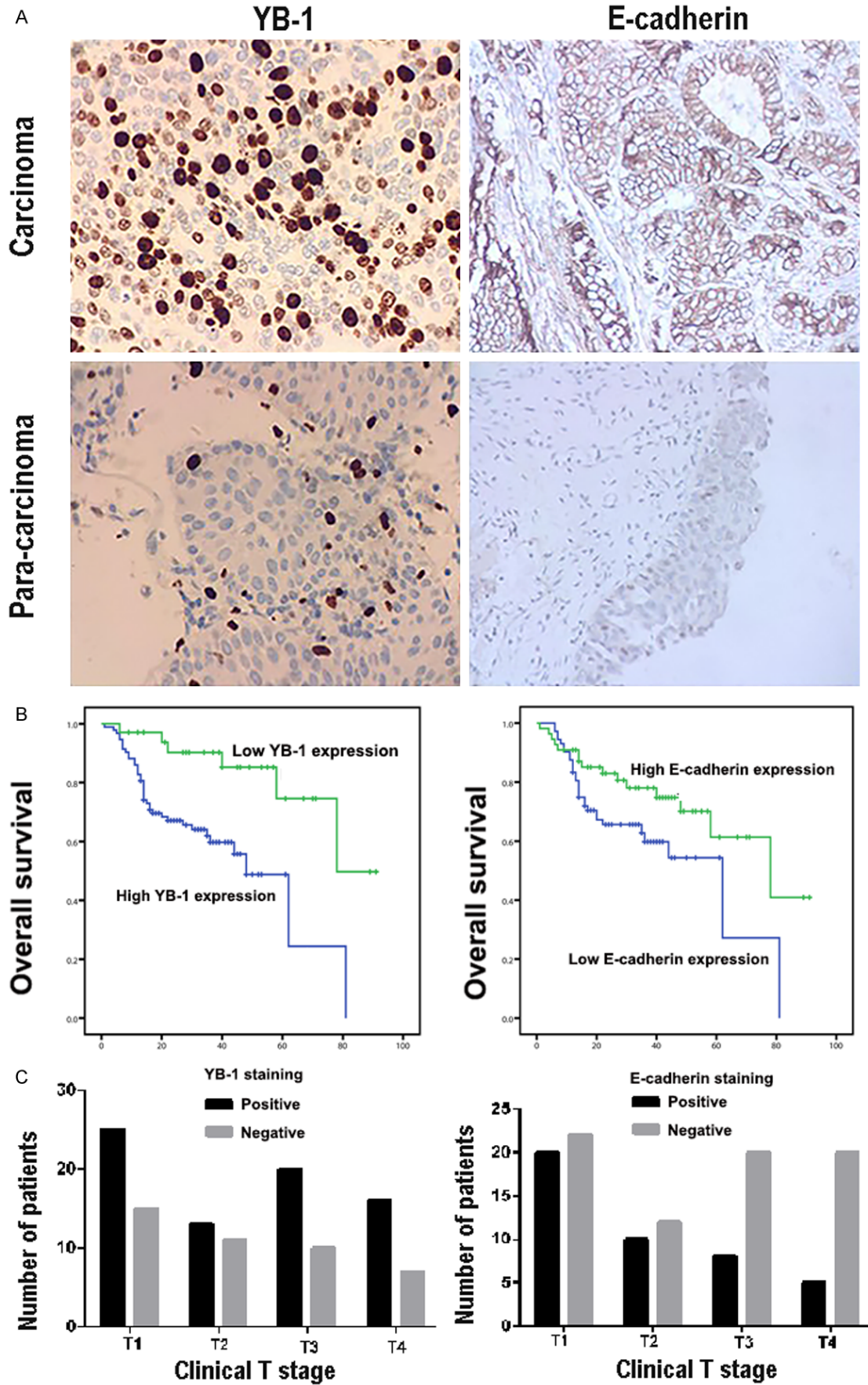
Figure 1. Representative immunohistochemical staining of YB-1 and E-caherin expression in the bladder carcinoma and para-carcinoma normal tissues. A. YB-1 expression was decreased and E-cadherin expression were elevated in bladder carcinoma tissues as compared with adjacent non-tumor tissues (×200). B. The patients with high YB-1 expression levels, low E-cadherin expression levels were positively associated with shorter overall survival for bladder carcinoma. C. Distribution of YB-1 and E-cadherin expression in different stages of bladder carcinoma.

Table 2. Univariate and multivariate analyses of characteristics associated with overall survival

Characteristics	Univariate			Multivariate		
	Hazard Ratio	95% CI	*P-value*	Hazard Ratio	95% CI	*P-value*
Age, years						
≥ 65 *vs* < 65	1.139	0.624-2.079	0.673	1.022	0.528-1.979	0.949
Gender						
Male *vs* Female	1.843	0.886-3.836	0.102	1.243	0.590-2.620	0.567
Clinical T stage, n (%)						
≥ T2 *vs* < T1	3.905	2.028-7.518	< 0.001	1.356	0.572-3.211	0.489
Pathologic T stage, n (%)						
≥ T2 *vs* < T1	4.199	2.306-7.648	< 0.001	2.791	1.495-5.210	0.001
Tumor size, n (%)						
≥ 3 cm *vs* < 3 cm	1.233	0.682-2.229	0.487	1.180	0.567-2.457	0.658
Tumor number, n (%)						
Multiple *vs* Single	0.836	0.401-1.740	0.632	0.954	0.256-3.559	0.944
LVI, n (%)						
Positive *vs* negative	1.086	0.601-1.965	0.784	0.709	0.374-1.345	0.293
LNM						
Yes *vs* No	5.267	2.884-9.619	< 0.001	3.546	1.889-6.659	< 0.001
Histological subtype						
UBC *vs* Non-UBC	0.918	0.441-1.914	0.820	0.681	0.312-1.487	0.335
YB-1 expression						
High *vs* Low	2.737	1.504-4.980	0.001	2.031	1.106-3.729	0.022
E-cadherin						
Low *vs* High	2.899	1.428-5.887	0.003	1.619	0.763-3.436	0.210

LNM = lymph node metastasis; LVI = lymphovascular invasion; pT = pathological tumor stage; UBC = Urothelial Bladder Cancer.

were correlated with a significantly higher risk of death (**Table 2**). In the multivariate analysis, YB-1 expression (HR = 4.823, P = 0.01) and LNM (HR = 3.546, P < 0.001) were found to be significantly associated with OS (**Table 2**).

Discussion

We evaluated the effect of YB-1 and E-cadherin expression on the OS of BC patients in this retrospective cohort study. In many tumors, including BC, EMT is associated with aggressive biological behavior and a poor prognosis [12, 13]. To date, only a small number of studies have explored the involvement of EMT in BC, and this study aimed to provide preliminary data on this topic.

YB-1 is a transcription/translation regulatory factor that belongs to the cold-shock domain family [14]. YB-1 overexpression triggers EMT,

an effect that is characterized by the loss of the epithelial marker E-cadherin and the induction of mesenchymal markers. YB-1 is also correlated with cancer aggressiveness [4, 15]. In our study, high levels of YB-1 protein were commonly observed in BC tissues. Patients with YB-1-high BC showed advanced clinical T stage, aggressive pathologic stage, positive LNM, and a short OS compared to those with YB-1-low BC. Moreover, our findings were in accordance with reports that described YB-1 transcriptional repressors in BC [16]. We observed that tumors with YB-1 overexpression exhibited reduced E-cadherin levels. These results may indicate that abnormal YB-1 expression is involved in BC invasion and metastasis, which is in agreement with Holm *et al.* [17]. This study also demonstrated that YB-1 negatively regulates E-cadherin levels in BC, raising the possibility that YB-1 plays a role in BC progression and progno-

sis by inhibiting E-cadherin expression, inducing the acquisition of mesenchymal properties, and promoting EMT.

E-cadherin is one of the most important mediators of cell-to-cell adhesion in epithelial tissues and has important barrier functions. Moreover, this protein maintains the cellular phenotype and apical-basal polarity of epithelial cells [18]. Downregulation of E-cadherin expression is also generally viewed as a key feature in the EMT process [19]. In our study, a significant relationship between low E-cadherin levels and poor prognosis was observed in BC patients, which is in line with Liu *et al.* [20], whom reported that E-cadherin-negative BC patients are more likely to be poorly differentiated. Moreover, E-cadherin protein levels were associated with pathologic T stage, with a higher proportion of low-stage tumors than high-grade tumors expressing E-cadherin (P = 0.002). Consistent with this result, Hu *et al.* [21] reported that the rate of reduced E-cadherin expression in early BC was significantly lower than that in advanced BC. In addition, reduced E-cadherin immunoreactivity was significantly associated with LNM (P < 0.01) and oncological outcome (P = 0.003) in BC. These results showed the important role of E-cadherin in maintaining an epithelial cell state and improving adhesion, which impedes EMT and prevents tumor progression. In addition, this model is consistent with the results of other studies (Raspollini et al. [22], Xie *et al.* [23]).

It has previously been demonstrated that YB-1 overexpression is associated with poor survival outcome in patients with solid tumors. Our findings supported this hypothesis, given that high YB-1 expression was associated with poor survival using both univariate and multivariate analyses to assess all the factors that influence survival. We did not find any relationship between the expression levels of YB-1 or E-cadherin and lymphovascular invasion (LVI). BC patients with LVI were more aggressive than those patients without LVI [24]. We also did not find any relationship between the expression of YB-1 or E-cadherin and tumor size. These results may be attributed to our immunohistochemistry methods.

Our study has several limitations, including the small sample size and the proportion of patients who were lost to follow-up due to many socio-economic factors. Second, we performed correlation analyses using prognostic information but were unable to consider metrics such as recurrence-free survival and progression-free survival, as these data were not available. Third, this study was a retrospective and single-center study. Therefore, additional studies with larger patient groups are needed to evaluate IHC markers.

In conclusion, this was the first prospective study to analyze the prognostic significance of two important EMT biomarkers in a large cohort of BC patients. We showed that YB-1 is a better predictor of OS in BC patients than E-cadherin. Our results highlight the potential role of YB-1 and E-cadherin as prognostic factors for BC and suggest that they may be useful molecular markers of progression in BC. Based on the findings of this study and others, YB-1 and E-cadherin may be predictive factors in BC and may also be novel targets for clinical therapy. Identifying new molecular markers could also be the first step to accurately defining a high risk-of-progression molecular profile for bladder carcinoma.

Disclosure of conflict of interest

None.

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