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The Role of *DBR1* as a Candidate Prognosis Biomarker in Esophageal Squamous Cell Carcinoma

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Abstract

Aims: Esophageal squamous cell carcinoma (ESCC) is one of the most prevalent malignancies with unfavorable clinical outcomes and limited therapeutic methods. As a key enzyme in RNA metabolism, debranching RNA Lariats 1 (DBR1) is involved in intron turnover and biogenesis of noncoding RNA. Although cancer cells often show disorder of nucleic acid metabolism, it is unclear whether *DBR1* has any effect on the carcinogenesis and progression of ESCC. **Methods:** Here we detected DBR1 expression in 112 ESCC samples by immunohistochemistry and analyzed its correlation with clinical parameters and survival. **Results:** DBR1 is mainly located in the nucleus of ESCC tissue. And DBR1 was associated with several malignant clinical features in patients, including tumor location ($\chi^2 = 9.687$, $P = .021$), pathologic T stage ($\chi^2 = 5.771$, $P = .016$), lymph node metastasis ($\chi^2 = 8.215$, $P = .004$) and N classification ($\chi^2 = 10.066$, $P = .018$). Moreover, Kaplan-Meier analysis showed that ESCC patients harboring lower DBR1 expression had a worse prognosis in comparison with those with higher DBR1 expression ($P = .005$). Univariate and multivariate Cox proportional hazards regression analyses indicated that decreased DBR1 might act as an independent predictor of poor prognosis for ESCC patients. **Conclusion:** Abnormal RNA metabolism might play a critical role in promoting the progression of ESCC, and *DBR1* may be a promising potential biomarker for predicting the prognosis of ESCC patients.

Keywords

DBR1, ESCC, prognosis, biomarker

Abbreviations

ALS, amyotrophic lateral sclerosis; ESCC, esophageal squamous cell carcinoma; DBR1, debranching RNA Lariats 1; MPE, metallophosphoesterase; NLS, nuclear localization signal; ROC, the receiver operating characteristic.

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Introduction

Esophageal squamous cell carcinoma (ESCC), characterized by aggressive clinical course and unfavorable prognosis, is the dominant type of esophageal cancer in China and remains the leading sixth incidence and the fourth mortality of malignancies.[1–3] Although chemotherapy and radiotherapy are beneficial for the disease survival outcome, frequent recurrence and limited therapeutic approaches result in poor prognosis with approximately 30% of 5-year survival rates in China.[4] Additionally, the highly heterogeneous characteristic of ESCC results in variable clinical outcomes and enhances the complexity of clinical options.[5] Currently, only a handful of

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patients can get a survival benefit from the standard treatment. Therefore, it is necessary to explore new biomarkers and novel targets for ESCC diagnosis, prognosis, and treatment.

Human debranching RNA Lariats 1(DBR1) is located in 3q22.3 and its protein consists of 544 amino acids with a conserved catalytic GNHE motif and a bipartite nuclear localization signal (NLS).[6] Functionally, as an RNA debranching enzyme, *DBR1* is involved in both the RNA splice process and lariat intron turnover pathway.[7,8] In general, introns are usually removed when RNA splicing to generate lariat structures harboring 2',5'-phosphodiester bonds. Importantly, *DBR1* cleaves the special 2′,5′ phosphodiester linkage and converts the lariat intron into a linear molecule, which is rapidly degraded in vivo or possibly forms multiple byproducts of lariat RNA processing like "mirtron" or snoRNAs.[9–11] *DBR1* is highly conserved metallophosphoesterase (MPE) in diverse species like yeast, homo species, and plays a significant role in the growth and development of a biological organism. It's reported that DBR1 mutants result in severe growth defects and abnormal cell morphology in *Schizosaccharomyces pombe*,[12] embryo-lethal effects in Arabidopsis,[13] and mice.[14] Furthermore, *DBR1* may be involved in retroviral replication. *DBR1* can affect HIV replication and inhibit cDNA synthesis by changing 5′ end conformation.[15] Bi-allelic *DBR1* mutations lead to significantly decreased *DBR1*, and then enhanced HSV viral infection of the brainstem in childhood.[16] Beyond the viral infection, *Dbr1* deficiency is also a stronger suppressor for *TDP-43* in amyotrophic lateral sclerosis (ALS) disease.[17] In human cancer, the modulation of *DBR1* expression is dependent on wild-type p53 when suffering from hypoxia, and decreased *DBR1* promoted tumor growth.[18]

However, the role and mechanism of *DBR1* in ESCC remains unclear. Here, we explored the expression of *DBR1* in ESCC and estimated the clinical significance of *DBR1* for favoring the management of ESCC.

Materials and Methods

Tissue Specimen and Clinical Data

The reporting of this study conforms to REMARK guidelines.[19] Our research was approved by Medical Ethics Committee of Affiliated Tumor Hospital of Shanxi Medical University (202113). The tissue chip included 112 tumor tissues and 68 matched paracancerous tissues with good quality, which were obtained from 112 ESCC patients (Shanghai Outdo Biotech Company). The samples in the tissue chip come from the National Human Genetic Resources Sharing Service Platform (ID: 2005DKA21300). Preparation and sales of the chips were approved by the Ethics Committee of Shanghai Outdo Biotech Company (NO. YB M-05-02). The clinical stage of all ESCC cases was judged based on the seventh TNM staging criteria of esophageal cancer proposed by the American Joint Commission on Cancer and the Union for International Cancer Control. The clinicopathological

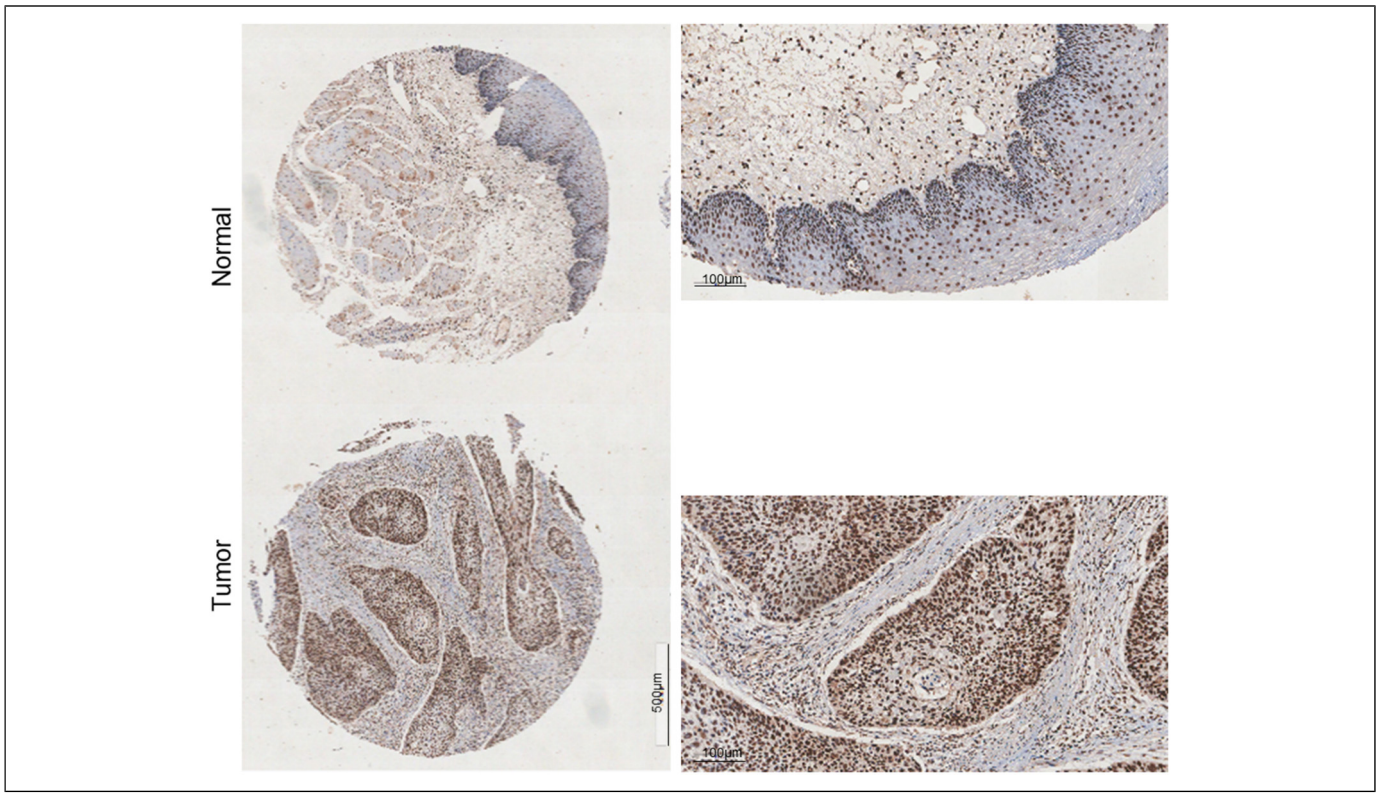

Figure 1. The subcellular location of DBR1 in ESCC tissue (left: × 40, right: × 200). The scale bar represents 500 μm (left) and 100 μm (right).
Abbreviations: ESCC, esophageal squamous cell carcinoma; DBR1, debranching RNA Lariats 1.

characteristics were displayed in Supplemental Table 1. All patient details have been de-identified. The follow-up date was July 2015.

Immunohistochemical (IHC) Analysis

The detection of DBR1 protein expression in tissue chips was performed by IHC staining. Briefly, the section was incubated with the special antibody (1:400, DBR1 rabbit polyclonal antibody, Cat No: 16019-1-AP, Lot: 00047263, Proteintech, USA) overnight at 4?. Then the slide was incubated with corresponding secondary antibody (MaxVision TM HRP-Polymer anti-Mouse/Rabbit IHC Kit, Kit-5020, MXB biotechnologies, Fuzhou, China) at 37? for 20 min and examined the interesting protein by the DAB kit (ZLI-9019, ZSGB-BIO, Beijing, China). Further, we counterstained them with hematoxylin. All section images were scanned and captured by Aperio Scan Scope (AperioTechnology Inc, USA) at 40×, 200×, respectively. Expression of the DBR1 protein was analyzed by Aperio image scope v 9.0 (Aperio, Vista, CA, USA). Histoscore (H-score) was calculated according to intensity score multiplied by the percentage of positive cells for a semi-quantitative assessment.

Table 1. The Correlation Between DBR1 Expression and Clinical Features in Tissue Microarray.

Clinical parameter	N = 112	DBR1 expression Low (n = 17)	DBR1 expression High (n = 95)	*F*	*P*
Gender					
Male	94	15 (16.0)	79 (84.0)	0.276	.600
Female	18	2 (11.1)	16 (88.9)		
Age					
<60 yr	35	6 (17.1)	29 (82.9)	0.153	.696
≥60 yr	77	69 (14.3)	26 (85.7)		
Tumor location					
Upper	4	0 (0.0)	4 (100.0)	9.687	.021*
Middle	25	1 (4.0)	24 (96.0)		
Lower	12	5 (41.7)	7 (58.3)		
Unkown	71	11 (15.5)	60 (84.5)		
TNM staging					
? + ?	66	8 (12.1)	58 (87.9)	1.167	.280
? + ?	46	9 (19.6)	37 (80.4)		
Tumor grade					
G1 + G2	82	14 (17.1)	68 (82.9)	0.854	.356
G3	30	3 (10.0)	27 (90.0)		
T staging					
T1 + T2	27	8 (29.6)	19 (70.4)	5.771	.016*
T3 + T4	85	9 (10.6)	76 (89.4)		
Lymph node metastasis					
No	62	4 (6.5)	58 (93.5)	8.215	.004**
Yes	50	13 (26.0)	37 (74.0)		
N classification					
N0	62	4 (6.5)	37 (93.5)	10.066	.018*
N1	29	7 (24.1)	22 (75.9)		
N2	14	3 (21.4)	11 (78.6)		
N3	7	3 (42.9)	4 (57.1)		
Survival status					
Live	77	7 (9.1%)	70 (90.9%)	7.093	.008**
Deceased	35	10 (28.6%)	25 (71.4%)		

*: $P < .05$, **: $P < .01$.

Statistical Analysis

All data were statistically analyzed by employing the SPSS 22.0 software package (IBS SPSS, Armonk, NY, USA). We selected the survival status of ESCC patients as status variable and DBR1 protein expression as test variable, perform the receiver operating characteristic (ROC) curve analysis, and then determine the optimal cut-off value by calculating Youden index. The best cutoff value of DBR1 protein classified the cases into 2 groups: $DBR1_{high}$ group and $DBR1_{low}$ group, respectively. Chi-square (χ^2) tests were applied to analyze the association of DBR1 expression with clinic-pathological parameters. Kaplan-Meier method and the log-rank test compared the difference in the cumulative survival rate within different DBR1-expression groups or with various clinicopathological characteristics. Cox proportional hazards regression model was employed to perform univariate and multivariate analyses. *P*-value less than .05 was regarded as a statistically significant difference.

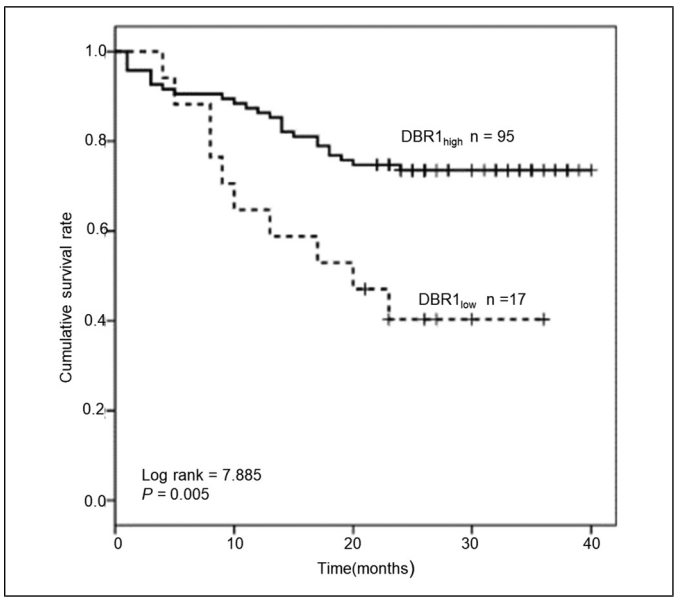

Figure 2. Kaplan-Meier analysis showed protein level of DBR1 acts as a predictor for the prognosis of ESCC patients. Solid line presents the cumulative survival rate of ESCC patients with high DBR1 expression. The dotted line indicates the cumulative survival rate of ESCC patients with low DBR1 expression. The difference in cumulative survival rates between the 2 groups was calculated by the Log-rank test.
Abbreviations: ESCC, esophageal squamous cell carcinoma; DBR1, debranching RNA Lariats 1.

Results

Expression and Subcellular Location of DBR1 in ESCC

To explore the potential clinical significance of DBR1, we performed and analyzed DBR1 protein levels from 112 cases of ESCC tissue by IHC stained with an anti-DBR1 antibody. It showed that DBR1 protein was largely located in the nucleus of ESCC tissues (Figure 1). It was consistent with previous reports.[8]

The Association Between DBR1 Expression and Relevant Clinicopathological Characteristics in ESCC Patients

We further analyzed the correlation between DBR1 and clinicopathological characteristics in ESCC patients. ROC analysis indicated the optimum cut-off value of DBR1 is the H-score of 78.1271, and thus the patients were divided into $DBR1_{low}$ and $DBR1_{high}$. The results showed that decreased DBR1 expression was remarkably associated with tumor location ($P < .05$), pathologic T stage ($P < .05$), lymphatic metastasis ($P < .01$), pathologic N classification ($P < .05$), and survival status ($P < .01$). And there were no statistical expression changes between age groups (<60 yr vs ≥60 yr, $P > .05$) and gender groups (male vs female, $P > .05$) (Table 1). Additionally, DBR1 expression didn't show a remarkable correlation with TNM staging ($P > .05$), but the $DBR1_{high}$ group had a much higher percentage in early-stage patients. All these results suggested that decreased DBR1 may be associated with the malignant features of ESCC.

DBR1 Predicts Survival of Patients With ESCC

We further compared the survival difference between the 2 groups. Kaplan-Meier plot analysis displayed that ESCC patients with low DBR1 had significantly worse overall

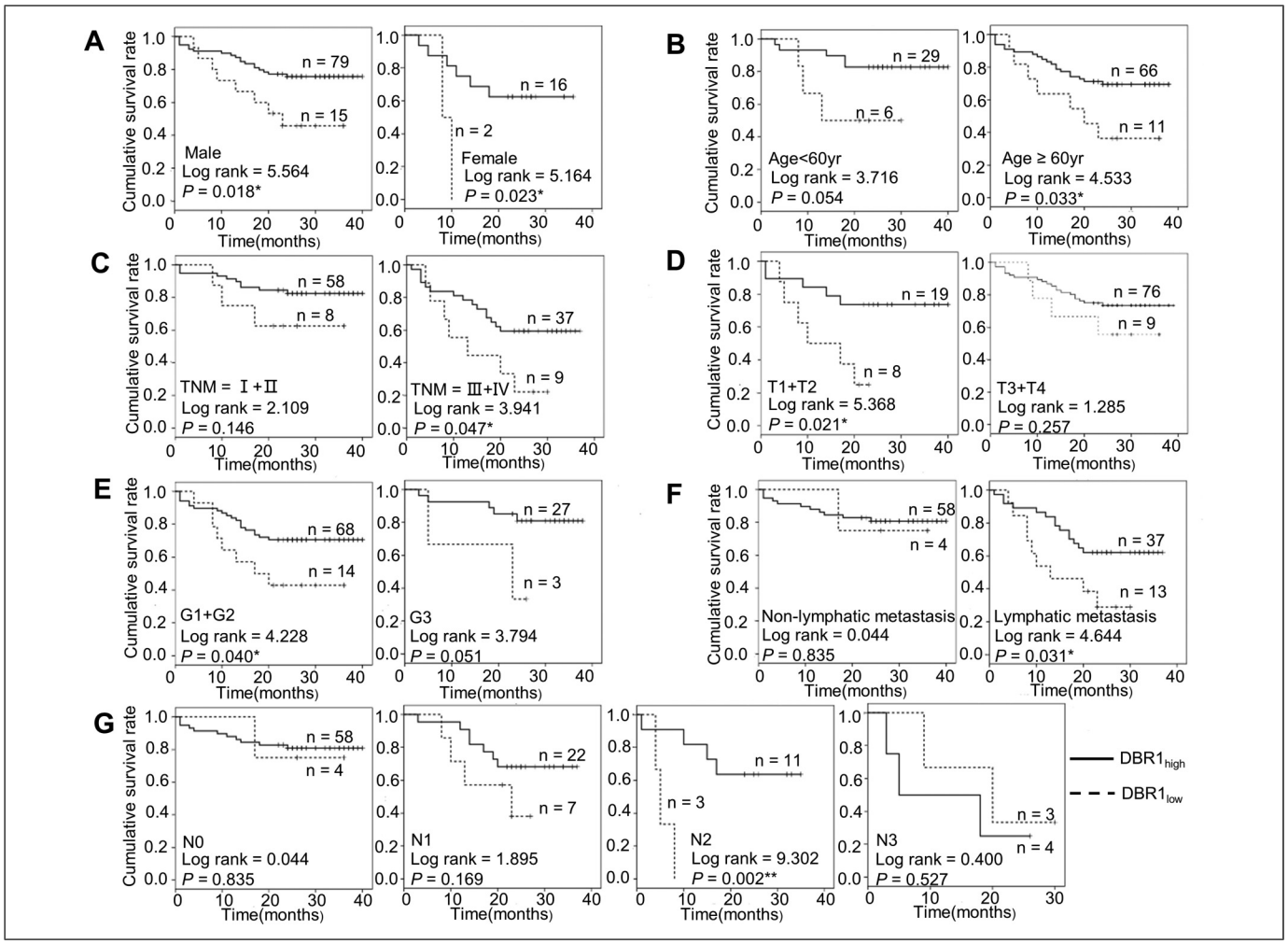

Figure 3. Predicating role of DBR1 protein expression for the OS of ESCC patients. (A-G) Kaplan-Meier survival curves of ESCC patients with different DBR1 protein expression levels combined with diverse features like gender (A), age (B), TNM staging (C), T staging (D), tumor grade (E), lymph node metastasis (F) and N classification (G). *: $P < .05$, **: $P < .01$.

Abbreviations: ESCC, esophageal squamous cell carcinoma; DBR1, debranching RNA Lariats 1; OS, overall survival.

survival (OS) time than those with highly expressed DBR1 ($P = .005$, Figure 2). Considering the heterogeneity of the tumor, we performed further strata analysis. It revealed that decreased DBR1 expression was a better indicator for an unfavorable prognosis in the ESCC patients with age ≥ 60 yr ($P < .05$), highly and moderately differentiated tumor ($P < .05$), pathologic T stage (T1 + T2) ($P < .05$), lymphatic metastasis and pathologic N2 group ($P < .01$) (Figure 3).

DBR1 Protein Level as an Independent Predicator for Prognosis for ESCC

To investigate the predicting action of genes expression for survival status of ESCC patients, we performed univariable and multivariable Cox proportional hazard regression analysis. The univariable analysis indicated that DBR1 expression ($P = .007$, $HR = 0.366$, 95% CI = 0.175-0.764), TNM stage ($P = .003$, $HR = 2.854$, 95% CI = 1.436-5.672), lymph node metastasis ($P = .005$, $HR = 2.729$, 95% CI = 1.356-5.493), were crucial risk factors for survival status (Figure 4A). The further result of multivariate analysis disclosed that DBR1 expression was a significant independent prognostic factor and protective factor for the survival status in ESCC ($P = .023$, $HR = 0.386$, 95% CI = 0.170-0.879) (Figure 4B). It indicated DBR1 might be an independent predictor for the outcomes of ESCC patients.

Discussion

In the present study, we examined the DBR1 protein expression level in ESCC tissues and evaluated its clinical significance in ESCC. We identified it was significantly associated with tumor location, the pathologic T stage, lymphatic metastasis, and the prognosis of ESCC patients.

In recent years, a growing number of pieces of evidences verified that DBR1 offers a critical rate-limiting step for the intron turnover, possibly adjusting the formation of translation competent mRNAs and noncoding RNA.[20] Following the identification and structural analysis of DBR1, it's reported that its protein was stable in the nucleus, but it can transfer between the nucleus and the cytoplasm.[8] In our study, we also uncovered that DBR1 exhibited a nucleic localization pattern in ESCC tissue, which is consistent with the role of DBR1 involved in nucleic acid metabolism.

Previous efforts have focused on the pathological mechanism of DBR1 in human diseases like viral infectious disease,[15] neurodegenerative disease ALS,[17] and so on. Currently, the responsibility of DBR1 in cancer development is explored at the initial stage. Han *et al*[18] advocated for the first time that DBR1 acts as a downstream target and is co-regulated by wild-type p53 and hypoxia-inducible factor-1 under hypoxic conditions and low DBR1 expression was associated with cancer. Meanwhile, deficient DBR1 possibly regulates the splicing process and promotes tumorigenesis due to

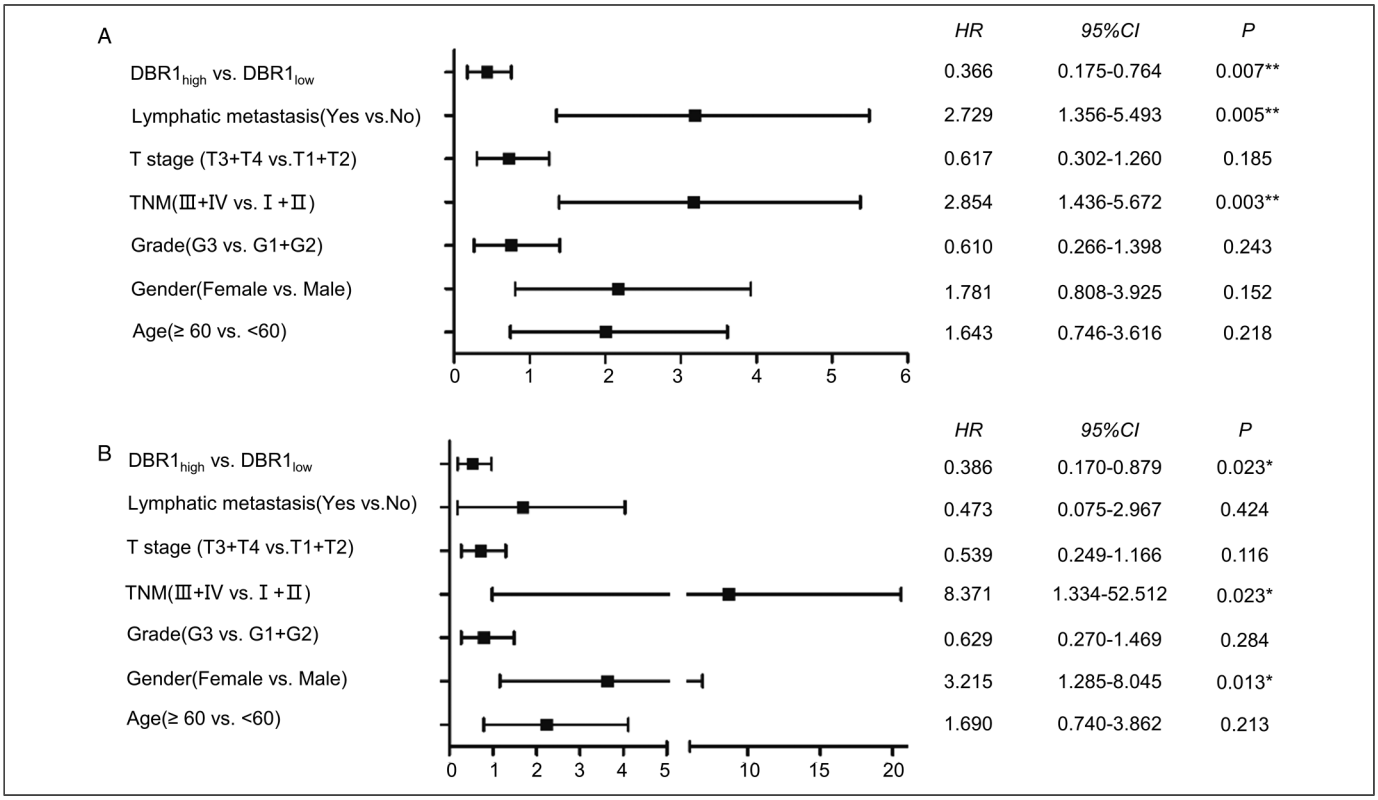

Figure 4. Cox proportional hazards regression analysis for debranching RNA Lariats 1 (DBR1) protein expression level. (A) and (B) represent univariable and multivariable analysis by Cox proportional hazard regression model, respectively. *: $P < .05$, **: $P < .01$.

abnormal snRNP recycling.[18] However, the involvement of DBR1 in ESCC has not been reported. Here, we presented the first report on DBR1 protein expression in a cohort of 112 ESCC patients. Our study figured out DBR1 expression was closely correlated with some malignant parameters. Ai *et al*[21] revealed that primary locations of esophageal tumor are responsible for the pattern of distant metastasis in 6812 patients. Upper esophageal cancer was more inclined to pulmonary metastasis, whereas lower esophageal cancer was more relevant to transferring to the liver. Additionally, the primary site of esophageal cancer also served as a primary hazard factor for distant metastasis like liver and lung metastasis. The lower segment showed a worse prognosis than the middle and upper locations. In our study, low DBR1 expression accounts for 41.7% in the lower location group, which is more than the upper and middle groups. On contrary, 58.3% of ESCC patients harbored highly expressed DBR1 in the lower location group, which is less than the other primary site groups. It's indicative of more ESCC with decreased DBR1 expression and lower site closely correlated with different distant metastasis. It may be useful for clinical evaluation at the time of diagnosis and for prediction in follow-up, especially for the ESCC cases that has presented no metastasis.

Expectedly, metastasis results in an unfavorable prognosis of ESCC. In general, lymph node metastasis can frequently be observed in ESCC, even in superficial ESCC.[22] Prediction of lymph node metastasis in ESCC is principal for prognosis.[23,24] In our study, ESCC patients with low DBR1 expression are more likely to have lymphatic metastasis. While highly expressed DBR1 showed the contrary tendency. We also found DBR1 showed a contrary correlation with T stage and lymphatic metastasis. Maybe it exerts a different role in early stage and advanced stage, implying that DBR1 may be involved in the onset and development of ESCC. We will explore its specific role and mechanism in further study. Strata analysis indicated that DBR1 might facilitate the accurate survival prediction of some special ESCC patients. These findings might assist clinicians to screen the high-risk populations for individualized treatment and clinical management.

To date, whether DBR1 has a correlation with the prognosis of cancer patients has not been reported in most types of malignancy. Our results showed ESCC patients with decreased DBR1 had an unfavorable prognosis. Multivariate analysis also revealed that DBR1 may act as an independent prognosis predictor and protective factor. All results suggest that it will be a powerful and promising predictor for the survival of ESCC patients. Our findings provided clues to explore the clinical value of DBR1 in cancer. What's more, DBR1 inhibited tumor growth, indicating functions as a tumor suppressor.[18] Importantly, this will give a clue to the relationship between RNA metabolism and cancer onset and development from another aspect.

Conclusions

Collectively, our study has shown that DBR1 acts as a candidate prognosis biomarker in ESCC and it vigorously predicts the prognosis of patients with ESCC. To the best of our knowledge, this is the first report to validate that DBR1 may be used in predicting survival in cancer patients. However, the sample size was limited and the statistical power was not precalculated in our study. It's indispensable to establish a multicenter prospective clinical investigation with enlarging sample size and longer time follow-up to examine the prognostic power of DBR1 for its future application clinically.

Ethics Statement

Our research was approved by Medical Ethics Committee of Affiliated Tumor Hospital of Shanxi Medical University (202113). The tissue chips used in our study were commercially available and obtained from Outdo Biotech Company (Shanghai). The application of tissue-chip in this research was approved by Ethics Committee of Shanghai Outdo Biotech Company (YB M-05-02).

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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