

# Correlation between reticulum ribosome-binding protein 1 (RRBP1) overexpression and prognosis in cervical squamous cell carcinoma

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## Research

**Keywords:** Cervical squamous cell carcinoma (SCC), Endoplasmic reticulum ribosome binding protein 1 (RRBP1), Immunohistochemistry, Western blotting, qRT-PCR, Diagnosis, Prognosis

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# Abstract

**Purpose:** The aim of this study was to investigate the relationship between endoplasmic reticulum ribosomal binding protein 1 (RRBP1) expression in cervical squamous cell carcinoma tissues and its poor prognosis. RRBP1 is a nascent transporter on the rough endoplasmic reticulum. Its function is to regulate the transport and secretion of proteins in cells, and to relieve endoplasmic reticulum (ER) stress, thereby promoting tumor cell proliferation.

**Methods:** The expression levels of RRBP1 in 96 cervical squamous cell carcinoma tissues were detected by immunohistochemistry. Western blot and qRT-PCR were used to compare the expression levels of RRBP1 in cervical squamous cell carcinoma tissues and normal cervical tissues. Then the chi-square test was used to analyze the results of immunohistochemistry. Finally, the Kaplan-Meier method was used to analyze the results by log-rank test and Cox regression (proportional risk model).

**Result:** The overexpression of RRBP1 in cervical squamous cell carcinoma tissues was related to FIGO stage ( $P=0.030$ ), tissue differentiation ( $P=0.047$ ), lymph node metastasis ( $P=0.031$ ), and was not related to the patient age ( $P=0.667$ ). Univariate survival analysis showed that the prognosis was associated to the expression level of RRBP1 ( $P=0.045$ ) and lymph node metastasis ( $P=0.001$ ). Analysis of multi-factor survival cox model proved that RRBP1 was an independent prognostic factor.

**Conclusion:** RRBP1 was overexpressed in cervical squamous cell carcinoma tissues, indicating that RRBP1 may be a new tumor marker for early diagnosis, treatment and prognosis of cervical squamous cell carcinoma, and it will provide help for individualized treatment and prognosis of cervical squamous cell carcinoma in the future.

## 1. Introduction

Cervical cancer is a common malignant tumor in gynecology. It was reported that in 2012, a total of 530,000 cases of cervical cancer were diagnosed worldwide, of which 85% occurred in underdeveloped areas.(1) Because of the distribution of medical source was uneven and the system of cervical cancer screening was unsound and it will be add 45 million new patients with cervical cancer in the next half century.(2) Cervical squamous cell carcinoma is a common pathological type of cervical cancer. Although conventional treatments for early cervical squamous cell carcinoma include surgery and chemotherapy, postoperative complications and poor prognosis of cervical squamous cell carcinoma still pose a threat to women's health. Therefore, we still need to continue to explore the prognostic factors and potential therapeutic targets of cervical squamous cell carcinoma.

Endoplasmic reticulum ribosomal binding protein 1 is a nascent transporter with a molecular weight of 180KD located on the rough endoplasmic reticulum membrane. Its primary structure contains three different domains, the most characteristic of which is 10 The amino acids were repeated in series 54 times in the vicinity of the -NH<sub>2</sub> terminus.(3)And Its function is to regulate the transport and secretion of proteins in some cells in mammalian cells. From previous studies, we found that in myogenic progenitor

cells, Low expression of RRBP1 contributes to excessive deposition of extracellular matrix (ECM).(4) RRBP1a, RRBP1b can be expressed in early embryonic development and adult tissues of *Xenopus laevis*. (5) At the same time, RRBP1 is also involved in the process of intestinal maturation and is expressed during the osteoblast differentiation and at the neuromuscular junction.(6–8) In recent years, as the deepening of the research, we found that the expression of cytosolic anaplastic lymphoma kinase (ALK) in the epithelioid inflammatory myofibroblastic sarcoma of RRBP1-ALK fusion increased with the nuclear week, and found a new carcinogenic mechanism of recurrent ALK.(9) Recently, we also learned that overexpression of RRBP1 in lung cancer may be involved in the regulation of mRNA stability of the unfolded protein response (UPR) component (ATF6 and GRP78), thereby reducing ER stress and helping tumor cell survival.(10) In addition, we also found that RRBP1 is overexpressed in a variety of tumor cells, including liver cancer, prostate cancer, colorectal cancer, lung cancer, Her-2 (+) breast cancer, esophageal cancer, endometrial cancer and ovarian cancer.(10–17)

However, to date, few has explored the relationship between RRBP1 overexpression and the prognosis of cervical squamous cell carcinoma. The purpose of the present study was to investigate whether RRBP1 was overexpressed in cervical squamous cell carcinoma through immunohistochemistry, Western blotting and qRT-PCR. Statistical methods were used to explore the correlation between the overexpression of RRBP1 and the prognosis of cervical squamous cell carcinoma.

## **2. Materials And Methods**

### **2.1 Patients and clinical samples**

Confirmed by the Department of Pathology for Harbin Medical University Cancer Hospital, gathered of 96 samples of patients with cervical squamous cell carcinoma who accepted cervical cancer staging surgery at the Harbin Medical University Cancer Hospital from January 2010 to December 2012. (not accepted any treatment before surgery, including immunotherapy, chemotherapy and radiotherapy). Clinical samples were processed by experienced pathologists through fixed, dehydrated, transparent, wax-transparent, embedded, sliced, patched, stained, transparent, sealed made into Paraffin embedded samples. Clinical pathological features including age, lymph node metastasis, tissue differentiation, and clinical stage (according to cervical cancer 2009 FIGO staging) were found by Hospital medical record system. The follow-up period was from the day of surgery to November 2018 (the follow-up date was 11–105 months, average 81 months). The study was ratified by the Harbin Municipal Ethics Committee and supported by the Harbin Medical University in China. All patients submit Informed consents.

### **2.2 Immunohistochemistry**

According to the manufacturer's instructions, the purchased RRBP1 antibody (1: 1000, RRBP1 antibody, Abcam, Ab95983, UK) was diluted. In short, 98 paraffin sections fixed from formalin of the Tumor Hospital of Harbin Medical University were dewaxed. In order to eliminate the effect of endogenous peroxidase, pretreatment with 3% H<sub>2</sub>O<sub>2</sub> solution for 10 minutes at room temperature. Antigen retrieval was achieved by microwave in citrate buffer pH 6.0 for 30 minutes. Block with the same source for serum

of the secondary antibody for 15–30 minutes, add all the samples to the primary antibody diluted with anti-RRBP1 antibody in a ratio of (1:1000), and incubate at 4 °C for overnight, and rewarmed, and rinse with phosphate buffered saline (PBS) 3 minutes × 5 times. Next the biotin-labeled secondary antibody (goat anti-rabbit IgG-HRP, wanleibio, WLA023, China) was further added dropwise and incubated at 37 °C for 30 minutes, and the cells were further washed with PBS for 3 minutes × 5 times. Then Immunoperoxidase staining was developed for 5 minutes using DAB chromogen and incubated at room temperature for 30 minutes to 1 hour. Finally, it was washed with PBS for 3 minutes x 5 times and counterstained with hematoxylin. We used a diluent instead of a primary antibody as a negative control. Immunohistochemistry results were analyzed by two experienced pathologists without understand of clinical information, and RRBP1 staining was analyzed by semi-quantitative methods. The staining intensity score was colorless (0), light yellow (1), brownish yellow (2), tan (3); Total percentage of positive cells scored 0 means < 5%; 1 means 5–25%; 2 means: 26–50%; 3 means 51–75%; 4 means > 75%. The score for each sample is calculated by the product of two values. The score ( $\geq 4$ ) was evaluated as high expression and the score ( $< 4$ ) was evaluated as low expression.

## 2.3 Western blot analysis

All fresh surgical specimen tissues (n = 36) were frozen in a -80 °C ultra-low temperature freezer, and the experimental reagents and polyacrylamide gels were prepared according to the manufacturer's instructions. Firstly, in order to extract the protein, aliquoted the lysate (containing 1% PMSF) according to the needed of the experiment, and added each sample to the lysate, and left it on ice for 5 minutes. The lysate was transferred to a centrifuge tube and centrifuged at 12000 rpm for 5 minutes at 4 °C, and took the supernatant and stored at -20 °C. Then total protein was quantified used the purchased BCA protein concentration determination kit (wanleibio, WLA004, China). The complex protein mixture was separated by SDS-PAGE (wanleibio, WLA013, China) and transferred to a PVDF membrane (Millipore, IPVH00010, USA) in a conventional manner. So as to incubate the primary antibody, the purchased RRBP1 antibody (1: 1000, RRBP1 antibody, Abcam, Ab95983, UK) was incubated in a buffer at 4 °C overnight. Then incubated the secondary antibody through goat anti-rabbit IgG-HRP (1:5000, Goat anti-rabbit IgG-HRP wanleibio, WLA023, China) was incubated for 45 minutes at 37° C. Finally, the configured chemiluminescent reagent was mixed with the membrane, shaken at room temperature for 5 minutes, the residual liquid was placed in the X-ray film at a dark room before final development, and the exposure time was appropriately adjusted according to the strength of the signal, and the image processing system (Gel-Pro-Analyzer software) analyzed the optical density value of the target band. The  $\beta$ -actin antibody (wanleibio, WL01845, China) was used as an internal reference antibody.

## 2.4 Real-time PCR analysis

First mRNA was extracted from fresh surgical samples (n = 36) according to the manufacturer's instructions and the concentration of RNA in each sample was determined used a UV spectrophotometer NANO 2000 (UV spectrophotometer NANO 2000, Thermo, USA). The cDNA was then synthesized in a PCR instrument (Real-Time PCR, Exicycler 96, BIONEER, Korea) used Super M-MLV reverse transcriptase (BioTeke, PR6502, Beijing), and the products were subjected to quantitative fluorescence analysis. This

experimental analysis method used the  $2^{-\Delta\Delta CT}$  method. PCR amplification conditions: Incubated at 94.00 °C, for 5 minutes, incubated at 94.00 °C, for 10 seconds, incubated at 60.00 °C, for 20 seconds, incubated at 72.00 °C, for 30 seconds. Scanned, went to line 2, Cycle 40, incubated at 72.00 °C, for 2 minutes 30 seconds, incubated at 40.00 °C, for 1 minutes 30 seconds, melted 60 °C to 94 °C, Every 1.0 °C, 1 seconds, incubated at 25.00 °C, for 1–2 minutes. The primer sequence of RRB1-F is 5'-TCCATCCAGAGTCTCACTTC-3', and the primer sequence of RRB1-R is 5'-GCCCTCGTTGAACACCAT-3'. The primer sequence of GAPDH-F is 5'-GGCACCCAGCACAATGAA-3', and the primer sequence of GAPDH-R is 5'-TAGAAGCATTTGCGGTGG-3'. The  $\beta$ -actin antibody (wanleibio, WL01845, China) was used as an internal reference antibody.

## 2.5 Statistical analysis

We utilized chi-square test to analyze the results of immunohistochemistry, and applied the Kaplan-Meier method to estimate the total survival (OS) and disease-free survival (DFS) of cervical cancer, and applied log rank test. Finally, multivariate analysis was assessed according to Cox regression (proportional risk model). A  $P < 0.05$  was deemed statistically implication. All the above statistical analysis applied Windows SPSS software V25.0 (IBM SPSS. Inc., Chicago, IL, USA).

## 3. Results

### 3.1 Patient pathological features

To analyze the immunity of RRB1 in cervical squamous cell carcinoma tissues, we used 96 samples without any treatment of cervical cancer tissues from Cancer Hospital of Harbin Medical University. In all samples a total of 45 samples were  $> 49$  years old, and 51 patients were aged  $\leq 49$  years old. Among the collected samples, 38 patients had stage I (according to the 2009 FIGO stage of cervical cancer), and 58 patients with stage II. The clinical and pathological features of the sample are shown in Table 1.

Table 1

Association analyses between the expression levels of RRB1 and the clinicopathological characteristics of Cervical squamous cell carcinoma(SCC)

Variables Patients n	RRBP1 expression		p <sup>a</sup>	
	Low	High		
All cases				
Age(years)				
>49	45	14	31	P = 0.667
≤ 49	51	19	32	
FIGO stage				
I	38	18	20	P=0.030
II	58	15	43	
Histological grade				
G1	23	12	11	P = 0.047
G2/G3	73	21	52	
lymph node metastasis				
No	83	32	51	P = 0.031
Yes	13	1	12	
FIGO, International Federation of Gynecology and Obstetrics; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; RRB1, Hematopoietic pre-B cell leukemia transcription factor (PBX)-interacting protein; <sup>a</sup> Chi-square test.				

### 3.2 Endoplasmic reticulum ribosomal binding protein 1 (RRBP1) in cervical squamous cell carcinoma tissue was overexpression

For the expression of RRB1 in cervical squamous cell carcinoma, we performed immunohistochemistry on 96 samples, and the results showed that RRB1 was located in the nucleus and cytoplasm of tumor cells (Fig. 2). As shown in Table 1, it total 12 patients showed low expression and 84 patients showed high expression. In addition, the high expression of RRB1 in cervical squamous cell carcinoma tissues was connected to FIGO stage (P = 0.030), tissue differentiation (P = 0.047), lymph node metastasis (P = 0.031), and was not related to the patient age (P = 0.667).

We used 36 fresh surgical specimen samples to analyze the differential expression of RRB1 among in cervical squamous cell carcinoma and normal cervical tissues through Western blot and qRT-PCR

(Fig. 1 and Fig. 2). Specifically, the expression of RRBP1 protein and the level of mRNA in cervical squamous cell carcinoma tissues was higher than that in normal cervical tissues. ( $P \leq 0.001$ )

### **3.3 The relationship between overexpression of RRBP1 in cervical squamous cell carcinoma and its poor prognosis**

We used the Kaplan-Meier method to analyze the overall survival (OS) and disease-free survival (DFS) of 96 samples. As shown in Table 2, univariate survival analysis showed that RRBP1 overexpression and lymph node metastasis were associated with poor prognosis of cervical squamous cell carcinoma. The RRBP1 overexpression overall survival (OS) (Table 2,  $P = 0.001$ ) and disease-free survival Period (DFS) (Table 2,  $P < 0.001$ ), and the lymph node metastasis overall survival (OS) (Table 2,  $P = 0.045$ ) and disease-free survival (DFS) (Table 2,  $P = 0.016$ ).

Table 2

Univariate survival analysis of OS and DFS in 96 patients with Cervical squamous cell carcinoma(SCC)

Variables	n	OS		p <sup>a</sup>	DFS		p <sup>a</sup>
		Mean ± SE(month) 95%CI			Mean ± SE(month) 95%CI		
Age(years)							
≥49	45	96 ± 3	91-102	P = 0.200	91 ± 4	82-99	P = 0.164
≤ 49	51	88 ± 4	81-95		80 ± 5	70-89	
FIGO stage							
I	38	93 ± 3	86-100	P = 0.767	85 ± 5	76-95	P = 0.667
II	58	91 ± 3	85-97		84 ± 4	76-93	
Histological grade							
G1	23	95 ± 3	90-100	P = 0.255	89 ± 6	77-100	P = 0.217
G2/ G3	73	91 ± 3	85-96		84 ± 4	76-91	
lymph node metastasis							
No	83	94 ± 2	89-99	P = 0.045	88 ± 3	82-95	P = 0.016
Yes	13	82 ± 5	71-92		65 ± 9	47-83	
RRBP1							
Low expression	38	97 ± 1	95-99	P = 0.001	96 ± 2	93-100	P < 0.001
High expression	58	87 ± 3	81-94		77 ± 5	68-86	
FIGO, International Federation of Gynecology and Obstetrics; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; RRBP1, Hematopoietic pre-B cell leukemia transcription factor (PBX)-interacting protein; OS, overall survival; DFS, disease-free survival; <sup>a</sup> Log-rank test.							

In addition, Multivariate survival analysis was assessed by Cox regression (proportional risk model), as show in Fig. 4, RRBP1 was an independent prognostic factor for cervical squamous cell carcinoma, and its overall survival (OS) (Table 3, 95% CI = 1.531–85.265, P = 0.018) and disease-free survival (DFS) (Table 3, 95% CI = 2.009-111.098, P = 0.008).

Table 3

Multivariate survival analysis of OS and DFS in 96 patients with Cervical squamous cell carcinoma(SCC)

Variables	OS			DFS		
	Exp(B)	95%CI	P <sup>a</sup>	Exp(B)	95%CI	P <sup>a</sup>
lymph node metastasis	1.770	0.697-4.495	P = 0.230	1.855	0.780-4.409	P = 0.162
RRBP1	11.424	1.531-85.265	P = 0.018	14.939	2.009-111.098	P = 0.008

FIGO, International Federation of Gynecology and Obstetrics; RRBP1, Hematopoietic pre-B cell leukemia transcription factor (PBX)-interacting protein; OS overall survival; DFS disease-free survival; CI confidence interval; <sup>a</sup> Cox regression test.

## 4. Discussion

In the present study, we analyzed the association of endoplasmic reticulum ribosomal binding protein 1 (RRBP1) expression in cervical squamous cell carcinoma and its poor prognosis. We proved that RRBP1 expression in cervical squamous cell carcinoma tissues was significantly higher than that in normal cervical tissues by Western blot and qRT-PCR analysis of RRBP1 protein expression and mRNA level analysis, and it indicates that RRBP1 may participated in the proliferation, invasion and metastasis of cervical squamous cell carcinoma. To the best of our knowledge, this is the first to publish evaluation of endoplasmic reticulum ribosomal binding protein 1 (RRBP1) overexpressing in cervical cancer tissue. In our study, we found that the overexpression of RRBP1 in cervical squamous cell carcinoma tissues was related to FIGO stage (P = 0.030), tissue differentiation (P = 0.047), and lymph node metastasis (P = 0.031), which was not related to patient age (P = 0.667). Univariate survival analysis showed that RRBP1 overexpression and lymph node metastasis affected the overall survival (OS) and disease-free survival (DFS) of patients. Multivariate survival analysis showed that RRBP1 was an independent prognostic factor.

Endoplasmic reticulum ribosomal binding protein 1 (RRBP1) was originally explored in *S. cerevisiae* and its primary structure with highly repetitive tandem sequences and demonstrated to be located on the endoplasmic reticulum membrane.(3) Because of its unusually long 51 UTR in its secondary structure, it may be directly involved in the recruitment of ribosomal subunits and initiate translation.(11) According to a large number of previous studies, it was found that RRBP1 may interact with kinesin family member 5B (KIF5B) to participate in the accumulation of autophagosomes in tumor cells, and overexpression of RRBP1 causes ER stress to be alleviated and stabilized, leads to the accumulation of unfolded and folded proteins (UPR) in the endoplasmic reticulum, resulting in abnormal protein function, which can lead to cancer.(18) Recently, overexpression of *rrbp1* was found in liver cancer, prostate cancer, colorectal cancer, lung cancer, Her-2 (+) breast cancer, esophageal cancer, in endometrial cancer and in ovarian cancer and was closely associated with poor prognosis.(9, 10, 12–17)

This study demonstrated the relationship between high expression of RRBP1 and its poor prognosis of cervical squamous cell carcinoma. We have the equally method as the previous study, which proved that RRBP1 plays a significant role in carcinogenesis and tumor progression, and it provides a powerful help for early diagnosis and individualized treatment of cervical squamous cell carcinoma from now on. Our samples were all squamous cell carcinoma, so more pathological types such as adenocarcinoma and adeno-squamous carcinoma will need to fully evaluate the relationship between the high expression of RRBP1 in cervical cancer and its prognosis.

## 5. Conclusion

In conclusion, this present research proves that endoplasmic reticulum ribosomal binding protein 1 (RRBP1) was overexpressed in cervical squamous cell carcinoma tissues and influence patients' disease-free survival (DFS) and overall survival (OS). RRBP1 as an independent prognostic factor for cervical squamous cell carcinoma, and It was indicated that RRBP1 may be become a new tumor marker for cervical squamous cell carcinoma, and will provide great help in early diagnosis, individualized treatment and monitoring of poor prognosis for cervical squamous cell carcinoma, and inhibitors of RRBP1 may be used to treat cancer to some extent.

## Abbreviations

RRBP1

Endoplasmic ribosome-binding protein 1

ER

Endoplasmic reticulum

SCC

Cervical squamous cell carcinoma

qRT-PCR

Quantitative real-time polymerase chain reaction

FIGO

Federation International of Gynecologie and Obstetrigue

OS

Overall survival

DFS

Disease-free survival

ECM

Extracellular matrix

ALK

Anaplastic lymphoma kinase

UPR

Unfolded protein response

PBS

Phosphate buffered saline

## Declarations

### Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of The Affiliated Tumor Hospital of Harbin Medical University. All procedures were performed by the ethical standards of our institutional research committee. Written informed consent was obtained from each participant by the institutional guidelines.

### Consent for publication

Informed consent was obtained from all participants for publication.

### Availability of data and materials

The data used and analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

JQZ: data acquisition and manuscript writing, RXZ: quality control of data review, WX and JM: follow up and quality control of data, NX: study concept and design.

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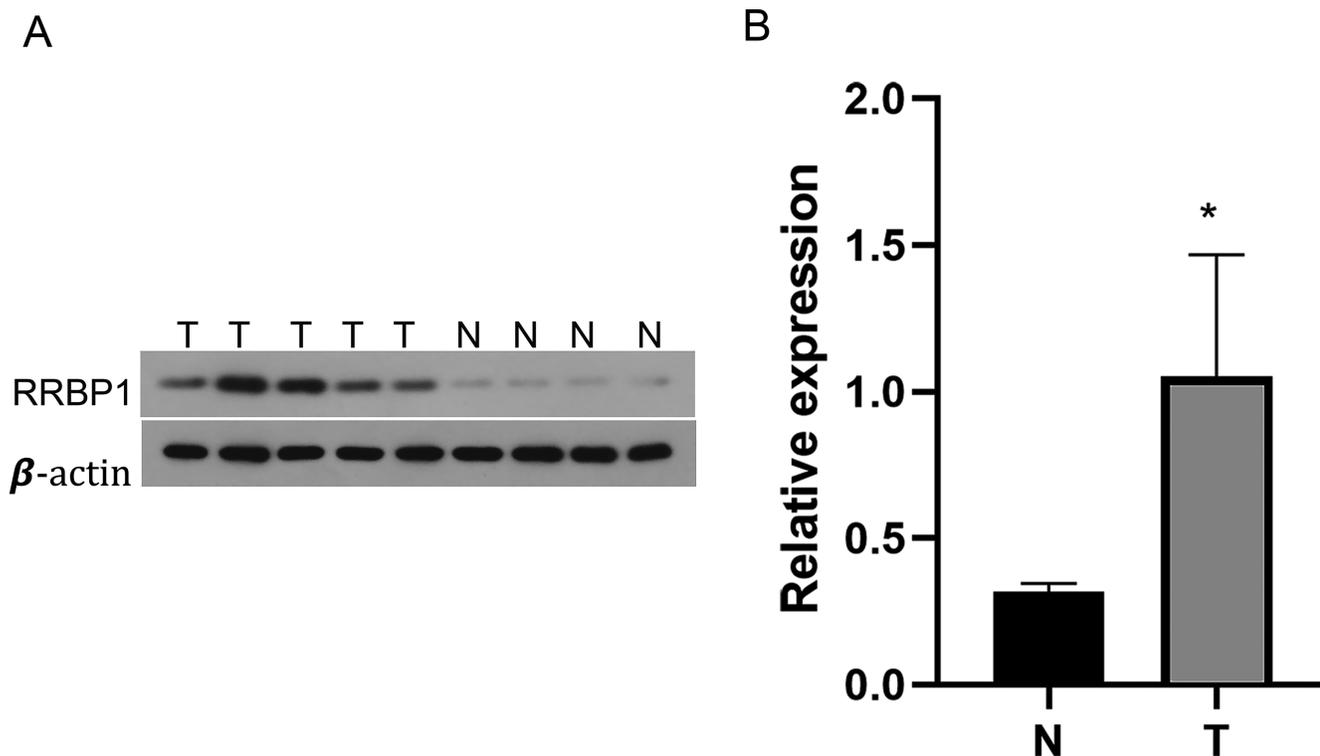
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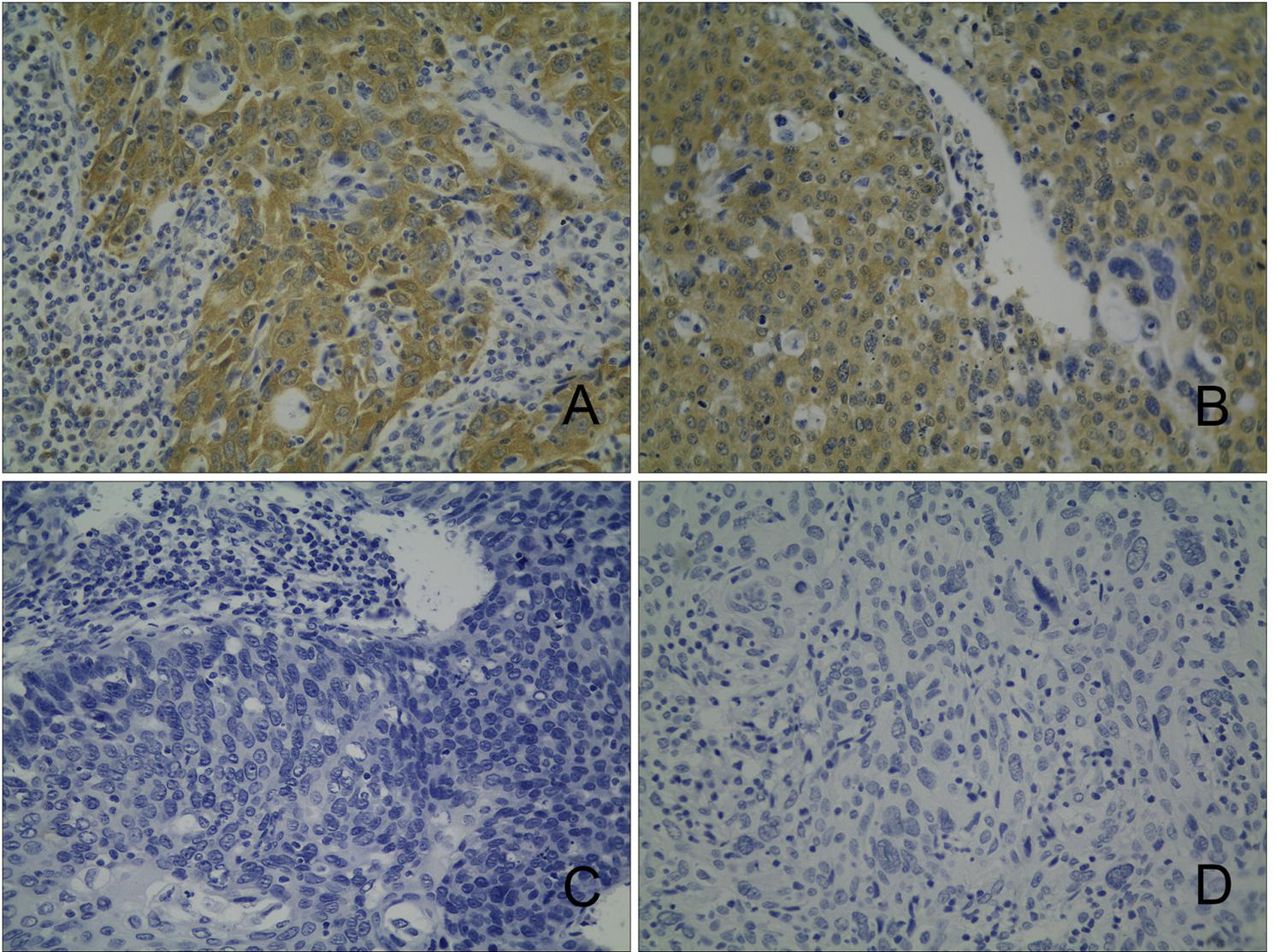
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## Figures



**Figure 1**

A, Representative protein samples obtained from frozen normal cervical tissues (N) and cervical squamous cell carcinoma tissues (T) were analyzed by Western blot. The levels of  $\beta$ -actin were used as an internal control; B, Histogram of pooled data from N (n=10) and cervical squamous cell carcinoma cells (SCCs) (n=26). RRBP1 expression was elevated in SCCs compared with that in N. The data are presented as the mean  $\pm$  s. d (\* P<0.001).



**Figure 2**

Immunohistochemical staining of RRBP1 in SCC specimens: A and B, High expression of RRBP1 in SCCs; C and D, Low expression of RRBP1 in SCCs.

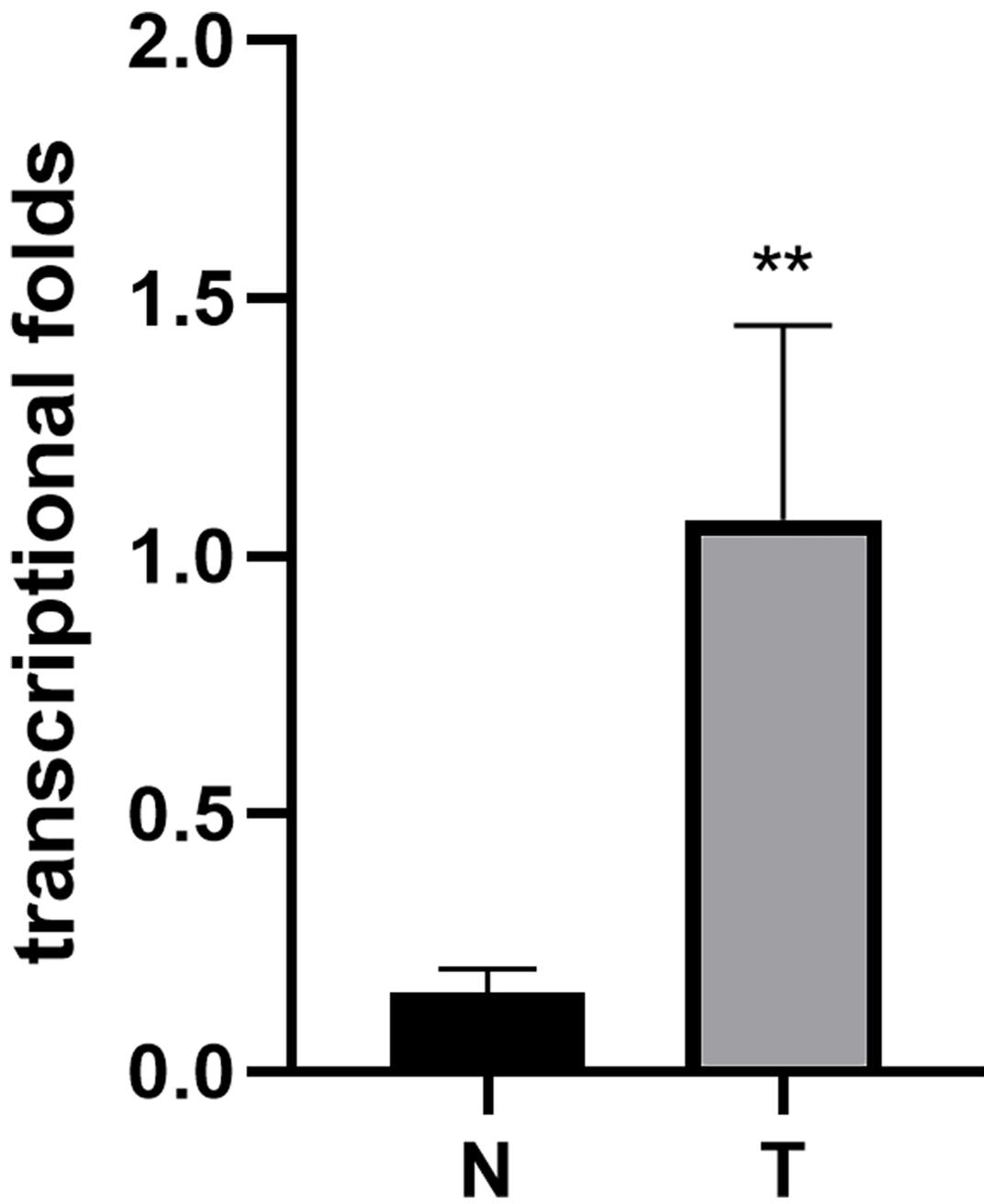
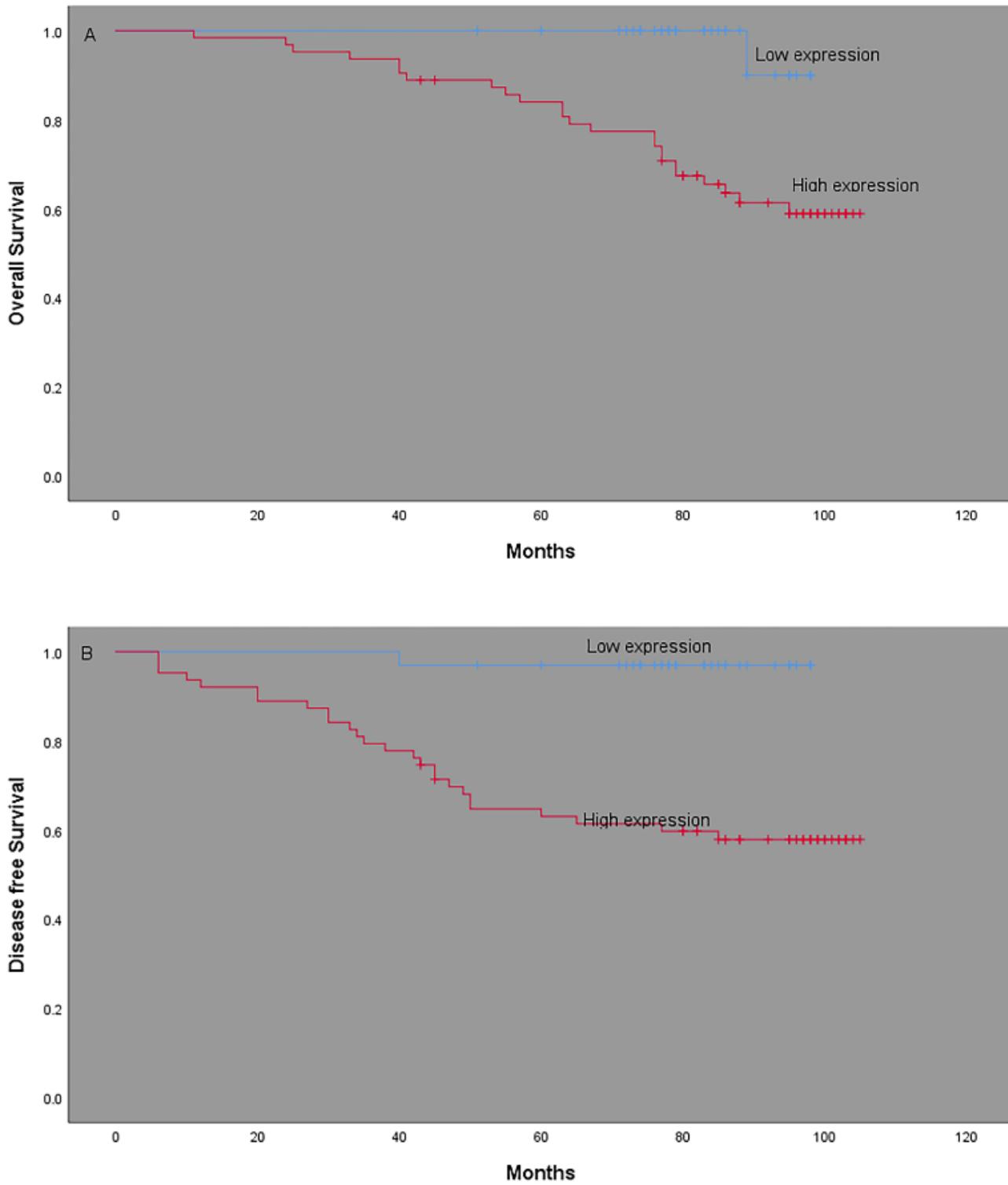


Figure 3

Histogram of RRBP1 mRNA expression in normal cervical tissues and cervical squamous cell carcinoma tissues (N, normal cervical tissues; T, cervical squamous cell carcinoma tissues). The levels of  $\beta$ -actin were used as an internal control, and the RRBP1 mRNA expression was calculated by  $2^{-\Delta\Delta Ct}$  method. RRBP1 mRNA expression was elevated in SCCs compared with normal cervical tissues. The data are presented as the mean  $\pm$  s. d (\*\*  $P < 0.001$ ).



**Figure 4**

Kaplan–Meier analysis of overall survival and disease-free survival related to the expression of RRBP1. Patients with high expression of RRBP1 had a poorer prognosis than those with low expression of RRBP1. A, overall survival curves of the SCC according to their RRBP1 expression status,  $P \leq 0.018$ ; B, disease-free survival curves of the SCC patients according to their RRBP1 expression status,  $P = 0.008$ .