# Increased expression of the RNA-binding motif protein 47 predicts poor prognosis in non-small-cell lung cancer

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**Abstract.** Lung cancer is the leading cause of cancer-associated mortality worldwide. In China, in particular, lung cancer mortality has markedly increased and is likely to continue to rise. RNA-binding proteins are pivotal to the development and progression of a variety of cancer types, including non-small cell lung cancer (NSCLC). RNA-binding motif protein 47 (RBM47) has been found to act as a tumor suppressor in breast cancer and NSCLC. However, to the best of our knowledge, RBM47 expression in NSCLC tissues has yet to be investigated. Analysis via the online database, Gene Expression Omnibus, revealed that RBM47 was upregulated in NSCLC and associated with pathological type, suggesting that RBM47 may play different roles in lung adenocarcinoma and lung squamous cell carcinoma. In the present study, the expression of RBM47 was examined by immunohistochemistry in 175 pairs of tumor and adjacent non-cancerous tissues resected from patients with NSCLC. The results indicated that the expression of RBM47 was significantly increased in NSCLC samples compared with that in the matched non-cancerous samples. Furthermore, RBM47 expression was higher in Xuanwei compared with that in non-Xuanwei NSCLC, suggesting that RBM47 is a more sensitive biomarker in Xuanwei NSCLC, and that it may serve as a candidate therapeutic target. In addition, RBM47 expression was associated with the pathological type, however not with the age, sex, lymph node metastasis, pT stage or pathological Tumor-Node-Metastasis stage of the patients. The increased expression level of RBM47 may indicate a worse overall survival rate for patients with NSCLC. In addition, multivariate survival analysis showed that the Xuanwei area is associated with poor prognosis for patients with NSCLC. In conclusion, the present study revealed that the upregulation of RBM47 accelerated the malignant progression of NSCLC, indicating that RBM47 may be a potential biomarker for NSCLC progression and a therapeutic target for NSCLC.

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## Introduction

Lung cancer is one of the most prevalent malignancies worldwide (1). In 2014, ~781,500 new cases of lung cancer were reported in China, with an incidence of $5713/10^5$, higher than the world standard rate of $3663/10^5$ (2). A notable increase in mortality rates of lung cancer was observed in China from $5.46/10^5$ in the 1970s, $17.54/10^5$ in the 1990s, to $30.83/10^5$ in the 2000s (3,4). Xuanwei has one of the highest mortality rates of lung cancer in China, and Xuanwei lung cancer (XWLC) is a term used to describe lung cancer cases recorded in Xuanwei City and Fuyuan County (5), which are known for extensive combustion of locally sourced bituminous (smoky) coal in Yunnan, China. XWLC is characterized by its high incidence and mortality rates, and a significant difference in incidence in terms of the sex of the patient (6), and a lower patient age at onset (7-9). The epidemiologic studies demonstrated that lung cancer mortality rates in Xuanwei were 4-5 times higher than the average in China (10). The mortality rate of lung cancer in Xuanwei in 2011-2013 was $82.53/10^5$ and $62.62/10^5$ for males and females, respectively. The age-standardized death rate (ASMR) of lung cancer among males in Xuanwei was three times of that in rural areas in China, and six times higher among females (6,11). A previous study demonstrated that indoor air pollution-releasing and cancer-causing substances, such as polycyclic aromatic hydrocarbons, also produce inorganic particulate matter that can lead to the damage of alveolar cells and activate NF-κB signaling pathways, ultimately leading to tumorigenesis (12). Non-small cell lung cancer

(NSCLC) accounts for 80-85% of all lung cancer cases and small cell lung cancer (SCLC) for the remaining 15-20% (13). Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the most common pathological types of NSCLC (14). In advanced NSCLC, due to its aggressiveness and resistance to treatment, the 5-year relative survival rate is ~17.4% (15), despite efforts to discover novel therapies, such as PD-1 inhibitors (16). In order to accurately diagnose and treat NSCLC, it is imperative that the molecular mechanisms of tumorigenesis and progression are accurately investigated, and novel therapeutic strategies are identified.

Previous investigations have indicated that RNA-binding proteins (RBPs) also serve a prominent role in tumorigenesis and cancer progression (17-19). Since they contain ≥1 RNA-binding domains, RBPs can bind to specific mRNA targets and participate in post-transcriptional RNA regulation, including splicing, polyadenylation and translation (20). For example, tumor spheres of lung cancer cells highly express ELAV-like RNA binding protein 1 (HuR), and lung cancer stem cells maintain stemness through the HuR/microRNA(miR)-873/cyclin-dependent kinase 3 (CDK3) and HuR/miR-125a-3p/CDK3 axes (21). Similarly, insulin-like growth factor 2 mRNA-binding protein 3 has been shown to promote lung tumorigenesis by attenuating p53 protein stability (22).

As a novel RBP, RNA-binding motif protein 47 (RBM47), located on chromosome 4p14, has a high affinity for poly-A, -C and -URNAs, and produces two transcripts due to alternative splicing during protein synthesis (23). In zebra fish embryogenesis, RBM47 serves a critical role in head formation and embryonic patterning through the Wnt8a signaling pathway (23). RBM47 also binds to Nanog transcript in mouse embryonic stem (ES) cells, regulating ES cell pluripotency (24). Regarding regulation, the basic machinery of C-to-U RNA editing comprised of RBM47 and apolipoprotein B mRNA editing enzyme catalytic subunit 1 (APOBEC1) to enable post-transcriptional modification (25).

Previous studies have demonstrated that RBM47 plays differing roles in breast cancer and lung cancer (26,27). It has been reported that RBM47 suppresses Wnt activity, inhibiting breast cancer progression and metastasis (26). It was also recently reported that RBM47 inhibits Nrf2 activity in LUAD, retarding tumor growth (27). However, to the best of our knowledge, the detection of RBM47 expression in lung cancer tissues has not been previously reported. The present study aimed to investigate the expression of RBM47 in tumor tissues, compared with matched adjacent non-neoplastic tissues from patients with NSCLC. The association between RBM47 expression and the clinicopathological characteristics of patients with NSCLC, including age, sex, pathological types, pT stage, lymph node metastasis, pathological Tumor-Node-Metastasis (pTNM) stage and overall survival rates were also analyzed.

## Materials and methods

*Gene expression omnibus (GEO) data from the Affymetrix platform*. RBM47 expression data were retrieved from the GEO database (http://www.ncbi.nlm.ih.gov/geo/), using the Affymetrix platform (https://www.thermofisher.com/cn/zh/home/life-science/microarray-analysis/affymetrix.html) for the following six datasets; GSE27262 (25 NSCLC samples and 25 marginal samples collected for microarray analysis), GSE7670 (27 paired NSCLC samples and the corresponding adjacent non-cancerous samples as a control), GSE19804 (60 NSCLC tumor samples and 60 adjacent normal tissues), GSE10245 (40 LUAD and 18 LUSC samples), GSE14814 (71 LUAD and 52 LUSC samples) and GSE21933 (11 LUAD and 10 LUSC samples). Paired Student's t-test was employed to compare RBM47 expression levels between the different groups. $P<0.05$ was considered to indicate a statistically significant difference. Additional details regarding the data are presented in Table I.

*Clinical data collection*. The clinical processes were approved by the Ethics Committee of the Third Affiliated Hospital of Kunming Medical University, and written informed consent was provided by all patients. Of the 175 cases, 107 were male and 68 were female, with a median age of 57 years (range, 27-78 years). XWLC fit the following criteria, three generations all residing in a coal region and >10 years of coal-burning history. Patients with NSCLC were divided into the XWLC group (72 patients) and non-XWLC group (103 patients). 175 pairs of samples were fixed in 10% formalin for 24 h at room temperature. Paraffin-embedded primary NSCLC tissues and paired non-cancerous lung tissues (5 cm away from the tumor edge) were obtained between December 2008 and October 2015 from the Third Affiliated Hospital of Kunming Medical University (Kunming, China). The stage of the tumor specimens was determined according to the 7th edition of the TNM Classification for Lung Cancer (28). American Joint Committee on Cancer (AJCC) staging (28) consists of clinical and pathological staging. Pre-operative CT examination was not performed on 20 patients due to economic reasons, resulting in an inaccurate reflection of the patient's clinical stage and therefore, the pathological stage was used. The patients included in the present study had been diagnosed with stage IA-IIIA NSCLC. Patients who had received preoperative radiation, chemotherapy or biotherapy were excluded from the study. None of the patients had been diagnosed with multiple primary cancers in other organs or tissues. The clinical data were derived from the patients' medical records. All patients had undergone complete surgical resection, including primary tumor plus mediastinal and bronchial lymph node dissection. All 175 patients with NSCLC had effective and accurate follow-up data (from 2-110 months ). The follow-up data of patients was updated using medical records, interviews and via telephone. According to the National Comprehensive Cancer Network guidelines (29), adjuvant chemotherapy is not recommended for stage IA, but may be useful in a subset of patients at stage IB. For stage II and IIIA, which is a therapeutically challenging and controversial subset of lung cancer, four cycles of platinum-based adjuvant chemotherapy to address micro-metastatic disease is recommended.

*Immunohistochemistry (IHC) and hematoxylin and eosin (H&E) staining*. IHC was performed in order to determine RBM47 expression levels and distribution patterns of 175 pairs of NSCLC tissues. IHC was performed in accordance with previous studies (30,31). Tissue samples were cut into 4-$\mu$m-thick sections and incubated in Rabbit polyclonal RBM7

Table I. Detailed information from the Affymetrix Human Genome platform for the Gene Expression Omnibus datasets.

| Series accession | Organism | Type | Affymetrix human genome platform | Reference |
|---|---|---|---|---|
| GSE7670 | Homo sapiens | Expression profiling by array | GPL96 [HG-U133A] Affymetrix Human Genome U133A Array | (55) |
| GSE19804 | Homo sapiens | Expression profiling by array | GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array | (56) |
| GSE27262 | Homo sapiens | Expression profiling by array | GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array | (57) |
| GSE10245 | Homo sapiens | Expression profiling by array | GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array | (58) |
| GSE14814 | Homo sapiens | Expression profiling by array | GPL96 [HG-U133A] Affymetrix Human Genome U133A Array | (59) |
| GSE21933 | Homo sapiens | Expression profiling by array | GPL6254 Phalanx Human OneArray | (60) |

antibody (1:500; cat. no. HPA006347; Sigma-Aldrich; Merck KGaA) for 12 h at 4˚C, in order to detect RBM47 protein expression. The sections were washed three times with PBS and incubated with avidin-biotin complex (Vector Laboratories, Inc.). 3-3'-diamino-benzidine was used as the chromogen for the color reaction. A total of 30 $\mu$l of the secondary antibody in the DAB kit (Dako; Agilent Technologies, Inc.), with original concentration, was added to the sections and incubated at 37˚C for 30 min. H&E staining was used to evaluate histology (32). Paraffin-embedded tissues samples were cut into 4-$\mu$m-thick sections and incubated at 60˚C for 1 h. The samples were subsequently deparaffinized in xylene at 60˚C for 15 min and rehydrated prior to incubation with hematoxylin (original concentration; Fuzhou Maixin Biotech Co., Ltd.) at room temperature for 2 min, followed by incubation with eosin (original concentration; Guangzhou RiboBio Co., Ltd.) at room temperature for 1 min. Subsequently, the slices were dehydrated in a step-up ethanol series at room temperature and mounted in neutral gum prior to analysis using an optical microscope (Leica Microsystems GmbH; magnification, x100 and x200). The negative controls were obtained by replacing the primary antibody with non-immunized sheep serum (Fuzhou Maixin Biotech Co., Ltd.).

*Evaluation of IHC*. RBM47-positive cells displayed brownish yellow granules in the cytoplasm and nucleus. Two independent pathologists from The Third Affiliated Hospital of Kunming Medical University, who were blinded to the clinical parameters, determined the scores using a fluorescent inverted microscope (magnification, x100 and x200). The extent and intensity of immunoreactivity was scored in a semi-quantitative manner. The extent of immunoreactivity was scored as follows: 0, 0% immunoreactive cells; 1, <5% immunoreactive cells; 2, 5-50% immunoreactive cells and 3, >50% immunoreactive cells. The intensity of immunoreactivity was scored as follows: 0, negative; 1, weak; 2, intermediate and 3, strong. The samples were grouped according to the sum of the immunoreactivity score as follows: 0, negative; 1-2, weak; 3, moderate and 4-6, strong (31). Final immunoreactivity scores >0 were considered positive, and those at 0 were considered negative.

*Statistical analysis*. All data are expressed as the mean ± standard deviation. All experiments were performed in triplicate and statistical analyses were conducted using SPSS (version 18.0; SPSS, Inc.). The paired Student's t-test was used in order to determine the differences in RBM47 protein expression levels between different groups in the GEO database. The $\chi^2$ test was used in order to determine the differences in the RBM47 protein expression levels between NSCLC and non-cancerous lung tissues. The Kaplan-Meier method was used to plot survival curves, and Cox's proportional hazards regression model was used to analyze multivariate survival. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Analysis of GEO data*. In order to examine the expression patterns of RBM47 in NSCLC, the GSE27262 dataset was first analyzed, which included 25 NSCLC and 25 marginal samples collected for microarray analysis. As presented in Fig. 1A, the RBM47 expression level was significantly higher in the cancerous samples compared with the marginal samples in GSE27262 ($P=0.0102$). In order to verify the result, two additional datasets (GSE7670 and GSE19804; Fig. 1B and C), were utilized and data were consistent (all $P<0.05$). These data revealed that RBM47 was upregulated in NSCLC. Furthermore, the potential association between RBM47 expression and the pathological type of NSCLC was investigated using the GSE10245 dataset in Fig. 1D, which included 40 LUAD and 18 LUSC samples. The data analysis results indicated that the expression level of RBM47 was significantly different between LUAD and LUSC samples ($P<0.001$). Furthermore, two additional datasets (GSE14814 and GSE21933; Fig. 1E and F), demonstrated similar results (all $P<0.05$). Collectively, RBM47 expression exhibited an association with NSCLC and pathological type, suggesting that RBM47 may serve different roles in LUAD and LUSC.

*Clinicopathological characteristics of patients with NSCLC*. A total of 175 histologically confirmed, paraffin-embedded NSCLC tissues were investigated. The clinicopathological

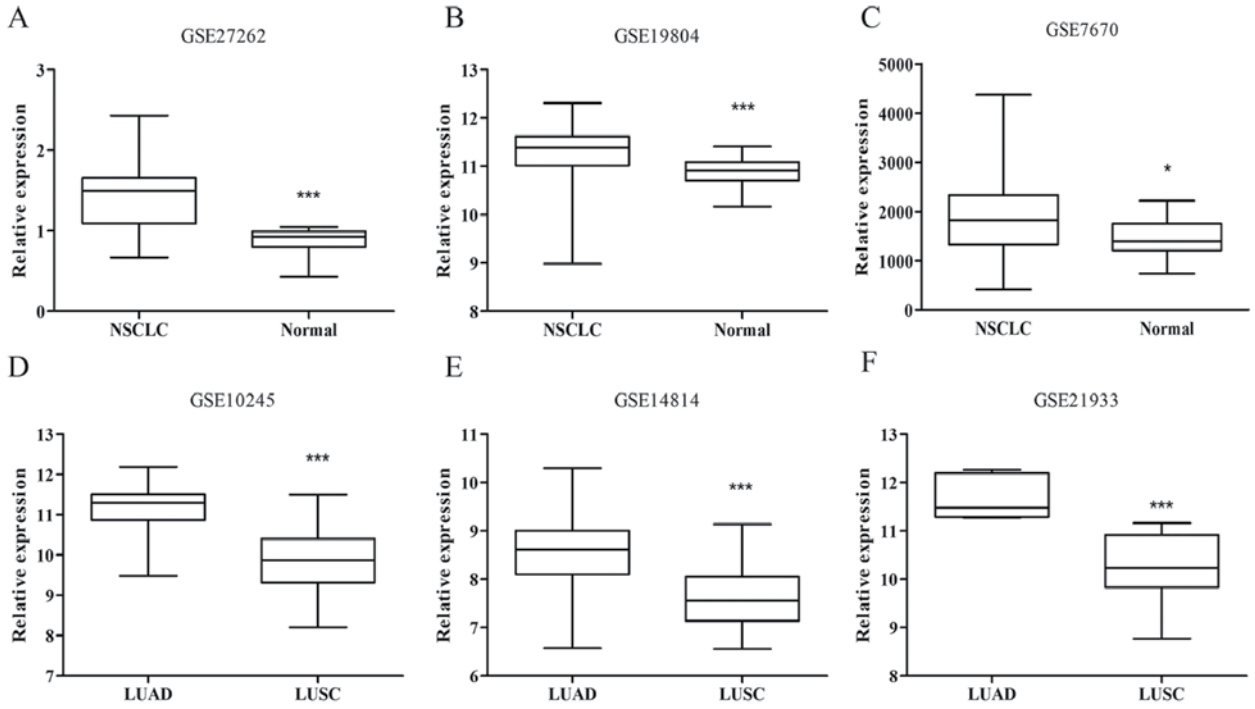


Figure 1. Analysis of RBM47 gene expression in NSCLC from GEO datasets. A total of six datasets, including (A) GSE27262, (B) GSE19804, (C) GSE7670, (D) GSE10245, (E) GSE14814 and (F) GSE21933 were acquired from the GEO repository and subjected to data analyses. (A-C) RBM47 expression was significantly increased in NSCLC compared with non-cancerous lung tissues. (D-F) GSE10245, GSE14814 and GSE21933 datasets were analyzed to confirm whether RBM47 protein exhibited significantly different expression levels between the LUAD and LUSC pathological types. $^{*}$P<0.05, $^{***}$P<0.001 vs. NSCLC; $^{***}$P<0.001 vs. LUAD. RBM47, RNA-binding motif protein 47; NSCLC, non-small-cell lung cancer; GEO, Gene Expression Omnibus; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

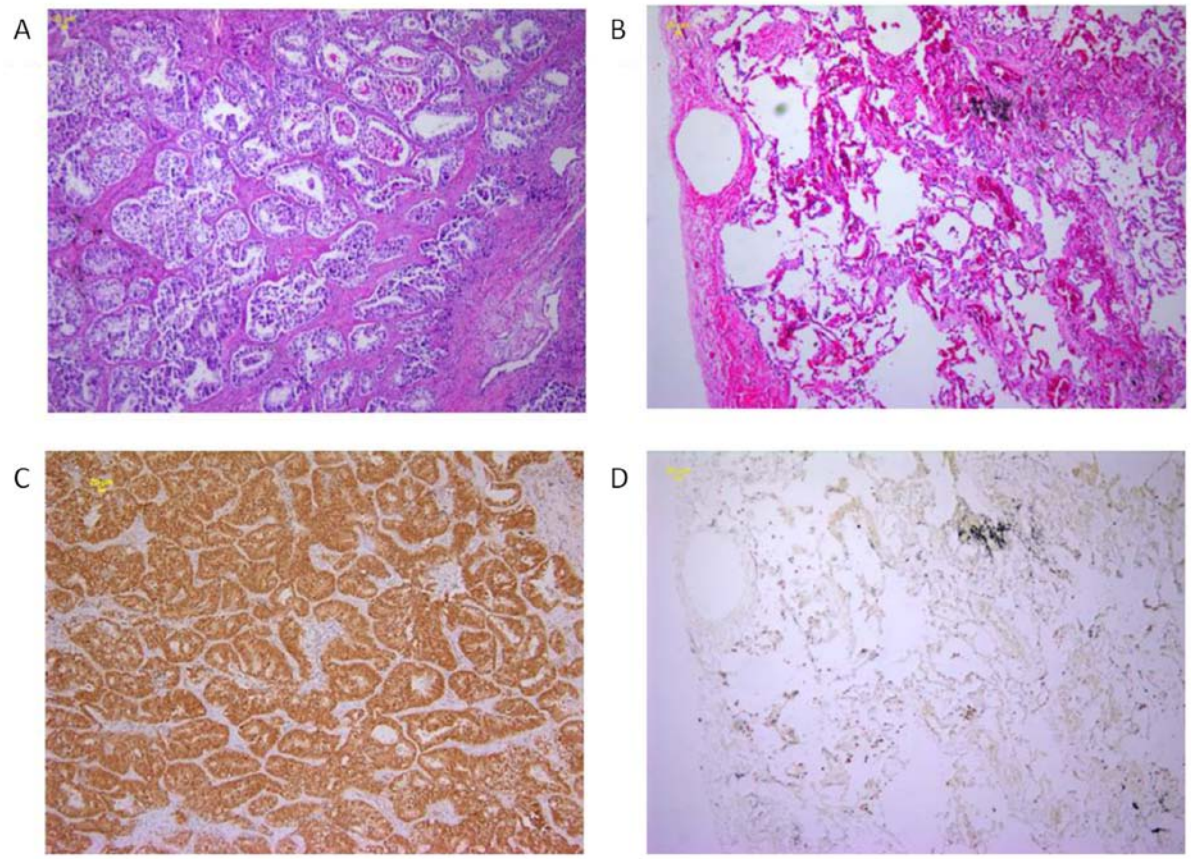


Figure 2. Representative immunohistochemistry images of RBM47 protein expression in NSCLC and non-cancerous lung tissues via hematoxylin & eosin staining. (A and C) RBM47 was positively expressed in NSCLC cells, but (B and D) negatively or weakly expressed in adjacent lung epithelial cells (all used magnification, x100). RBM47, RNA-binding motif protein 47; NSCLC, non-small-cell lung cancer.

Table II. Clinicopathological characteristics of patients with non-small cell lung cancer.

| Characteristics | Patient, n (%) |
|---|---|
| Total | 175 |
| Sex | |
| Male | 107 (61.1) |
| Female | 68 (38.9) |
| Age, years | |
| <60 | 99 (56.6) |
| ≥60 | 76 (43.4) |
| Area | |
| Xuanwei | 72 (41.1) |
| Non-Xuanwei | 103 (58.9) |
| Pathological type | |
| Adenocarcinoma | 131 (74.9) |
| Squamous carcinoma | 44 (25.1) |
| Tumor size (pT stage) | |
| T1 | 94 (53.7) |
| T2 | 60 (34.3) |
| T3/T4 | 21 (12.0) |
| Lymph node metastasis (pN stage) | |
| Negative | 104 (59.4) |
| Positive | 71 (40.6) |
| AJCC stage at diagnosis (pTNM) | |
| I | 86 (49.1) |
| II | 34 (19.4) |
| IIIA | 55 (31.5) |

pT, pathological tumor size; pN, pathological lymph node stage; AJCC, American Joint Committee on Cancer; TNM, Tumor-Node-Metastasis.

characteristics are presented in Table II. The majority of the samples were adenocarcinoma (131 cases, 74.9%) and the remaining were squamous cell carcinoma (44 cases, 25.1%). Tumor stage was determined according to the 7th edition of the AJCC cancer staging manual (19): 86 cases (49.1%) were stage I, 34 (19.4%) stage II and 55 (31.5%) stage III. XWLC fit the following conditions: i) Three generations all residing in a coal region; and ii) >10 years of coal-burning history. The patients were divided into 72 cases of XWLC (41.1%) and 103 cases of non-XWLC (58.9%). The median follow-up time was 39 months (range, 2-110). Of the 175 patients, 71 (40.6%) succumbed to NSCLC; the remaining 104 (59.4%) were still alive at the end of the follow-up. Detailed data are presented in Table II.

*RBM47 is upregulated in NSCLC tissues*. The GEO dataset indicated increased expression of the RBM47 gene in NSCLC cases. The present study, to the best of our knowledge, was the first to measure RBM47 protein expression and subcellular localization by IHC staining, in an independent cohort of 175 patients with NSCLC with cancer tissues and their matched adjacent non-cancerous tissues. IHC staining revealed that RBM47 was localized to the nucleus and cytoplasm. In addition, it was observed that the staining density of RBM47 in the NSCLC tissues was more intense and had a broader distribution than that observed in non-cancerous tissues. In summary, RBM47 was upregulated in NSCLC. Representative IHC staining images of RBM47 protein expression in the tumor and non-cancerous lung tissues are shown in Fig. 2, and the difference in staining intensity between specimens is presented in Fig. 3. These results were consistent with the analysis of the GEO database.

*RBM47 expression is associated with the pathological type of NSCLC*. To further determine the role of RBM47 expression in NSCLC, the association between RBM47 and the clinical characteristics of patients with NSCLC was analyzed. Table III shows the number and percentage of RBM47-positive samples in the two groups. It was also observed that RBM47 stained positive in 75.4% (132/175) of NSCLC tissues, but only 21.7% (38/175) in the matched adjacent non-cancerous tissues, which in turn was significantly lower compared with that in the NSCLC samples (P<0.001). The expression of RBM47 was associated with pathological type (P<0.001), as it was detected in 84% (110/131) of the samples collected from LUAD tissues, but only 50% (22/44) of LUSC tissues. No association was observed between RBM47 and the age, sex, tumor size, pT stage, lymph node metastasis and pTNM stage in patients with NSCLC (Table III). This result indicated that RBM47 expression was associated with the pathological type of NSCLC, which was consistent with the results of the GEO profiles.

In order to compare RBM47 expression between XWLC and non-XWLC, patients with NSCLC were divided into the Xuanwei and non-Xuanwei groups. Patients in the Xuanwei group were all from Xuanwei city and Fuyuan County of the Yunnan Province. The association between the RBM47 expression and the clinicopathological characteristics of the two groups was subsequently investigated (Tables IV and V). The expression of RBM47 was only associated with the pathological type (P<0.001) in both the Xuanwei and non-Xuanwei groups. Samples of the Xuanwei group had a significantly higher RBM47 expression level than those in the non-Xuanwei group (P=0.031) (Table VI), suggesting that RBM47 was a more sensitive biomarker in Xuanwei NSCLC.

*Association between RBM47 expression, and overall survival rates and prognosis in NSCLC*. The association between RBM47 expression and overall survival in NSCLC was analyzed. The present data revealed that a high RBM47 expression level in patients with NSCLC was significantly associated with poor prognosis compared with a low RBM47 expression level (P=0.009; Fig. 4A). Furthermore, the potential differences in the prognostic roles of RBM47 between patients with Xuanwei and non-Xuanwei NSCLC were investigated (Fig. 4B and C). It was indicated that RBM47 did not serve as a prognostic factor for patients with Xuanwei NSCLC, since its quantity variance affected the statistical analysis. However, RBM47 was significantly associated with poor survival in patients with non-Xuanwei NSCLC (P=0.004).

The independent prognostic factors, including RBM47, location, age, sex, tumor size, pT stage, lymph node metastasis and pTNM stage, were analyzed in association with NSCLC patient survival using multivariate Cox's proportional hazards

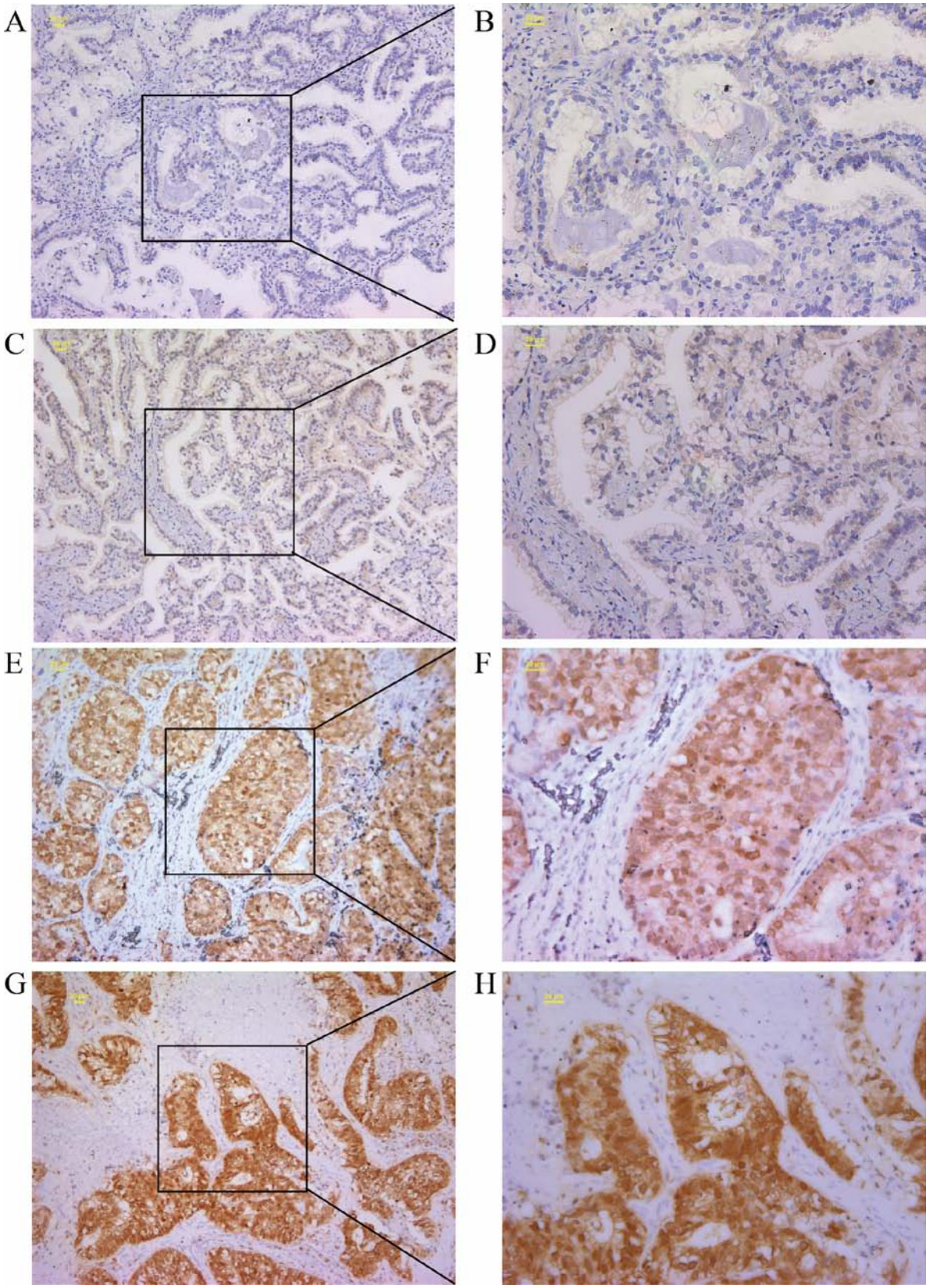


Figure 3. Staining intensity of RBM47 differed between the specimens. Representative images demonstrated different staining intensities of RBM47 expression, as follows: (A and B) Negative staining, (C and D) weak staining, (E and F) moderate staining and (G and H) strong staining (A, C, E and F magnification, x100; B, D, F and H magnification, x200). RBM47, RNA-binding motif protein 47.

regression analysis. It was found that location, however not RBM47 expression, was an independent prognostic factor for NSCLC (P=0.001; Table VII). In conclusion, the present data demonstrated that the Xuanwei area is associated with poor prognosis in patients with NSCLC, and that RBM47 is a more sensitive prognostic biomarker in Xuanwei NSCLC.

Table III. RBM47 expression in patients with NSCLC and its associations with different characteristics.

| Variables | Patient, n | RBM47 expression | | P-value[a] |
|---|---|---|---|---|
| | | Negative expression, n (%) | Positive expression, n (%) | |
| Tissues | | | | <0.001 |
| Normal lung tissues | 175 | 137 (78.3) | 38 (21.7) | |
| Primary tumors of NSCLC | 175 | 43 (24.6) | 132 (75.4) | |
| Sex | | | | 0.329 |
| Male | 107 | 29 (27.1) | 78 (72.9) | |
| Female | 68 | 14 (20.6) | 54 (79.4) | |
| Age, years | | | | 0.125 |
| <60 | 99 | 20 (20.2) | 79 (79.8) | |
| ≥60 | 76 | 23 (30.3) | 53 (69.7) | |
| Pathological type | | | | <0.001 |
| Adenocarcinoma | 131 | 21 (16.0) | 110 (84.0) | |
| Squamous carcinoma | 44 | 22 (50.0) | 22 (59.0) | |
| Tumor size (pT stage) | | | | 0.784 |
| T1 | 94 | 25 (26.6) | 69 (73.4) | |
| T2 | 60 | 13 (21.7) | 47 (73.8) | |
| T3/T4 | 21 | 5 (23.8) | 16 (76.2) | |
| Lymph node metastasis (pN stage) | | | | 0.578 |
| Negative | 104 | 24 (23.1) | 80 (76.9) | |
| Positive | 71 | 19 (26.8) | 52 (73.2) | |
| AJCC stage at diagnosis | | | | 0.366 |
| I | 86 | 19 (22.1) | 67 (77.9) | |
| II | 34 | 12 (35.3) | 24 (64.7) | |
| IIIA | 55 | 12 (21.8) | 43 (78.2) | |

[a]According to the $\chi^2$ test. RBM47, RNA-binding motif protein 47; NSCLC, non-small cell lung cancer; pT, pathological tumor size; pN, pathological lymph node stage; AJCC, American Joint Committee on Cancer.

## Discussion

An increasing number of recent reports have indicated that RBPs serve an important role in cancer development (33-35). A number of studies have investigated RBPs using IHC staining, and demonstrated that they were abnormally expressed in cancer, compared with adjacent normal tissues, which was associated with patient prognosis (36-38). The RBP La-related protein 1 is a post-transcriptional regulator of ovarian cancer progression and chemotherapy resistance (39). Furthermore, the expression level of RBM4 is significantly lower in gastric cancer compared with adjacent non-cancerous tissues, and it's also associated with poor differentiation, lymph node status and distant metastasis (40). In addition, it was identified as a novel biomarker and an independent prognostic marker (40). In addition to protein expression levels, the biological functions of RBPs are varied, including RNA alternative splicing, stability, subcellular localization and translation (41,42). The roles of RBPs in cancer can therefore be diverse. RBM10 was reported to be consistently downregulated in LUAD, promoting NUMB mRNA exon 9 skipping to generate a NUMB isoform that blocks cell proliferation and inhibited Notch activity (43,44). Regarding mRNA stability, in multiple types of cancer (21,45,46), overexpressed Hu-Antigen R contributes to the accelerated proliferation of cancer cells by enhancing the stability of cell-cycle regulators containing cyclinA and cyclinB1 encoded by AU-rich element-containing mRNAs (47,48). Furthermore, RBM5, as a tumor suppressor in NSCLC, splices the pre-mRNA of multiple target genes, including the tumor suppressor protein p53, which participates in the induction of cell cycle arrest and apoptosis (49). Therefore, further examination on the functional role of RBPs may contribute to the diagnosis and treatment of human cancers.

In the present study, RBM47 expression was investigated in 175 paired tissue samples from patients with NSCLC. IHC data indicated that RBM47 was more highly expressed in cancer tissues compared with the matched adjacent tissues. In addition, the RBM47 expression levels were also elevated in the data from the three GEO datasets, suggesting that RBM47 may be involved in the development of NSCLC. The next investigation indicated that enhanced RBM47 expression was significantly associated with the pathological type of NSCLC, however not with the sex, age, tumor size, pT stage, lymph

Table IV. RBM47 expression in patients with NSCLC from the Xuanwei area.

| | | RBM47 expression | | |
|---|---|---|---|---|
| Variables | Patient, n | Negative expression, n (%) | Positive expression, n (%) | P-value[a] |
| Tissue | | | | <0.001 |
| Normal lung tissues | 72 | 58 (80.6) | 14 (19.4) | |
| Primary tumors of NSCLC | 72 | 12 (16.7) | 60 (83.3) | |
| Sex | | | | 0.329 |
| Male | 42 | 7 (16.7) | 35 (83.3) | |
| Female | 30 | 5 (16.7) | 25 (83.3) | |
| Age, years | | | | 0.385 |
| <60 | 55 | 8 (14.5) | 47 (85.5) | |
| ≥60 | 17 | 4 (23.5) | 13 (76.5) | |
| Pathological type | | | | 0.007 |
| Adenocarcinoma | 73 | 8 (11.0) | 65 (89.0) | |
| Squamous carcinoma | 9 | 4 (44.4) | 5 (55.6) | |
| Tumor size (pT stage) | | | | 0.296 |
| T1 | 44 | 9 (20.5) | 35 (79.5) | |
| T2 | 19 | 1 (5.3) | 18 (94.7) | |
| T3/T4 | 9 | 2 (22.2) | 7 (77.8) | |
| Lymph node metastasis (pN stage) | | | | 0.814 |
| Negative | 52 | 9 (17.3) | 43 (82.7) | |
| Positive | 20 | 3 (15.0) | 17 (85.0) | |
| AJCC stage at diagnosis | | | | 0.720 |
| I | 45 | 9 (20.0) | 67 (80.0) | |
| II | 12 | 2 (16.7) | 10 (83.3) | |
| III | 15 | 1 (6.7) | 14 (93.3) | |

[a]According to the $\chi^2$ test. RBM47, RNA-binding motif protein 47; NSCLC, non-small cell lung cancer; pT, pathological tumor size; pN, pathological lymph node stage; AJCC, American Joint Committee on Cancer.

node metastasis or pTNM stage of patients with NSCLC. Subsequently, a higher expression level of RBM47 was detected in Xuanwei compared with non-Xuanwei NSCLC, which suggested that RBM47 was a more sensitive prognostic marker of Xuanwei NSCLC. As candidate oncogenes are generally associated with poor prognosis in patients with cancer (50), it was inferred that RBM47 may also be associated with the prognosis of those with NSCLC. Furthermore, the association between RBM47 expression and the overall survival of patients with NSCLC was analyzed. The data revealed that a high RBM47 expression was associated with a worse prognosis compared with a low RBM47 expression level in patients with NSCLC, including non-Xuanwei NSCLC. For patients with Xuanwei NSCLC, RBM47 did not serve as a prognostic factor, because its quantity variance affected the statistical analysis. However, a trend in prognostic differences was revealed by the survival curve. Unfortunately, due to the limited number of available specimens, the number of cases of XWLC could not be increased to improve the results of the statistical analyses. The results provided a greater understanding of the underlying role of RBPs in the development and progression of NSCLC. However, one limitation of the study was the lack of investigation into the role of RBM47 in lung cancer cell proliferation and other malignant processes, *in vivo* and *in vitro*. Further investigation is necessary to determine whether RBM47 promotes lung cancer and the exact underlying mechanisms.

Patients with breast cancer with high expression levels of RBM47 tend to achieve a relatively good clinical outcome, as demonstrated in a previous study (26). In experimental models, RBM47 regulated the target mRNA to inhibit the reinitiation and growth of breast cancer, rather than acting directly as a tumor suppressive gene (26). RBM47 bound to the 3'UTR of Dickkopf-related protein 1 mRNA to enhance its expression levels, which has been shown to inhibit breast cancer progression by antagonizing Wnt. Consistent with this observation, Sakurai *et al* (27) used a collection of published microarray data to show that the RBM47 expression may be associated with a longer survival time in patients with lung and gastric cancer. In addition to its tumor-suppressive role in lung cancer, RBM47 bound to the mRNA of kelch-like ECH-associated protein 1 and Cullin 3 to inhibit Nrf2 activity. Meanwhile, RBM47-knockdown accelerated tumor formation and metastasis in mouse xenograft models. The present study focused on the function of RBM47, without detecting the RBM47 expression levels in human lung cancer tissue samples. Taking the above into consideration, further research is required to

Table V. RBM47 expression in patients with NSCLC from the non-Xuanwei area.

| Variables | Patient, n | RBM47 expression | | P-value[a] |
|---|---|---|---|---|
| | | Negative expression, n (%) | Positive expression, n (%) | |
| Tissue | | | | <0.001 |
| Normal lung tissues | 103 | 80 (77.7) | 33 (22.3) | |
| Primary tumors of NSCLC | 103 | 32 (31.1) | 71 (68.9) | |
| Sex | | | | 0.278 |
| Male | 65 | 22 (33.8) | 43 (66.2) | |
| Female | 38 | 9 (23.7) | 29 (76.3) | |
| Age, years | | | | 0.589 |
| <60 | 44 | 12 (27.3) | 32 (72.7) | |
| ≥60 | 59 | 19 (32.2) | 40 (67.8) | |
| Pathological type | | | | 0.001 |
| Adenocarcinoma | 68 | 13 (19.1) | 55 (80.9) | |
| Squamous carcinoma | 35 | 18 (51.4) | 17 (48.6) | |
| Tumor size (pT stage) | | | | 0.605 |
| T1 | 50 | 16 (32.0) | 34 (68.0) | |
| T2 | 41 | 12 (29.3) | 18 (70.7) | |
| T3/T4 | 12 | 3 (25.0) | 9 (75.0) | |
| Lymph node metastasis (pN stage) | | | | 0.623 |
| Negative | 52 | 15 (28.8) | 37 (71.1) | |
| Positive | 51 | 17 (33.3) | 34 (66.7) | |
| AJCC stage at diagnosis | | | | 0.223 |
| I | 41 | 10 (24.4) | 31 (75.6) | |
| II | 22 | 10 (45.5) | 12 (54.5) | |
| III | 40 | 12 (30.0) | 28 (70.0) | |

[a]According to the $\chi^2$ test. RBM47, RNA-binding motif protein 47; NSCLC, non-small cell lung cancer; pT, pathological tumor size; pN, pathological lymph node stage; AJCC, American Joint Committee on Cancer.

Table VI. Association between RBM47 expression and Xuanwei region in patients with NSCLC.

| Variables | Patient, n | RBM47 expression | | P-value[a] |
|---|---|---|---|---|
| | | Negative expression, n (%) | Positive expression, n (%) | |
| Xuanwei NSCLC | 72 | 12 (16.7) | 60 (83.3) | 0.031 |
| Non-Xuanwei NSCLC | 103 | 32 (31.1) | 71 (68.9) | |

[a]According to the $\chi^2$ test. RBM47, RNA-binding motif protein 47; NSCLC, non-small cell lung cancer.

clarify whether RBM47 indicates good or poor prognosis in lung cancer.

A recent study reported that miR-25 could directly target RBM47 to upregulate PI3K/Akt/mTOR signaling in melanoma (51). Interleukin (IL)-8, another target of RBM47, exhibited increased expression in buccal epithelial cells collected from healthy, non-smoking female residents of Xuanwei and Fuyuan, who burnt smoky and smokeless coal, as indicated in the genome-wide gene expression profiles. This suggested that the physiological response to smoky coal modulated pro-inflammatory events in smoky coal users (52). Moreover, RBM47 has been reported to serve an important role in promoting the regulatory functions of B cells by regulating IL-10 at the post-transcriptional level (53). Since RBM47 may serve various roles in different types of cancer, future studies should focus on the exact regulatory functions and the mechanism of RBM47 in the proliferation, migration and invasion of NSCLC cells. In addition to RBM47, other RBPs have been identified as crucial biomarkers or prognostic factors for NSCLC. For instance, the increased expression of hnRNPA2/B1

Table VII. Multivariate analysis of prognostic factors for patients with non-small cell lung cancer.

| Prognostic factor | Hazard ratio | 95% CI | P-value |
|---|---|---|---|
| Area (Xuanwei vs. Non-Xuanwei) | 2.519 | 1.456-4.357 | 0.001 |
| RBM47 expression (Positive vs. Negative) | 0.721 | 0.408-1.275 | 0.261 |
| Sex (Male vs. Female) | 1.232 | 0.732-2.076 | 0.432 |
| Age, years (≥60 vs. <60) | 1.333 | 0.775-2.291 | 0.299 |
| Pathological type (AC vs. SCC) | 1.570 | 0.816-3.022 | 0.177 |
| pT stage (T1-2 vs. T3-4) | 1.220 | 0.62-2.401 | 0.564 |
| Lymph node metastasis (Yes vs. No) | 0.549 | 0.23-1.313 | 0.178 |
| pTNM (I-II vs. IIIA) | 1.480 | 0.574-3.82 | 0.417 |

CI, confidence interval; RBM47, RNA-binding motif protein 47; AC, adenocarcinoma; SCC, squamous cell carcinoma; pT, pathological tumor size; TNM, Tumor-Node-Metastasis.

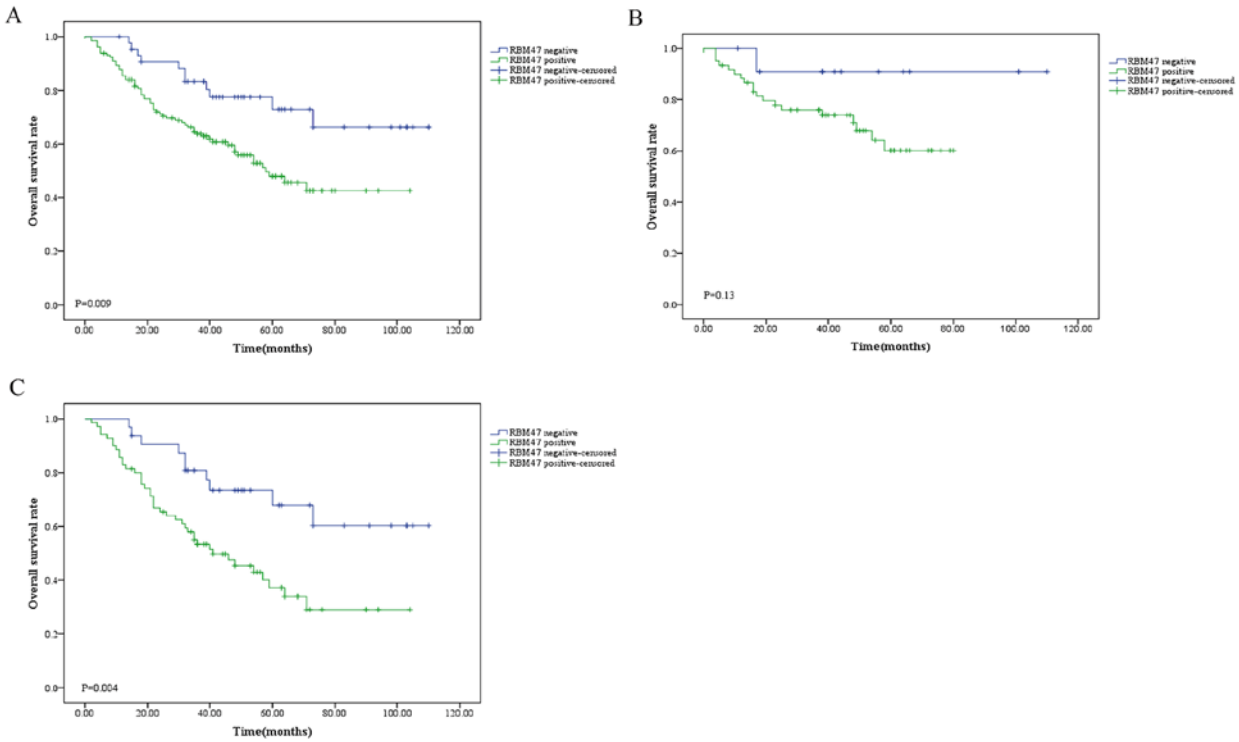


Figure 4. Analysis of overall survival for all patients by log-rank test according to RBM47 expression. (A) Survival curves of 175 patients with NSCLC. Negative RBM47 expression, n=43 and positive RBM47 expression, n=132. (B) Survival curves of 72 patients with Xuanwei NSCLC. Negative RBM47 expression, n=12 and positive RBM47 expression, n=60. (C) Survival curves for 103 patients with non-Xuanwei NSCLC. Negative RBM47 expression, n=32 and positive RBM47 expression, n=71. RBM47, RNA-binding motif protein 47; NSCLC, non-small-cell lung cancer.

is associated with a poor prognosis in patients with NSCLC, and hnRNPA2/B1 activates prostaglandin G/H synthase 2 signaling in NSCLC cells to promote tumor cell growth (54).

In conclusion, the present study indicated that RBM47 is significantly upregulated in NSCLC tissues, as well as being a more sensitive biomarker in Xuanwei NSCLC, and that RBM47 expression is significantly associated with pathological type. Furthermore, the increased expression level of RBM47 may predict poor prognosis in patients with NSCLC. Taking the aforementioned into consideration it was therefore suggested that RBM47 promotes the malignant progression of NSCLC and may become a crucial biomarker and prognostic factor for patients with NSCLC.

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## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

## Authors' contribution

XS and GL contributed to the conception and design of the study. RL, HL, CG, QF and ZL contributed to the acquisition of data. YJ, QT, ZTZ, ZWZ and SD contributed to the analysis and interpretation of data. RL, HL wrote the manuscript. RL, HL, SD and ZWZ collected the clinical samples. All authors contributed to the intellectual content of the article and read and approved the submitted manuscript.

## Ethics approval

The present study was approved by the Ethics Committee of the Department of Science and Technology of Kunming Medical University and written informed consent was obtained from all patients.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2019. CA Cancer J Clin 69: 7-34, 2019.
2. Sun KX, Zheng RS, Zeng HM, Zhang SW, Zou XN, Gu XY, Xia CF, Yang ZX, Li H, Chen WQ, He J: The incidence and mortality of lung cancer in China, 2014. Zhonghua Zhong Liu Za Zhi 40: 805-811, 2018 (In Chinese).
3. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
4. Chen W, Zheng R, Zeng H and Zhang S: Epidemiology of lung cancer in China. Thorac Cancer 6: 209-215, 2015.
5. Nakanishi Y, Chen S, Inutsuka S, Ma Y, Jiang X, Hara N, Sera N and Tokiwa H: Possible role of indoor environment and coal combustion emission in lung carcinogenesis in Fuyuan County, China. Neoplasma 44: 69-72, 1997.
6. Chen G, Sun X, Ren H, Wan X, Huang H, Ma X, Ning B, Zou X, Hu W and Yang G: The mortality patterns of lung cancer between 1990 and 2013 in Xuanwei, China. Lung Cancer 90: 155-160, 2015.
7. Wu H, Meng S, Xu Q, Wang X, Wang J, Gong R, Song Y, Duan Y and Zhang Y: Gene expression profiling of lung adenocarcinoma in Xuanwei, China. Eur J Cancer Prev 25: 508-517, 2016.
8. Mumford JL, He XZ, Chapman RS, Cao SR, Harris DB, Li XM, Xian YL, Jiang WZ, Xu CW, Chuang JC, *et al*: Lung cancer and indoor air pollution in Xuan Wei, China. Science 235: 217-220, 1987.
9. Jiang CL, He SW, Zhang YD, Duan HX, Huang T, Huang YC, Li GF, Wang P, Ma LJ, Zhou GB and Cao Y: Air pollution and DNA methylation alterations in lung cancer: A systematic and comparative study. Oncotarget 8: 1369-1391, 2017.
10. He XZ, Chen W, Liu ZY and Chapman RS: An epidemiological study of lung cancer in Xuan Wei County, China: Current progress. Case-control study on lung cancer and cooking fuel. Environ Health Perspect 94: 9-13, 1991.
11. Lan Q, Chapman RS, Schreinemachers DM, Tian LW and He XZ: Household stove improvement and risk of lung cancer in Xuanwei, China. J Natl Cancer Inst 94: 826-835, 2002.
12. Yang J, Li G, Huang Y, Ye L, Zhou Y, Zhao G, Lei Y, Chen X, Wang K, Chen Y, *et al*: Association of inorganics accumulation with the activation of NF-κB signaling pathway and the iNOS expression of lung tissue in xuanwei lung cancer patients. Zhongguo Fei Ai Za Zhi 19: 30-37, 2016 (In Chinese).
13. Wakelee H, Kelly K and Edelman MJ: 50 Years of progress in the systemic therapy of non-small cell lung cancer. Am Soc Clin Oncol Educ Book: 177-189, 2014.
14. Ettinger DS, Aisner DL, Wood DE, Akerley W, Bauman J, Chang JY, Chirieac LR, D'Amico TA, Dilling TJ, Dobelbower M, *et al*: NCCN guidelines insights: non-small cell lung cancer, version 5.2018. J Natl Compr Canc Netw 16: 807-821, 2018.
15. Chen Z, Fillmore CM, Hammerman PS, Kim CF and Wong KK: Non-small-cell lung cancers: A heterogeneous set of diseases. Nat Rev Cancer 14: 535-546, 2014.
16. Aguilar EJ, Ricciuti B, Gainor JF, Kehl KL, Kravets S, Dahlberg S, Nishino M, Sholl LM, Adeni A, Subegdjo S, *et al*: Outcomes to first-line pembrolizumab in patients with non-small-cell lung cancer and very high PD-L1 expression. Ann Oncol 30: 1653-1659, 2019.
17. Grelet S and Howe PH: hnRNP E1 at the crossroads of translational regulation of epithelial-mesenchymal transition. J Cancer Metastasis Treat 5: pii: 16, 2019.
18. Sherman EJ, Mitchell DC and Garner AL: The RNA-binding protein SART3 promotes miR-34a biogenesis and G1 cell cycle arrest in lung cancer cells. J Biol Chem 294: 17188-17196, 2019.
19. Jung JH, Lee H, Cao B, Liao P, Zeng SX and Lu H: RNA-binding motif protein 10 induces apoptosis and suppresses proliferation by activating p53. Oncogene, Oct 7, 2019 (Epub ahead of print).
20. Pereira B, Billaud M and Almeida R: RNA-binding proteins in cancer: Old players and new actors. Trends Cancer 3: 506-528, 2017.
21. Zhang Y, Yang L, Ling C and Heng W: HuR facilitates cancer stemness of lung cancer cells via regulating miR-873/CDK3 and miR-125a-3p/CDK3 axis. Biotechnol Lett 40: 623-631, 2018.
22. Zhao W, Lu D, Liu L, Cai J, Zhou Y, Yang Y, Zhang Y and Zhang J: Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) promotes lung tumorigenesis via attenuating p53 stability. Oncotarget 8: 93672-93687, 2017.
23. Guan R, El-Rass S, Spillane D, Lam S, Wang Y, Wu J, Chen Z, Wang A, Jia Z, Keating A, *et al*: rbm47, a novel RNA binding protein, regulates zebrafish head development. Dev Dyn 242: 1395-1404, 2013.
24. Yeganeh M, Seyedjafari E, Kamrani FA and Ghaemi N: RNA-binding protein Rbm47 binds to Nanog in mouse embryonic stem cells. Mol Biol Rep 40: 4391-4396, 2013.
25. Fossat N, Tourle K, Radziewic T, Barratt K, Liebhold D, Studdert JB, Power M, Jones V, Loebel DA and Tam PP: C to U RNA editing mediated by APOBEC1 requires RNA-binding protein RBM47. EMBO Rep 15: 903-910, 2014.
26. Vanharanta S, Marney CB, Shu W, Valiente M, Zou Y, Mele A, Darnell RB and Massagué J: Loss of the multifunctional RNA-binding protein RBM47 as a source of selectable metastatic traits in breast cancer. Elife 3, 2014.
27. Sakurai T, Isogaya K, Sakai S, Morikawa M, Morishita Y, Ehata S, Miyazono K and Koinuma D: RNA-binding motif protein 47 inhibits Nrf2 activity to suppress tumor growth in lung adenocarcinoma. Oncogene 36: 5083, 2017.
28. Edge SB and Compton CC: The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 17: 1471-1474, 2010.
29. Ettinger DS, Wood DE, Aggarwal C, Aisner DL, Akerley W, Bauman JR, Bharat A, Bruno DS, Chang JY, Chirieac LR, *et al*: NCCN guidelines insights: Non-small cell lung cancer, version 1.2020: J Natl Compr Canc Netw 17: 1464-1472, 2019.
30. Fu QF, Liu Y, Fan Y, Hua SN, Qu HY, Dong SW, Li RL, Zhao MY, Zhen Y, Yu XL, *et al*: Alpha-enolase promotes cell glycolysis, growth, migration, and invasion in non-small cell lung cancer through FAK-mediated PI3K/AKT pathway. J Hematol Oncol 8: 22, 2015.

31. Suwei D, Liang Z, Zhimin L, Ruilei L, Yingying Z, Zhen L, Chunlei G, Zhangchao L, Yuanbo X, Jinyan Y, *et al*: NLK functions to maintain proliferation and stemness of NSCLC and is a target of metformin. J Hematol Oncol 8: 120, 2015.
32. Zeng B, Ge C, Li R, Zhang Z, Fu Q, Li Z, Lin Z, Liu L, Xue Y, Xu Y, *et al*: Knockdown of microsomal glutathione S-transferase 1 inhibits lung adenocarcinoma cell proliferation and induces apoptosis. Biomed Pharmacother 121: 109562, 2019.
33. Lian S, Li L, Zhou Y, Liu Z and Wang L: The co-expression networks of differentially expressed RBPs with TFs and LncRNAs related to clinical TNM stages of cancers. PeerJ 7: e7696, 2019.
34. Liu G, Zhang Q, Xia L, Shi M, Cai J, Zhang H, Li J, Lin G, Xie W, Zhang Y and Xu N: RNA-binding protein CELF6 is cell cycle regulated and controls cancer cell proliferation by stabilizing p21. Cell Death Dis 10: 688, 2019.
35. Hong YG, Xu GS, Yu GY, Zhou JD, Liu QZ, Ni JS, Yan HL, Zhang W and Hao LQ: The RNA binding protein neuro-oncological ventral antigen 1 (NOVA1) regulates IL-6 mRNA stability to enhance JAK2-STAT3 signaling in CRC. Surg Oncol 31: 67-74, 2019.
36. Busà R, Paronetto MP, Farini D, Pierantozzi E, Botti F, Angelini DF, Attisani F, Vespasiani G and Sette C: The RNA-binding protein Sam68 contributes to proliferation and survival of human prostate cancer cells. Oncogene 26: 4372-4382, 2007.
37. Vo DT, Subramaniam D, Remke M, Burton TL, Uren PJ, Gelfond JA, de Sousa Abreu R, Burns SC, Qiao M, Suresh U, *et al*: The RNA-binding protein Musashi1 affects medulloblastoma growth via a network of cancer-related genes and is an indicator of poor prognosis. Am J Pathol 181: 1762-1772, 2012.
38. Wurth L, Papasaikas P, Olmeda D, Bley N, Calvo GT, Guerrero S, Cerezo-Wallis D, Martinez-Useros J, García-Fernández M, Hüttelmaier S, *et al*: UNR/CSDE1 drives a post-transcriptional program to promote melanoma invasion and metastasis. Cancer Cell 30: 694-707, 2016.
39. Hopkins TG, Mura M, Al-Ashtal HA, Lahr RM, Abd-Latip N, Sweeney K, Lu H, Weir J, El-Bahrawy M, Steel JH, *et al*: The RNA-binding protein LARP1 is a post-transcriptional regulator of survival and tumorigenesis in ovarian cancer. Nucleic Acids Res 44: 1227-1246, 2016.
40. Yong H, Zhu H, Zhang S, Zhao W, Wang W, Chen C, Ding G, Zhu L, Zhu Z, Liu H, *et al*: Prognostic value of decreased expression of RBM4 in human gastric cancer. Sci Rep 6: 28222, 2016.
41. Glisovic T, Bachorik JL, Yong J and Dreyfuss G: RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett 582: 1977-1986, 2008.
42. Biswas J, Patel VL, Bhaskar V, Chao JA, Singer RH and Eliscovich C: The structural basis for RNA selectivity by the IMP family of RNA-binding proteins. Nat Commun 10: 4440, 2019.
43. Bechara EG, Sebestyén E, Bernardis I, Eyras E and Valcárcel J: RBM5, 6, and 10 differentially regulate NUMB alternative splicing to control cancer cell proliferation. Mol Cell 52: 720-733, 2013.
44. Hernández J, Bechara E, Schlesinger D, Delgado J, Serrano L and Valcárcel J: Tumor suppressor properties of the splicing regulatory factor RBM10. RNA Biol 13: 466-472, 2016.
45. Zhang Z, Huang A, Zhang A and Zhou C: HuR promotes breast cancer cell proliferation and survival via binding to CDK3 mRNA. Biomed Pharmacother 91: 788-795, 2017.
46. Mitsunari K, Miyata Y, Asai A, Matsuo T, Shida Y, Hakariya T and Sakai H: Human antigen R is positively associated with malignant aggressiveness via upregulation of cell proliferation, migration, and vascular endothelial growth factors and cyclooxygenase-2 in prostate cancer. Transl Res 175: 116-128, 2016.
47. Wang W, Caldwell MC, Lin S, Furneaux H and Gorospe M: HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. EMBO J 19: 2340-2350, 2000.
48. Wu M, Tong CWS, Yan W, To KKW and Cho WCS: The RNA binding protein HuR: A promising drug target for anticancer therapy. Curr Cancer Drug Targets 19: 382-399, 2019.
49. Prabhu VV and Devaraj N: Regulating RNA binding motif 5 gene expression-A novel therapeutic target for lung cancer. J Environ Pathol Toxicol Oncol 36: 99-105, 2017.
50. Kavianpour M, Ahmadzadeh A, Shahrabi S and Saki N: Significance of oncogenes and tumor suppressor genes in AML prognosis. Tumour Biol 37: 10041-10052, 2016.
51. Jiang QQ and Liu WB: miR-25 Promotes Melanoma Progression by regulating RNA binding motif protein 47. Med Sci (Paris) 34 Focus Issue F1: 59-65, 2018.
52. Wang TW, Vermeulen RC, Hu W, Liu G, Xiao X, Alekseyev Y, Xu J, Reiss B, Steiling K, Downward GS, *et al*: Gene-expression profiling of buccal epithelium among non-smoking women exposed to household air pollution from smoky coal. Carcinogenesis 36: 1494-1501, 2015.
53. Wei Y, Zhang F, Zhang Y, Wang X, Xing C, Guo J, Zhang H, Suo Z, Li Y, Wang J, *et al*: Post-transcriptional regulator Rbm47 elevates IL-10 production and promotes the immunosuppression of B cells. Cell Mol Immunol 16: 580-589, 2019.
54. Xuan Y, Wang J, Ban L, Lu JJ, Yi C, Li Z, Yu W, Li M, Xu T, Yang W, *et al*: hnRNPA2/B1 activates cyclooxygenase-2 and promotes tumor growth in human lung cancers. Mol Oncol 10: 610-624, 2016.
55. Wei TY, Juan CC, Hisa JY, Su LJ, Lee YC, Chou HY, Chen JM, Wu YC, Chiu SC, Hsu CP, *et al*: Protein arginine methyltransferase 5 is a potential oncoprotein that upregulates G1 cyclins/cyclin-dependent kinases and the phosphoinositide 3-kinase/AKT signaling cascade. Cancer Sci 103:1640-1650, 2012.
56. Su LJ, Chang CW, Wu YC, Chen KC, Lin CJ, Liang SC, Lin CH, Whang-Peng J, Hsu SL, Chen CH and Huang CY: Selection of DDX5 as a novel internal control for Q-RT-PCR from microarray data using a block bootstrap re-sampling scheme. BMC Genomics 8: 140, 2007.
57. Lu TP, Tsai MH, Lee JM, Hsu CP, Chen PC, Lin CW, Shih JY, Yang PC, Hsiao CK,Lai LC and Chuang EY. Identification of a novel biomarker, SEMA5A, for non-smallcell lung carcinoma in nonsmoking women. Cancer Epidemiol Biomarkers Prev 19: 2590-2597, 2010.
58. Kuner R, Muley T, Meister M, Ruschhaupt M, Buness A, Xu EC, Schnabel P, Warth A, Poustka A, Sültmann H and Hoffmann H: Global gene expression analysis revealsspecific patterns of cell junctions in non-small cell lung cancer subtypes. Lung Cancer. 63: 32-38, 2009.
59. Zhu CQ, Ding K, Strumpf D, Weir BA, Meyerson M, Pennell N, Thomas RK, Naoki K, Ladd-Acosta C, Liu N, *et al*: Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. J Clin Oncol 10: 28: 4417-4424, 2010
60. Lo FY, Chang JW, Chang IS, Chen YJ, Hsu HS, Huang SF, Tsai FY, Jiang SS, Kanteti R, Nandi S, *et al*: The database of chromosome imbalance regions and genes resided in lung cancer from Asian and Caucasian identified by array-comparative genomic hybridization. BMC Cancer 12: 235, 2012.