

**CLINICAL SIGNIFICANCE OF MIR-3178 IN NON-SMALL  
CELL LUNG CANCER PATIENTS**KLINIČKI ZNAČAJ miR-3178 KOD PACIJENATA SA  
NEMIKROCELULARNIM KARCINOMOM PLUĆADeng Huang<sup>1#</sup>, Yingfang Ma<sup>2#</sup>, Haizhen Yu<sup>3</sup>, Shizhen Li<sup>3</sup>, Ling Zhou<sup>4\*</sup><sup>1</sup>Department of Respiratory and Critical Care Medicine, Yangxin People's Hospital, Huangshi City 435200, China<sup>2</sup>Interventional Radiology Department, Heilongjiang Provincial Daqing Hospital of Traditional Chinese Medicine, Heilongjiang 163311, China<sup>3</sup>Department of Laboratory, Zhucheng People's Hospital, Zhucheng 262299, China<sup>4</sup>Pulmonary and Critical Care Medicine, Wuhan Pu Ren Hospital, Wuhan 430000, China**Summary**

**Background:** Non-small cell lung cancer (NSCLC) is a malignant tumour with high morbidity and mortality, with a low survival rate and poor prognosis. This study aims to explore the diagnostic and prognostic value of miR-3178 in NSCLC.

**Methods:** The study included 118 NSCLC patients and 97 healthy subjects. RT-qPCR measured miR-3178 and MIB2 in serum or cell lines.  $\chi^2$  tests linked miR-3178 to clinico-pathological features. ROC, Kaplan-Meier, and Cox analyses evaluated the diagnostic and prognostic significance of miR-3178 and MIB2.

**Results:** Compared to the control group, the expression level of miR-3178 was significantly reduced, while MIB2 expression was notably elevated in NSCLC patients. The expression of miR-3178 is significantly correlated with the TNM stage and LNM in NSCLC patients. NSCLC patients with higher miR-3178 expression levels exhibit significantly higher 5-year overall survival rates. Furthermore, TNM stage, miR-3178 expression, and MIB2 expression are independent risk factors that influence the prognosis of NSCLC. MIB2 serves as a target gene of miR-3178, and its expression levels display a significant negative correlation. When diagnosing NSCLC solely based on miR-3178, the AUC is 0.838, the diagnostic sensitivity and specificity were 83.1% and 72.2%, respectively; for MIB2 alone, the AUC

**Kratak sadržaj**

**Uvod:** Nemikrocelularni karcinom pluća (NSCLC) je maligni tumor sa visokom stopom oboljevanja i smrtnosti, niskom stopom preživljavanja i lošom prognozom. Cilj ove studije je da istraži dijagnostičku i prognostičku vrednost miR-3178 kod pacijenata sa NSCLC.

**Metode:** Studija je obuhvatila 118 pacijenata sa NSCLC i 97 zdravih ispitanika. RT-qPCR metodom mereni su nivoi miR-3178 i MIB2 u serumu ili ćelijskim linijama.  $\chi^2$  testom analizirana povezanost miR-3178 sa kliničko-patološkim karakteristikama. ROC analiza, Kaplan-Majer i Cox analiza su korišćene za procenu dijagnostičke i prognostičke vrednosti miR-3178 i MIB2.

**Rezultati:** U poređenju sa kontrolnom grupom, kod pacijenata sa NSCLC nivo miR-3178 je bio značajno smanjen, dok je ekspresija MIB2 bila značajno povišena. Ekspresija miR-3178 bila je značajno povezana sa TNM stadijumom i LNM kod pacijenata sa NSCLC. Pacijenti sa višim nivoom miR-3178 su pokazali značajno veću petogodišnju stopu ukupnog preživljavanja. Pored toga, TNM stadijum, ekspresija miR-3178 i ekspresija MIB2 predstavljaju nezavisne faktore rizika koji utiču na prognozu NSCLC. MIB2 je identifikovan kao ciljani gen miR-3178, a njihova ekspresija pokazuje značajnu negativnu korelaciju. Pri dijagnostikovanju NSCLC samo na osnovu miR-3178, AUC je 0,838, dijagnostička osetljivost i specifičnost su iznosile 83,1% i

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is 0.818, the sensitivity and specificity were 76.3% and 72.2%; however, when miR-3178 and MIB2 are combined for diagnosis, the AUC increases to 0.903, the sensitivity and specificity were 84.8% and 83.5%.

**Conclusions:** The downregulation of miR-3178 and upregulation of MIB2 are associated with the progression and deterioration of NSCLC patients, indicating poor prognosis. miR-3178 targets MIB2, and their combined diagnosis can enhance the diagnostic value for NSCLC, potentially reducing the risk of adverse outcomes.

**Keywords:** non-small cell lung cancer, miR-3178, MIB2

## Introduction

Lung cancer ranks as the second most common malignancy globally and constitutes a significant contributor to cancer-related fatalities in China (1, 2). As one of the most common types of lung cancer, non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases. It is a heterogeneous disease that encompasses different histological subtypes, primarily adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (3, 4). NSCLC has no obvious early symptoms, leading to late diagnosis and poor prognosis (5). Despite rapid advances in precision oncology and some progress made in the treatment and prognosis of NSCLC patients, it remains a clinically challenging disease, with 5-year survival rates of 15% and 83% for advanced and stage I NSCLC patients, respectively (6). Therefore, it is of great importance to identify new biomarkers that aid in the diagnosis, prognosis, and treatment of NSCLC.

Currently, molecular screening is a research hotspot in early screening for NSCLC, with circulating microRNAs (miRNAs) demonstrating significant effectiveness in early cancer screening (7, 8). miRNAs are widely present in eukaryotic cells and stably exist in human circulation. Compared to other markers, miRNAs possess the characteristics of stability, repeatability, and consistency, and have gradually been used for early cancer screening in recent years (9). It is reported that miRNAs in NSCLC patients have oncogenic or tumour suppressor effects through their target genes. For example, the let-7 family, miR-34, miR-486, and miR-200 act as tumour suppressors, while miR-196b, miR-21, and miR-224 act as oncogenes (10). miR-1231 plays a suppressive role in prostate cancer and is accompanied by significant downregulation (11). miR-532 is regarded as a prognostic biomarker, and its upregulation promotes migration and invasion of gastric cancer (12). According to current research, abnormally expressed miRNAs in serum may serve as diagnostic markers for NSCLC (13).

miR-3178 is abnormally expressed in many tumours, but there is relatively little research on miR-3178 in lung cancer. Some studies have found that miR-3178 is down-regulated in lung cancer (14, 15). However, the involvement and regulatory mecha-

nisms of miR-3178 in NSCLC pathogenesis remain largely unexplored. MIB2 is a RING-type ubiquitin ligase that regulates the Notch signalling pathway through ubiquitination of Notch ligands and is also involved in the regulation of NF- $\kappa$ B signalling pathways and type I interferon responses (16). Current research indicates that MIB2 plays an essential role in various malignant tumours, such as cervical cancer, breast cancer, and neuroblastoma (17–19). The role of serum matrix-derived miR-3178 and MIB2 in the early screening and diagnosis of NSCLC warrants further investigation.

**Zaključak:** Smanjena ekspresija miR-3178 i povećana ekspresija MIB2 su povezane sa progresijom i pogoršanjem stanja kod pacijenata sa NSCLC, što ukazuje na lošu prognozu. miR-3178 cilja MIB2, a njihova kombinovana dijagnostika može poboljšati dijagnostičku vrednost za NSCLC i potencijalno smanjiti rizik od nepovoljnih ishoda.

**Ključne reči:** nemikrocelularni karcinom pluća, miR-3178, MIB2

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In the study, the differential expression of miR-3178 and MIB2 in NSCLC was investigated. The correlation between miR-3178 expression and NSCLC severity was evaluated. The Kaplan-Meier survival curve has been used to assess the predictive value of miR-3178 for the 5-year prognosis of NSCLC patients. Furthermore, the downstream target of miR-3178 was identified through a database, and the interaction between miR-3178 and MIB2 was explored by dual luciferase activity. The combined diagnostic value of miR-3178 and MIB2 for NSCLC was analysed, aiming to provide an effective diagnostic basis for NSCLC in clinical practice.

## Materials and Methods

**Study subjects:** A total of 118 patients admitted to Zhucheng People's Hospital from January 2017 to December 2018 were selected as the experimental group, and 97 healthy individuals who underwent physical examination during the same period were selected as the control group. Inclusion criteria: (1) All patients in the experimental group were confirmed to have NSCLC through cytological or pathological examinations; (2) Aged between 50 and 75 years, regardless of gender; (3) All participants signed an informed consent form; (4) Relevant examinations were complete, and data were available. Exclusion criteria: (1) Patients who had undergone radiotherapy, chemotherapy, or targeted therapy before sample collection for the study indicators; (2) Patients with infection or acute inflammatory diseases; (3) Patients with other malignancies; (4) Patients with haemato-

logical or immune system diseases; (5) Pregnant or lactating women. For the healthy control group, the inclusion criteria are as follows: (1) matched with the NSCLC patients in age, gender, BMI, and smoking and drinking history; and (2) confirmed by the medical examination center that the individual's lung function and biochemical indicators are normal, no viral pneumonia, and no history of lung or other malignant diseases. This study has obtained the informed consent of patients and their close relatives, as well as the approval of the hospital ethics committee. All patients were followed up for five years or until their death, and the time of death was recorded through telephone or an outpatient visit.

**Sample collection:** Blood samples were collected from both groups, centrifuged at 4,000 r/min for 10 minutes, and the supernatants were collected and stored in a -80 °C freezer for future testing.

**Cell transfection:** Three NSCLC cell lines, including A549, H1299, and MES-1, as well as normal human lung epithelial cells BEAS-2B from the Chinese Academy of Sciences Shanghai Cell Bank, were purchased. Cells were cultured in a 37 °C humidified incubator with 5% CO<sub>2</sub> in RPMI1640 medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NE, USA). In addition, the miR-3178 mimics and their negative controls (NC) were acquired from GenePharma (Shanghai, China) and transfected into A549 or H1299 cells via Lipofectamine 2000 (Invitrogen, USA). The miR-3178 mimics sequence was 5'-GGGGCGCGCCCGAUCG-3' and the miR-NC sequence was 5'-CCTTGGATGGCCTAGGAGATAG-3'. QRT-PCR verified the transduction efficiency.

**Dual-Luciferase reporter assay:** The relationship between miR-3178 and MIB2 was evaluated using a dual-luciferase reporter assay. The binding sites of miR-3178 and MIB2 were predicted by TargetScan, and wild-type (WT) and mutant (MT) MIB2 were established. The mutant type of MIB2 was established through point mutations in the binding sites. The reporter plasmid was co-transfected with miR-3178 mimics or negative controls into NSCLC cell lines for 48 hours. The luciferase activity was detected using the dual-luciferase reporting kit (Promega, Shanghai, China) according to the instructions. The activity of

Renilla luciferase was considered an internal control for the calculation of relative firefly luciferase activity.

**RNA extraction and qRT-PCR:** Total RNA was extracted from cells and serum using Trizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was synthesised by reverse transcription using reverse transcriptase (TOYOBO, Japan). PCR amplification was performed using SYBR premix (TOYOBO, Japan) at 60 °C for 10 min, 95 °C and 72 °C for 30 s, and 95 °C for 5 min. This experiment used specific primers of miR-3178 (forward primer 5'-TCG-GCAGGGGGCG-CGGCC-3' and reverse primer 5'-GCGTGTCTGGAGTCGGC-3'). Using U6 (GENEWIZ) as an internal control (forward primer 5'-CATCAC-CATCACTCAGG-AGAGTCG-3' and reverse primer 5'-TGACGCTTGCCACAGCCTT-3'), the expression levels of miR-3178 and MIB2 were calculated by the 2<sup>-ΔΔCt</sup> method.

**Data processing:** Data were analysed using SPSS 22.0 software. The results are expressed as mean ±SD, and comparisons between groups were performed using independent sample t-tests. The Bonferroni method was a post hoc test for comparing differences. A chi-square test identified the clinicopathological characteristics that correlated with miR-3178 expression. Pearson correlation coefficients were used for correlation analysis, and ROC analysis was employed to assess the diagnostic value of miR-3178 and MIB2 in NSCLC. Kaplan-Meier analysis and Cox regression analysis were conducted to determine the prognostic significance of miR-3178. *P*<0.05 was considered statistically significant.

## Results

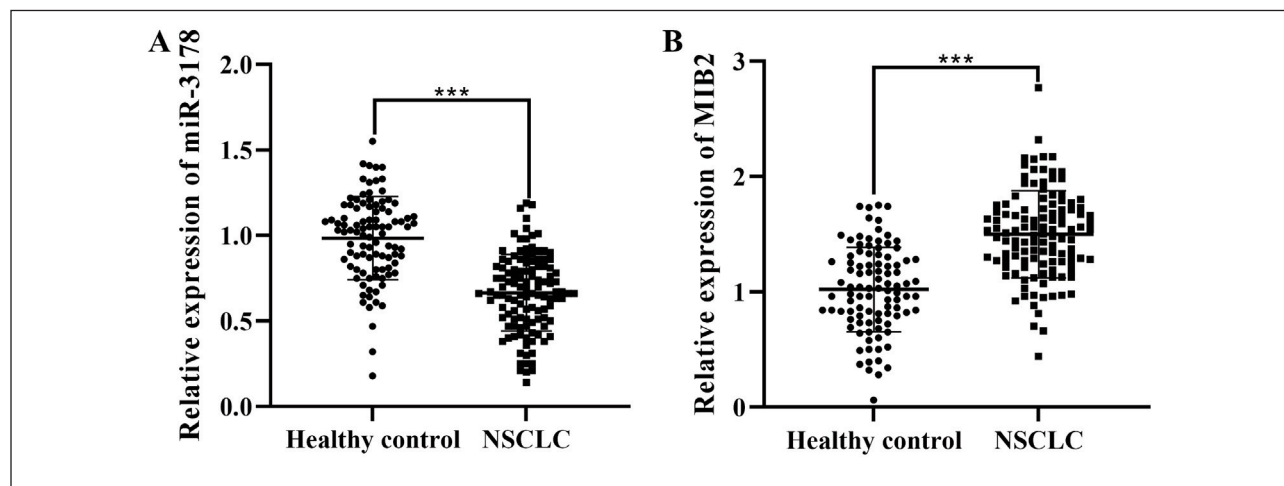
### *General information on healthy controls and NSCLC patients*

The study included 60 male and 37 female healthy controls with an average age of 61.58±4.57 years and a BMI of 22.59±1.32 kg/m<sup>2</sup>. The NSCLC patients comprised 71 males and 47 females, with an average age of 61.22±4.72 years and a BMI of 22.87±1.37 kg/m<sup>2</sup>. No significant differences were observed between healthy controls and NSCLC patients in terms of age, gender, BMI, smoking history, or drinking history (*P*>0.05, *Table 1*).

**Table 1** Comparison of baseline data between the two groups of people.

|                      | Healthy control (n=97) | NSCLC patients (n=118) | <i>P</i> -value |
|----------------------|------------------------|------------------------|-----------------|
| Age                  | 61.58±4.57             | 61.22±4.72             | 0.576           |
| Gender (male/female) | 60/37                  | 71/47                  | 0.801           |
| BMI                  | 22.59±1.32             | 22.87±1.37             | 0.134           |
| Smoking history      | 46                     | 57                     | 0.307           |
| Drinking history     | 45                     | 63                     | 0.897           |

Note: BMI, body mass index



**Figure 1** Expression levels of miR-3178 and MIB2 in the serum of NSCLC patients. A) The expression level of miR-3178 B) The expression level of MIB.

**Table II** The correlation between the expression level of miR-3178 and the clinical characteristics of patients.

| Variables               | NSCLC patients (n=118) | miR-3178 expression |             | P-value |
|-------------------------|------------------------|---------------------|-------------|---------|
|                         |                        | Low (n=59)          | High (n=59) |         |
| Age (years)             |                        |                     |             |         |
| <60                     | 39                     | 22                  | 17          | 0.328   |
| ≥60                     | 79                     | 37                  | 42          |         |
| Gender                  |                        |                     |             |         |
| Male                    | 71                     | 37                  | 34          | 0.573   |
| Female                  | 47                     | 22                  | 30          |         |
| Smoking history         |                        |                     |             |         |
| No                      | 55                     | 27                  | 28          | 0.854   |
| Yes                     | 63                     | 32                  | 31          |         |
| Drinking history        |                        |                     |             |         |
| No                      | 61                     | 28                  | 33          | 0.357   |
| Yes                     | 57                     | 31                  | 26          |         |
| Histology type          |                        |                     |             |         |
| Adenocarcinoma          | 79                     | 38                  | 41          | 0.557   |
| Squamous cell carcinoma | 39                     | 21                  | 18          |         |
| Differentiation         |                        |                     |             |         |
| High and moderate       | 101                    | 49                  | 52          | 0.432   |
| Poor                    | 17                     | 10                  | 7           |         |
| Tumour size             |                        |                     |             |         |
| <3                      | 89                     | 46                  | 43          | 0.521   |
| ≥3                      | 29                     | 13                  | 16          |         |
| TNM stage               |                        |                     |             |         |
| I                       | 91                     | 40                  | 51          | 0.016   |
| II-III                  | 27                     | 19                  | 8           |         |
| LNM                     |                        |                     |             |         |
| Negative                | 99                     | 45                  | 54          | 0.024   |
| Positive                | 19                     | 14                  | 5           |         |

Note: TNM, tumour-nodes-metastasis; LNM, lymph node metastasis (negative, positive)

### Expression levels of miR-3178 and MIB2

Compared to the control group, NSCLC patients exhibited significantly reduced miR-3178 expression levels ( $P < 0.01$ , Figure 1A) and considerably elevated MIB2 expression levels ( $P < 0.01$ , Figure 1B).

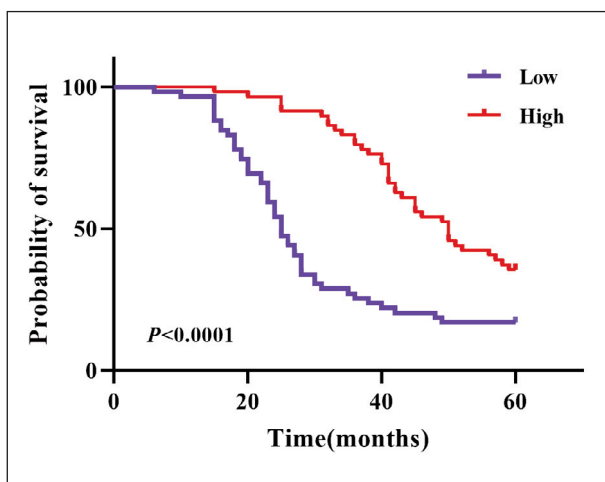
### Correlation of miR-3178 expression with clinical characteristics

Using the mean expression value of miR-3178 (0.66), 118 patients were divided into two groups: the high miR-3178 expression group and the low miR-3178 expression group. As shown in Table II, the

expression level of miR-3178 was significantly correlated with tumour-nodes-metastasis stage (TNM,  $P = 0.016$ ) and lymph node metastasis (LNM,  $P = 0.024$ ) in NSCLC patients, while no significant correlation was observed with other characteristics, including age, gender, smoking history, drinking history, histology type, differentiation, and tumour size ( $P > 0.05$ ).

### Prognostic analysis of NSCLC patients

A five-year follow-up and Kaplan-Meier survival analysis based on miR-3178 expression levels revealed that NSCLC patients with higher miR-3178 expression had significantly better 5-year overall survival rates compared to those with lower miR-3178 expression ( $P < 0.0001$ , Figure 2). Univariate analysis showed that TNM stage (HR=1.985, 95% CI=1.222–3.226,  $P = 0.006$ ), LNM (HR=1.863, 95% CI=1.081–3.209,  $P = 0.025$ ), miR-3178 expression (HR=0.372, 95% CI=0.242–0.573,  $P < 0.001$ ), and MIB2 expression (HR=1.92, 95% CI=1.252–2.947,  $P = 0.003$ ) were significantly associated with 5-year survival in NSCLC patients. Multivariate analysis confirmed TNM stage (HR=1.952, 95% CI=1.183–3.22,  $P = 0.009$ ), miR-3178 expression (HR=0.418, 95% CI=0.267–0.654,  $P < 0.001$ ), and MIB2 expression (HR=1.576, 95% CI=1.017–2.443,  $P < 0.042$ ) as independent prognostic factors (Table III).

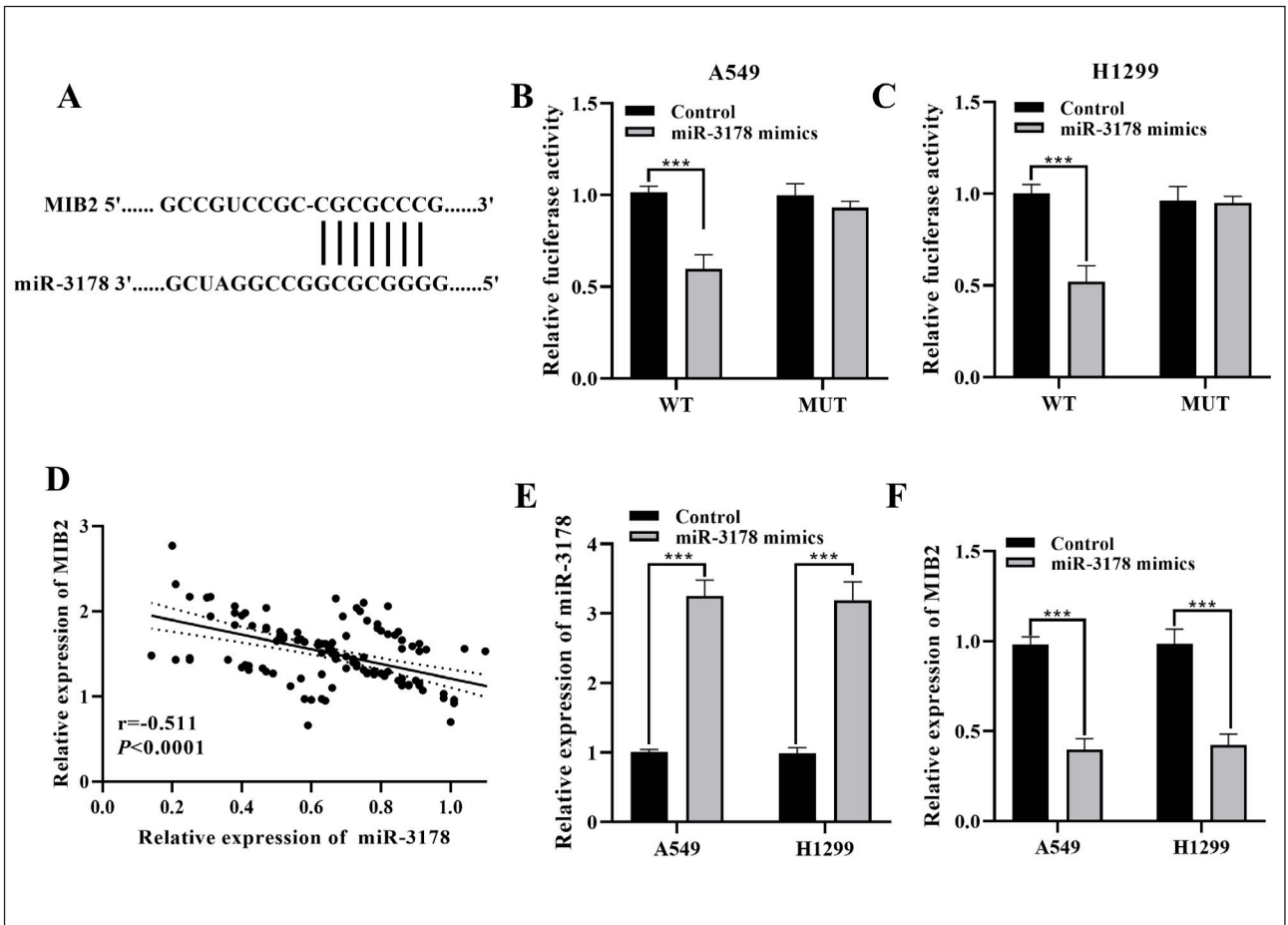


**Figure 2** Kaplan-Meier Curves for patients with low or high miR-3178 expression.

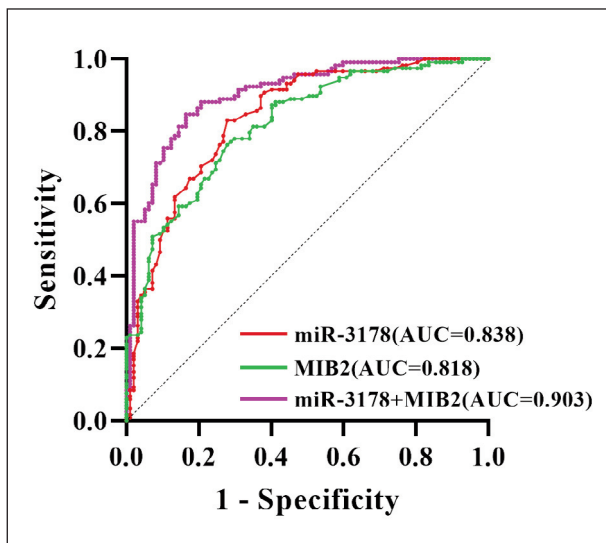
**Table III** The correlation between the expression level of miR-3178 and the clinical characteristics of patients.

| Variables           | Univariate analysis |             |         | Multivariate analysis |             |         |
|---------------------|---------------------|-------------|---------|-----------------------|-------------|---------|
|                     | HR                  | 95% CI      | P value | HR                    | 95% CI      | P value |
| Age                 | 0.977               | 0.938–1.018 | 0.273   |                       |             |         |
| Gender              | 0.843               | 0.436–0.843 | 0.436   |                       |             |         |
| Smoking history     | 0.918               | 0.603–1.398 | 0.69    |                       |             |         |
| Drinking history    | 0.995               | 0.653–1.517 | 0.982   |                       |             |         |
| Histologic type     | 1.187               | 0.763–1.848 | 0.447   |                       |             |         |
| Differentiation     | 0.928               | 0.504–1.707 | 0.81    |                       |             |         |
| Size                | 1.121               | 0.696–1.806 | 0.639   |                       |             |         |
| TNM stage           | 1.985               | 1.222–3.226 | 0.006   | 1.952                 | 1.183–3.22  | 0.009   |
| LNM                 | 1.863               | 1.081–3.209 | 0.025   | 1.714                 | 0.978–3.003 | 0.06    |
| miR-3178 expression | 0.372               | 0.242–0.573 | <0.001  | 0.418                 | 0.267–0.654 | <0.001  |
| MIB2 expression     | 1.92                | 1.252–2.947 | 0.003   | 1.576                 | 1.017–2.443 | 0.042   |

Note: TNM, tumour-nodes-metastasis; LNM, lymph node metastasis



**Figure 3** Luciferase reporter gene assay to verify the targeting relationship between miR-3178 and MIB2. A) Predicted targeting sites between miR-3178 and MIB2 B) Luciferase reporter gene assay in A549 cells C) Luciferase reporter gene assay in H1299 cells D) Correlation analysis between miR-3178 and MIB2 E) Expression levels of miR-3178 in cell lines after transfection F) Expression levels of MIB2 in cell lines after transfection.



**Figure 4** Diagnostic value of miR-3178 and MIB2 alone and in combination.

*Target relationship between miR-3178 and MIB2*

Bioinformatic analyses using TargetScan ([https://www.targetscan.org/vert\\_72/](https://www.targetscan.org/vert_72/)) predicted MIB2 as a target gene of miR-3178. miR-3178 could directly bind to the 3' UTR of MIB2 (Figure 3A). Overexpression of miR-3178 significantly reduced the luciferase activity of MIB2-WT in both A549 (Figure 3B) and H1299 (Figure 3C) cell lines ( $P < 0.01$ ), while no significant change was observed in MIB2-MUT ( $P > 0.05$ ). The expression levels of miR-3178 and MIB2 in the serum of NSCLC patients exhibit a significant negative correlation ( $P < 0.01$ ), with a correlation coefficient of  $-0.511$ . ( $P < 0.01$ , Figure 3D). Overexpression of miR-3178 significantly increased miR-3178 expression ( $P < 0.01$ , Figure 3E) and decreased MIB2 expression ( $P < 0.01$ , Figure 3F) in both A549 and H1299 cell lines.

### *Diagnostic value of miR-3178 and MIB2 alone and in combination*

Figure 4 presents the diagnostic performance. The area under the curve (AUC) for miR-3178 alone was 0.838 (95% CI=0.784–0.892), with a sensitivity (positive true rate) of 83.1%, specificity of 72.2%, and false rate of 27.8%. For MIB2 alone, the AUC was 0.818 (95% CI=0.763–0.874), sensitivity of 76.3%, specificity of 72.2%, and false rate of 27.8%. When miR-3178 and MIB2 were combined, the AUC increased to 0.903 (95% CI=0.863–0.944), sensitivity of 84.8%, specificity of 83.5%, and false rate of 16.5%.

### **Discussion**

NSCLC, as the primary type of lung cancer, most patients have reached the middle or advanced stage at the time of treatment, thus missing the best opportunity for surgical intervention. Despite significant progress in treatment methods in recent years, the therapeutic effects and prognosis of patients remain poor (20, 21). Identifying miRNAs as biomarkers facilitates early diagnosis and targeted rapid treatment for NSCLC, thereby reducing patient mortality. miRNAs have emerged as biomarkers for various types of cancer. In the context of gastric cancer, the dysregulation of miR-19a and miR-199a has provided new diagnostic avenues for this malignancy (22).

Furthermore, miR-203 has been significantly downregulated and correlates strongly with multiple myeloma-related parameters (23). A variety of miRNAs have been reported as potential biomarkers for NSCLC, with distinct characteristics shown by miR-630, miR-654-3p, and miR-199a-3p/5p (24–26). Therefore, abnormal expression of miRNAs in the serum of NSCLC patients has been found in many studies to be useful for auxiliary diagnosis (27).

It has been reported that miR-3178 is a tumour-associated miRNA linked to various cancers, including lung cancer, prostate cancer, and hepatocellular carcinoma. In these cancers, the expression of miR-3178 is dysregulated (15, 28, 29). Previous studies have shown that miR-3178 expression is significantly downregulated in tumour tissues of pancreatic cancer patients (30). Downregulation of miR-3178 has been detected in patients with primary gastric cancer accompanied by positive LNM (31). In gastric stromal tumours, miR-3178 expression is significantly downregulated, leading to speculation that it may serve as a tumour suppressor gene for use in the diagnosis and prognosis of gastric stromal tumours (32). Our results showed that the serum miR-3178 levels in NSCLC patients were significantly lower than those in healthy individuals, consistent with previous gene chip findings (14). Studies have found that miR-3178 is downregulated in gastric cancer and significantly

correlated with the patient's TNM stage and LNM (33). This is similar to the findings of our study, low expression of miR-3178 was related to advanced clinical features of NSCLC, such as advanced TNM stage and higher incidence of LNM, which ultimately led to a poorer prognosis. Therefore, the expression of miR-3178 is closely related to the tumour progression and severity of NSCLC patients, and can potentially predict the progression of their condition.

Studies have demonstrated that tumour markers, such as ALK and PD-L1, serve as important prognostic indicators and overall survival assessment markers in spinal metastases of NSCLC (34). However, while the use of standard tumour markers may improve the accuracy of identifying suitable surgical candidates, their effectiveness in predicting overall survival rates and diagnosing early-stage disease remains limited. Therefore, the exploration of new biomarkers is significant. Studies have found that the downregulation of miR-3178 is associated with poor prognosis, and miR-3178 and the patient's TNM stage are considered two independent prognostic indicators for gastric cancer (28, 32). Similar to the results of this study, which observed that NSCLC patients with low serum miR-3178 expression levels had lower survival rates and poor prognosis via Kaplan-Meier analysis. Numerous studies have also identified LNM and TNM staging as playing crucial roles in the diagnosis and prognosis of NSCLC (35, 36). Our study also confirms these findings. Through Cox regression analysis, we further discovered that the expression levels of miR-3178 and MIB2 remained independent prognostic predictors for NSCLC patients after adjusting for confounding factors.

In recent years, studies have found that E3 ligase is related to lung cancer cell proliferation and metastasis. MIB2, a member of the E3 ligase family, has attracted increasing attention in recent years. The expression trend of MIB2 varies across different types of tumours. For example, MIB2 has been reported to be up-regulated in leiomyomas (37), whereas in melanoma, it functions as a tumour suppressor (38–40). MIB2 acts as a suppressor in lung cancer, where it has been found to inhibit the proliferation, migration, and invasion of NSCLC through ubiquitination of Notch1 (41). MIB2 levels were significantly elevated, also aligning with prior research (42). These findings suggest that miR-3178 and MIB2 have the potential to serve as diagnostic biomarkers for NSCLC. miR-3178 also has multiple targets during cancer progression. For instance, miR-3178 inhibits the proliferation, migration, and invasion of hepatocellular carcinoma by suppressing EGR3 expression while promoting its apoptosis (29). Notch1 is one of the targets of miR-3178, as miR-3178 inhibits the progression of triple-negative breast cancer (28). According to the prediction made by TargetScan, MIB2 is a target gene of miR-3178, and miR-3178 negatively reg-

ulates MIB2. Studies have shown that serum miR-145 is an excellent biomarker for distinguishing NSCLC patients from healthy control groups, with an AUC of 0.84, and optimal sensitivity and specificity were 92.75% and 61.43% (43). Serum CRP demonstrates significant diagnostic value for NSCLC, with an AUC of 0.795 and diagnostic sensitivity and specificity of 81.8% and 66.8%, respectively (44). Our study found that the AUC for miR-3178 alone in diagnosis is 0.838, with a sensitivity and specificity were 83.1% and 72.2% (44). This indicates that compared to established biomarkers, miR-3178 exhibits higher specificity for the diagnosis of NSCLC. Furthermore, the AUC for MIB2 alone was 0.818, with a sensitivity and specificity were 76.3% and 72.2%. When miR-3178 was used in combination with MIB2 for diagnosis, the AUC value reached 0.903, with sensitivity and specificity increasing to 84.8% and 83.5%, respectively. Therefore, the combined diagnostic approach of miR-3178 and MIB2 can be clinically adopted for the diagnosis of NSCLC.

However, this study still has certain limitations. For instance, we did not explore the roles of miR-3178 and MIB2 in the cellular biological functions of NSCLC, which could help reveal novel regulatory axes and molecular mechanisms, thereby providing new targets for the diagnosis and treatment of NSCLC. Although this study did not directly address this issue, we will pursue follow-up research in this promising direction.

In conclusion, we discovered that miR-3178 is significantly downregulated in NSCLC serum and cell lines. The downregulation of miR-3178 is correlated with the progression of NSCLC patients and indicates a poor prognosis. Therefore, miR-3178 has the potential to serve as a molecular marker for assessing the prognosis of NSCLC patients. Given that miR-3178 targets and regulates MIB2, the combined diagnosis of these two factors can enhance the diagnostic value for NSCLC. Monitoring the dynamic

expression levels of both miR-3178 and MIB2 holds significant importance for analysing clinical pathological changes.

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*Authors' contributions*

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*Data availability*

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Conflict of interest statement**

All the authors declare that they have no conflict of interest in this work.

## References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; 71(3): 209–49.
2. Wu F, Wang L, Zhou C. Lung cancer in China: current and prospect. *Curr Opin Oncol* 2021; 33(1): 40–6.
3. Mithoowani H, Febraro M. Non-Small-Cell Lung Cancer in 2022: A Review for General Practitioners in Oncology. *Curr Oncol* 2022; 29(3): 1828–39.
4. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, et al. Non-Small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2022; 20(5): 497–530.
5. Walters S, Maringe C, Coleman MP, Peake MD, Butler J, Young N, et al. Lung cancer survival and stage at diagnosis in Australia, Canada, Denmark, Norway, Sweden and the UK: a population-based study, 2004–2007. *Thorax* 2013; 68(6): 551–64.
6. Sun J, Wu S, Jin Z, Ren S, Cho WC, Zhu C, et al. Lymph node micrometastasis in non-small cell lung cancer. *Biomed Pharmacother* 2022; 149: 112817.
7. Zou R, Loke SY, Tang YC, Too HP, Zhou L, Lee ASG, et al. Development and validation of a circulating microRNA panel for the early detection of breast cancer. *Br J Cancer* 2022; 126(3): 472–81.

8. Pan G, Liu Y, Shang L, Zhou F, Yang S. EMT-associated microRNAs and their roles in cancer stemness and drug resistance. *Cancer Commun (Lond)* 2021; 41(3): 199–217.
9. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterisation of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; 18(10): 997–1006.
10. Zhu X, Kudo M, Huang X, Sui H, Tian H, Croce CM, et al. Frontiers of MicroRNA Signature in Non-small Cell Lung Cancer. *Front Cell Dev Biol* 2021; 9: 643942.
11. Wang Y, Zhang Q, Guo B, Feng J, Zhao D. miR-1231 Is Downregulated in Prostate Cancer with Prognostic and Functional Implications. *Oncol Res Treat* 2020; 43(3): 78–86.
12. Hu S, Zheng Q, Wu H, Wang C, Liu T, Zhou W. miR-532 promoted gastric cancer migration and invasion by targeting NKD1. *Life Sci* 2017; 177: 15–9.
13. Chen X, Hu Z, Wang W, Ba Y, Ma L, Zhang C, et al. Identification of ten serum microRNAs from a genome-wide serum microRNA expression profile as novel noninvasive biomarkers for non-small cell lung cancer diagnosis. *Int J Cancer* 2012; 130(7): 1620–8.
14. Su H, Lv Q, Wang L, Hu J, Lv Y, Jia L, Qi Q. Expressions of shox2, RASSF1A and PTGER4, and the relationship between their methylation and clinicopathological characteristics in patients with lung cancer. *J Med Biochem* 2025; 44(2): 211–20.
15. Wang H, Li K, Mei Y, Huang X, Li Z, Yang Q, et al. Sp1 Suppresses miR-3178 to Promote the Metastasis Invasion Cascade via Upregulation of TRIOBP. *Mol Ther Nucleic Acids* 2018; 12: 1–11.
16. Li X, Liao Y, Dong Y, Li S, Wang F, Wu R, et al. Mib2 Deficiency Inhibits Microglial Activation and Alleviates Ischemia-Induced Brain Injury. *Aging Dis* 2020; 11(3): 523–35.
17. Ding W, Liu S, Zhang H, Ding H, Zhou S, Ye H, et al. Identification and validation of a thirteen-gene signature based on ubiquitin related genes in cervical cancer. *Discov Oncol* 2025; 16(1): 1000.
18. Du P, Han A, Liu J, Li W, Feng X, Chen L, et al. Taraxerol induces ferroptosis in breast cancer by targeting Nrf2 transcriptional activity to promote MIB2-mediated GPX4 ubiquitination. *Phytomedicine* 2025; 145: 157024.
19. Qin X, Chen B. Comprehensive analysis and validation reveal potential MYCN regulatory biomarkers associated with neuroblastoma prognosis. *J Biomol Struct Dyn* 2023; 41(18): 8902–17.
20. Clevers MR, Kastelijjn EA, Peters BJM, Kelder H, Schramel F. Evaluation of Serum Biomarker CEA and Ca-125 as Immunotherapy Response Predictors in Metastatic Non-small Cell Lung Cancer. *Anticancer Res* 2021; 41(2): 869–76.
21. Nasralla A, Lee J, Dang J, Turner S. Elevated preoperative CEA is associated with subclinical nodal involvement and worse survival in stage I non-small cell lung cancer: a systematic review and meta-analysis. *J Cardiothorac Surg* 2020; 15(1): 318.
22. Liu HN, Wu H, Tseng YJ, Chen YJ, Zhang DY, Zhu L, et al. Serum microRNA signatures and metabolomics have high diagnostic value in gastric cancer. *BMC Cancer* 2018; 18(1): 415.
23. Gupta N, Kumar R, Seth T, Garg B, Sati HC, Sharma A. Clinical significance of circulatory microRNA-203 in serum as novel potential diagnostic marker for multiple myeloma. *J Cancer Res Clin Oncol* 2019; 145(6): 1601–11.
24. Liu X, Wang X, Chai B, Wu Z, Gu Z, Zou H, et al. miR-199a-3p/5p regulate tumorigenesis via targeting Rheb in non-small cell lung cancer. *Int J Biol Sci* 2022; 18(10): 4187–202.
25. Pang M, Jiang Y, Huang Y, Ren B, He L, Jiang L. MiR-654-3p targets SRC to suppress tumor growth in non-small cell lung cancer. *Cell Mol Biol (Noisy-le-grand)* 2023; 69(4): 157–63.
26. Wang B, Li J, Li Y, Liang T, Chu X. MiR-630 suppresses non-small cell lung cancer by targeting vimentin. *J Clin Lab Anal* 2022; 36(9): e24536.
27. Zhang H, Mao F, Shen T, Luo Q, Ding Z, Qian L, et al. Plasma miR-145, miR-20a, miR-21 and miR-223 as novel biomarkers for screening early-stage non-small cell lung cancer. *Oncol Lett* 2017; 13(2): 669–76.
28. Kong P, Chen L, Yu M, Tao J, Liu J, Wang Y, et al. miR-3178 inhibits cell proliferation and metastasis by targeting Notch1 in triple-negative breast cancer. *Cell Death Dis* 2018; 9(11): 1059.
29. Li W, Shen S, Wu S, Chen Z, Hu C, Yan R. Regulation of tumorigenesis and metastasis of hepatocellular carcinoma tumor endothelial cells by microRNA-3178 and underlying mechanism. *Biochem Biophys Res Commun* 2015; 464(3): 881–7.
30. Lin MS, Chen WC, Huang JX, Gao HJ, Sheng HH. Aberrant expression of microRNAs in serum may identify individuals with pancreatic cancer. *Int J Clin Exp Med* 2014; 7(12): 5226–34.
31. Yang B, Jing C, Wang J, Guo X, Chen Y, Xu R, et al. Identification of microRNAs associated with lymphangiogenesis in human gastric cancer. *Clin Transl Oncol* 2014; 16(4): 374–9.
32. Xiao J, Wang QX, Zhu YQ. Altered expression profile of microRNAs in gastric stromal tumor. *J Huazhong Univ Sci Technolog Med Sci* 2015; 35(6): 842–50.
33. Wu JC, Liu ZH, Ding X, Ke RS. miR-3178 as a prognostic indicator and tumor suppressor of gastric cancer. *Ir J Med Sci* 2022; 191(1): 139–45.
34. Foresi B, Shah A, Meade S, Krishnaney A. Tumor markers in non-small cell lung cancer spine metastasis: an assessment of prognosis and overall survival. *Eur Spine J* 2024; 33(11): 4346–52.
35. Fortea-Sanchis C, Martínez-Ramos D, Escrig-Sos J. The lymph node status as a prognostic factor in colon cancer: comparative population study of classifications using the logarithm of the ratio between metastatic and non-metastatic nodes (LODDS) versus the pN-TNM classification and ganglion ratio systems. *BMC Cancer* 2018; 18(1): 1208.

36. Nwogu CE, Groman A, Fahey D, Yendamuri S, Dexter E, Demmy TL, et al. Number of lymph nodes and metastatic lymph node ratio are associated with survival in lung cancer. *Ann Thorac Surg* 2012; 93(5): 1614–9; discussion 9–20.
37. Sahar T, Nigam A, Anjum S, Waziri F, Biswas S, Jain SK, et al. Interactome Analysis of the Differentially Expressed Proteins in Uterine Leiomyoma. *Anticancer Agents Med Chem* 2019; 19(10): 1293–312.
38. Takeuchi T, Heng HH, Ye CJ, Liang SB, Iwata J, Sonobe H, et al. Down-regulation of a novel actin-binding molecule, skeletrophin, in malignant melanoma. *Am J Pathol* 2003; 163(4): 1395–404.
39. Takeuchi T, Adachi Y, Ohtsuki Y. Skeletrophin, a novel RING molecule controlled by the chromatin remodeling complex, is downregulated in malignant melanoma. *DNA Cell Biol* 2005; 24(5): 339–44.
40. Takeuchi T, Adachi Y, Ohtsuki Y. Skeletrophin, a novel ubiquitin ligase to the intracellular region of Jagged-2, is aberrantly expressed in multiple myeloma. *Am J Pathol* 2005; 166(6): 1817–26.
41. Guo Y, Chi X, Qu S, Sun Y, Liu J, Zhang L, et al. E3 ubiquitin-protein ligase 2 inhibits cell proliferation, migration, and invasion of non-small cell lung cancer through ubiquitination of Notch1. *Acta Histochem* 2022; 124(1): 151818.
42. Kong Y, Li J, Liang X, Zhou X. MIB2 promotes the progression of non-small cell lung cancer by regulating cell cycle control pathways. *Genes Genomics* 2023; 45(9): 1143–52.
43. Wang RJ, Zheng YH, Wang P, Zhang JZ. Serum miR-125a-5p, miR-145 and miR-146a as diagnostic biomarkers in non-small cell lung cancer. *Int J Clin Exp Pathol* 2015; 8(1): 765–71.
44. Zhou J, Diao X, Wang S, Yao Y. Diagnosis Value of Combined Detection of Serum SF, CEA and CRP in Non-Small Cell Lung Cancer. *Cancer Manag Res* 2020; 12: 8813–9.

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