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*Original Article*

# The diagnostic and prognostic role of circulating miR-141 expression in non-small-cell lung cancer patients

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**Abstract:** MicroRNAs (miRNA) play a crucial role in pathogenesis, progress, and prognosis of non-small-cell lung cancer (NSCLC). The purpose of this present study was to investigate the correlation of serum miR-141 expression with risk, clinicaopathologic features and OS in NSCLC patients, and further to explore its prognostic value in subtypes of NSCLC. 108 patients diagnosed with primary NSCLC and 54 age and gender matched health volunteers were recruited in this prospective cohort study. Blood serum was collected from all patients before treatment and health controls. Total RNA was extracted from serum and miRNA expression was measure by PCR methods. Serum miR-141 expression was evaluated in NSCLC patients (4.124 (3.259-4.944)) than in health controls (2.181 (1.036-2.946)), P<0.001, with area under curve (AUC) 0.856 (95% CI: 0.798-0.913) by Receiver operating characteristic (ROC) curve. And miR-141 expression was associated with differentiation (P=0.017), lymphatic metastasis (P=0.015), distant metastasis (P=0.025) and overall survival (OS) (P=0.002). Besides, miR-141 level was independent risk factor for OS (P=0.019) in NSCLC patients. Further subtype's analysis showed that serum miR-141 level could only predict OS in lung adenocarcinoma patients but not in SCC patients. Circulating miR-141 may be a novel and promising biomarker for susceptibility, clinicopathologic features of NSCLC patients, as well as an independent prognosis factor in lung adenocarcinomas patients but not in SCC patients.

**Keywords:** MiR-141, NSLCL, adenocarcinomas, diagnosis, prognosis

## Introduction

Lung cancer, as the leading cause of tumor death worldwide, leads to 1.8 million people diagnosed and 1.6 million deaths per year [1, 2]. Non-small-cell lung cancer (NSCLC), accounts for 85% primary lung cancers approximately, including adenocarcinoma, squamous cell carcinoma (SCC) and large cell carcinoma [3].

Although much progress has been made in screening, chemotherapy, surgery and so on, the prognosis of NSCLC is still unsatisfied, late diagnosis remains one of the most important reasons for the high mortality rate as well as lacking promising prognostic biomarkers [3-5]. Therefore, the identification of novel, feasible and convincing diagnostic and prognostic biomarkers is critical and essential for the treatment of NSCLC.

MicroRNAs (miRNA), small non-coding RNAs composed of 21-25 nucleotides, play a crucial role in pathogenesis, progress, treatment and prognosis of NSCLC, mainly through the regulation by targeting mRNAs for translational repression or degradation [6-10].

MicroRNA-141 (miR-141), located in chromosome 12, has been reported to play an important role in oncogenesis of various cancers [11, 12]. MiR-141 was reveal to be reduced and regarded as a potential tumor suppressor in gastric cancer; while in colorectal cancer, miR-141 acts as a functional oncogene [13, 14]. In NSCLC cells, miR-141 is reported to promote proliferation by regulation of PH domain leucine-rich-repeats protein phosphatase 1 (PHLPP1) and PHLPP2 [12]. And miR-141 expression in lung cancer tissue is increased and associated with overall survival (OS) [15]. However, the role of serum miR-141 expression

in diagnosis and prognosis in NSCLC patients is still not clear.

The purpose of this present study was to investigate the correlation of serum miR-141 expression with risk, clinicaopathologic features and OS in NSCLC patients, and further to explore its prognostic value in subtypes of NSCLC.

## Materials and methods

### *Participants*

108 patients diagnosed with primary NSCLC at Respiratory Department, in Shanghai Chest Hospital, from Sep. 2008 to Aug. 2010, were recruited in this prospective cohort study. Before peripheral blood sampling, none of the patients received radiotherapy, chemotherapy, surgery or other treatment. In addition, 54 age and gender matched health volunteers were enrolled as health controls.

This study was approved by Ethics committee of Shanghai Chest Hospital. All patients and health volunteers provided written informed consent.

### *Blood sample*

Peripheral blood (4 ml) was collected from NSCLC patients and health controls. After standing at room temperature for 1 hour, peripheral blood was centrifuged at 1000 g for 10 minutes at 4°C. The supernatant were subsequently acquired and further centrifuged at 16000 g for 10 minutes at 4°C. The supernatant were then collected and stored at -80°C.

### *Real-time PCR*

Total RNA was extracted from serum using TRIzol Reagent (TaKaRa Bio Inc., Otsu, Shiga, Japan), and a spectrophotometer was used to assess the concentration and purity of RNA. RNA was then subjected to reverse transcription with the PrimerScript Real-time reagent kit (TaKaRa Bio Inc., Otsu, Shiga, Japan) according to the manufacturer's instructions. Quantitative analysis of miR-141 expression was performed using SYBR Premix Ex TaqTM II (TaKaRa Bio Inc., Otsu, Shiga, Japan). Expression level of miR-141 was calculated utilizing the $2^{-\Delta\Delta t}$ method with U6 as the internal reference. The primers of miR-141 and U6 were listed as follows: miR-141 (Forward: 5'-CGCCAGGATAAATTGACGCACCATCTTTAC-3', Reverse: 5'-CCGCCTTAACACTGTCTGGTAATCGCCAGGATAAATTGACGCA-3'); U6 (Forward: 5'-CGCTTCGGCAGCACATATAC-3'; Reverse: 5'-TTCACGAATTTGCGTGTCAT-3').

### *Follow up*

NSCLC Patients were followed up at out-patient clinic or by telephone calls, and OS was calculated from the time of recruiting to the date of death or last follow-up, the last day of follow up was Dec. 2015 with median time 44 months.

### *Statistics*

Wilcoxon rank sum test was used to compare expression levels of miR-141 between two groups, while Kruskal-Wallis H rank sum test was performed among three groups. Receiver operating characteristic (ROC) curve was drawn for predictive value of miR-141 for risk of NSCLC.

Kaplan-Meier curves were drawn for OS and compared by log-rank test between miR-141 high expression and low expression groups in all NSCLC patients, then in lung adenocarcinoma patients and SCC patients. Univariate Cox's proportional hazards regression was performed to evaluate factors that influence the risk of OS, and all factors with a $p$-value ≤0.1 were further included in the multivariate Cox's proportional hazards regression analyses. All statistical analyses were performed using SPSS software, Version 19, and a P≤0.05 was considered as a statistically significant.

## Results

### *Characteristics of NSCLC patients*

Among 108 NSCLC patients, 45 cases (42%) ≤60 years and 63 cases (58%) >60 years, with 42 cases (39%) female and 66 (61%) male. 49 cases (45%) were adenocarcinoma, 52 cases (48%) were SCC and 7 case (7%) as others. The other detailed clinical and pathological characteristics of the patients were presented in **Table 1**.

### *Serum miR-141 expression was evaluated in NSCLC patients than in health controls*

The expression level of serum miR-141 was increased in NSCLC patients (4.124 (3.259-

**Table 1.** Clinical and pathological characteristics of NSCLC patients

| Parameters | | Cases (%) | Serum miR-141 | *p* value |
|---|---|---|---|---|
| Age | ≤ 60 | 45 (42%) | 4.002 (3.497-4.751) | 0.812 |
| | > 60 | 63 (58%) | 4.384 (3.329-5.021) | |
| Gender | Female | 42 (39%) | 3.973 (3.384-5.156) | 0.773 |
| | Male | 66 (61%) | 4.193 (3.186-4.883) | |
| Histology | Adenocarcinoma | 49 (45%) | 3.728 (2.759-4.105) | 0.285 |
| | Squamous cell carcinoma | 52 (48%) | 4.353 (3.992-5.541) | |
| | Others | 7 (7%) | 3.914 (2.699-4.163) | |
| Pathological stage | Highly-moderately differentiated | 84 (78%) | 3.638 (3.112-4.365) | 0.017 |
| | Poorly differentiated | 24 (22%) | 5.146 (4.039-5.962) | |
| Tumor Size | ≤ 50 mm | 81 (75%) | 3.841 (2.984-4.392) | 0.138 |
| | > 50 mm | 27 (25%) | 4.839 (3.662-5.834) | |
| Lymphatic metastasis | Negative | 76 (70%) | 3.695 (2.984-4.405) | 0.015 |
| | Positive | 32 (30%) | 5.075 (4.364-5.801) | |
| Distant metastasis | Negative | 95 (88%) | 3.973 (3.016-5.107) | 0.025 |
| | Positive | 13 (12%) | 5.471 (4.537-5.962) | |
| TNM Stage | I/II | 72 (67%) | 3.672 (2.904-4.528) | 0.069 |
| | III/IV | 36 (33%) | 5.021 (4.292-5.503) | |

Data are presented as counts (%) or median ($25^{th}$-$75^{th}$), A *p* Value <0.05 was considered statistically significant. Significance of the comparison was determined by wilcoxon rank sum test or Kruskal-Wallis H rank sum test.

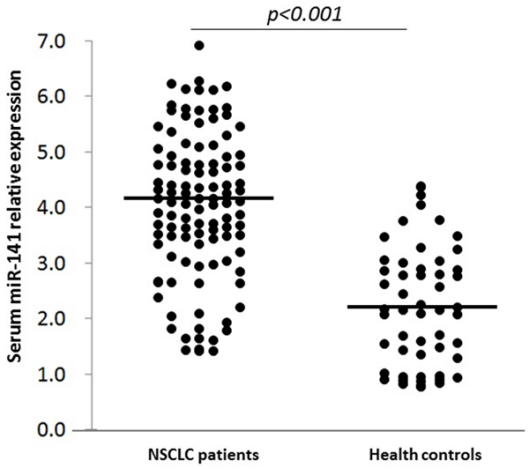


**Figure 1.** Serum miR-141 expression in NSCLC patients and health controls.

4.944)) compared with health controls (2.181 (1.036-2.946)), P<0.001, **Figure 1**. And ROC curve showed that serum miR-141 level predicted risk of NSCLC patients with area under curve (AUC) 0.856 (95% CI: 0.798-0.913), **Figure 2**.

*The correlation of serum miR-141 expression with clinical and pathological features*

Patients with poor differentiation showed a higher serum miR-141 level (5.146 (4.039-5.962)) than those with high-moderate differentiation (3.638 (3.112-4.365)), P=0.017. While serum miR-141 level was increased in lymphatic metastasis positive patients (5.075 (4.364-5.801)) compare to negative patients (3.695 (2.984-4.405)), P=0.015, so as to distant metastasis positive patients with evaluated miR-141 expression (5.471 (4.537-5.962)) than negative patients (3.973 (3.016-5.107)), P=0.025. However, no correlations were found between serum miR-141 expression level with age, gender, histology, tumor size and TNM stage, as presented in **Table 1**.

*The correlation of serum miRNA-141 expression with OS in NSCLC patients*

Patients were divided into two groups according to level of serum miR-141 level: High expression group (miR-141 level >4.124) and Low expression group (miR-141 level <4.124), the cut off value was regarded as median level of serum miR-141 in NSCLC patients.

As shown in **Figure 3**, NSCLC patients with miR-141 low expression exhibited a longer OS compared with patients with high expression analyzed by Kaplan-Meier method, with a P=0.002 by Log Rank test.

Univariate Cox's proportional hazards regression was subsequently used to evaluate factors

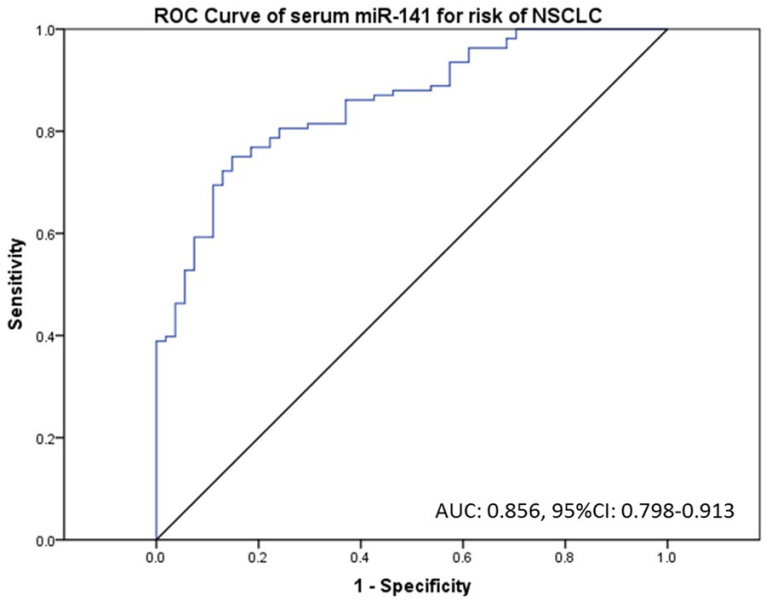


**Figure 2.** ROC curve of serum miR-141 level for risk of NSCLC.

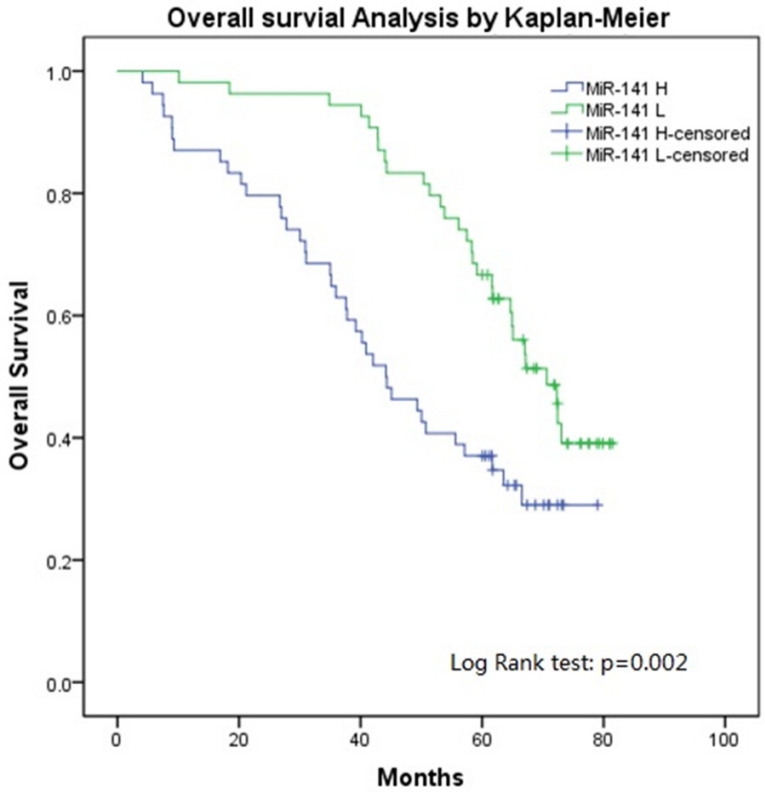


**Figure 3.** Overall survival analysis of serum miR-141 expression by Kaplan-Meier curve in NSCLC patients.

that influence the risk of OS, as presented in **Table 2**, which indicated high expression of miR-141 (P=0.002), poor differentiation (P=0.016), lymphatic metastasis positive (P=0.047) and distant metastasis positive (0.024) were associated with worse OS.

The factors with *p*-value ≤0.1 were further analyzed by multivariate Cox's proportional hazards regression, as shown in **Table 3**, which demonstrated that high expression of miR-141 was an independent risk factor for OS (P=0.019), as well as lymphatic metastasis positive (P=0.018) and distant metastasis positive (P=0.005).

*Serum miR-141 expression predicts OS in lung adenocarcinoma patients but not in SCC patients*

In order to further identify the prognostic role of serum miR-141 expression in NSCLC. Kaplan-Meier curves were drawn in both lung adenocarcinoma patients (N=49) and lung SCC patients (N=52).

Lung adenocarcinoma patients with miR-141 low expression displayed a longer OS compared with those with high expression (P=0.001), as shown in **Figure 4A**. While no differences were found between groups in lung SCC patients (P=0.273), as shown in **Figure 4B**. These indicated serum miR-141 expression affects the prognosis of lung adenocarcinoma patients but not lung SCC patients.

Subsequently, Factors which influenced OS in lung adenocarcinoma patients were analyzed, and we found that high expression of serum miR-141 (P=0.002), poor differentiation (0.038), lymphatic metastasis

**Table 2.** Univariate and multivariate analysis of the risk factors for overall survival in NSCLC patients

| | Univariate Cox's | | | | Multivariate Cox's | | | |
|---|---|---|---|---|---|---|---|---|
| | *p* value | HR | 95% CI | | *p* value | HR | 95% CI | |
| | | | Lower | Higher | | | Lower | Higher |
| MiR-141 (High vs Low) | 0.002 | 2.167 | 1.324 | 3.547 | 0.019 | 1.757 | 1.096 | 2.817 |
| Age (>60 vs ≤60) | 0.241 | 1.366 | 0.811 | 2.301 | - | - | - | - |
| Gender (Male vs Female) | 0.597 | 1.142 | 0.698 | 1.867 | - | - | - | - |
| Histology (Adenocarcinoma vs Squamous cell carcinoma and others) | 0.759 | 1.080 | 0.662 | 1.760 | - | - | - | - |
| Pathological stage (Poorly vs Highly-moderately differentiated) | 0.016 | 1.827 | 1.119 | 2.983 | 0.176 | 1.401 | 0.860 | 2.282 |
| Tumor Size (>50 mm vs ≤50 mm) | 0.129 | 1.480 | 0.892 | 2.458 | - | - | - | - |
| Lymphatic metastasis (Positive vs Negetive) | 0.047 | 1.643 | 1.006 | 2.682 | 0.018 | 1.806 | 1.106 | 2.950 |
| Distant metastasis (Positive vs Negetive) | 0.024 | 1.925 | 1.089 | 3.401 | 0.005 | 2.191 | 1.264 | 3.797 |
| TNM stage (III/IV vs I/II) | 0.115 | 1.488 | 0.908 | 2.438 | - | - | - | - |

A *p* Value <0.05 was considered statistically significant. Significance was determined by univariate and multivariate Cox's proportional hazards regression analysis.

**Table 3.** Univariate and multivariate analysis of the risk factors for overall survival in lung adenocarcinoma patients

| | Univariate Cox's | | | | Multivariate Cox's | | | |
|---|---|---|---|---|---|---|---|---|
| | *p* value | HR | 95% CI | | *p* value | HR | 95% CI | |
| | | | Lower | Higher | | | Lower | Higher |
| MiR-141 (High vs Low) | 0.002 | 3.268 | 1.529 | 6.984 | 0.015 | 2.607 | 1.203 | 5.650 |
| Age (>60 vs ≤60) | 0.377 | 1.275 | 0.744 | 2.182 | - | - | - | - |
| Gender (Male vs Female) | 0.521 | 1.184 | 0.707 | 1.982 | - | - | - | - |
| Pathological stage (Poorly vs Highly-moderately differentiated) | 0.038 | 1.753 | 1.032 | 2.979 | 0.070 | 1.637 | 0.960 | 2.794 |
| Tumor Size (>50 mm vs ≤50 mm) | 0.102 | 1.545 | 0.917 | 2.605 | - | - | - | - |
| Lymphatic metastasis (Positive vs Negetive) | 0.040 | 1.722 | 1.026 | 2.890 | 0.093 | 1.561 | 0.929 | 2.624 |
| Distant metastasis (Positive vs Negetive) | 0.028 | 1.944 | 1.075 | 3.518 | 0.004 | 2.234 | 1.287 | 3.879 |
| TNM stage (III/IV vs I/II) | 0.090 | 1.569 | 0.932 | 2.644 | 0.118 | 1.496 | 0.903 | 2.481 |

A *p* Value <0.05 was considered statistically significant. Significance was determined by univariate and multivariate Cox's proportional hazards regression analysis.

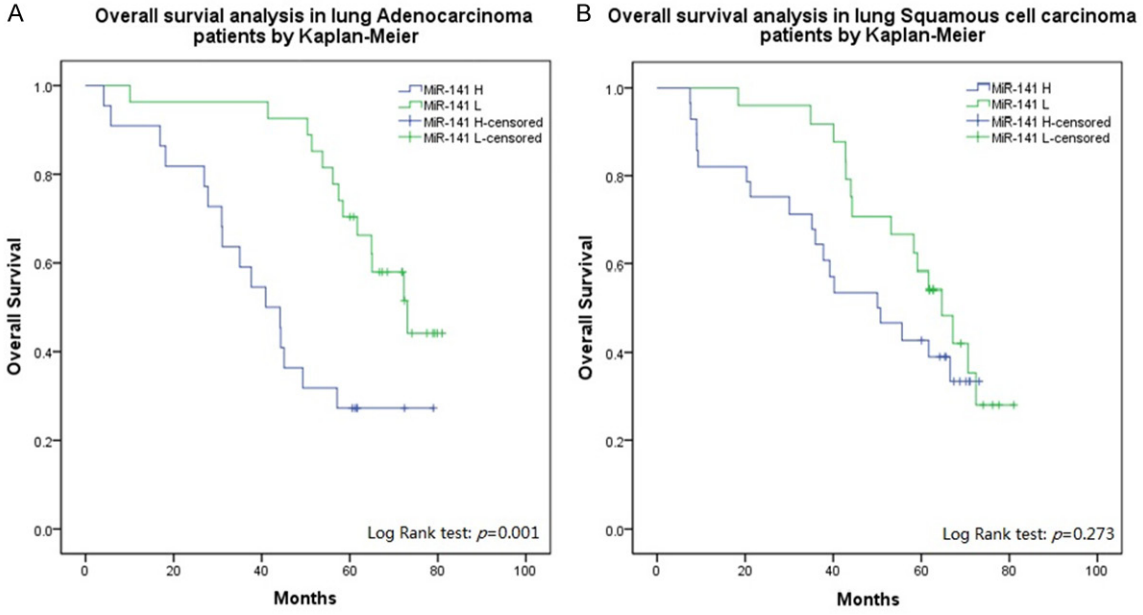


**Figure 4.** Overall survival analysis of serum miR-141 expression by Kaplan-Meier curve. A: In lung adenocarcinoma patients; B: In lung squamous cell carcinoma patients.

positive (P=0.040) and distant metastasis positive (0.028) predicted poor OS by univariate Cox's proportional hazards regression. In addition, miR-141 high expression was an independent risk factor for OS (P=0.015), as well as distant metastasis (P=0.004) by multivariate Cox's proportional hazards regression.

## Discussion

In the present study, we illuminated that serum miR-141 expression was evaluated in NSCLC patients than in health controls, and correlated with differentiation, lymphatic metastasis, distant metastasis and OS, and could be served as an independent risk factor for OS. In addition, we found miR-141 expression could only predict OS in lung adenocarcinoma patients but not SCC patients.

A growing number of evidence indicates various roles of microRNAs in development, progression, prognosis of NSCLC patients [6, 16]. Seven up-regulated and eight down-regulated miRNAs are found in tumor tissues of NSCLC patients by meta-analysis [17]. While in body fluids, miR-21 is overexpressed in sputum specimens of NSCLC patients; plasma miR-21, miR-126, miR210, miR-486-5p expressions are associated with risk of NSCLC [18, 19]. MiR-135a expression is reported to be related with pathological stage, TNM stage and lymphatic metastasis and could predict prognosis of NSCLC patients [20].

MiR-141, belonging to miR-200 family, has been described to be correlated with various cancers [11, 12]. MiR-141 expression is down-regulated in pancreatic ductal adenocarcinoma [21] and renal cell carcinoma [22] while up-regulated in ovarian cancer [11], which indicates its dual roles in different cancers. Our study presented serum miR-141 were overexpressed in NSCLC patients compared with health controls, in consist of previous study which showed higher miR-141 expression in cancer tissues than in normal tissues, suggested its oncogenic role in NSCLC [23].

MiR-141 promotes the proliferation of NSCLC cells by regulating expression of PHLPP1 and PHLPP2, antagonists of PI3K/AKT signaling [12]. And inhibition of miR-141 reverses cisplatin resistance in NSCLC cells by up-regulating programmed cell death protein 4 [24]. These indicate miR-141 expression might be related with the clinical pathological features and prognosis of NSCLC. In our study, we found poor differentiation, lymphatic metastasis positive and distant metastasis positive were correlated with higher serum miR-141 expression in NSCLC patients, and serum miR-141 high expression was independent risk factor for OS, partly in line with previous study that cancer tissue miR-141 expression is negatively associated with OS while the association with clinicopathologic feature is not analyzed [15].

In addition, we further explored the prognostic role of serum miR-141 expression in subtypes of NSCLC. We found higher serum miR-141 expression was associated with shorter OS only in lung adenocarcinoma patients but not in SCC patients. Compared with SCC, lung adenocarcinoma is more mesenchymal-like type, with vimentin overexpressed in adenocarcinoma but not detected in SCC tissues [25]. MiR-141 expression improves the secretion of vascular endothelial growth factor A (VEGFA) through down-regulation of KLF6 level, and VEGFA was found to be related with cancer-associated fibroblasts (CAFs) in murine lung adenocarcinomas and associated with tumor invasion [15, 26]. Which indicates miR-141 expression may be of critical importance in progress and prognosis in lung adenocarcinomas.

Further studies with large sample of participants are needed to further confirm the diagnostic and prognostic value of circulating miR-141 expression in NSCLC patients, especially in lung adenocarcinomas.

In conclusion, Circulating miR-141 may be a novel and promising biomarker for susceptibility, clinicopathologic features of NSCLC patients, as well as an independent prognosis factor in lung adenocarcinomas patients but not in SCC patients.

## Disclosure of conflict of interest

None.

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