

# MicroRNA-449a Plays an Indicative Role in Diagnosing Lung Cancer

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## Primary research

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# Abstract

**Background:** Lung cancer is one of the most common causes of cancer death among all the malignancies worldwide. Evidences suggest that the incidence and mortality of lung cancer has been on the rise. *MicroRNA-449a* (*miR-449a*) as one important member of microRNAs, has been demonstrated acting as a tumor suppressor in lung cancer. In this study, we sought to assess the relationship between *miR-449a* expression level and diagnostic value of lung cancer.

**Methods:** In this present research, quantitative Real-Time PCR was applied to detect the *miR-449a* expression in 116 lung cancer patients and 41 healthy volunteers. The diagnostic value of *miR-449a* in lung cancer patients was determined by receiver operating characteristic (ROC) curve.

**Results:** *MiR-449a* was significantly down-regulated in lung cancer patients compared with healthy control ( $P<0.05$ ). In addition, *miR-449a* expression was associated with sex ( $P=0.004$ ), tumor size ( $P=0.000$ ), TNM stage ( $P=0.006$ ) and metastasis ( $P=0.036$ ). However, there was no correlation with age, smoking history and histological type of lung cancer patients (all  $P\geq 0.05$ ). In the ROC analysis, the results showed that the area under the ROC curve (AUC) was 0.902 with the sensitivity of 94.8% and specificity of 78.0%, and the optimum cutoff value was 2.255.

**Conclusion:** *MiR-449a* expression was down-regulated in lung cancer patients, and it could be an efficient diagnostic biomarker in lung cancer patients.

## Background

Lung cancer is one of the leading causes of cancer related death around the world, and has an increasing incidence and mortality [1]. There is no obvious clinical symptoms at the early stage of lung cancer, with the evidences suggest that almost 75% of lung cancer patients are already at an advanced stage at the time of diagnosis [2, 3]. However, few effective test methods of lung cancer are available so far. Several protein markers, such as carcinoembryonic antigen, cytokeratin 19 fragment, cancer-associated antigen (CA) 125, CA 19-9, neuron-specific enolase, and tissue polypeptide specific antigen have been used to diagnose lung cancer without a surgical procedure, but the sensitivity is limited [3, 4]. Therefore, novel sensitive and specific diagnostic biomarkers are needed for the detection of lung cancer.

MicroRNAs (miRNAs) are endogenous non-coding RNA molecules of 22 nucleotides in length, which play important roles in regulation of pathological and biological processes [5]. Cellular miRNAs are released into different body fluids, such as blood [6]. They are presented in human serum in a highly stable form that is resistant to harsh conditions and RNase digestion [7, 8]. It is generally considered that miRNAs target the 3'-untranslated region (3'-UTR) of the targeted mRNAs, causing translation inhibiting or degradation of the mRNA. There are approximately half of miRNA genes are located at genomic regions which frequently amplified or deleted in cancer [9-11]. Recently studies have found that miRNAs possess regulatory functions in tumorigenesis and closely correlated with tumor differentiation [12-15]. *MicroRNA-449a* (*miR-449a*) is one of these miRNAs, which expressed in wide types of cancers, such as ovarian

cancer, gastric carcinoma, bladder cancer and lung cancer [16-20]. Recent evidences suggest that *miR-449a* performs suppression role in non-small cell lung cancer (NSCLC), which could inhibit tumor cell proliferation and promote tumor cell apoptosis [21]. However, no related studies have reported the diagnostic value of *miR-449a* in lung cancer.

In this present study, we examined the expression level of *miR-449a* in lung cancer patients with Quantitative Real-Time PCR (qRT-PCR) method and further evaluated the diagnostic potential of *miR-449a* for lung cancer patients.

## Methods

### Patients and serum specimens' collection

The study was approved by Ethics Committee of the The First Affiliated Hospital of Xinxiang Medical University. All the serum specimens and clinical materials in this research were obtained with the informed consents of all the participants.

Serum specimens were obtained from 116 cases with lung cancer and 41 healthy control cases without any malignancy before the study. These serum specimens were collected from patients on the day of diagnosis and before tumor surgery and therapy. All samples taken from lung cancer patients and healthy volunteers were put into blood collection tube of EDTA and stored at -80°C until RNA extraction. At the same time, clinicopathological features, which including age, sex, smoking history, histological type, tumor size, TNM stage and metastasis were also collected.

### Plasma preparation and RNA extraction

A volume of 5 ml of EDTA-anticoagulated blood was obtained from each participant. Serum was separated by centrifugation. Total RNA, including miRNA, was extracted from the serum using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA with OD A260/A280 ratio closed to 2.0 was subsequently used, which suggested that the RNA were pure.

### Quantitative Real-Time PCR (qRT-PCR)

The RNA was reverse transcribed into cDNA by AMV reverse transcription system (Promega, USA) and stored at -20°C. Quantitative real-time PCR was performed to evaluate the expression level of *miR-449a* using SYBR Green PCR master mix (Applied Biosystems, USA) by the 7300 Real-Time PCR System (Applied Biosystems, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as the endogenous control. Primer sequences were as follow, *miR-449a* forward: 5'-TGCGGTGGCAGTGTATTGTTAGC-3', reverse: 5'-CCAGTGCAGGGTCCGAGGT-3'; GAPDH forward: 5'-TGCACCACCAACTGCTTAGC-3' reverse: 5'-GGCATGCACTGTGGTCATGAG-3' [22]. After the reaction, all the data were quantitated with  $2^{-\Delta\Delta C_t}$  method.

### Statistical analysis

Statistical analysis was performed using SPSS 19.0 software. Measurement data were expressed as means  $\pm$  SD. T-test was used to compare the expression of *miR-449a* between the lung cancer patients and the healthy group. Chi-square test was used to analyze the differences between *miR-449a* expression and various clinicopathological characteristics. The diagnostic value of *miR-449a* in distinguishing lung cancer patients from healthy controls was performed using receiver operating characteristic (ROC) analysis. All tests with *P* values  $<0.05$  were considered as statistically significant.

## Results

### The expression level of miR-449a

The present analysis used qRT-PCR to estimate the miR-449a expression level in 116 lung cancer patients and 41 healthy volunteers. The result showed that the expression of *miR-449a* was significantly reduced in lung cancer patients compared with the healthy controls ( $P=0.05$ ; **Figure 1**).

### Relationship between miR-449a and clinicopathological characteristics of lung cancer

In this study, we used statistical analysis to explore the relationship between miR-449a expression level and the clinicopathological data of lung cancer patients. In Table 1, the analysis results showed that miR-449a expression was associated with sex ( $P=0.004$ ), tumor size ( $P=0.000$ ), TNM stage ( $P=0.006$ ) and metastasis ( $P=0.036$ ). However, there was no significant association between miR-449a expression and age, smoking history as well as histological type of lung cancer patients (all  $P>0.05$ ).

### Diagnostic value of miR-449a for lung cancer

ROC curves were constructed to evaluate the diagnostic value of miR-449a expression level in lung cancer patients. In Figure 2, the ROC curve showed that the lung cancer patients were distinguished from the healthy volunteers with sensitivity of 94.8%, specificity of 78.0% and associated area under the curve (AUC) value of 0.902. The cutoff value for miR-449a expression level is 2.255. This result indicated that the miR-449a expression has efficient diagnostic value for lung cancer.

## Discussion

Lung cancer, as a major death cause, is reported to be with no clinically apparent symptoms until it has reached an advanced stage [23]. Recently evidences demonstrated that the incidence and mortality of lung cancer has been on the rise [9]. The development of lung cancer is a complicated process that involves multiple genes, factors and pathways. Although there are several biomarkers for lung cancer such as SCC antigen, carcinoembryonic antigen and pro-gastrin-releasing peptide, none of them are perfect in terms of sensitivity or specificity. Therefore, we sought to find an efficient biomarker to achieve the earlier diagnosis for lung cancer patients.

MiRNAs are a class of small noncoding RNAs. There are several researches find that miRNAs are associated with a wide range of cellular processes, such as cellular proliferation, differentiation, apoptosis, and play a critical role in cancer [24-26]. Recently, *miR-449a* as a member of miRNAs has been found down-regulated in several types of cancers including prostate cancer, gastric cancer, bladder cancer as well as lung cancer [17, 19, 27-29]. These studies have showed that *miR-449a* is an important miRNA that has been identified as tumor suppressor, and it plays important roles in the occurrence, development, and prognosis of multiple malignancies. However, the role of *miR-449a* in the diagnosis of lung cancer still needs to be explored.

In our study, we observed that the *miR-449a* expression level was lower in lung cancer patients than that in healthy volunteers. This finding is consistent with previous reports. For example, Ding *et al.* demonstrated that the *miR-449a* expression levels in non-small cell lung cancer are significantly lower than those in the surrounding tissue [21]. Ren *et al.* confirmed that *miR-449a* acts as a tumor suppressor and is mostly down-regulated in lung cancer patients [29]. In addition, the present study assessed the relationship between *miR-449a* expression and the clinical pathological features of lung cancer patients and found that the expression of *miR-449a* was significantly associated with sex, tumor size, TNM stage and metastasis. However, there were no relation with age, smoking history and histological type of the lung cancer patients. Luo *et al.* [30] have proved that low expression level of *miR-449a* appeared to be correlated with various clinical features such as advanced pathological stage, and lymph node metastasis. What's more, they also suggested that *miR-449a* might be a useful prognostic predictor for NSCLC patients. In the present study, we evaluated the diagnostic value of *miR-449a* in lung cancer patients, and the results of ROC analysis showed that down-regulation of *miR-449a* might serve as a novel biomarker to diagnose lung cancer.

Currently, the molecular mechanisms for the expression of *miR-449a* in tumors still remain unclear. According to the previous reports, single miRNAs with multiple functions are potential candidates for gene therapy. For example, Noonan *et al.* [28] have showed that HDAC1 was a target of *miR-449a* and *miR-449a* regulates cell growth and viability in part by repressing the expression of HDAC1. Moreover, in the study of Jeon HS *et al.* [20] showed that the down expression of *miR-449a* might be one mechanism for over-expression of HDAC1 in lung cancer, and *miR-449a* could be a potential therapeutic candidate in lung cancer patients.

## Conclusions

In summary, the results provided evidences that *miR-449a* was significantly down-regulated in lung cancer patients compared with the healthy controls. The ROC curves indicated that the down-regulation of *miR-449a* could be used as a novel biomarker to distinguish patients with lung cancer from the healthy ones. However, there are still some limitations, for example, the sample size is small and we did not give subdivision of lung cancer. Therefore, more detailed research will be needed in the further studies.

## List Of Abbreviations

MicroRNA-449a (miR-449a)

Receiver operating characteristic (ROC)

Cancer-associated antigen (CA)

MicroRNAs (miRNAs)

3'-untranslated region (3'-UTR)

Non-small cell lung cancer (NSCLC)

Quantitative Real-Time PCR (qRT-PCR)

Area under the curve (AUC)

## **Declarations**

### **Ethics approval and consent to participate**

This study was supported by the Ethics Committee of The First Affiliated Hospital of Xinxiang Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

### **Consent for publication**

We obtaining permission from participants to publish their data.

### **Availability of data and materials**

All data generated or analysed during this study are included in this published article.

### **Competing interests**

The authors declare that they have no competing interests.

### **Funding**

Not applicable.

### **Authors' contributions**

Jun.Y design of the work; H.Z. and X.L. the acquisition, analysis, Q.Y. interpretation of data; Jian.Y. the creation of new software used in the work; C.J. have drafted the work or substantively revised it. All

authors read and approved the final manuscript.

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Not applicable.

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Tables

**Table 1.** The expression of *miR-449a* and clinicopathological features in lung cancer patients

Features	No. N=116	<i>MiR-449a</i> expression		<i>P</i> values
		Low (n=76)	High (n=40)	
Age (years)				0.053
<60	64	37	27	
≥60	52	39	13	
Sex				0.004
Male	65	50	15	
Female	51	26	25	
Smoking history				0.390
Yes	32	19	13	
No	84	57	27	
Tumor size				0.000
<3cm	47	21	26	
≥3cm	69	55	14	
Histologic type				0.633
Adenocarcinoma	90	60	30	
Squamous carcinoma	8	4	4	
Others	18	12	6	
TNM stage				0.006
I-II	52	41	11	
III-IV	64	35	29	
Metastasis				0.036
Yes	73	53	20	
No	43	23	20	

## Figures

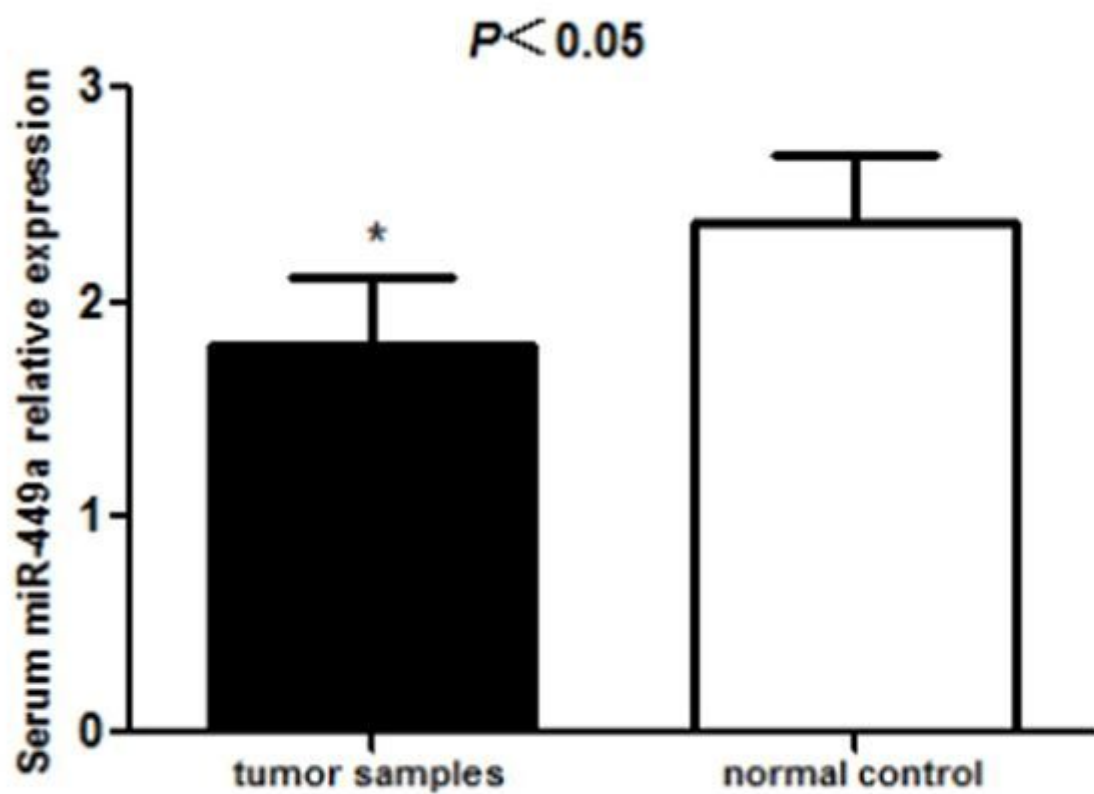
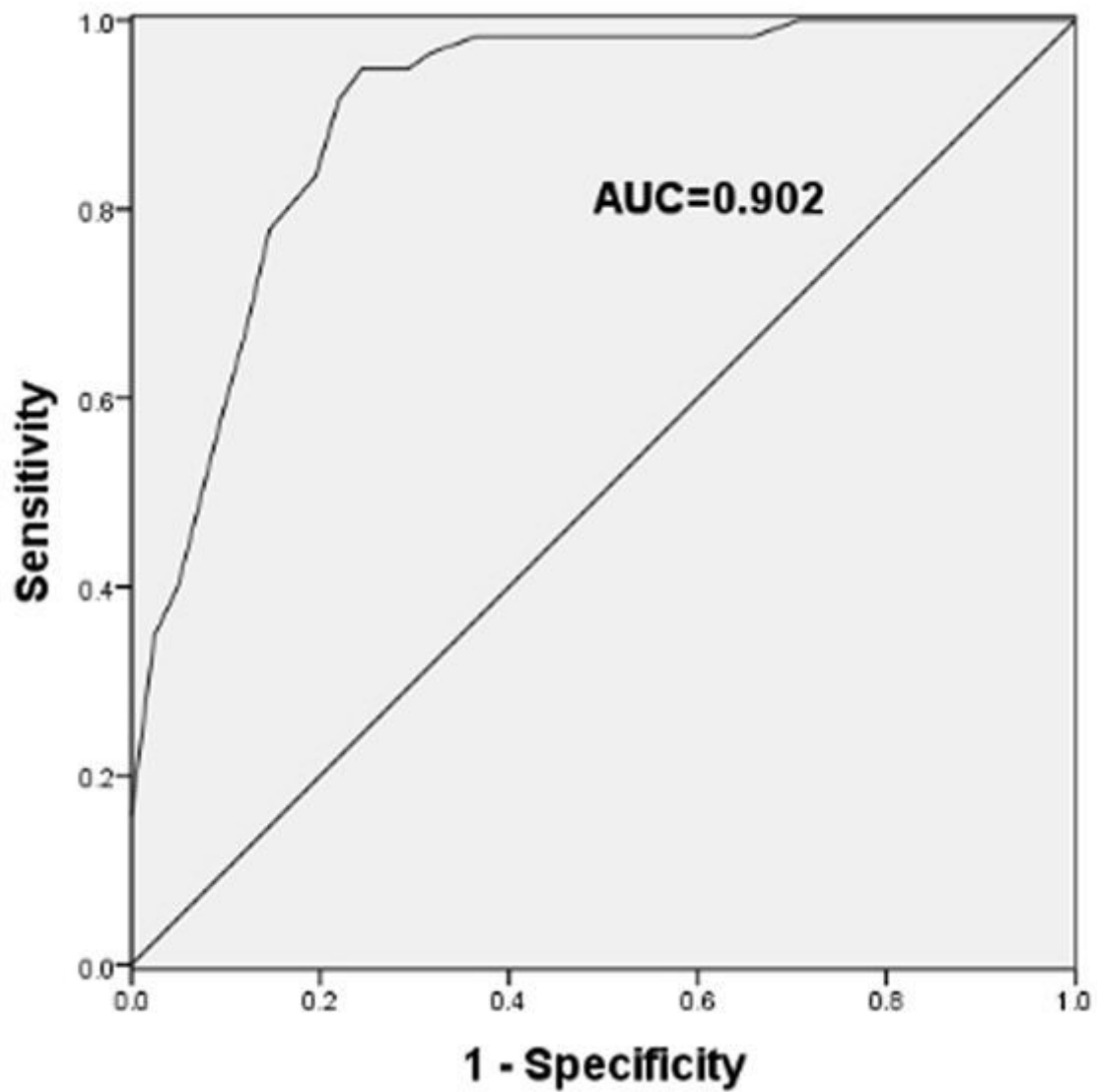


Figure 1

Serum miR-449a expression levels was evaluated by qRT-PCR in lung cancer patients (n=116) and healthy controls (n=41) \*,  $P < 0.05$ .



**Figure 2**

ROC analysis based on serum miR-449a expression for lung cancer patients. ROC: receiver operating characteristic, AUC: area under the curve.