Original Article

A test of miR-128-3p and miR-33a-5p in serum exosome as biomarkers for auxiliary diagnosis of non-small cell lung cancer

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Background: Lung cancer is the malignant tumor with the highest incidence and mortality rate in the world today, and non-small cell lung cancer (NSCLC) is its most common type. However, there is still a paucity of specific tumor markers for lung cancer screening. Herein, we detected and compared the levels of miR-128-3p and miR-33a-5p in serum exosomes of NSCLC patients and healthy volunteers, with the aim of identifying suitable exosomal microRNAs (miRNAs) as tumor biomarkers, and explored their value in the auxiliary diagnosis of NSCLC.

Methods: All participants were recruited from September 1, 2022 to December 30, 2022, and met the inclusion criteria. The case group included 20 patients with lung nodules who were highly suspected of having lung cancer (two cases were excluded). A total of 18 healthy volunteers (control group) were also enrolled. Blood samples were collected in both the case group before surgery and in the control group. Quantitative real-time polymerase chain reaction method was used to detect the expression of miR-128-3p and miR-33a-5p in serum exosomes. The main indicators of statistical analysis included the area under the receiver operating characteristic curve (AUC), sensitivity, and specificity.

Results: Compared with the healthy control group, the NSCLC case group had significantly lower expression levels of serum exosome miR-128-3p and miR-33a-5p ($P<0.01$, $P<0.001$), and there was a significant positive correlation between the two exosome miRNAs ($r=0.848$, $P<0.01$). The AUC values of miR-128-3p alone and miR-33a-5p alone in distinguishing case group and control group were 0.789 [95% confidence interval (CI): 0.637–0.940; sensitivity: 61.1%; specificity: 94.4%; $P=0.003$] and 0.821 (95% CI: 0.668–0.974; sensitivity: 77.8%; specificity: 83.3%; and $P=0.001$), respectively. The combination of miR-128-3p and miR-33a-5p had an AUC of 0.855 (95% CI: 0.719–0.991; $P<0.001$) for distinguishing case group and control group, which was greater than the AUC values of miR-128-3p alone and miR-33a-5p alone (cut-off value: 0.034; sensitivity: 83.3%; and specificity: 88.9%). However, there was no significant difference in the AUC among these three groups ($P>0.05$).

Conclusions: Serum exosome miR-128-3p and miR-33a-5p showed good performance in NSCLC screening and may be used as new biomarkers for large-scale NSCLC screening.

Keywords: Non-small cell lung cancer (NSCLC); exosomes; microRNA (miRNA); liquid biopsy

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Introduction

Lung cancer is one of the most common malignancies and remains the leading cause of cancer-related deaths worldwide (1-3). According to global statistics published in *The Lancet*, there were 3.3 million new lung cancer cases and 1.7 million deaths in 2015, suggesting lung cancer is a major threat to human health in the 21st century, while non-small cell lung cancer (NSCLC) is the most common type of lung cancer with the highest mortality rate (4). The stage at diagnosis is a major determinant of lung cancer prognosis, and resection is the primary mode of treatment for lung cancer. However, there is still a lack of specific tumor markers for NSCLC screening. Although related studies have confirmed that carcinoembryonic antigen, cancer antigen 125 and cytokeratin fragment 21-1 are serum antigenic biomarkers associated with lung cancer. And only by incorporating some clinical variables (nodule size, smoking history, age, etc.) can these biomarkers improve the diagnostic accuracy of lung cancer and reduce the invasive diagnosis and treatment of benign nodules, but also delay the treatment of malignant nodules to a certain extent, and the clinical application is limited.

Highlight box

Key findings

- MiR-128-3p and miR-33a-5p demonstrated advantages in diagnosis of NSCLC, and the combined detection of miR-128-3p and miR-33a-5p showed higher sensitivity.

What is known and what is new?

- MiR-128-3p and miR-33a-5p were significantly down-regulated in NSCLC serum exosomes compared with paired normal tissues.
- Serum exosomes miR-128-3p and miR-33a-5p showed good performance in NSCLC screening with excellent sensitivity and specificity.

What is the implication, and what should change now?

- Both miR-128-3p and miR-33a-5p may be potential novel biomarkers for distinguishing NSCLC patients and healthy individuals. Now, what we need is to build high-quality NSCLC prediction models including miRNA to improve the diagnosis rate of NSCLC. And, testing the function of miR-128-3p and miR-33a-5p in NSCLC is an essential step.

Besides, the value of circulating tumor DNA (ctDNA) as a biomarker of advanced tumor has been well established. However, its role in lung cancer screening and auxiliary diagnosis is still uncertain (5-8). In addition, conventional diagnostic methods have certain disadvantages that limit their application in the screening of malignant tumors. For example, imaging examinations are less specific, have certain requirements on lesion size, and are not easy to perform. Although histopathology is the gold standard for tumor diagnosis, it requires tissue sampling and accurate lesion locating prior to sampling (9,10). Therefore, it is of great significance to develop more efficient auxiliary diagnostic methods for NSCLC to improve prognosis, prolong survival, and increase the quality of life in NSCLC patients.

Liquid biopsy is a promising new technology for the early screening of malignant tumors by obtaining tumor information through non-invasive blood sampling to assist the diagnosis and treatment of malignant tumors (11). MicroRNAs (miRNAs) are a family of 21–25 nucleotide-long non-coding small RNA molecules that play key roles in important life processes such as embryonic development, cell cycle regulation, proliferation/differentiation, and apoptosis. There is increasing evidence that there are abnormalities in the expressions of a variety of miRNAs in human tumors, suggesting that miRNAs may play important roles in the pathogenesis of malignant tumors by regulating the expressions of oncogenes or tumor suppressor genes. A recent study on miRNA subtypes revealed the potential of blood miRNAs in indicating complex diseases and thus miRNAs may be novel clinical parameters for liquid biopsy (12). Exosomes are 40–100 nm membrane vesicles secreted by most cell types, which exist in serum, urine, saliva and other body fluids. Exosomes can carry RNA to shuttle freely between cells and tissues, connect the communication network between cells, and widely participate in many biological processes such as immune response, antigen presentation, cell differentiation, tumor growth and invasion (13-15). Relevant studies have shown that lung cancer-related exosomes can affect the occurrence and progression of lung cancer by regulating the physiological functions of surrounding tissue cells and microenvironment, and are considered to be an important part of lung cancer fluid biopsy. For example, exosomes regulate the function

of immune cells such as T lymphocytes, dendritic cells, and natural killer cells by transferring immunosuppressive factors such as exocrine miRNAs. Furthermore, it affects the occurrence and development of lung cancer: the high level of miR-660-5p in exocrine promotes the progression of NSCLC by targeting KLF9, miR-21/29a initiates the growth and metastasis of lung cancer by activating TLR7 and TLR8 on immune cells, and the communication between lung cancer cells and $CD4^+$ lymphocytes through exosome mir-214, which effectively reduces the expression of PTEN and promotes the expansion of regulatory T cells and tumor growth (13,16-18). Therefore, with the advancement of RNA sampling and detection technology, miRNA in exosome is relatively stable and can be effectively recovered in biological fluid, making people increasing interests in using miRNA as biomarkers for NSCLC screening or for monitoring tumor recurrence or metastasis after surgery (19).

Relevant studies have shown that miR-128-3p and miR-33a-5p, as tumor suppressor genes, can affect the occurrence, invasion, and metastasis of gastric cancer, esophageal cancer, breast cancer, colorectal cancer, melanomas, and other cancers, through different signaling pathways (20-23). In lung cancer, miR-33a-5p has been reported to inhibit the proliferation and invasion of lung cancer cells. In addition, the expression of miR-128-3p in lung cancer was significantly downregulated, and the repair of miR-128-3p *in vivo* could significantly suppress the tumorigenesis of A549 cells in nude mice models and inhibit angiogenesis and lymphangiogenesis in tumour xenografts (24-26). More importantly, the reason why we pay attention to the possibility that miR-128-3p and miR-33a-5p can be used as biomarkers for NSCLC screening is that relevant studies have shown that these two miRNAs are significantly down-regulated in NSCLC tissues than in matched normal tissues, indicating that their content is associated with lung cancer cells (27). However, due to the limitations of lung tissue collection, the clinical application of these two miRNAs in the diagnosis of lung cancer is very difficult. Compared with the acquisition of lung tissue samples, blood samples are easier to collect. Therefore, in this present study, by detecting the expressions of miR-128-3p and miR-33a-5p in the serum exosome of NSCLC patients and healthy volunteers using quantitative real-time polymerase chain reaction (qRT-PCR), we explored the relationships of these two miRNAs with the pathogenesis of NSCLC and assessed the sensitivity and specificity of different diagnostic models, with a view to providing potential and reliable biomarkers for early diagnosis and monitoring of NSCLC. We present this article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-398/rc).

Methods

Subjects

The inclusion criteria for the case group were as follows: (I) inpatients scheduled for surgery; (II) computed tomography (CT) of the lungs using a 64-slice spiral CT scanner (Philips, Amsterdam, the Netherlands) showed peripheral pulmonary nodules sized 0.5–5.0 cm, which were highly suspected as lung cancer; and (III) postoperative pathological results confirmed the diagnosis of NSCLC. The inclusion criteria for the control group included the following: (I) no pulmonary nodule was seen on CT of the lungs using a 64-slice spiral CT scanner (Philips); and (II) no history of malignancy. All subjects were treatment-naive and had not received neoadjuvant therapy such as chemotherapy or radiotherapy. In addition, all participants must have complete medical records and imaging examination information.

The exclusion criteria included the following: (I) patients with severe coronary heart disease, severe bronchial asthma, cardiomyopathy, malignant tumors other than NSCLC, allergic diseases, severe liver and kidney dysfunction, hematopoietic system diseases, neurological diseases, psychiatric diseases, autoimmune diseases, active tuberculosis, and/or other diseases; (II) individuals with communication problems; and (III) pregnant and lactating women.

A total of 20 patients with lung nodules who were highly suspected of having lung cancer were recruited. Two cases were excluded, with one patient failing to undergo surgery and the other patient presenting with a benign lesion. Thus, the case group included 18 patients who received surgical treatment and were pathologically confirmed with NSCLC (postoperative pathological diagnosis is the gold standard for the diagnosis of lung cancer). A total of 18 healthy volunteers were recruited as the control group. All participants were recruited from our center from September 1, 2022 to December 30, 2022. Among these 36 subjects, there were 20 females and 16 males, with an average age of 60.94±6.08 years (*Figure 1*).

Before the initiation of the study, all subjects were evaluated and screened by the researchers to determine

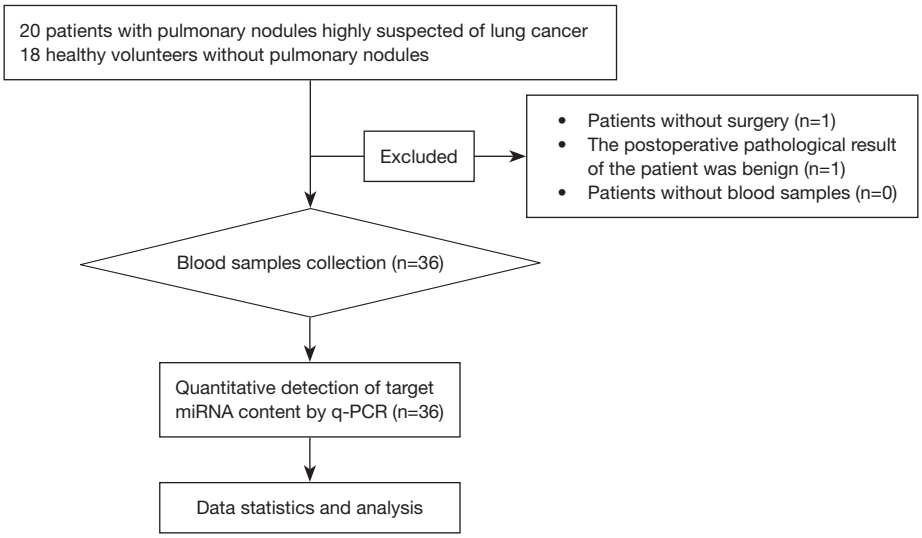

Figure 1 An overview of patients' enrollment and samples collection process. miRNA, microRNA; q-PCR, quantitative polymerase chain reaction.

whether they were eligible for the study and to finalize the grouping based on the tests received and the postoperative pathological results. The subject data were registered in the thoracic surgeons' offices. The clinical data were extracted from the patients' medical records.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Changhai Hospital (No. 2020021) and informed consent was taken from all the participants.

Reagents and equipment

Reagents and equipment used included the following: serum/plasma miRNA extraction kits and primers (Shanghai Puxi Biotech, Shanghai, China); grinder (MP Bio, Santa Ana, CA, USA); spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); refrigerated centrifuge (Beckman Coulter, Brea, CA, USA); Ultra-highspeed centrifuge (Thermo Fisher Scientific); gel scanner (Bio-Rad, Hercules, CA, USA); and real-time PCR machine (Bio-Rad).

Collection of serum exosomes

Venous blood (10 mL) was collected 1 day before surgery using ordinary vacuum blood-collection tubes. After 10–60 min of clotting at 4 ℃, the blood samples were centrifuged at 300×g for 10 min. The upper layer of the serum (yellow) was carefully transferred to a new 1.5 mL centrifuge tube and centrifuged at 2,000×g for 10 min at 4 ℃. Take the new supernatant and continue to increase the centrifugal force to 10,000×g for 30 min, and then the cell fragments were removed. After treatment, the supernatant was centrifuged for 70 min with 100,000×g centrifugal force to obtain crude exocrine sediment (containing a small amount of foreign protein). Use phosphate buffer solution (PBS) to resuspend the exocrine precipitate, and then 100,000×g again, centrifuge for 70 min to obtain pure exocrine, and stored at −80 ℃ until use. None of the patients experienced significant adverse effects after blood collection.

Measurements

To determine of the expression levels of miR-128-3p and miR-33a-5p, total RNA was extracted from the treated samples using the RNA extraction kit (9109, TAKARA, Beijing, China) and reversed into cDNA using the reverse transcription kit (Bio-Rad, CFX Connect). The experimental procedures were conducted in strict accordance with kit instructions. The miR-128-3p, miR-33a-5p, and their internal reference U6 were amplified by qRT-PCR instrument. The detailed primer sequences are shown in *Table 1*. The qRT-PCR reaction system included the following: 2× iTaqTM universal SYBR Green supermix (5 μL), forward and reverse primers (1 μL), and DNA template (2 μL). In order to reduce experimental error, all

Table 1 Primer sequences for the quantitative reverse transcription polymerase chain reaction experiments

Primer name	Sequence (5' to 3')
hsa-miR-33a-5p -F	GCGCGTGCATTGTAGTTGC
hsa-miR-128-3p -F	GCGCTCACAGTGAACCGGT
Universal primer R	CGA GGAAG AAGA CGG AAGAAT
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT

Table 2 Patient characteristics

Variables	Control (n=18)	Lung cancer (n=18)
Age (years)		
Median [range]	61.5 [50–69]	58.5 [53–72]
Gender, n (%)		
Female	11 (61.1)	9 (50.0)
Male	7 (38.9)	9 (50.0)
Tobacco and alcohol use, n (%)		
Both	2 (11.1)	3 (16.7)
Tobacco only	3 (16.7)	2 (11.1)
Alcohol only	4 (22.2)	2 (11.1)
None	9 (50.0)	11 (61.1)
Family history of cancer, n (%)		
Yes	1 (5.6)	2 (11.1)
No	17 (94.4)	16 (88.9)
Tumor location, n (%)		
Left		8 (44.4)
Right		10 (55.6)
Maximum tumor diameter (cm)		
Median [range]		3.1 [0.8–5.7]
Lymph node stage, n (%)		
pN0		16 (88.9)
pN1		1 (5.6)
pN2–pN3		1 (5.6)
TNM stage, n (%)		
0–I		14 (77.8)
II		3 (16.7)
III		1 (5.6)

TNM, tumor-node-metastasis

experiments were performed in triplicate wells. The relative expressions of the serum exosome miR-128-3p and miR-33a-5p were calculated using the Bio-Rad CFX Manager software. Laboratory staffs were blinded to the patient information corresponding to the blood samples throughout the tests, and they were only responsible for sample testing and data reporting.

Statistical analysis and visualization

All data were analyzed using the SPSS 25.0 software package and visualized using the Graphpad Prism 9 software. Measurement data are expressed as mean ± standard deviation, and count data are expressed as frequency or rate (%). Inter-group comparisons were based on *t*-tests. The Pearson method was used to analyze the correlation between the expression levels of miR-128-3p and miR-33a-5p in the serum exosome. Application of logistics regression analysis method to establish combined diagnosis model. The receiver operating characteristic (ROC) curves were drawn using the IBM SPSS Statistics 26 software package, and the area under the ROC curves (AUC), sensitivity, and specificity of different indicators in diagnosing NSCLC were analyzed, and the cut-off value was the threshold to determine the sensitivity and specificity. The R studio software was used to analyze the difference in AUCs. A P value <0.05 was considered statistically significant. Patients with missing or inconclusive data were excluded from the final analysis.

Results

Clinical features

The clinical features of the participants are shown in *Table 2*. The median age was 59.5 years (range, 50–72 years), and the median age of patients in the case group was 58.5 years (range, 53–72 years). There were 9 males (50%) and 9 females (50%) in the case group, with 5 patients (27.8%) presenting with a history of smoking. According to the 8th edition of AJCC/UICC staging criteria, there were 8 cases with stage IA tumors (44.4%), 6 cases (33.3%) with stage IB, 3 cases (16.7%) with stage IIB, and 1 case (5.6%) with stage IIIA tumor. The maximum tumor diameter ranged from 0.8 to 5.7 cm (median: 3.1 cm). All enrolled patients were naive to neoadjuvant radiochemotherapy or traditional Chinese medicine treatments. The median age in the control group

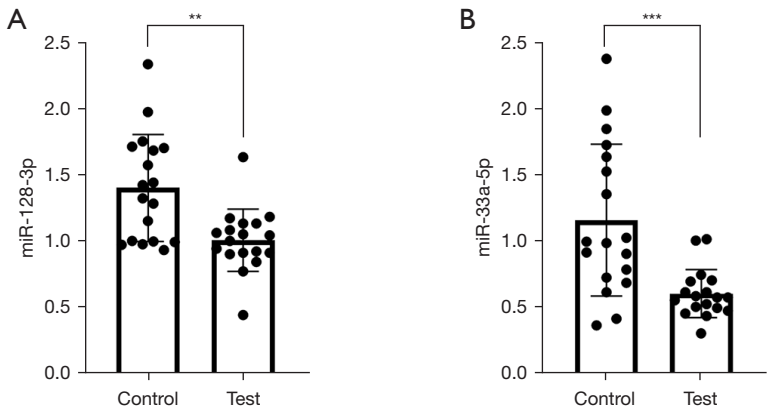

Figure 2 A comparison of the expression levels of miR-128-3p and miR-33a-5p in the control group and the NSCLC (test) group. **, P<0.01; ***, P<0.001. NSCLC, non-small cell lung cancer.

Table 3 The correlation between miR-128-3p and miR-33a-5p content

	miR-128-3p	miR-33a-5p
miR-128-3p		
Pearson correlation	1	0.848**
Sig. (2-tailed)	–	0.000
N	36	36
miR-33a-5p		–
Pearson correlation	0.848**	1
Sig. (2-tailed)	0.000	–
N	36	36

**, P<0.01.

was 61.5 years (range, 50–69 years). No pulmonary nodule was detected by chest CT in the control group.

Differential expression levels of miR-128-3p and miR-33a-5p levels in serum exosomes

A comparison of the differential expression levels of miR-128-3p and miR-33a-5p in the serum exosomes showed that the expression of miR-128-3p and miR-33a-5p were significantly lower in the case group than in the control group (P<0.05; *Figure 2*).

The correlation between miR-128-3p and miR-33a-5p expression levels in subjects

The mean values of three measurements for miR-128-3p and miR-33a-5p in each sample were taken as the observation data, and the correlation between these two genotypes was analyzed. These mean values were normally distributed, and the results of Pearson correlation analysis are shown in *Table 3*. The correlation coefficient between miR-128-3p and miR-33a-5p in the serum was 0.848 (r=0.848, 2-tailed), suggesting that the levels of miR-128-3p and miR-33a-5p were positively correlated at the level of 0.01.

miR-128-3p and miR-33a-5p are effective biomarkers for distinguishing non-small cell NSCLC patients from healthy individuals

ROC curves were drawn to evaluate the ability of miR-128-3p or miR-33a-5p to distinguish between NSCLC patients and healthy controls. The AUC value of miR-128-3p alone in distinguishing case group and control group was 0.789 (95% CI: 0.637–0.940; sensitivity: 61.1%; specificity: 94.4%; P=0.003), with a cut-off value of 1.240 (*Figure 3A*). The AUC value of miR-33a-5p alone in distinguishing case group and control group was 0.821 (95% CI: 0.668–0.974; sensitivity: 77.8%; specificity: 83.3%; P=0.001), with a cut-off value of 0.720 (*Figure 3B*). Thus, both miR-128-3p and miR-33a-5p could be used to distinguish NSCLC patients

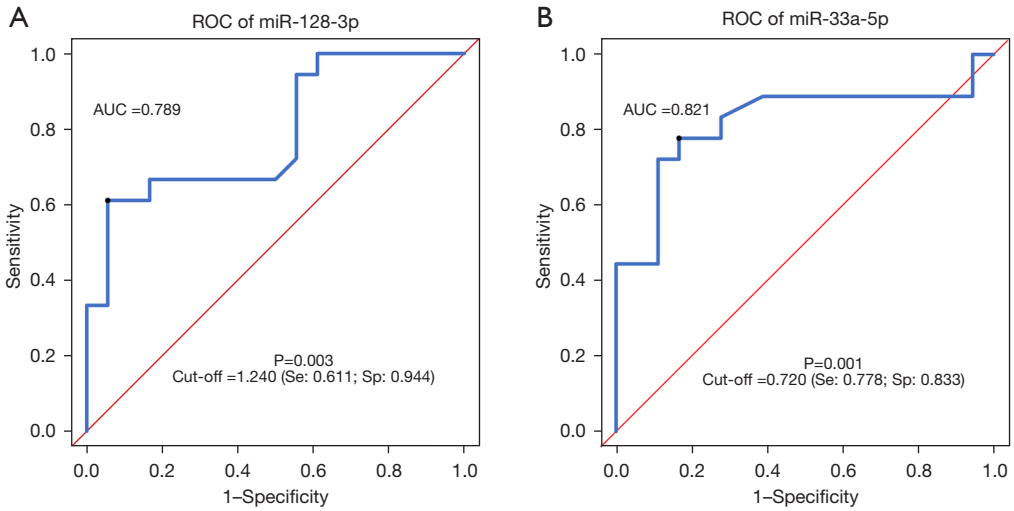

Figure 3 Differential ROC curves of serum exosomes miR-128-3p (A) and miR-33a-5p (B). ROC, receiver operating characteristic; AUC, area under the ROC curve; Se, sensitivity; Sp, specificity.

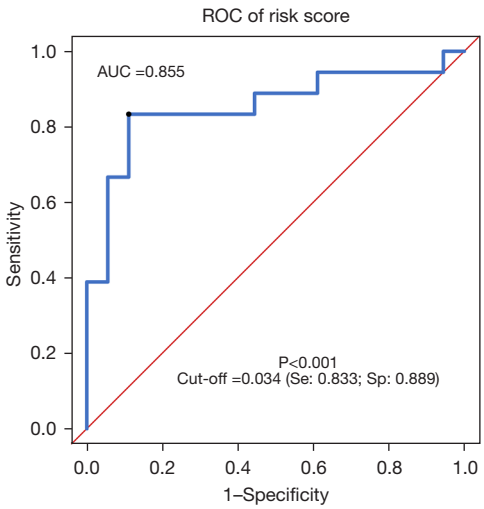

Figure 4 The ROC curve of testing the combination of miR-128-3p and miR-33a-5p. ROC, receiver operating characteristic; AUC, area under the ROC curve; Se, sensitivity; Sp, specificity.

and healthy controls, with miR-128-3p showing a higher specificity (94.4%) and miR-33a-5p showing a higher sensitivity (77.8%).

The combined use of miR-128-3p and miR-33a-5p as a biomarker for distinguishing NSCLC patients from healthy individuals

Based on a logistics model, the risk score of the combination of miR-128-3p and miR-33a-5p was calculated using the following formula: risk score = miR-128-3p × (–2.223) + miR-33a+5p × (–3.678) + 5.428. This risk score model was used to make ROC curve to evaluate the ability of combining miR-128-3p and miR-33a+5p to predict NSCLC. We called this model "model-combination A". The AUC calculated according to the risk score was 0.855 (95% CI: 0.719–0.991; P<0.001), and the optimal cut-off value was 0.034. A diagnosis of NSCLC was made if the risk score was larger than 0.034, with the sensitivity being 83.3% and the specificity being 88.9% at this point (*Figure 4*). The ROC curves of serum exosome miR-128-3p, miR-33a-5p, and model-combination A were compared using the R studio software. The AUCs of miR-128-3p and miR-33a-5p showed no significant differences to that of model-combination A (P=0.3664 and P=0.2142, respectively; *Table 4*), suggesting that the performance of the combined detection of serum exosome miRNAs was not superior to that of serum exosome miR-128-3p alone nor miR-33a-5p alone.

In pursuit of higher sensitivity, 1.240 was selected as the diagnostic criterion for miR-128-3p and 0.720 was selected for miR-33a-5p. A diagnosis of NSCLC would be made if any of these two indicators decreased. Based on this criterion, the prediction model had a sensitivity of 0.833 (15/18) and a specificity of 0.833 (15/18) (*Table 4*) (referred to as model-combination B). Analysis using R studio software found no statistically significant difference between model-combination B and the previous three models (P=0.5814, P=0.776, and P=0.583, respectively; *Table 4*).

Table 4 A comparison of area difference under the receiver operating characteristic curve

Model name	Model-miR-33a-5p	Model-miR-128-3p	Model-combination A	Model- combination B
Model-miR-33a-5p	–			
Model-miR-128-3p	0.7186	–		
Model-combination A	0.2142	0.3664	–	
Model- combination B	0.776	0.5814	0.583	–

Discussion

Lung cancer is the malignant tumor with the highest incidence in the world today, which poses a serious threat to human health. If lung cancer can be treated with thoracoscopic resection at the time of discovery, lung cancer metastasis and mortality can be reduced (10,28). Therefore, it is of great significance to find suitable biomarkers for lung cancer screening and auxiliary diagnosis. Advances in liquid biopsy may greatly contribute to the auxiliary diagnosis and treatment of lung cancer. To date, liquid biopsy has been widely used in three clinical areas: (I) tumor detection and progression monitoring (29,30); (II) diagnosis of heart attack (31); and (III) prenatal diagnosis (32). In addition, it can be applied to cancer research in two main aspects: (I) detection and monitoring of circulating tumor cells (33); and (II) identification of cell-free tumor DNA (34). These applications of liquid biopsy in cancer research confirmed their applicability for clinical identification, long-term monitoring, and prognosis prediction of multiple tumor types (e.g., lung cancer, liver cancer, and colorectal cancer) (30,33,35). Relevant studies have found that miR-21, miR-30d, miR-451, miR-10a, miR-30e-5p, and miR-128 are potential biomarkers for lung cancer diagnosis, suggesting that miRNAs may play a prominent role in clinical diagnosis and monitoring (35,36). Due to their great potential, exosomal miRNAs can be used as an excellent non-invasive tool for diagnosis, prognosis and prediction of treatment success or drug resistance in this pathology. However, there are still challenges to be achieved. Firstly, because of the heterogeneity in size (different subtypes of vesicles), standardized methodologies must be established for isolation, characterization and study of exosomes cargo. Secondly, identifying and validating the unique clinical value of each candidate miRNA is expensive and time-consuming (13,35,37-39). Therefore, the mature and stable testing process is indispensable, and in order to lower the economic burden of patients, we selected only a single or a few miRNAs that affect the development and progression of NSCLC for detection and analysis.

In our present case-control study, the levels of miR-128-3p and miR-33a-5p in serum exosomes were measured in both NSCLC patients and healthy controls, and the value of miR-128-3p alone, miR-33a-5p alone, and the combination of miR-128-3p and miR-33a-5p as biomarkers for NSCLC screening was compared. The serum exosome levels of both miR-128-3p and miR-33a-5p were significantly lower in NSCLC patients than those in the healthy controls, and the miR-128-3p expression was positively correlated with miR-33a-5p expression. Both the detection of serum exosome miR-128-3p alone or the combined detection of miR-128-3p and miR-33a-5p can be used to distinguish NSCLC patients from healthy controls. The combined use of miR-128-3p and miR-33a-5p had the largest AUC and the highest sensitivity compared with either miR-128-3p alone or miR-33a-5p alone, however, the difference in AUC was not statistically significant, indicating that the combined detection of these two miRNAs was not superior to the detection of either miR-128-3p or miR-33a-5p alone. However, this study was limited by its sample size and subgroup analyses on NSCLC at different stages and with different degrees of differentiation were not performed. It is speculated that miR-128-3p or miR-33a-5p may become auxiliary indicators for determining the stage and degree of malignancy of NSCLC.

In summary, exosomes miR-128-3p and miR-33a-5p showed good performance for NSCLC screening, and both can be used as biomarkers for NSCLC screening, and the combined detection of exosomes miR-128-3p and miR-33a-5p has higher sensitivity. Therefore, these two miRNAs may be widely used in clinical screening and auxiliary diagnosis of NSCLC in the future, and it may be more cost-effective to detect a single miRNA for the economic benefit of patients. However, this study still has some limitations, and the clinical application of these two miRNAs still needs further research. Firstly, the sample size of this study is small, because of various reasons, only 36 patients can be included

for data analysis, but the value of these biomarkers should be verified in studies with larger sample sizes, which is also what we will do in the future. Since stability determines whether a miRNA can be used as a biomarker (5), the stabilities of miR-128-3p and miR-33a-5p should be further verified and analyzed under various harsh conditions (e.g., radiotherapy, chemotherapy, and immunotherapy). Secondly, to avoid false positives, serum exosome expressions of miR-33a-5p and miR-128-3p in NSCLC patients should also be distinguished from those in benign lung diseases (e.g., pneumonia and tuberculosis). Besides, a more stable and accurate NSCLC screening model should be established by integrating general features including tumor indexes, gender, age, family history, smoking history, and occupational history. It is also necessary to compare the levels of miR-128-3p and miR33a-5p in different NSCLC stages or in different NSCLC subgroups, and to establish a prognosis prediction model in combination with tumor indicators, pathological stages, tissue differentiation degree and other factors, so as to strengthen postoperative monitoring, management and timely intervention of NSCLC patients. However, more importantly, we have not yet studied the mechanism of exocrine miR-128-3p and miR-33a-5p regulating the occurrence and development of NSCLC, which limits the clinical value of miRNA. Therefore, in the follow-up research, we will study the mechanism of the effects of these two miRNAs on NSCLC, in order to find the key targets to regulate different biological processes such as lung cancer growth, progression, invasion, angiogenesis, metastasis and drug resistance.

Conclusions

From this study, we learned that miR-128-3p and miR-33a-5p demonstrated advantages in the auxiliary diagnosis of NSCLC, and the combined detection of miR-33a-5p and miR-128-3p showed higher sensitivity. Both miR-128-3p and miR-33a-5p may be potential novel biomarkers for distinguishing NSCLC patients and healthy individuals. This discovery encourages the progress of miRNAs research related to NSCLC, not only as a new biomarker, but also may lead to the development of new pharmacological drugs to assist the treatment of NSCLC.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-398/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-398/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Review Board of Changhai Hospital (No. 2020021) and informed consent was taken from all the participants.

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