

ORIGINAL ARTICLE

Plasma miR-1247-5p, miR-301b-3p and miR-105-5p as potential biomarkers for early diagnosis of non-small cell lung cancer

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Keywords

Biomarkers; miR-105-5p; miR-1247-5p; miR-301b-3p; non-small cell lung cancer.

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Abstract

Background: Accumulating evidence shows that microRNAs are aberrantly expressed and exert essential roles in the tumorigenesis and tumor progression of non-small cell lung cancer (NSCLC).

Methods: The plasma miRNAs from five healthy donors and four NSCLC patients were profiled by miRNA microarray. The differentially expressed miRNAs from 154 primary NSCLC patients and 146 healthy donors were subjected to RNA isolation and verified by quantitative PCR (qPCR).

Results: The miRNA microarray analysis revealed that 40 differential miRNAs between NSCLC patients and healthy donors were selected. We found that the plasma miR-1247-5p, miR-301b-3p and miR-105-5p levels of patients were significantly higher than those of healthy controls. The receiver operating characteristic curve (ROC) analyses revealed higher area under the ROC curve (AUC) values and higher sensitivity/specificity of carcinoembryonic antigen (CEA) in combination with miR-1247-5p, miR-301b-3p, or miR-105-5p were superior to that of CEA alone.

Conclusions: High miR-1247-5p, miR-301b-3p and miR-105-5p expression have been demonstrated to accelerate tumorigenesis, and these three miRNAs in plasma act as novel biomarkers for the early diagnosis of NSCLC patients.

Key points

Plasma miR-1247-5p, miR-301b-3p and miR-105-5p act as novel biomarkers for early NSCLC and NSCLC.

Introduction

Non-small cell lung cancer (NSCLC) contributes to approximately 80% of lung cancer subtypes which is acknowledged as the leading cause of cancer associated incidence and mortality for both sexes combined worldwide.¹ The disappointing survival rate is mainly determined by late diagnosis and most patients already have local or distant metastasis which leads to the opportunity of surgical excision being lost.² Therefore, the discovery of early specific biomarkers for diagnosis and potential

therapeutic targets may significantly improve the clinical intervention and survival rate of NSCLC patients.

The molecular regulatory pathway alterations that affects the biological behavior of NSCLC cells are multiple and these molecular networks may lead to an imbalance that generates a malignant phenotype at the very beginning. These molecular modifications include changes in gene expression, copy number alterations, different methylation patterns, but also changes in the expression of non-coding RNAs.³ MicroRNAs (miRNAs), containing about 22 nucleotides, are the most important category of the non-coding

part of the genome.⁴ It has been proven that miRNAs are stably contained within biofluid medium such as plasma⁵ and exert modulatory functions upon target genes or molecules that further affect important signaling pathways in lung cancer cells development, epithelial–mesenchymal transition and response to therapy.⁶ The essential role of miRNAs results from the fact that one single miRNA is able to target multiple mRNAs in a post-transcriptional manner, thus simultaneously affecting the expression of hundreds of genes.⁷

Specific miRNAs have been implicated in blood specimens of patients with NSCLC, suggesting that these markers could act as potential diagnostic indicators for NSCLC. There is evidence that miR-146a-5p has a significantly higher expression in the serum and tissue samples of NSCLC patients than that of the healthy controls. Its effects on NSCLC cell survival and migration have previously been reported to be mediated by direct binding to, and suppression of, TRAF6.⁸ Additionally, multiple studies reported miR-21 as an oncogene was upregulated in NSCLC patients, and closely associated with a worse outcome.⁹ All these suggested miRNAs measured in body fluids can reflect altered physiological conditions, and may serve as a noninvasive and sensitive object able to detect early neoplastic changes.

In the current study, we found and verified three miRNAs (miR-1247-5p, miR-301b-3p and miR-105-5p) from plasma between NSCLC patients and healthy donors using miRNA microarray analysis. We evaluated the expression signature and diagnostic power of these three miRNAs in NSCLC and early-stage patients. The aim of this study was to assess whether plasma miR-1247-5p, miR-301b-3p and miR-105-5p can be used as biomarkers for identifying NSCLC, especially for early-stage NSCLC.

Methods

Patients and clinical samples

Whole blood samples of 154 NSCLC patients and 146 healthy volunteers were obtained from Shandong Cancer Hospital and Institute between November 2019 and June 2020. None of the involved patients had received surgery or antitumor treatments, including chemo-, radio-

targeted therapy. The healthy controls were selected from a healthy population who participated in the physical examination. Written informed consent was obtained from each individual participant and the study protocol was approved by the Ethics Committee of Shandong Cancer Hospital and Institute.

RNA extraction and complementary DNA (cDNA) synthesis

Whole blood was collected in EDTAK2-coated purple-cap Vacutainer tubes (BD, Franklin Lakes, NJ, USA) until centrifugation twice at $3000 \times g$ for 10 minutes (4°C) to separate plasma from cells and debris. Total RNA was extracted from plasma using TRIzol LS reagent (Thermo Fisher Scientific, Carlsbad, CA, USA), and stored at -80°C until use. The concentration and purity of extracted RNA samples were determined with the NanoDrop (Thermo Fisher Scientific, Wilmington, DE). Thereafter, cDNA was synthesized by reverse transcription using Mix-X miRNA first-strand synthesis kit (TaKaRa Bio, Nojihigashi, Kusatsu, Japan).

Identification of differentially expressed genes (DEGs) in miRNA microarray

Plasma was obtained from 10 mL of peripheral blood from four NSCLC patients and five healthy donors, and analyzed using an Agilent 2100 Bioanalyzer (Agilent Biotechnologies, Palo Alto, CA, USA). The prepared libraries were finally sequenced using Illumina NextSeq 500/550 V2 kit (#FC-4042005, Illumina, USA), according to the manufacturer's instructions. $P < 0.05$ and $|\log_2\text{fold change (FC)}| \geq 1.5$ were set as the cutoff criteria for differentially expressed miRNAs. Hierarchical clustering, scatter plot and volcano plot were constructed with the differentially expressed miRNAs in either R or Python.

Quantitative PCR (qPCR) assay

A quantitative PCR assay was performed using TB-Green Premix Ex Taq II reagent (Takara Bio) in a volume of 20 μL . The expression of miRNAs was detected on LightCycler 480 qPCR system (Roche Diagnostics, Germany) and evaluated by the comparative cycle threshold (ΔCt) method: ($\Delta\text{Ct} = \text{Ct}^{\text{miRNA}} - \text{Ct}^{\text{U6}}$) as described previously.¹⁰ U6 acted as an endogenous control as described previously.¹¹ The primers designed to amplify the miRNA transcripts are listed in Table 1.

Table 1 Sequence information of the primers for qPCR

Gene	Primer
hsa-miR-1247-5p	GTCCCGTTCGTCCTCCGGAAA
hsa-miR-301b-3p	GCAGTGCAATGATATTGTCAAAGC
hsa-miR-105-5p	TCAAATGCTCAGACTCCTGTGGT
U6 forward	TGGAACGCTTCACGAATTTGCG
U6 reverse	GGAACGATACAGAGAAGATTAGC

Table 2 miRNAs expression profiling (28 upregulated and 12 downregulated)

miRNA	log ₂ fold change	Description	miRNA	log ₂ fold change	Description
hsa-miR-512-5p	9.766 150 787	up	hsa-miR-520d-5p	6.627 214 059	up
hsa-miR-376c-3p	9.503 468 956	up	hsa-miR-877-3p	6.574 761 579	up
hsa-miR-548n	9.243 849 325	up	hsa-miR-548ab	6.534 445 097	up
hsa-miR-106a-3p	9.107 295 079	up	hsa-miR-520f-3p	6.502 631 988	up
hsa-miR-548p	8.807 415 444	up	hsa-miR-18a-3p	5.224 509 517	up
hsa-miR-548ah-3p	8.807 415 444	up	hsa-miR-181a-2-3p	3.794 524 717	up
hsa-miR-485-5p	8.681 734 057	up	hsa-miR-215-5p	1.815 606 041	up
hsa-miR-1247-5p	8.101 421 637	up	hsa-miR-122-5p	1.545 033 385	up
hsa-miR-501-5p	7.893 508 413	up	hsa-miR-30c-1-3p	7.706 989 298	down
hsa-miR-301b-3p	7.717 498 37	up	hsa-miR-548ay-3p	7.651 187 735	down
hsa-miR-5187-5p	7.639 762 596	up	hsa-miR-6722-3p	7.620 853 852	down
hsa-miR-655-3p	7.577 037 146	up	hsa-miR-521	7.517 812 815	down
hsa-miR-517c-3p	7.572 255 215	up	hsa-miR-187-3p	7.090818229	down
hsa-miR-1273h-3p	7.402 062 921	up	hsa-miR-581	7.025694494	down
hsa-miR-195-3p	7.207 760 832	up	hsa-miR-580-3p	6.404 865 374	down
hsa-miR-3940-3p	7.165 152 739	up	hsa-miR-384	5.477 500 265	down
hsa-miR-365b-5p	7.104 387 651	up	hsa-miR-873-5p	5.254 075 151	down
hsa-miR-377-5p	7.046102123	up	hsa-miR-147b-3p	5.133 807 356	down
hsa-let-7b-3p	6.965 388 803	up	hsa-miR-214-5p	5.114 376 122	down
hsa-miR-105-5p	6.827 985 131	up	hsa-miR-454-3p	2.774 020 807	down

Bioinformatic analysis

TargetScan¹² (http://www.targetscan.org/vert_72/) and miRDB¹³ (<http://mirdb.org/>) were employed for the prediction of potential miRNA targets. The target genes of miR-1247-5p, miR-301b-3p and miR-105-5p were predicted by these two databases, respectively and the top 200 target genes were selected according to their respective database scores. We used the Database for Annotation Visualization and Integrated Discovery¹⁴ (DAVID 6.8 online, <https://david.ncifcrf.gov/home.jsp>) to analyze the gene ontology (GO) enrichment and KEGG pathway of these target genes to identify the possible biological function of the three miRNAs. $P < 0.05$ was considered statistically significant.

Statistical analysis

The statistical analyses were carried out using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) and SPSS 22.0 (SPSS Inc., Chicago, IL, US). An unpaired *t*-test or Mann-Whitney test was used to assess the statistical significance between groups, while multiple group analysis was tested by Kruskal-Wallis test. Chi-square test was employed to evaluate the relationship between miRNAs and clinical characteristics. The diagnostic power of miRNAs was evaluated by sensitivity, specificity, and ROC curve. P -values < 0.05 were considered statistically significant, and all tests were set as two-tailed.

Results

Differentially expressed miRNAs identified by miRNA microarray

To determine the differential expression of miRNAs, we analyzed the RAW miRNA expression profiling data of plasma from four NSCLC patients and five healthy controls. In total, 40 miRNAs were identified to be differentially expressed between the two groups, of which 28 were upregulated and 12 were downregulated in plasma relative to NSCLC, and listed in Table 2. Hierarchical clustering analysis was performed using these significantly differential miRNAs, indicating a distinguishable miRNA expression profiling among these samples (Fig 1a). As shown in Fig 1b, c, the scatter and volcano plots were constructed to visualize the variation in miRNA expression between the two groups.

Validation of miRNAs expression using qPCR

To validate the reliability of the miRNA microarray data, 11 upregulated miRNAs which varied significantly between patients and controls in the microarray were chosen to be verified by qPCR. Several miRNAs were ruled out due to no statistical difference, low expression and low specificity of primers. Intriguingly, miR-1247-5p, miR-301b-3p and miR-105-5p were identified due to their remarkable differences and then subjected to a larger scale validation cohort including 146 healthy donors and 154 NSCLC patients naive to any anticancer treatment.

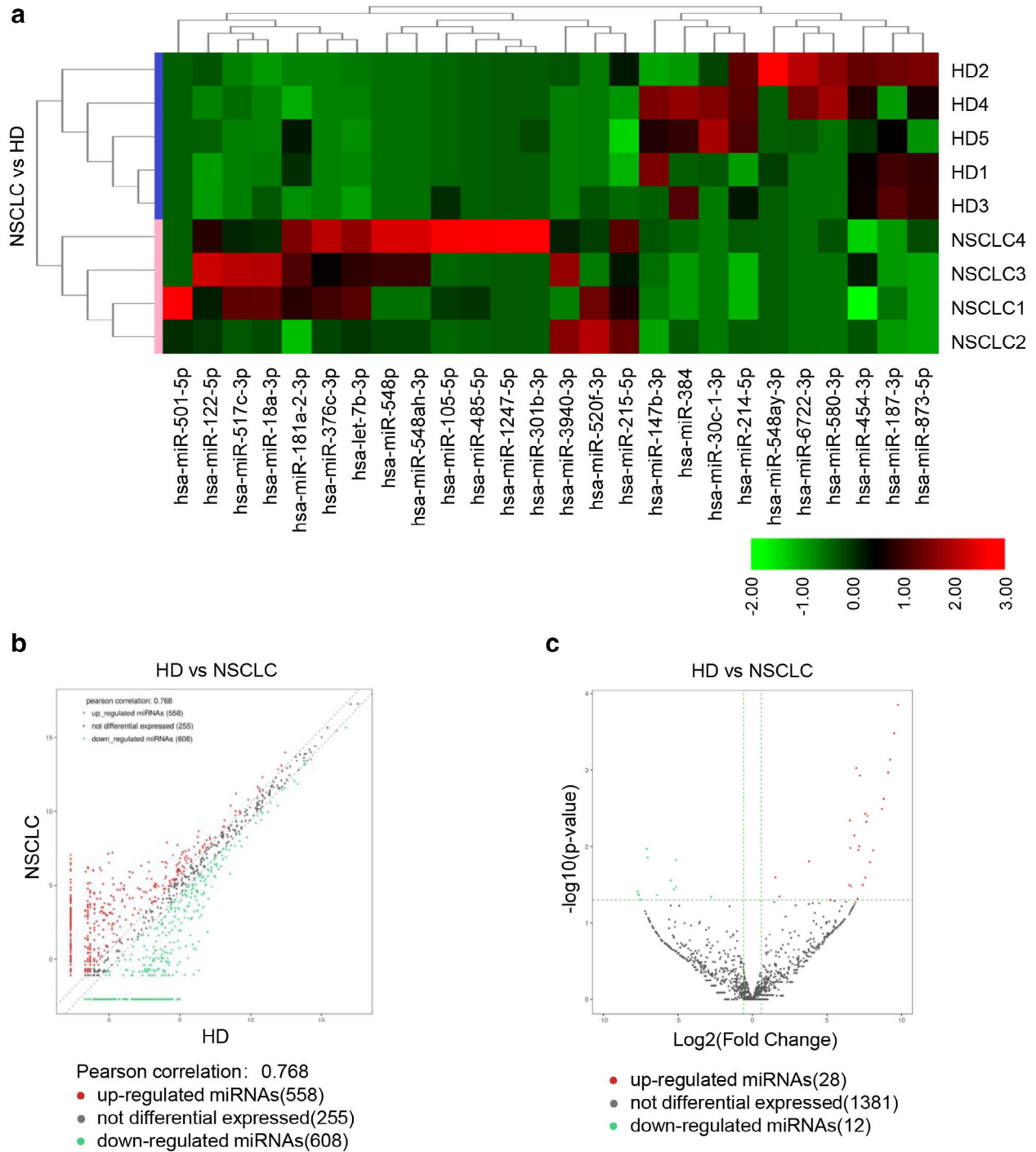


Figure 1 Plasma miRNA profile of the non-small cell lung cancer (NSCLC) patients. **(a)** A heat map was generated after supervised hierarchical cluster analysis. The differential miRNA expression is shown in red (upregulation) versus green (downregulation) ($P < 0.05$). **(b)** The scatter plot between two groups for NSCLC patients and healthy donors. **(c)** Volcano plot compared the expression \log_2 -fold-change of miRNA in NSCLC patients and healthy donors. The red dots represent the upregulated miRNAs, and the green dots represent the downregulated miRNAs.

To explore the potential roles of miR-1247-5p, miR-301b-3p and miR-105-5p in NSCLC, we identified their target genes which were subjected to relevant biological

annotation analysis as described in Methods. As shown in Fig S1a-c, GO enrichment analysis demonstrated they were significantly enriched in several biological processes.

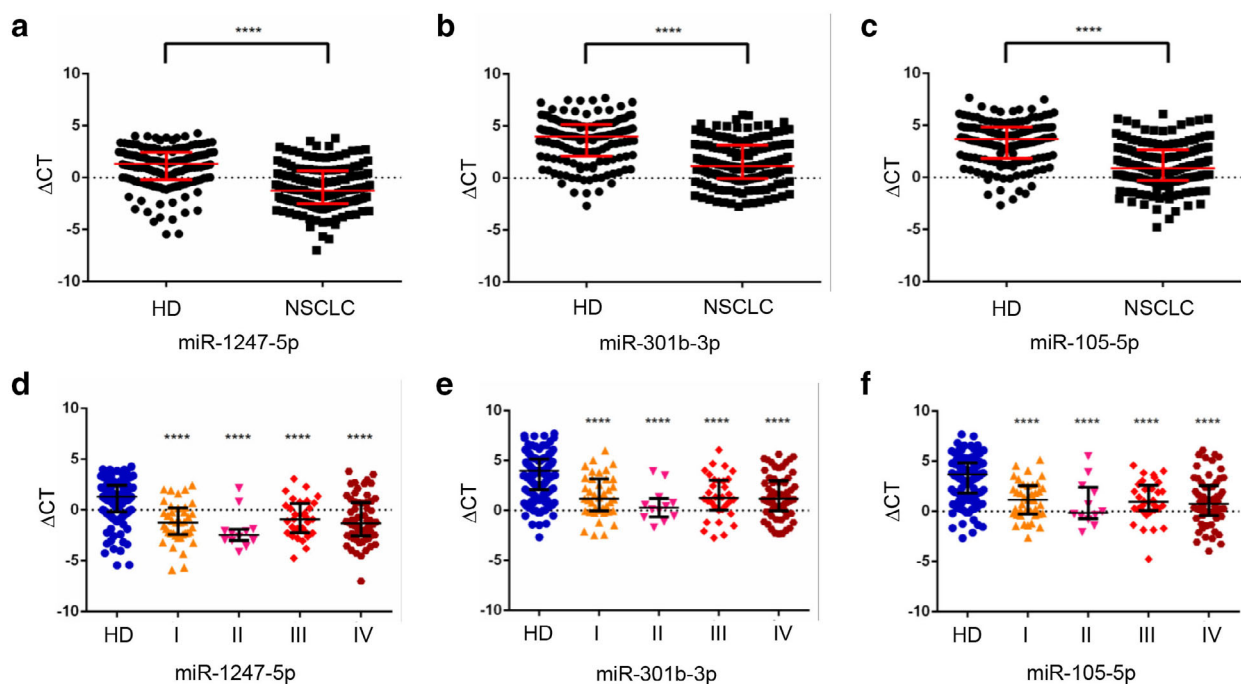


Figure 2 miR-1247-5p, miR-301b-3p and miR-105-5p were upregulated in non-small cell lung cancer (NSCLC). (a–c) miR-1247-5p, miR-301b-3p and miR-105-5p were upregulated in NSCLC patients ($n = 154$) compared with healthy controls ($n = 146$); (d–f) miR-1247-5p, miR-301b-3p and miR-105-5p were upregulated in various TNM stages. HD, healthy donors; NSCLC, non-small cell lung cancer patients. **** $P < 0.0001$.

The target genes were enriched in utero embryonic development for miRNA-1247-5p; negative regulation of transcription for miR-301b-3p, and peptidyl–threonine phosphorylation for miR-105-5p, respectively. Due to the lack of predicted target genes, only miR-301b-3p was subjected to KEGG analysis and more than three pathways were enriched. As shown in Fig S1d, the target genes of miR-301b-3p were enriched in the mTOR signaling pathway, implying that it might be involved in mTOR signaling dependent tumorigenesis.

miR-1247-5p, miR-301b-3p and miR-105-5p were upregulated in NSCLC

As shown in Fig 2a–c, miR-1247-5p, miR-301b-3p and miR-105-5p were significantly expressed in NSCLC patients compared with normal individuals, and elevated significantly from NSCLC plasma samples ($P < 0.0001$, $P < 0.0001$ and $P < 0.0001$, respectively). Furthermore, we analyzed the differential levels of miR-1247-5p, miR-301b-3p and miR-105-5p in patients with different TNM stage. As shown in Fig 2d–f, when compared with the healthy donors, the miRNA levels were significantly upregulated in various TNM stage.

The relationship between miRNA expression and clinical pathological characteristics of NSCLC patients are summarized in Table 3. However, none of these were correlated with gender, age, smoking, drinking, histological type, lymph node metastasis, or distant metastasis.

Diagnostic role of miR-1247-5p, miR-301b-3p and miR-105-5p in NSCLC patients

ROC curves were plotted to evaluate the diagnostic values of miR-1247-5p, miR-301b-3p and miR-105-5p in distinguishing patients with NSCLC from cancer-free individuals. As shown in Fig 3a–c, AUCs were 0.769 with 87.7% sensitivity and 57.8% specificity, 0.761 with 65.8% sensitivity and 79.9% specificity, as well as 0.777 with 66.4% sensitivity of and 79.9% specificity for miR-1247-5p, miR-301b-3p and miR-105-5p, respectively. These data provide compelling evidence that miR-1247-5p, miR-301b-3p and miR-105-5p potentially act as relatively high diagnostic accuracies for NSCLC.

Because the carcinoembryonic antigen (CEA) is the most commonly accepted blood biomarker for NSCLC,¹⁵ we estimated plasma CEA levels and analyzed the ROC curves of CEA of the subjects. Compared with healthy controls, NSCLC patients showed significantly increased CEA

Table 3 Correlation between miRNA-1247-5p, miRNA-301b-3p, miRNA-105-5p expression and clinicopathological characteristics of lung cancer patients

Characteristics	Cases No. (%)	miRNA-1247-5p			miRNA-301b-3p			miRNA-105-5p		
		Low	High	<i>P</i> -value*	Low	High	<i>P</i> -value*	Low	High	<i>P</i> -value*
Gender										
Male	91 (59.1)	46	45	0.87	41	50	0.14	42	49	0.251
Female	63 (40.9)	31	32		36	27		35	28	
Age (years)										
<62	73 (47.4)	39	34	0.42	38	35	0.628	37	36	0.872
≥62	81 (52.6)	38	43		39	42		40	41	
Smoking										
No	99 (64.3)	48	51	0.614	50	49	0.866	51	48	0.614
Yes	55 (35.7)	29	26		27	28		26	29	
Drinking										
No	112 (72.7)	53	59	0.278	54	58	0.469	57	55	0.717
Yes	42 (27.3)	24	18		23	19		20	22	
Histology										
NSCLC										
AC	108 (70.1)	55	53	0.407	56	52	0.223	57	51	0.189
SCC	35 (22.7)	15	20		14	21		14	21	
Others	11 (7.1)	4	3		4	3		4	3	
Tumor size										
<8.788 cm ³	65 (42.2)	36	29	0.256	35	30	0.337	33	32	0.664
≥8.788 cm ³	66 (42.9)	30	36		30	36		31	35	
Unknown	23 (14.9)									
T stage										
Tis	2 (1.3)	0	2	0.562	0	2	0.587	0	2	0.528
T1	50 (32.5)	27	23		26	24		26	24	
T2	46 (29.9)	22	24		21	25		19	27	
T3	22 (14.3)	12	10		12	10		11	11	
T4	23 (14.9)	10	13		10	13		12	11	
Unknown	11 (7.1)									
TNM stage										
Stage 0	2 (1.3)	0	2	0.075	0	2	0.273	0	2	0.481
Stage I	39 (25.3)	20	19		19	20		19	20	
Stage II	12 (7.8)	10	2		9	3		8	4	
Stage III	32 (20.8)	13	19		15	17		15	17	
Stage IV	62 (40.3)	32	30		31	31		31	31	
Unknown	7 (4.5)									
Lymph node metastasis										
No	56 (36.4)	30	26	0.536	28	28	0.84	26	30	0.726
Yes	87 (56.5)	42	45		42	45		43	44	
Unknown	11 (7.1)									
Distant metastasis										
No	85 (55.2)	43	42	0.902	43	42	0.944	42	43	0.944
Yes	62 (40.3)	32	30		31	31		31	31	
Unknown	7(4.5)									

*Chi-square test was used.

levels ($P < 0.0001$), and plasma CEA levels were able to distinguish NSCLC patients from healthy individuals with an AUC of 0.793. The corresponding sensitivity and specificity were 53.9% and 96.6%, respectively (Fig 3d,e).

Importantly, based on the previously reported methods,¹⁶ we estimated diagnostic performance for the

combination of CEA with miR-1247-5p, miR-301b-3p, or miR-105-5p in NSCLC. As shown in Fig 3f–h, AUCs were 0.872 with 72.1% sensitivity and 91.1% specificity, 0.873 with 68.2% sensitivity and 95.2% specificity, as well as 0.883 with 79.2% sensitivity and 84.2% specificity, respectively, higher than that for CEA alone.

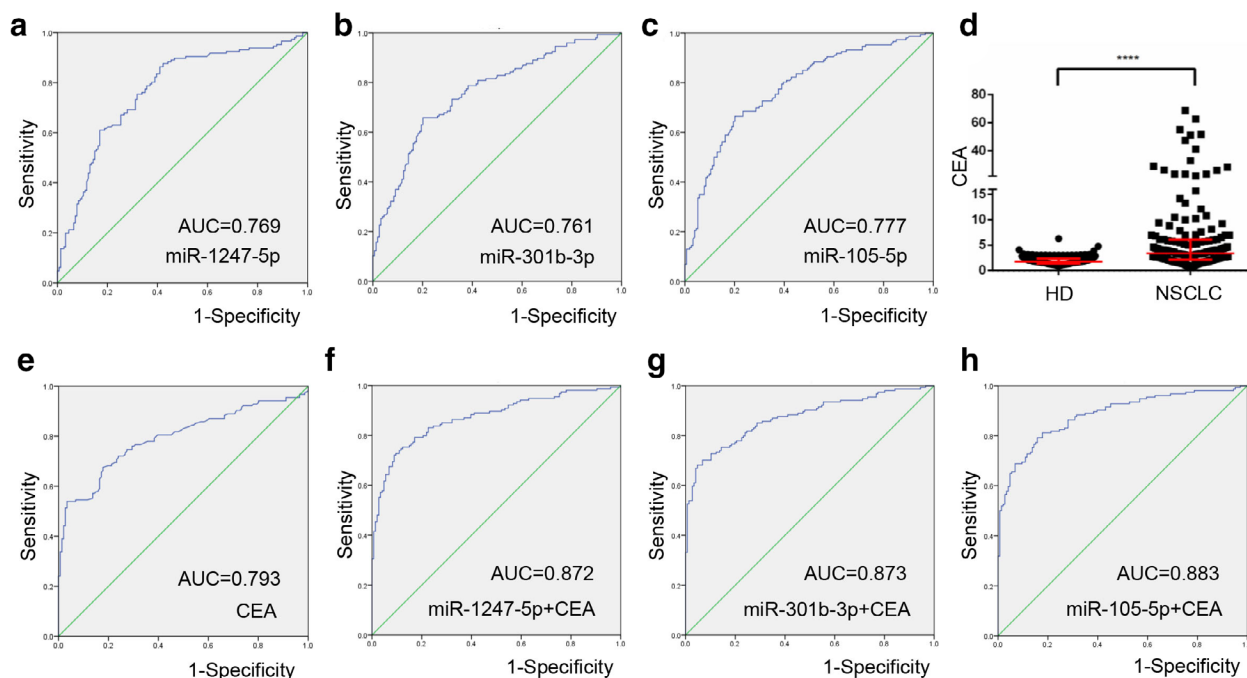


Figure 3 Diagnostic role of miR-1247-5p, miR-301b-3p and miR-105-5p in non-small cell lung cancer (NSCLC) patients. (a–c) The ROC curve analysis of miR-1247-5p, miR-301b-3p and miR-105-5p for NSCLC. (d) The levels of CEA were increased in NSCLC patients compared with healthy controls. (e) The ROC curve analysis of CEA for NSCLC patients. (f–h) The ROC curve analysis of CEA in combination with miR-1247-5p, miR-301b-3p, or miR-105-5p for NSCLC patients. **** $P < 0.0001$.

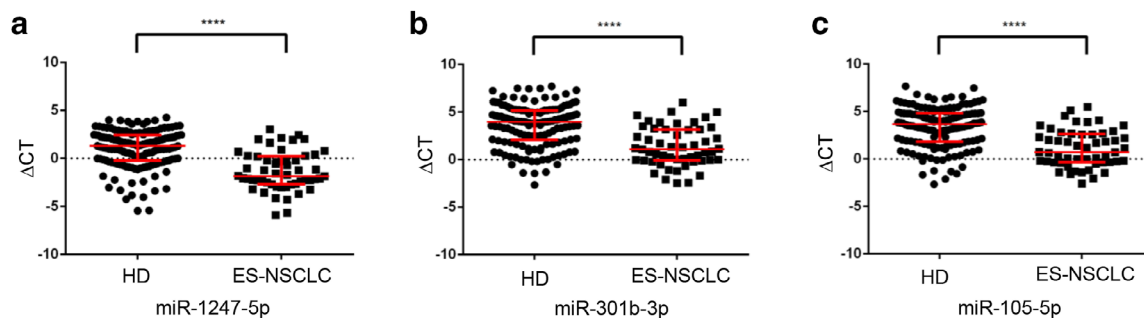


Figure 4 miR-1247-5p, miR-301b-3p and miR-105-5p were upregulated in ES-NSCLC. (a–c) miR-1247-5p, miR-301b-3p and miR-105-5p were upregulated in early-stage non-small cell lung cancer (NSCLC) patients ($n = 53$) compared with healthy controls ($n = 146$). HD, healthy donors; ES-NSCLC, early-stage NSCLC patients. **** $P < 0.0001$.

miR-1247-5p, miR-301b-3p, miR-105-5p function as early diagnostic biomarkers

We then analyzed the differential expression of miR-1247-5p, miR-301b-3p, and miR-105-5p in 53 early-stage NSCLC patients (Tis stage = 2, I stage = 39, II stage = 12) as well as in 146 healthy donors. As shown in Fig 4a–c, significantly higher levels of miR-1247-5p, miR-301b-3p and miR-105-5p were detected in early-stage NSCLC patients than in healthy donors. To explore the potential of miR-1247-5p, miR-301b-3p, and miR-105-5p as circulating biomarkers for early NSCLC, ROC curves were applied to

analyze favorable diagnostic efficiencies between stages I & II and healthy controls, possessing AUCs of 0.812 with 88.4% sensitivity and 64.7% specificity, 0.788 with 65.8% sensitivity and 82.4% specificity, and 0.793 with 68.5% sensitivity and 82.4% specificity, respectively (Fig 5a–c). Moreover, the diagnostic performance for their combination demonstrated the AUC was 0.815 with a relative sensitivity of 72.5% and relative specificity of 82.2% (Fig 5d). As shown in Fig 5e,f, the levels of CEA were greatly increased in stage I & II patients ($P < 0.0001$), with AUC of 0.803, 78.4% sensitivity and 70.5% specificity for early NSCLC diagnosis.

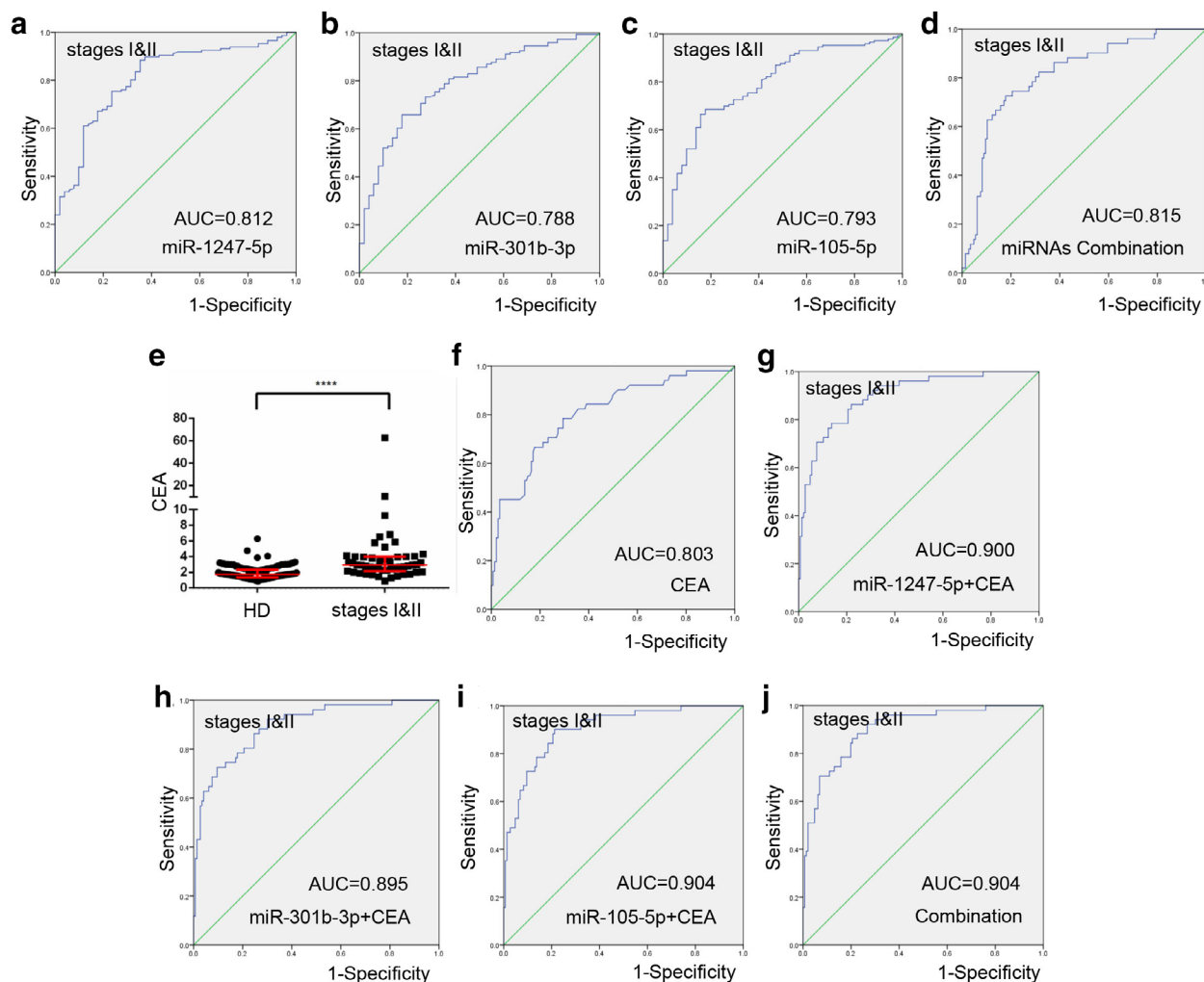


Figure 5 miR-1247-5p, miR-301b-3p, miR-105-5p function as early diagnostic biomarkers. (a–d) The ROC curve analysis of miR-1247-5p, miR-301b-3p, miR-105-5p, and combination of miRNAs for stage I & II non-small cell lung cancer (NSCLC). (e) The levels of CEA were increased in stage I & II patients compared with healthy controls. (f) The ROC curve analysis of CEA for stage I & II NSCLC patients. (g–j) The ROC curve analysis of CEA in combination with miR-1247-5p, miR-301b-3p, miR-105-5p, or miRNA combination for stage I & II NSCLC patients. **** $P < 0.0001$.

More importantly, diagnosis of NSCLC was greatly improved by using CEA in combination with miR-1247-5p, miR-301b-3p, miR-105-5p, or a combination of all miRNAs (Fig 5f–h).

In addition, we also generated ROC for III & IV including a combination of all miRNAs compared with healthy donors as shown in Fig S2a–d. However, no significant difference in the three miRNA expression was observed between patients with stages I & II and III & IV (Fig S2e–g).

Discussion

In the era of precision oncology, molecular profiling of individual patients’ tumors can determine treatment decisions, monitor subsequent responses, and provide early warning of treatment resistance and neoplasm

recurrence.¹⁷ Traditional biomarkers, such as carcinoembryonic antigen (CEA), a broad-spectrum tumor marker, have been in routine clinical use for decades, but are increasingly unable to satisfy the requirements of liquid biopsy in today’s precision oncology era due to their poor specificity and sensitivity in the early diagnosis of NSCLC. In the current study, we screened miR-1247-5p, miR-301b-3p, and miR-105-5p using microarrays and validated their expression pattern in a large cohort, thus providing evidence that miR-1247-5p, miR-301b-3p, and miR-105-5p are biomarkers for NSCLC.

Several studies have previously indicated that miR-1247-5p, miR-301b-3p, and miR-105-5p play different roles in various types of cancer cells. MiR-1247-5p has been reported to be overexpressed in castration-resistant

prostate cancer (CRPC)¹⁸ and pancreatic cancer.¹⁹ However, it has been found to be downregulated in several malignancies, functioning as a tumor suppressor in hepatocellular carcinoma (HCC),²⁰ malignant glioma,²¹ as well as being associated with poor prognosis and facilitating tumor cell growth in breast cancer.^{22,23} It has previously been reported that miR-301b-3p serves as a tumor promoter which drives the growth of gastric cancer (GC)²⁴ and HCC.²⁵ It also facilitates tumor metastasis in high-grade ovarian serous cancer by targeting CPEB3/EGFR axis.²⁶ In addition, other studies have confirmed that miRNA-105-5p has been found to inhibit cell proliferation in glioma cells²⁷ and HCC.²⁸ The inconsistent results are probably due to the different tumor microenvironment(s) which act on tumor cells affecting miR-105-5p expression.

In our recent study, we reported that miR-1247-5p, miR-301b-3p, and miR-105-5p were potential biomarkers for NSCLC. First, miR-1247-5p, miR-301b-3p, and miR-105-5p expression levels were found to be significantly increased in patients with NSCLC or early stage NSCLC. ROC curve analysis showed miR-1247-5p, miR-301b-3p, and miR-105-5p could distinguish patients from healthy donors, possessing favorable diagnostic efficiencies. Our study showed that CEA could be combined for the diagnosis of early-stage NSCLC patients. miR-1247-5p, miR-301b-3p, and miR-105-5p in combination with CEA could improve the diagnostic power for early-stage NSCLC to 0.880, 0.877 and 0.888, respectively. Taken together, miR-1247-5p, miR-301b-3p, and miR-105-5p are specifically present in the plasma and could spill into the circulation at an early stage of NSCLC, thus acting as early and specific biomarkers for NSCLC.

Several limitations should be carefully considered in the present study. First, our results included 154 NSCLC patients, and therefore the total sample sizes were small and might lack statistically vigorous power. Second, we did not detect the expression of these three miRNAs beyond NSCLC, resulting in a lack of tissue-specificity. Finally, the underlying mechanisms in which those miRNAs studied contribute to LC development remain unclear, and require further assessment in future. Nevertheless, our study and others have also demonstrated miR-1247-5p, miR-301b-3p and miR-105-5p in plasma act as novel biomarkers in the early diagnosis of NSCLC patients. Notably, our group and others have identified kinds of non-coding RNAs in circulation, which also serve as promising biomarkers for lung cancer, such as lncRNAs,²⁹ tumor educated platelet small nuclear RNAs (snRNAs)³⁰ and exosomal miRNAs,³¹ indicating the complex mechanisms involved in the tumorigenesis of lung cancer. Combined detection of these kinds of non-coding RNAs might be a shortcut to increase the accuracy of the diagnoses of lung cancer.

In conclusion, our results indicate that miR-1247-5p, miR-301b-3p, and miR-105-5p are candidate biomarkers of NSCLC, which perform even better when combined with CEA levels. These findings also prompted us to hypothesize that the early dysregulation of miR-1247-5p, miR-301b-3p and miR-105-5p in the NSCLC disease process, together with the requirement of only a small amount of plasma, render miR-1247-5p, miR-301b-3p and miR-105-5p suitable as early warning biomarkers for NSCLC.

Acknowledgments

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Disclosure

The authors declare that they have no competing interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Bioinformatic analysis for miR-1247-5p, miR-301b-3p and miR-105-5p. (a–c) Gene ontology enrichment analyses showed that the target genes of miRNA-1247-5p, miR-301b-3p, miR-105-5p. (d) KEGG pathway analysis for miR-301b-3p target genes. The vertical axis shows the annotated functions of the target genes, and the horizontal axis the enrichment score. GO, gene ontology; MF, molecular function; CC, cellular component; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genome.

Figure S2. Analyses of miR-1247-5p, miR-301b-3p and miR-105-5p in stages III & IV NSCLC. (a–d) The ROC curve analysis of miR-1247-5p, miR-301b-3p, miR-105-5p, and combination of miRNAs for stages III & IV NSCLC. (e–g) No significant difference of miR-1247-5p, miR-301b-3p, or miR-105-5p was observed in either stages I & II or in stages III & IV.