Prognostic implications of decreased microRNA-101-3p expression in patients with non-small cell lung cancer

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Abstract. To investigate the expression level of microRNA-101-3p (miR-101-3p) and its possible association with progression, prognosis and chemotherapy in patients with non-small cell lung cancer (NSCLC), the Gene Expression Omnibus (GEO) database was used. Quantitative polymerase chain reaction was used to verify the expression in 327 NSCLC and 42 adjacent normal lung tissues, of which 42 viable tissues were paired with nearby normal lung tissues. Based on the Cox regression model, univariate and multivariate analyses were used to address the factors that had effects on overall survival (OS) and disease-free survival (DFS) rate. Data from the GEO database demonstrated that the miR-101-3p expression in NSCLC was downregulated, compared with normal lung cancer. Survival analysis through univariate and multivariate models indicated that the miR-101-3p expression level was a crucial risk factor for OS and DFS in patients with NSCLC. A number of clinical parameters were determined to be associated with miR-101-3p expression, including tumor diameter, lymph node metastasis and tumor-node-metastasis stage. Adjuvant chemotherapy with high expression of miR-101-3p was determined to increase OS and DFS in patients with NSCLC, compared with patients with de novo or low expression of miR-101-3p. The present results demonstrated that miR-101-3p expression levels were associated with NSCLC progression and prognosis, which indicated that miR-101-3p may serve as a biomarker for patients with NSCLC who have received adjuvant chemotherapy.

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Introduction

Lung cancer is the most frequently diagnosed cancer and was reported in 2015 as the leading cause of cancer-associated mortalities globally, as its incidence and mortality rate have been increasing in numerous countries, including China (1). Of the lung cancer instances, ~80% are non-small cell lung cancer types (NSCLC), which are clinically and pathologically different from SCLC types (2). Treatment options for lung cancer include surgery, radiation therapy, chemotherapy and targeted therapy. Therapeutic modalities depend on a number of factors, including the type and stage of cancer (3). Despite ongoing therapeutic efforts, patients with lung cancer have a poor prognosis with an arithmetic average 5-year survival rate of 15% (4). This is primarily due to inadequate knowledge regarding tumor progression and its associated molecular

alterations, which delay diagnosis (5); therefore, improvements in molecular genetics diagnosis and prediction of prognosis for targeted treatments and clinical decisions are required.

microRNAs (miRNAs) are a class of non-coding single stranded RNA molecules of ~22 nucleotides, which are encoded by endogenous genes (6). miRNAs are important regulators of gene expression in plants and animals (7). Recent studies have determined that miRNAs are associated with the formation and suppression of tumors (8-10). Changes in miRNA expression may serve an essential role in tumorigenesis and cancer inhibition. A number of miRNAs act as tumor suppressors, while others stimulate tumor growth. For example, there is reduced miR-143 expression in patients with colorectal cancer (11), miR-15-a and miR-16-1 are reduced in patients with B cell chronic lymphocytic leukemia (12), precursor miR-155 is highly expressed in Burkitt lymphoma, and the miR-17/92 cluster has been determined to be highly expressed in lung cancer, particularly in patients with SCLC (13,14). Additionally, miR-608 regulates apoptosis in human lung cancer via the regulation of AKT serine/threonine kinase (Akt)2, and miR-99a suppresses the invasion and migration of NSCLC cells (15). miR-101-3p is a member of the miR-101 family, and a recent study indicated that it has tumor suppressor effects in patients with NSCLC (16). However, to the best of our knowledge, a limited number of studies have addressed the association between miR-101-3p and adjuvant chemotherapy in patients with NSCLC (17-19).

In the present study, the miR-101-3p expression was investigated using the Gene Expression Omnibus (GEO) database and the expression levels of miR-101-3p in NSCLC tissues was evaluated using quantitative polymerase chain reaction (qPCR). Additionally, the association between miR-101-3p and prognosis following adjuvant chemotherapy was investigated in patients with NSCLC.

Materials and methods

Compliance with ethical standards. The present study was approved by the Ethics Committee of Shanghai Tenth People's Hospital, Tongji University School of Medicine (approval no. SHSY-IEC-pap-15-18; Shanghai, China). Each participant provided signed informed consent prior to participate in the present study. Patients or their legal surrogates provided signed informed consent for the surgical procedures. All specimens were handled and anonymized according to ethical and legal standards.

miRNA expression in NSCLC from the GEO database. The expression levels of miRNAs were assessed in NSCLC tissues and normal tissue samples from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) (20-23) using the following keywords: 'Homo sapiens'; 'NSCLC' and 'miRNA'. All datasets used the Illumina or Agilent Array platform to detect signals. For quality control, exclusion criteria for probes were as follows: i) had a low bead count of <3 in at least 5% of samples and ii) indicated a detection-P>0.05 in at least 5% of samples (20). The raw data set GSE61741 (21) was downloaded, which provided the peripheral blood miRNA profiles from 94 healthy controls and 73 patients with lung cancer. Additionally, GSE24709 (22) (including 19 healthy controls and 28 patients with lung cancer) and GSE56036 (23) (including 29 health controls and 23 patients with NSCLC) were downloaded in the GEO datasets to identify differentially expressed miRNAs in NSCLC samples and adjacent non-tumor tissues. Fold change (FC ≥2) and P<0.05 served as basic screening parameters. Hierarchical clustering was performed using the multiple experiment viewer 4.7.1 software programs (http://www.tm4.org/).

Clinical specimens. A total of 327 lung cancer tissues were collected from 206 male patients and 121 female patients with lung cancer who underwent surgery in Shanghai Tenth People's Hospital of the Tongji University School of Medicine (Shanghai, China) between January 2004 and December 2016. Inclusion criteria consisted of the following: ≤75 years with histologically proven NSCLC; no severe major organ dysfunction; World Health Organization (WHO) performance status of 0 or 1 and no prior cancer chemotherapy. Exclusion criteria consisted of the following: Age ≥76; severe major organ dysfunction; WHO performance status of >1 or prior cancer chemotherapy. Clinical information of the patients were recorded, including sex, age, smoking history, the diameter and differentiation of the tumor, lymph node metastasis, stage of Tumor-Node-Metastasis (TNM), histological grade, degree of invasion of the lung membrane, degree of vascular invasion, whether chemotherapy had been administered, overall survival (OS) rate, disease-free survival (DFS) rate and miR-101-3p expression status. Each participant provided signed informed consent prior to participation in the present study. Patients or their legal surrogates provided signed informed consent for the surgical procedures.

RNA extraction and detection of miR-101-3p expression by qPCR. RNA, including miRNAs, from NSCLC and normal tissue samples, were extracted using TRIzol® reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocols and an optimized protocol (13). RNA concentration was measured using NanoDrop ND-1000 (Thermo Fisher Scientific, Inc.) and the quality was assessed using electrophoresis in 1.5% denaturing agarose gels and viewed on a Kodak Gel Logic 2200 imaging system (Kodak, Rochester, NY, USA). TaqMan probe-based qPCR was carried out using a commercial kit (cat. no., A25576; Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the protocol of the manufacturer (24). RT reactions were performed using AMV Reverse Transcriptase (Takara Biotechnology Co., Ltd., Dalian, China) and qPCR was performed using a standard TaqMan PCR kit protocol on the Applied Biosystems 7900HT Sequence Detection system (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. qPCR for miR-101-3p was executed using the TaqMan® universal PCR kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. Thermocycling conditions were as follows: Initial denaturation at 94˚C for 10 min, followed by 35 cycles of 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec, with a final extension at 72˚C for 10 min. Each reaction was independently tested in duplicate at a minimum of three times. The following primers were used: miR-101-3p, forward, 5'-GGTCACTAAGGCGGT-3' and reverse, 5'-CAGTCGTTGCGTCGGAGT-3'; U6, forward, 5'-CTGGTTAGTACTTGGACGGGAGAC-3' and reverse, 5'-GTGCAGGGTCCGAGGT-3'. U6 was used as the endogenous control and the $2^{-\Delta\Delta Cq}$ method was used to analyze expression levels (25).

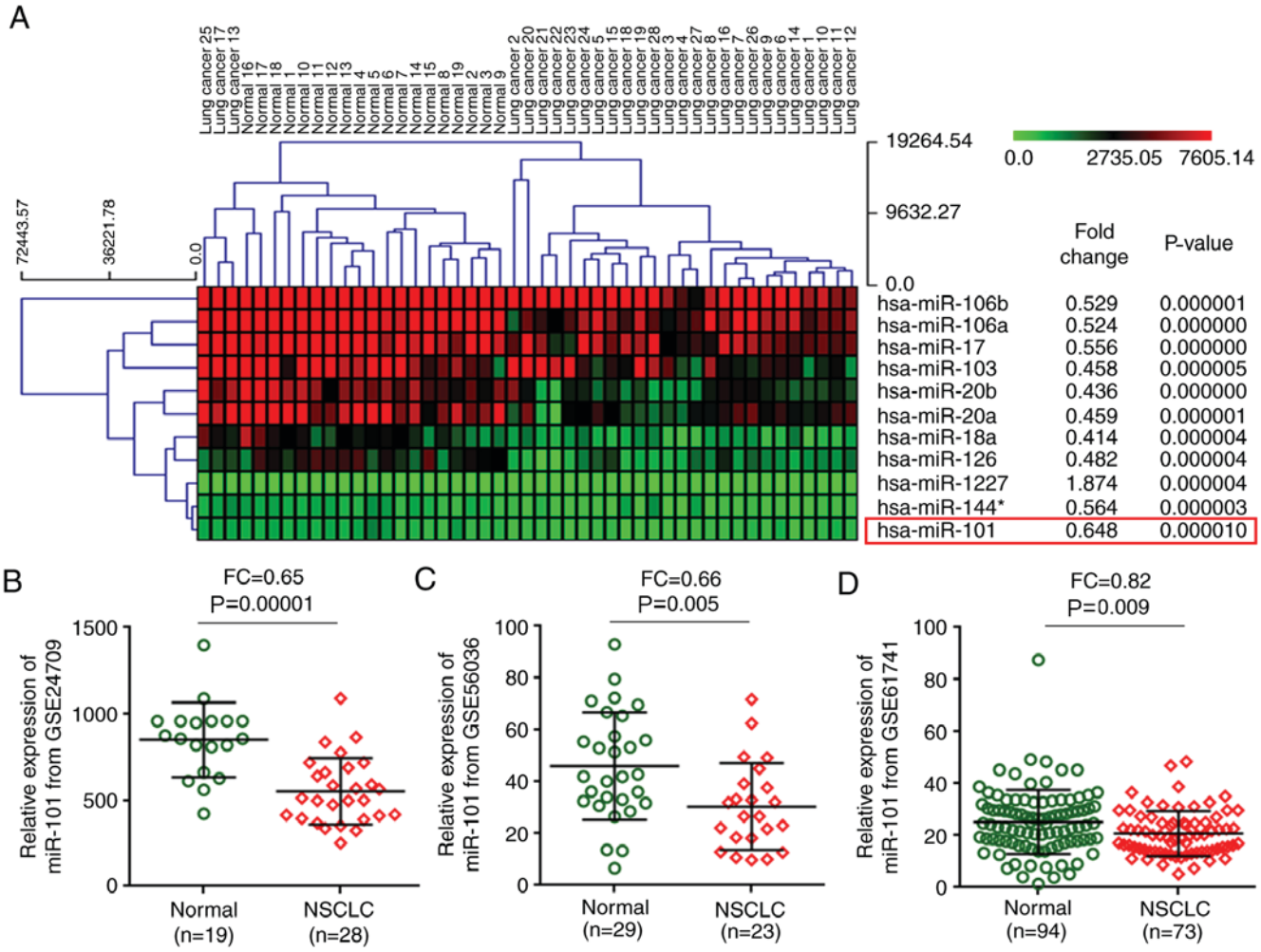

Figure 1. Analysis of miR-101-3p expression in NSCLC using the GEO database. (A) Data from the GEO dataset (GSE24709) was clustered using the multiple experiment viewer 4.7.1 software. (B) The miR-101-3P expression level in normal tissues, compared with NSCLC tissues, from the GEO database (GSE24709). (C) The miR-101-3p expression level in normal tissues, compared with NSCLC tissues, from the GEO database (GSE56036). (D) The miR-101-3p expression level in normal tissues, compared with NSCLC tissues, from the GEO database (GSE61741). miR, microRNA; NSCLC, non-small cell lung cancer; GEO, Gene Expression Omnibus; FC, fold change.

Statistical analysis. Expression levels of miR-101-3p were summarized and presented as the mean ± standard deviation. All statistical analyses were performed with IBM SPSS statistics software version 20.0 for Windows (IBM Corp., Armonk, NY, USA). The paired Student's t-test was used to determine the difference between two groups of data. The χ^2 test was used to evaluate the differences among groups. Kaplan-Meier estimator curves and the log-rank test were performed to analyze the OS or DFS of patients with NSCLC. Hierarchical clustering was performed using the multiple experiment viewer 4.7.1 software programs: (http://www.tm4.org/mev/), according to the manufacturer's protocols. Univariate and multivariate Cox proportional hazards regression models were used to investigate the multiple characteristics associated with the prognosis of patients with NSCLC. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of miR-101-3p using the GEO database and clustering analysis. Firstly, miR-101-3p expression analysis was performed using data from the GEO database. GSE24709 included the miRNA profile in peripheral blood samples from patients with lung diseases and healthy controls. The present analysis demonstrated that miR-101-3p expression in NSCLC tissues was significantly reduced, compared with non-tumor tissue (FC, 1.5; P<0.00001; Fig. 1A and B). The miR-101-3p expression was validated in NSCLC tissues in two datasets (GSE56036 and GSE61741) and indicated that miR-101-3p levels were significantly reduced in NSCLC tissues, compared with normal tissues (P=0.005, Fig. 1C; P=0.009, Fig. 1D).

miR-101-3p expression in NSCLC and adjacent non-tumor tissues. Expression of miR-101-3p was evaluated in NSCLC samples (n=327) and compared with adjacent non-tumor tissue (n=42) by qPCR. The present results indicated that miR-101-3p expression levels were significantly reduced in NSCLC tissues, compared with adjacent non-tumor tissues (FC, 0.36; P=0.002; Fig. 2A). The level of miR-101-3p expression was 0.72±0.04 in 327 NSCLC tissues, which was significantly reduced compared with normal tissues (n=42; 1.28±0.16) (FC, 0.56; P=0.117; Fig. 2B).

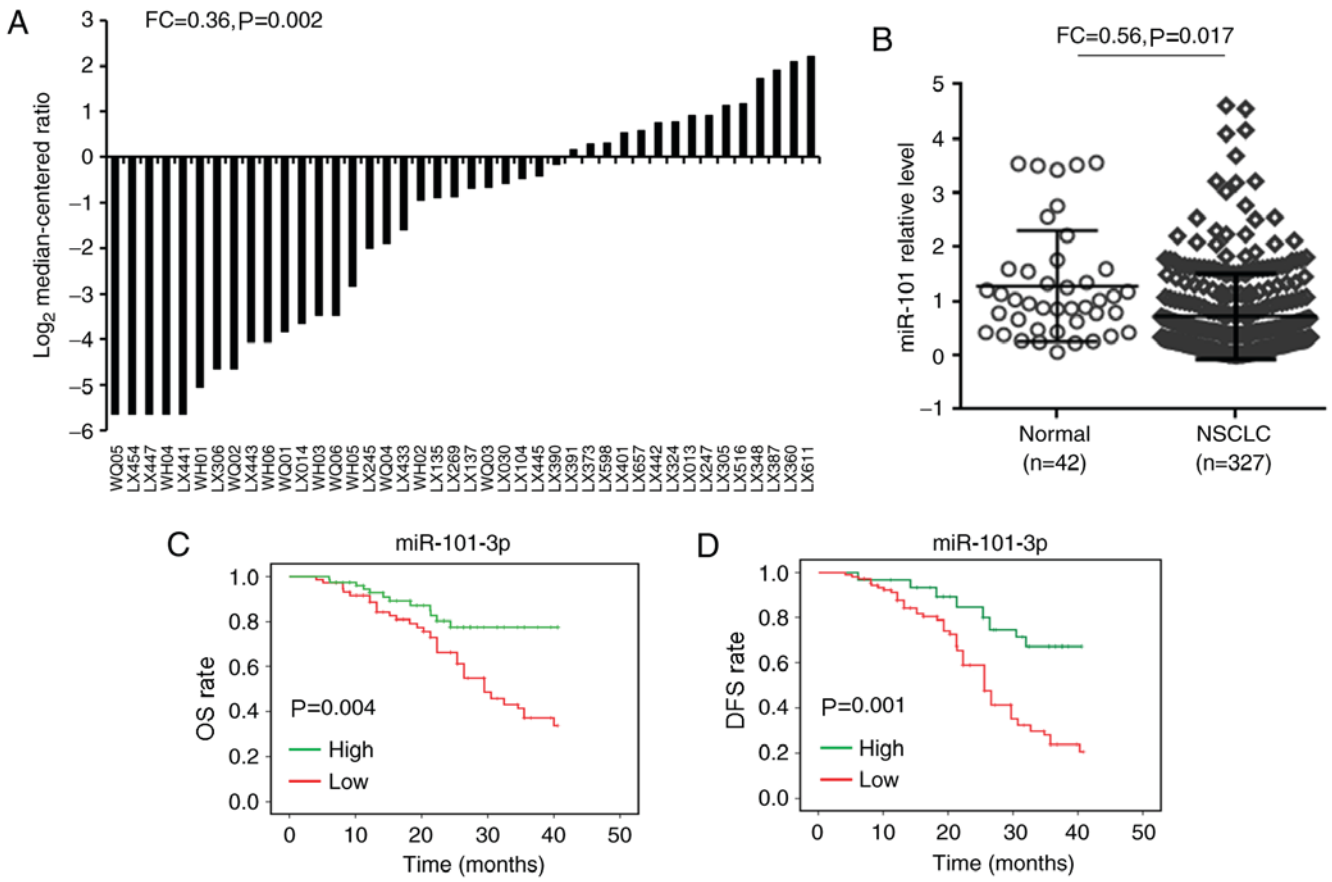

Figure 2. Analysis of miR-101-3p expression in NSCLC and normal lung cancer tissues via quantitative polymerase chain reaction. (A) The expression levels of miR-101-3p in 42 paired NSCLC and adjacent non-cancerous tissues. (B) The miR-101-3p expression in NSCLC (n=327) and paired adjacent non-tumor tissues (n=42) was analyzed. Univariate survival analysis of (C) OS and (D) DFS in NSCLC as determined by Kaplan-Meier plots based on miR-101-3p expression. miR, microRNA; NSCLC, non-small cell lung cancer; FC, fold change; OS, overall survival; DFS, disease-free survival.

Association between clinical characteristics and miR-101-3p expression. Additionally, miR-101-3p expression was analyzed in NSCLC samples based on various clinical characteristics, including age, sex, smoking history, lymph node metastasis, tumor differentiation, histology, TNM stage, invasion of lung membrane, vascular invasion and tumor diameter. Univariate analysis demonstrated that miR-101-3p expression was significantly associated with lymph node metastasis (P=0.08), tumor diameter (P=0.019) and TNM stage (P=0.036) in all patients with NSCLC (Table I). However, no significant association was determined between miR-101-3p expression in NSCLC samples and age, sex, smoking history, tumor differentiation, histology, invasion of the lung membrane or vascular invasion (P>0.05; Table I).

Univariate analysis of prognosis based on various clinical characteristics in patients with NSCLC. To ascertain whether the prognosis of patients with NSCLC was influenced by age, sex, smoking history, lymph node metastasis, tumor differentiation, histology, TNM stage, invasion of lung membrane, vascular invasion, tumor diameter or miR-101-3p expression, univariate analysis with the Kaplan-Meier estimator method was performed. The results demonstrated that lymph node metastasis (P=0.042), TNM stage (P=0.018), tumor diameter (P=0.013) and miR-101-3p expression were significantly associated with OS (P=0.004) and DFS (P=0.001) (Table II; Fig 2).

Univariate Cox regression analysis revealed that lymph-node metastasis [hazard ratio (HR), 1.743; 95% confidence interval (CI), 1.191-2.421; P=0.032], TNM stage (HR, 1.562; 95% CI, 1.124-1.962; P=0.036), tumor diameter (HR, 2.125; 95% CI, 1.563-3.346; P=0.005), chemotherapy (HR, 0.778; 95% CI, 0.469-0.968; P=0.026) and miR-101 expression (HR, 0.687; 95% CI, 0.498-0.952; P=0.003) were also positively significantly associated with a poor prognosis. No significant associations were observed for age, sex, smoking history, tumor differentiation, histology, invasion of lung membrane or vascular invasion (Table III).

Cox regression model analysis of prognosis based on various clinical characteristics in patients with NSCLC. To determine whether miR-101-3p expression levels, in combination with lymph-node metastasis, TNM stage or tumor diameter had prognostic value, multivariate analysis with a Cox regression model was used (Table II). This analysis also indicated that lymph node metastasis (HR, 1.924; 95% CI, 1.386-3.405; P=0.014), TNM stage (HR, 1.967; 95% CI, 1.544-2.325; P=0.018) and tumor diameter (HR, 2.869; 95% CI, 2.025-3.396; P=0.002) were significantly associated with reduced prognosis (Table III). This analysis initially included all of the parameters that were predictive of OS in the univariate analysis of the entire study group as presented in Table II (age, sex, smoking history, lymph-node metastasis, tumor

Table I. Association between miR-101 expression and clinical characteristics.

Factor	No. of patients	miR-101 expression (mean ± SD)	P-value
Age (years)			0.668
≥60	167	0.703±0.021	
<60	160	0.728±0.059	
Sex			0.239
Male	206	0.732±0.045	
Female	121	0.699±0.091	
Smoking history			0.078
Smoked	136	0.682±0.028	
Never smoked	119	0.735±0.071	
Unknown	72	0.728±0.031	
Lymph node metastasis			0.008[a]
Positive	105	0.632±0.084	
Negative	166	0.753±0.054	
Unknown	56	0.721±0.029	
Tumor differentiation			0.097
Poorly	136	0.691±0.063	
Moderately	111	0.714±0.036	
Well	80	0.722±0.089	
Histology			0.604
Adenocarcinoma	185	0.705±0.039	
Squamous cell carcinoma	142	0.711±0.057	
TNM stage (38)			0.036[a]
III-IV	158	0.681±0.067	
I-II	169	0.736±0.055	
Invasion of lung membrane			0.063
Positive	109	0.686±0.029	
Negative	168	0.725±0.013	
Unknown	50	0.721±0.066	
Vascular invasion			0.269
Positive	65	0.692±0.057	
Negative	189	0.721±0.033	
Unknown	73	0.708±0.037	
Tumor diameter (cm)			0.019[a]
≥5	186	0.677±0.054	
<5	141	0.743±0.065	

[a]P<0.05. miR, microRNA; TNM, Tumor-Node-Metastasis; SD, standard deviation.

differentiation, histology, vascular invasion, tumor diameter and invasion of the lung membrane).

Prediction of OS and DFS for patients with NSCLC based on chemotherapy alone or chemotherapy and miR-101-3p expression. There was a statistically significant association between chemotherapy and OS (27.624±3.858 vs. 31.457±2.924, respectively; P=0.012) and DFS (26.985±2.247 vs. 31.004±3.357, respectively; P=0.005) in patients with NSCLC. Chemotherapy is the primary adjuvant treatment for the majority of patients with NSCLC undergoing surgery. In the present study, it was determined that adjuvant chemotherapy and high expression of miR-101-3p increased the OS and DFS of patients (32.738±3.574 and 31.946±3.789, respectively) compared with non-therapeutic patients with low expression of miR-101-3p (25.352±2.568 and 25.004±2.876, respectively) (Table IV). These data indicated that patients with NSCLC with a high expression of miR-101-3p may have an increased benefit from chemotherapy.

Discussion

Lung cancer is the most common type of malignant tumor and was reported in 2015 as the leading cause of cancer-associated

Table II. Univariate analysis of OS and DFS based on patients stratified by clinical characteristics.

		OS time			Progression-free survival time		
Factor	No. of patients	Months (mean)	95% CI (mean)	P-value (log-rank test)	Months (mean)	95% CI (mean)	P-value (log-rank test)
Age (years)							0.331
≥60	167	28.065	26.945-30.253	0.289	28.069	26.889-30.453	
<60	160	29.331	28.314-31.846		29.564	28.651-31.754	
Sex							0.822
Male	206	29.652	28.773-31.325	0.806	30.594	28.797-31.393	
Female	121	31.068	28.962-32.618		31.056	28.845-32.648	
Smoking history							0.115
Smoked	136	27.849	26.152-29.086	0.109	27.654	26.465-29.754	
Never smoked	119	29.659	27.854-30.554		29.435	27.784-30.058	
Unknown	72	28.381	26.989-31.062		28.675	27.095-31.365	
Lymph node metastasis							0.041[a]
Positive	105	26.731	24.377-29.501	0.042[a]	26.456	24.943-29.063	
Negative	166	31.229	28.815-33.056		31.854	28.326-33.064	
Unknown	56	28.056	26.053-30.434		28.06	26.366-30.582	
Tumor differentiation (38)							0.274
Poorly	136	28.851	26.826-31.058	0.246	28.821	26.487-31.348	
Moderately	111	29.348	27.854-31.854		29.815	27.145-31.487	
Well	80	31.857	29.581-32.783		31.825	29.747-33.045	
Histology (38)							0.867
Adenocarcinoma	185	29.744	26.451-31.845	0.865	28.745	26.787-31.165	
Squamous cell carcinoma	142	29.043	25.624-32.434		29.257	25.474-32.345	
TNM stage (38)							0.012[a]
III-IV	158	26.748	24.285-30.647	0.018[a]	26.778	24.548-30.487	
I-II	169	31.049	28.834-34.415		32.091	28.674-34.358	
Invasion of lung membrane							0.067
Positive	109	27.995	25.453-29.454	0.086	27.123	25.748-29.486	
Negative	168	31.412	28.542-32.966		31.545	28.364-32.684	
Unknown	50	28.095	26.354-30.888		28.157	26.387-30.054	
Vascular invasion							0.066
Positive	65	27.849	25.446-29.354	0.072	27.048	25.487-29.954	
Negative	189	31.069	26.878-33.147		31.157	26.748-33.444	
Unknown	73	29.534	27.956-31.259		29.348	27.248-31.187	
Tumor diameter (cm)							0.009[a]
≥5	186	25.386	23.685-27.913	0.013[a]	24.886	23.065-26.648	
<5	141	31.553	27.456-33.546		30.661	28.446-32.596	
miR-101 expression (median)							0.001[a]
Low	163	24.154	22.314-26.043	0.004[a]	24.365	22.625-26.443	
High	164	32.317	28.414-34.916		33.053	28.456-34.994	

[a]P<0.05. miR, microRNA; TNM, Tumor-Node-Metastasis; OS, overall survival; CI, confidence interval.

mortality worldwide (26). Drug resistance has remained the primary factor influencing prognosis, therefore the treatment of advanced and metastatic NSCLC remains a notable challenge (27). The recognition of novel biomarkers for prediction of patient response to chemotherapy and prognosis is essential for improving OS and DFS in patients with NSCLC.

As molecular biomarkers, miRNAs serve significant roles in the selection of therapeutic schedules. Their functions as tumor suppressors and oncogenes in human cancer have been previously reported (28). In lung cancer, miRNAs exhibit alterations in expression that predict survival and relapse (29). miR-101 inhibits ovarian cancer cell invasion and

Table III. Cox regression model analysis for prognosis based on various clinical characteristics in patients with NSCLC.

	miR-101 univariate analysis			miR-101 multivariate analysis		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.153	0.681-1.554	0.093	1.176	0.699-1.568	0.087
Sex	0.887	0.525-1.115	0.801	0.867	0.504-1.089	0.542
Smoking history	1.196	0.723-1.587	0.087	1.198	0.825-1.589	0.061
Lymph-node metastasis	1.743	1.191-2.421	0.032[a]	1.924	1.386-3.405	0.014[a]
Tumor differentiation	1.154	0.961-1.224	0.224	1.168	0.979-1.231	0.201
Histology	0.835	0.698-1.142	0.324	0.831	0.624-1.104	0.265
TNM stage	1.562	1.124-1.962	0.036[a]	1.967	1.544-2.325	0.018[a]
Invasion of lung membrane	1.164	1.006-1.453	0.089	1.196	1.075-1.469	0.066
Vascular invasion	1.169	1.021-1.468	0.088	1.182	1.069-1.494	0.059
Tumor diameter	2.125	1.563-3.346	0.005[a]	2.869	2.025-3.396	0.002[a]
Chemotherapy	0.778	0.469-0.968	0.026[a]	0.559	0.374-0.786	0.006[a]
miR-101 expression	0.687	0.498-0.952	0.003[a]			

[a]P<0.05. miR, microRNA; CI, confidence interval; TNM, Tumor-Node-Metastasis.

Table IV. OS and DFS of patients with NSCLC based on chemotherapy alone or chemotherapy and miR-101 expression.

		OS			DFS		
Characteristics	No. of patients	Mean ± SD	95% CI	P-value	Mean ± SD	95% CI	P-value
Chemotherapy				0.012			0.005
Yes	231	31.457±2.924	28.728-34.632		31.004±3.357	28.244-33.148	
No	96	27.624±3.858	25.741-29.478		26.985±2.247	25.311-28.634	
Chemotherapy & miR-101 expression				0.001			<0.001
P & H	65	32.738±3.574	30.469-34.774		31.946±3.789	28.158-33.564	
N & L	87	25.352±2.568	22.914-27.353		25.004±2.876	23.043-26.965	

P & H, chemotherapy and high miR-101 expression; N & L, no chemotherapy and low miR-101 expression. OS, overall survival; DFS, disease-free survival; miR, microRNA; SD, standard deviation; CI, confidence interval.

proliferation by downregulating the expression of suppression of cytokine signaling 2 (30), and also has implications in suppressing the spread of a number of tumor types, including chondrosarcoma (31), thyroid cancer (32), breast cancer (33) and hepatocellular carcinoma (34). It has been reported that miR-101 inhibits cell proliferation and invasion of lung cancer by regulating cyclooxygenase-2 (35). Additionally, low expression of miR-101 in lung cancer has been demonstrated to inhibit the invasion of lung cancer by regulating its target gene enhancer of zerte 2 polycomb repressive complex 2 subunit (36).

miR-101-3p is a member of the miR-101 family and inhibits the metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1)-induced activation of the phosphoinositide 3-kinase/Akt signaling pathway, resulting in the inhibition of NSCLC growth and metastasis (16). It is notable that miR-101-3p expression in NSCLC cells was significantly reduced, whereas MALAT-1 expression was significantly increased. Furthermore, high expression of miR-101-3p inhibits the proliferation, migration and invasion of NSCLC (37). In the present study, significantly reduced levels of miR-101-3p was observed in patients with NSCLC, compared with healthy controls. The level of miR-101-3p expression in NSCLC was determined to be associated with OS and DFS, indicating that miR-101-3p may serve as a prognostic biomarker in patients with NSCLC. The analysis of a number of clinical factors, including tumor diameter, TNM stage and lymph node metastasis, demonstrated associations with OS and DFS. Notably, as an independent parameter, miR-101-3p expression levels in NSCLC also affected prognosis and survival.

miR-101-3p is a valuable prognostic predictor and therapeutic target of clinical chemotherapy. The present analysis determined that a high level of expression of miR-101-3p in patients with NSCLC who had undergone routine

chemotherapy was positively associated with OS and DFS rates. This indicated that patients with NSCLC with high levels of miR-101-3p expression may have improved benefit from chemotherapy. In brief, miR-101-3p was significantly downregulated in NSCLC, which increased the OS and DFS rates of patients receiving adjuvant chemotherapy. Based on these results, whether the miR-101-3p expression level may serve as a biomarker for chemotherapy use in patients with NSCLC should be investigated, and therefore may be a valuable and promising biomarker for this disease. However, the molecular and pathophysiological mechanisms of miR-101-3p in NSCLC are not fully understood, and will be assessed in subsequent studies. The present study had a number of limitations. For example, this is a retrospective study and only one marker was addressed. There are other markers or associated pathways that must be studied. In the future, multicenter studies regarding miR-101-3p in NSCLC should be performed.

In conclusion, the present data confirmed that with adjuvant chemotherapeutic treatment the median OS and DFS rate of patients improved. The analyses demonstrated that low expression of miR-101-3p, together with adjuvant chemotherapy, notably improved the OS and DFS of patients with NSCLC. The use of miR-101-3p as a specific and sensitive biomarker may be suitable for prediction of therapeutic responses in patients with advanced NSCLC, which may result in a superior level of personalized therapy. Therefore, miR-101-3p may be considered as a potential biomarker for chemosensitivity in the tumors of patients with NSCLC.

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Availability of data and materials

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

Authors' contributions

HML, WWY, YSM, FY, JBL, and DF designed the study. HML, WWY, FY, YSM, WTX, HWF, ZWL, LKH, WW, JJJ, ZYC, MXS, YCS, LC, CYJ, GXL and DF performed the qPCR experiments. HML, WWY, FY, YSM, WTX, LKH, WW, MXS, HQY, CZ, LC, CYJ, GXL, CYW and DF performed the statistical analyses and interpreted the data. FY, YSM, ZWL, LKH, WW, HML, JJJ, MXS, LC, CYJ, GXL, CYW, XJZ, JBL, and DF are involved in patient recruitment. FY, YSM, ZWL, CYW, JBL, and DF contributed to study materials and consumables. FY, YSM, ZWL, WTX and DF wrote the manuscript. HML, WWY, YSM, WW and FY contributed equally to this work. All authors agreed with the results and conclusions.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shanghai Tenth People's Hospital, Tongji University School of Medicine (approval no. SHSY-IEC-pap-15-18). Each participant provided signed informed consent prior to participate in the present study. Patients or their legal surrogates provided signed informed consent for the surgical procedures.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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