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Predictive value of microRNA-10b expression in peripheral blood mononuclear cells in evaluating short- and long-term efficacy of chemotherapy for patients with advanced non-small-cell lung cancer

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The expression profile in peripheral blood mononuclear cells (PBMCs) from advanced non-small-cell lung cancer (NSCLC) patients and the role of microRNAs (miRs) in NSCLC remain unclear. Herein, the present study aims to investigate predictive value of miR-10b expression in PBMCs in evaluating short- and long-term efficacy of chemotherapy for NSCLC patients. A total of 194 advanced NSCLC patients were selected as the NSCLC group and 199 healthy individuals were recruited as a control group. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed to determine the miR-10b expression in PBMCs. The patients with advanced NSCLC were treated with chemotherapy, and the relationship between miR-10b expression in PBMCs and the clinicopathological characteristics, clinical response to chemotherapy and prognosis were analyzed. The values of miR-10b in diagnosis and prediction of clinical response to chemotherapy before chemotherapy were detected by receiver operating characteristic (ROC) curve analysis, the clinical response to chemotherapy by logistic regression analysis, and the risk factors of prognosis in NSCLC patients by Cox regression analysis. Compared with the control group, miR-10b in PBMCs was highly expressed in the NSCLC group. The area under the curve (AUC) of miR-10b expression in the diagnosis of NSCLC was 0.967, with sensitivity and specificity of 85.10% and 99.5%, respectively. MiR-10b expression was related to lymph node metastasis (LNM), distant metastasis, pathological types and differentiation. The AUC of miR-10b expression in the prognosis of advanced NSCLC was 0.793, with sensitivity and specificity of 75.20% and 76.62%, respectively. Logistic regression analysis showed that clinical response to chemotherapy was significantly influenced by miR-10b expression, distant metastasis and LNM. Cox regression analysis showed that the high miR-10b expression, smoking, LNM and distant metastasis were risk factors of prognosis for advanced NSCLC patients. The present study offers intriguing new perspectives based on evidence that miR-10b expression in PBMCs has predictive value for the tumor response to chemotherapy and prognosis for advanced NSCLC patients.

Key words: microRNA-10b, advanced non-small-cell lung cancer, peripheral blood mononuclear cells, chemotherapy, differentiation, prognosis

Lung cancer, a prominent malignant tumor, has become the first leading cause of death throughout the world, and can be classified into two subtypes, small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) [1, 2]. NSCLC becomes the most commonly seen type of lung cancer as it accounts for 80% in all lung cancer cases [3]. Treatment methods for patients with stage III NSCLC consist of surgery, radiotherapy and chemotherapy, with cure rates reaching 10% to 25%. For patients with stage IV NSCLC, it is primarily palliative chemotherapy combined with palliative radiotherapy or surgery controlling symptomatic distant tumor localization [4]. Additionally, smoking, operative methods, lymph node dissection, and blood loss are significantly related to postoperative complications [5]. The 5-year survival rate of patients with advanced NSCLC stage is very poor, and advanced stage NSCLC patients show poor results in long-term survival [6]. Even with the disappointing clinical results, there is an urging demand for effective diagnostic strategies to early identify high-risk populations faster and earlier, thus decreasing the mortality rate in NSCLC patients, as well as predictive factors for prognosis to manage the outcomes of the treatment [7].

Nowadays, upcoming evidence indicates that microRNAs (miRs), especially conserved non-coding RNAs, are diffusely used in the NSCLC occurrence and development [8].

MiRs are small, single-stranded non-coding RNA molecules with a length of 19–23 nucleotides, which can modulate gene expression [9]. MiRs, acting as tumor suppressors or oncogenes, play decisive roles in the occurrence and development of cancer with abnormal expressions [10]. It has been proven that MiR-10b plays critical roles in cancer progression in a variety of cancers, such as colorectal cancer, breast cancer and pancreatic cancers [11–13]. As a tumor enhancer, miR-10b is expressed in the NSCLC cell line A549, reported as a potential target for interventional therapy for NSCLC [14]. Furthermore, miR-10b is over-expressed in metastatic cancer cells, suggesting that miR-10b overexpression may enhance the invasion and metastatic potential of cancer [11]. In addition, miRs in peripheral blood mononuclear cells (PBMCs) in patients are found to be associated with malignant tumors and might be therefore used as cancer biomarkers [15, 16]. However, limited researches pay attention to the value of miR-10b expression in PBMCs for the prognosis of NSCLC. Thus, our study aims to investigate the predictive value of miR-10b expression in PBMCs in tumor response to chemotherapy and prognosis for patients with advanced NSCLC, which mainly refers to the short- and long-term efficacy with a 5-year follow-up.

Patients and methods

Ethical statement. This study was approved by the Ethics Committee of the Affiliated Hospital of Beihua University. All patients signed informed consent in written form for this research.

Study subjects. From August 1998 to July 2006, 194 patients diagnosed with advanced NSCLC in the Affiliated Hospital of Beihua University were selected as the NSCLC group. Inclusion criteria: 1) patients with advanced NSCLC through pathological diagnosis; 2) patients without any other organ dysfunction, or disease requiring hospitalization; 3) patients in stage IV and those who had not received surgery or chemotherapy in stage III, based on the World Health Organization (WHO) histologic classification system of tumor and the American Joint Committee on Cancer (AJCC) staging manual [17]; 4) patients without contraindications to chemotherapy; 5) patients with complete medical records, and all patients without receiving chemotherapy and biotherapy before sampling. Exclusive criteria: 1) patients with other malignant tumors or with a history of malignant tumors; 2) patients who had received surgery and chemotherapy; 3) patients with congenital diseases, hereditary diseases, autoimmune diseases and cardiovascular diseases; 4) patients who were unable to get in touch. According to the clinical and pathological data in the NSCLC group, there were 120 patients aged <60 years and 74 patients aged ≥60 years with mean age of 59.9±10.1 years; the patients consisted of 113 males and 81 females, 117 patients with smoking history and 77 patients without smoking history; there were 80 patients with low and moderate differentiation, 114 patients with highly differentiated tumors according to the degree of tumor differentiation; there were 102 patients with distant metastases, 92 patients without distant metastasis, 86 patients with lymph node metastasis (LNM), and 108 patients without LNM; 119 patients with squamous cell carcinoma, 61 patients with adenocarcinoma and 14 patients with other types of cancer on the basis of different pathological types. Beside, NSCLC tissues and paracancerous tissues located more than 3 cm from the tumor tissue of 48 advanced NSCLC patients (30 males and 18 females) were collected for waxing and storage. There were 199 healthy individuals randomly selected in the same area as the control group, 87 patients aged <60 years and 112 patients aged ≥60 years with mean age of 59.2±9.2 years. There were 139 males and 60 females, 106 patients with smoking history and 93 patients without smoking history. No significant difference in age, gender and smoking history between the NSCLC and control groups (all $p>0.05$).

Sample collection. Before and after four cycles of chemotherapy, peripheral blood samples (5 ml each case) were collected by ethylenediamine tetra-acetic acid (EDTA) anticoagulant tube in the NSCLC and control groups. PBMCs were isolated from peripheral blood with lymphocyte separating liquid (07801, StemCells, Inc. California, USA), fully lysed with 1 ml Trizol (15596-026, Invitrogen, Carlsbad, CA, USA) and kept at –80 °C. The RNA was extracted from PBMCs when being used for avoiding RNA degradation caused by repeated freezing and thawing.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the PBMCs using the miRNeasy Mini Kit (217004, Qiagen, Hilden, Germany). The concentration and purity of the RNA were detected by using ultraviolet spectrophotometry through optical density (OD) values at wavelengths of 260 nm and 280 nm. The OD260/OD280 ratio between 1.7 and 2.1 indicates a high purity of the RNA. The cDNA template was synthesized by reverse transcription using a PCR amplification instrument (ABI Company, Oyster Bay, NY, USA). The RT-qPCR was conducted using ABI 7500 Real-Time PCR instrument. The reaction conditions were as follows: pre-denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, extension at 72 °C for 34 s. The reaction system consisted of 10 μl of the SYBR Premix Ex Taq$^{TM\,II}$, 0.8 μl of Forward Primer (10 μM), 0.8 μl of Reverse Primer (10 μM), 0.4 μl of the ROX Reference Dye, 2.0 μl of cDNA template and 6.0 μl of sterile distilled water. The forward primer sequence of miR-10b was 5'-GGATACCCTGTAGAACCGAA-3', the reverse primer sequence of miR-10b was 5'-CAGTGCGTGTCGTGGAGT-3'. The forward primer sequence of reference gene U6 was 5'-TGGGGTTATACATTGTGAGAGGA-3', the reverse primer sequence of U6 was 5'-GTGTGCTACGGAGTTCA-

GAGGTT-3'. PCR results were analyzed by the Opticon Monitor software version 3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The threshold was manually set in the lowest point of parallel rise in the logarithmic amplification curve, and the threshold cycle (Ct) value was analyzed using $2^{-\Delta\Delta Ct}$ method. The proportions of the target gene expression between the NSCLC group and the control group were expressed by $2^{-\Delta\Delta Ct}$, the formula is as follows: $\Delta\Delta Ct=\Delta Ct_{experimental\ group}-\Delta Ct_{control\ group}$, which is $\Delta Ct=Ct_{miR}-Ct_{U6}$. The experiment was repeated three times.

Chemotherapy regimens and efficacy evaluation. The first-line chemotherapy regimen was used for the patients with advanced NSCLC. Patients received a paclitaxel + cisplatin regimen with intravenous infusion of paclitaxel (135–175 mg/m^2) and cisplatin (70–80 mg/m^2) on day 2, 3 and 4, respectively; and navelbine (NVB) (25 mg/m^2) on day 1 and 8; which was repeated every three weeks in one chemotherapy cycle [18].

After four cycles of chemotherapy [18], according to the Response Evaluation Criteria In Solid Tumors (RECIST 1.1) [19, 20], the results were categorized as complete response (CR), partial response (PR), progressive disease (PD) and stable disease (SD). CR and PR were defined as overall response (OR), PD and SD were treated as non-remission or ineffective. Computed tomography (CT) scan was performed after four cycles of chemotherapy to evaluate the clinical response to chemotherapy.

Follow-up. The follow-up was conducted by telephone, outpatient and medical records and ended in September 2016 with 5 cases lost during the time. The follow-up rate was 97.42%. Progression free survival (PFS) is defined as the time between treatment initiation and tumor recurrence or death from any cause.

Statistical analysis. The statistical analysis was conducted using SPSS 21.0 (IBM Corp. Armonk, NY, USA). The measurement data were presented by the mean±standard deviation (SD). Differences between two groups were compared by t-test. Receiver operating characteristic (ROC) curve was used to evaluate the prognostic value of miR-10b expression in PBMCs with chemotherapy for advanced NSCLC patients. The logistic regression analysis was conducted to analyze the influencing factors of the clinical response to chemotherapy for advanced NSCLC patients. Probability of patients' survival time was presented by using Kaplan-Meier curve and Log-rank test was applied to detect the survival curve difference between groups. The Cox regression model was applied to analyze the prognostic factors of advanced NSCLC patients.A p-value <0.05 was considered statistically significant.

Results

MiR-10b is highly expressed in NSCLC tissues and PBMCs of advanced NSCLC patients. The relative expression of miR-10b in NSCLC tissues was obviously higher while miR-100 was lower than that in paracancerous tissues (both $p<0.05$). The relative expression of miR-10b in PBMCs of the NSCLC group was higher while the miR-100 was lower than that in the control group (both $p<0.05$) (Figure 1).

Correlation between miR-10b expression in PBMCs and clinicopathological features of advanced NSCLC patients. Association between miR-10b expression in PBMCs and the clinicopathologic features of the patients with advanced NSCLC is depicted in Table 1. Patients with LNM showed significantly higher miR-10b expression than those without (3.39±0.97 vs. 2.59±0.94) ($p<0.05$), miR-10b expression in patients with distant metastasis was evidently upregulated than in patients without distant metastasis (3.62±0.85 vs. 2.51±0.91) ($p<0.05$), miR-10b expression in patients with low-moderate tumor differentiation was prominently enhanced than in patients with high differentiation (2.85±1.04 vs. 3.30±0.98), miR-10b expression in patients with squamous cell carcinoma was remarkably escalated than in patients with adenocarcinoma and other pathological types (3.29±0.98 vs. 2.66±1.02, 3.29±0.98 vs. 2.51±0.94), and miR-10b expression in patients with smoking history was markedly increased than in patients without smoking history (3.17±0.99 vs. 2.84±1.08). There was no significant

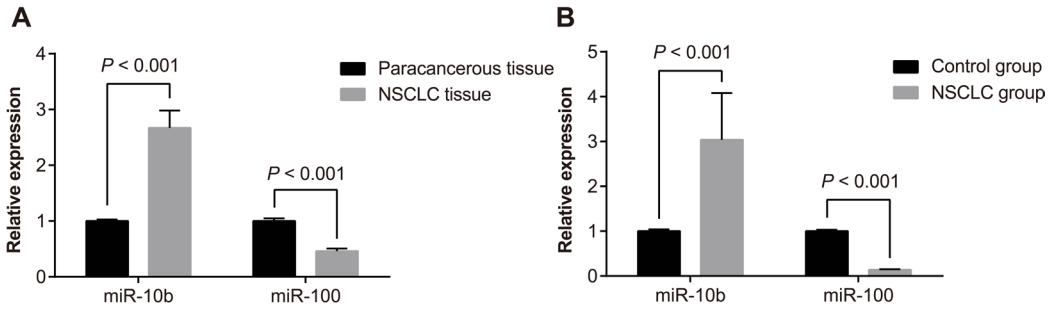

Figure 1. Expression of miR-10b and miR-100 in PBMCs in the NSCLC and control groups, NSCLC tissues and paracancerous tissues. A) expression of miR-10b and miR-100 in the NSCLC tissues and paracancerous tissues; B) expression of miR-10b and miR-100 in PBMCs; NSCLC; non-small-cell lung cancer; PBMCs, peripheral blood mononuclear cells; miR-10b, microRNA-10b; miR-100, microRNA-100.

association between miR-10b expression and age, gender (all p>0.05).

Correlation between miR-10b expression in PBMCs and the tumor response to chemotherapy. The relative expression of miR-10b in PBMCs before and after four cycles of chemotherapy was 3.04±1.04 and 2.71±0.99, respectively. After four cycles of chemotherapy, miR-10b expression was markedly diminished when compared with the time before chemotherapy (p<0.05). Among 194 patients, there were 23 patients with CR, 54 patients with PR, 48 patients with PD, 69 patients with SD, which indicated that chemotherapy was effective for 77 patients (39.69%). The miR-10b expression in the effective group (CR+PR) before chemotherapy and after four cycles of chemotherapy was 2.28±0.86 and 1.94±0.79, respectively, and in the ineffective group (SD+PD) it was 3.54±0.82 and 3.22±0.75, respectively. The results indicated that miR-10b expression in the effective group was dramatically elevated after four cycles of chemotherapy than before chemotherapy (p<0.05). The relative expression of miR-10b was significantly lower in the effective group before chemotherapy and after four cycles of chemotherapy than in the ineffective group (all p<0.05) (Figure 2).

Predicative values of miR-10b for the diagnosis and efficacy of chemotherapy for patients with advanced NSCLC. ROC curve of miR-10b expression in the PBMCs of the NSCLC and control groups before chemotherapy is shown in Figure 3A. The area under the curve (AUC) was calculated to assess the diagnostic efficacy of miR-10b in patients with NSCLC. The ROC curve demonstrated that AUC value of miR-10b in the diagnosis of NSCLC was 0.967 (95% CI: 0.952–0.982), suggesting a good predicative value. The cutoff value was 1.98, the diagnostic sensitivity and specificity of miR-10b in the diagnosis of NSCLC were 85.10% and 99.50%.

Figure 3B shows the ROC curve of miR-10b expression in the PBMCs of the effective and ineffective groups before chemotherapy. The AUC value of miR-10b was 0.793 (95% CI: 0.725–0.861), indicating a good predictive value of miR-10b for the efficacy of chemotherapy. The cutoff value was 2.87, the diagnostic sensitivity was 75.20% and specificity was 76.62%.

Influencing factors for the chemotherapy efficacy for advanced NSCLC patients by logistic regression analysis. Logistic regression analysis was performed with the efficacy of chemotherapy for advanced NSCLC patients as the dependent variable, and the clinicopathological features and the expression of miR-10b as the independent variable (Table 2). The results proved that distant metastasis, LNM, smoking history and miR-10b expression had a significant influence on the efficacy of chemotherapy (p<0.05). There was no significant difference in age, gender, tumor differentiation and pathological types (all p>0.05).

Table 1. Association between the relative expression of miR-10b and the clinicopathological features for advanced NSCLC patients.

Clinicopathological data	Number	miR-10b expression	p-value
Age (years)			
<60	120	3.04±1.02	0.948
≥60	74	3.03±1.07	
Gender			
Male	113	2.93±1.10	0.084
Female	81	3.19±0.92	
Smoking History			
Yes	117	3.17±0.99	0.030
No	77	2.84±1.08	
Differentiation			
Low-moderate	80	3.30±0.98	0.003
High	114	2.85±1.04	
Distant metastasis			
Yes	102	2.51±0.91	<0.001
No	92	3.62±0.85	
Lymph node metastasis			
No	86	2.59±0.94	<0.001
Yes	108	3.39±0.97	
Pathological types			
Squamous cell carcinoma	61	2.66±1.02	
Adenocarcinoma	119	3.29±0.98	<0.001
Other	14	2.51±0.94	

Notes: NSCLC, non-small cell lung cancer; miR-10b, microRNA-10b.

Table 2. Influencing factors for the clinical response to chemotherapy for advanced NSCLC patients.

Factor	B	SE	Wald	p-value	OR	95% CI	
						Lower	Upper
Age	0.297	0.503	0.350	0.554	1.346	0.502	3.608
Gender	0.380	0.512	0.549	0.459	1.462	0.535	3.991
Differentiation	–0.740	0.558	1.760	0.185	0.477	0.160	1.424
Distant metastasis	1.922	0.537	12.813	<0.001	6.833	2.386	19.568
Lymph node metastasis	1.871	0.499	14.088	<0.001	6.495	2.445	17.255
Smoking history	1.515	0.497	9.312	0.002	4.550	1.719	12.042
Pathological types	–0.304	0.413	0.540	0.462	0.738	0.328	1.659
MiR-10b expression	2.795	0.579	23.282	<0.001	16.361	5.257	50.919

Notes: B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; NSCLC, non-small cell lung cancer; miR-10b, microRNA-10b.

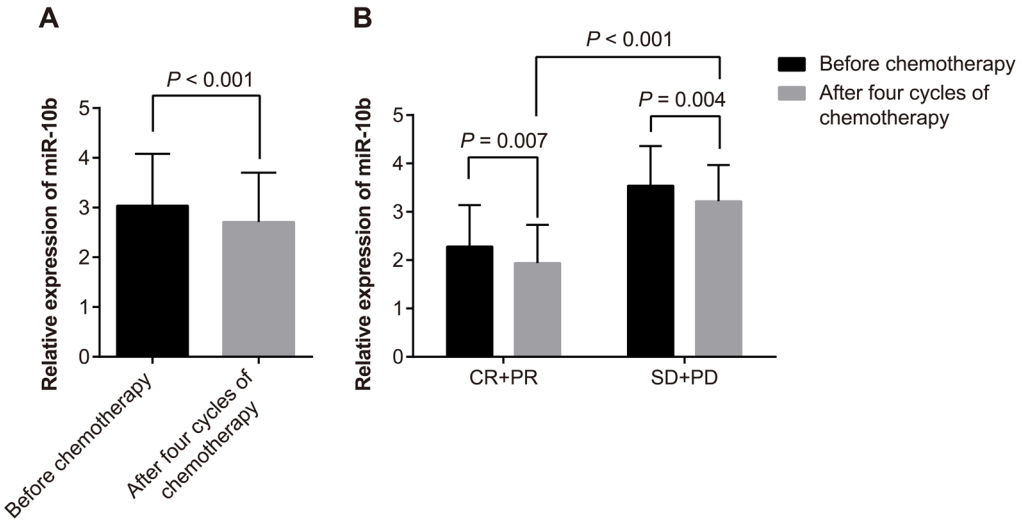

Figure 2. Expression of miR-10b in PBMCs of advanced NSCLC before chemotherapy and after four cycles of chemotherapy. A) expression of miR-10b in PBMCs before chemotherapy and after four cycles of chemotherapy; B) expression of miR-10b in PBMCs in the effective group (CR+PR) and the ineffective group (PD+SD) before chemotherapy and after four cycles of chemotherapy, paired t-test was used to analyze the difference before chemotherapy and after four cycles of chemotherapy, unpaired t-test was used to analyze the difference between the effective group (CR+PR) and the ineffective group (PD+SD) after chemotherapy; NSCLC; non-small-cell lung cancer; PBMCs: peripheral blood mononuclear cells; CR, complete response; PR, partial response; PD, progressed disease; SD, stable disease.

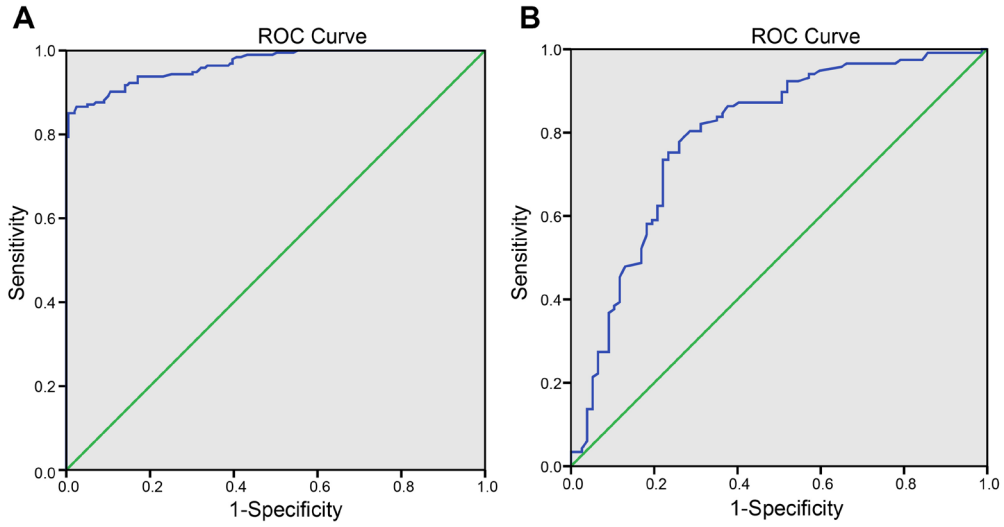

Figure 3. Predictive value of miR-10b expression in PBMCs for advanced NSCLC patients with chemotherapy. A) ROC curve of the predictive value of miR-10b expression for advanced NSCLC in the NSCLC and control groups; B) ROC curve of the predictive value of miR-10b expression in the clinical response to chemotherapy for advanced NSCLC in the effective and ineffective groups; ROC, receiver operating characteristic; miR-10b, microRNA-10b; NSCLC, non-small-cell lung cancer; PBMCs: peripheral blood mononuclear cells.

Kaplan-Meier univariate analysis of influencing factors for the prognosis of patients with advanced NSCLC. Univariate analysis was performed with Kaplan-Meier survival curves, and Log-Rank test was applied to detect the difference of Kaplan-Meier survival curve between groups (Table 3). When the threshold value (2.87) of ROC curve served as the cutoff value of miR-10b expression, the NSCLC patients were classified into high expression group and low expression group. Kaplan-Meier survival analysis curve of miR-10b expression, smoking history, LNM and distant metastasis are shown in Figure 4. The results show that median PFS and 5-year survival rate were 12.0 months and 0% in patients with high miR-10b expression, respectively, which was prominently reduced compared to patients with low miR-10b expression (median PFS: 18.0 months; the 5-year survival rate: 19.1%) (all $p<0.05$). Median PFS

Table 3. Kaplan-Meier univariate analysis of influencing factors for the prognosis of patients with advanced NSCLC.

Clinicopathological data	Number	PFS		95% CI		p-value
		Estimated value	SD	Lower	Upper	
Age (years)						
<60	120	14	0.855	12.324	15.676	0.956
≥60	74	13	0.644	11.737	14.263	
Gender						
Male	113	14	0.874	12.287	15.713	0.050
Female	81	13	1.038	10.966	15.034	
Smoking history						
Yes	117	12	0.773	10.486	13.514	< 0.001
No	77	16	1.194	13.660	18.340	
Differentiation						
Low-moderate	114	13	1.051	10.940	15.060	0.096
High	80	14	0.720	12.589	15.411	
Distant metastasis						
No	102	17	1.407	14.242	19.758	<0.001
Yes	92	11	0.684	9.660	12.340	
Lymph node metastasis						
No	86	17	1.604	13.857	20.143	<0.001
Yes	108	12	0.647	10.732	13.268	
Pathological types						
Squamous cell carcinoma	61	14	1.259	11.533	16.467	
Adenocarcinoma	119	13	0.818	11.397	14.603	0.228
Others	14	15	1.195	12.657	17.343	
MiR-10b						
High	89	12	0.590	10.843	13.157	<0.001
Low	105	18	2.495	13.111	22.889	

Notes: PFS, progression free survival; CI, confidence interval; SD, standard deviation; NSCLC, non-small cell lung cancer; miR-10b, microRNA-10b.

Table 4. Influencing factors for the prognosis of advanced NSCLC patients.

Factors	B	SE	Wald	p-value	RR	95% CI	
						Lower	Upper
Distant metastasis	0.532	0.205	6.772	0.009	1.703	1.140	2.543
Lymph node metastasis	0.416	0.175	5.672	0.017	1.515	1.076	2.133
Smoking history	0.425	0.164	6.713	0.010	1.529	1.109	2.108
MiR-10b	0.571	0.210	7.358	0.007	1.769	1.172	2.672

Notes: B, regression coefficient; SE, standard error; RR, relative risk; CI, confidence interval; NSCLC, non-small cell lung cancer; miR-10b, microRNA-10b.

and 5-year survival rate were 15.0 months and 14.3% in patients without smoking history, respectively, which were dramatically elevated compared to patients with smoking history (median PFS: 13.0 month; the 5-year survival rate: 5.1%) (all $p<0.05$). Median PFS and 5-year survival rate were 17.0 month and 17.4% in patient without LNM, respectively, which were evidently enhanced compared to patients with LNM (median PFS: 12.0 month; the 5-year survival rate: 1.9%) (all $p<0.05$). Median PFS and 5-year survival rate were 17.0 months and 16.7% in patients without distant metastasis, respectively, and both were obviously ascended compared to patients with distant metastasis (median PFS: 11.0 month; the 5-year survival rate: 0%) (all $p<0.05$). Therefore, miR-10b expression, smoking history, LNM and distant metastases were predictive factors for PFS for advanced NSCLC patients after chemotherapy.

Cox proportional hazard regression analysis for the risk factors of the prognosis for patients with advanced NSCLC. Statistically significant factors (miR-10b expression, smoking history, LNM and distant metastasis) were included in the Cox proportional hazard model as seen in Table 4. The results showed that the over-expression of miR-10b, smoking history, LNM and distant metastasis were prognosis risk factors for patients with advanced NSCLC (all $p<0.05$).

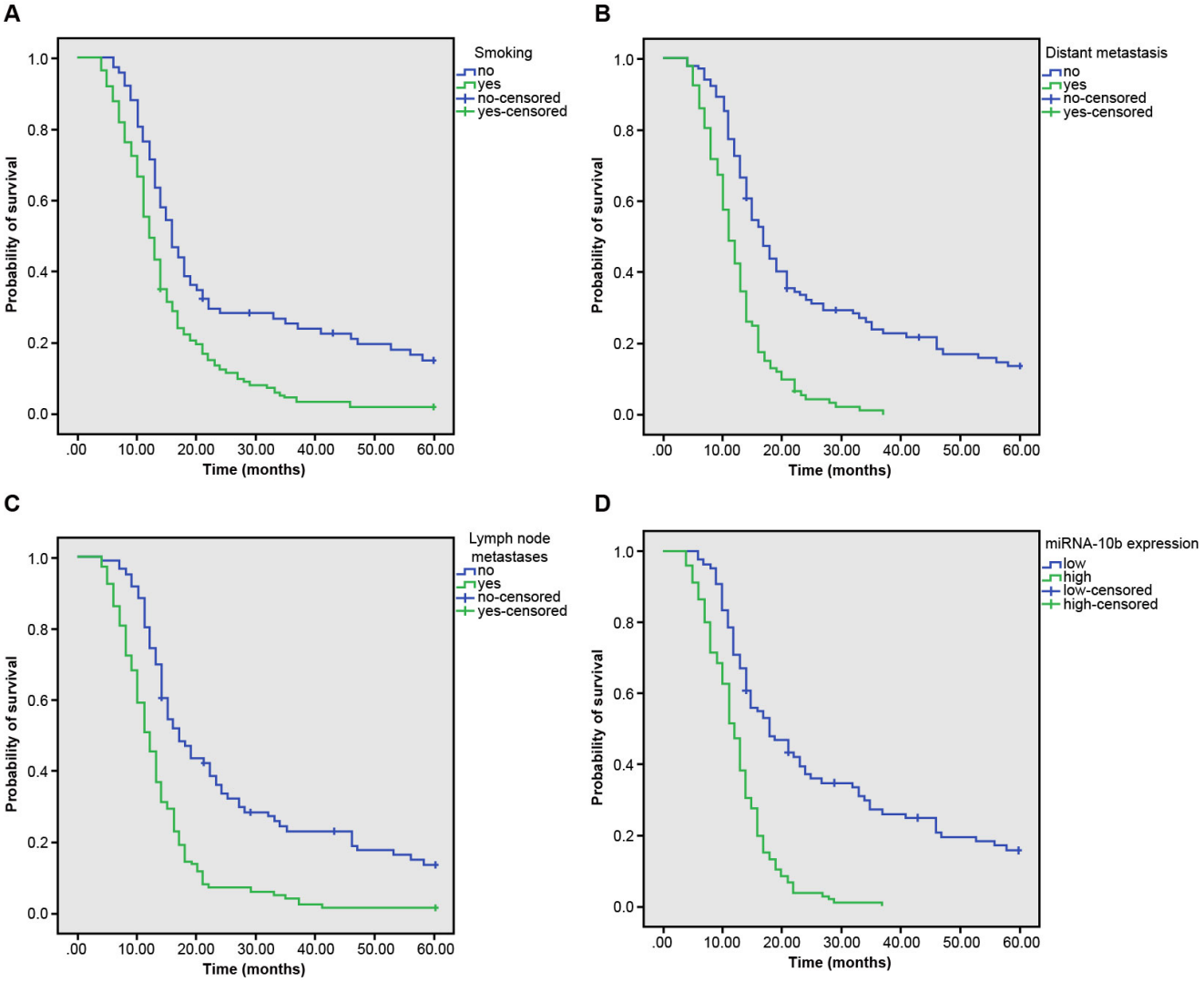

Figure 4. Kaplan-Meier survival curves of influencing factors for the PFS of advanced NSCLC patients. A) Kaplan-Meier survival curves of the PFS of advanced NSCLC patients with smoking history; B) Kaplan-Meier survival curves of the PFS of advanced NSCLC patients with distant metastasis; C) Kaplan-Meier survival curves of the PFS of advanced NSCLC patients with LNM; D) Kaplan-Meier survival curves of the PFS of advanced NSCLC patients in the low expression and high expression miR-10b groups; LNM, lymph node metastasis; miR-10b, microRNA-10b; PFS, progression free survival; NSCLC, non-small cell lung cancer.

Discussion

NSCLC is one of the most common lung cancers, and finding effective methods of treatment is very important for NSCLC patients [21]. Further research is necessary to improve the diagnosis, early prevention, and treatments to cure this cancer and more and more studies have shown that miRs may play a decisive role in NSCLC pathogenesis [14, 22, 23]. In our study, we investigated predictive value of miR-10b expression in PBMCs in evaluating clinical response to chemotherapy and prognosis for patients with advanced NSCLC. The results of present study indicate that over-expression of miR-10b in PBMCs could predict worse tumor response to chemotherapy and prognosis for advanced NSCLC patients.

Initially, our study demonstrated that miR-10b expression in PBMCs in the NSCLC group was significantly higher than that in the control group and over-expression of miR-10b was closely related to poorer outcomes. The abnormal expression of several miRs have been proven to serve as important biomarkers for the diagnosis, prognosis and survival [24]. Consistent with our results, Zhang et al. reported that miR-10b, which is involved in cancer invasion and metastasis, is up-regulated in the NSCLC cells and

tissues, and even the overexpression predicts poor prognosis [25]. Besides, it has been proven that up-regulated miR-10b in breast cancer cells promotes the invasion and metastasis of tumor cells with an increase in the metastatic potential, thereby leads to a poor prognosis [26, 27]. Furthermore, miR-10b is over-expressed in pancreatic cancer cells and is related to prognosis of pancreatic cancer patients [13]. As an oncogene, the overexpression of miR-10b in the metastatic lymph node tissues results in metastasis development in the NSCLC, indicating a key role of miR-10b in the lung cancer cell metastasis [28]. As reflected in our study, over-expressed miR-10b in the PBMCs of patients with advanced NSCLC is strongly correlated with LNM, distant metastasis, low-moderate differentiation, squamous cell carcinoma and stage IV cancer. In line with the finding, miR-10b was differently expressed in squamous cell carcinoma (SCC) and AC, indicating a correlation of miR-10b with the clinical stage of NSCLC [29]. Additionally, the report suggests the up-regulated expression of miR-10b is closely related to a larger tumor size as well as advanced pathological stage of NSCLC, and the inhibition of miR-10b might provide a novel way for the clinical diagnosis and targeted therapy [30]. A previous study has revealed that overexpression of miR-10b enhances cell proliferation, migration, and invasion capacities in NSCLC, and Kruppel-like factor 4 (KLF4) may be indirectly targeted by miR-10b during the increased proliferation of NSCLC cells [14]. Another relevant study has identified that miR-10b targets KLF4 to affect the biological function of cells in human esophageal cancer, thereby affects the progression, metastasis and prognosis of tumor [31]. By downregulating the tumor suppressor gene KLF4, miR-10b can promote cancer cell proliferation and invasive metastasis, possibly by preventing the KLF4-induced G1/S cell cycle arrest through the regulation of the extracellular matrix proteins, cyclin D1 or p21 expression [32]. Meanwhile, the miR-10b expression might negatively regulate the E-cadherin expression (a potent suppressor of lung cancer metastasis), which directly affects the tumor grade and stage, to function as an independent factor for the prognosis of NSCLC patients [25]. Yang et al. have also reported that miR-10b expression in the PBMCs isolated from NSCLC patients showed high diagnostic sensitivity and specificity; high miR-10b expression, patients' age, LNM and distant metastases were independent risk factors for worse prognosis of NSCLC patients [7], which is consistent with the results of the logistic regression analysis, univariate analysis and ROC curves in the presented paper. All the data above contribute to a conclusion that miR-10b is an important diagnostic and prognostic factor for patients with advanced NSCLC.

Additionally, compared with the expression of miR-10b before chemotherapy, miR-10b expression was significantly decreased after four cycles of chemotherapy; and the effective group exhibited strikingly lower miR-10b expression than the ineffective group. The plasma miRNAs (hsa-miR-98-5p, hsa-miR-302e, hsa-miR-495-3p, and hsa-miR-613) in NSCLC patients are important predictors of the radiosensitivity, and became novel biomarkers for radiotherapy response [33]. Besides, the circulating cell-free miRs in blood plasma and serum are noted to be associated with the tumor progression and prognosis of lung cancer, and serve as tumor biomarkers and prognosticators in patients with NSCLC or other types of lung cancers [34]. In the presented study, we showed that miR-10b is not only associated with tumor response to chemotherapy after a four-course treatment, but is also related to the PFS and survival rate after follow-ups. Although several miRs are involved in the chemoresistance of NSCLC cells to cisplatin, such as miR-182 and miR-192 [35, 36], miR-10b was demonstrated to be a potential indicator of chemosensitivity in colorectal cancer [11]. It still remains to determine the value of miR-10b in predicting the efficacy of chemotherapy for other cancers. We suggest that miR-10b expression in PBMCs has a high predictive value for the efficacy of chemotherapy for patients with advanced NSCLC, both in the short-term tumor response and long-term survival.

In summary, we provide strong evidence that high miR-10b expression in PBMCs of NSCLC patients correlates with poor prognosis and it can effectively predict tumor response to chemotherapy (short-term efficacy) and prognosis (long-term efficacy) for advanced NSCLC patients. Nevertheless, a larger population is needed to further identify whether other aberrantly expressed miRs interfere with these results and the specific mechanisms of miR-10b in the chemosensitivity may be further explored in future studies, including critical signaling pathways or essential factors related to the treatment of lung cancer, such as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), etc.

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