miR-486 as an unfavorable prognostic biomarker for patients with non-small cell lung cancer

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Background: Non-small cell lung cancer (NSCLC) is the most common malignant tumor in China. miR-486 was found to be associated with many tumors. In previous study, we aimed to investigate the expression and prognostic value of miR-486 in patients with NSCLC.

Methods: In order to measure the expression of miR-486 in 140 NSCLC patients, in situ hybridization (ISH) was made. The staining of miR-486 was scored by two independent investigators. Then, the prognostic value of miR-486 was evaluated by plotting Kaplan-Meier survival curves and making multivariate analysis.

Results: miR-486 was mainly expressed in the cytoplasm of tumor cells. miR-486 expression was corrected with tumor differentiation (P=0.011) but not with any other clinicopathological characteristics. However, high expression of miR-486 was significantly correlated with shortened overall survival (OS) in NSCLC patients (P=0.001), especially in stage I patients (P=0.005). Multivariate analysis revealed that miR-486 was an independent prognostic factor in NSCLC patients (P=0.002).

Conclusions: miR-486 high expression predicts poor survival in patients with NSCLC. miR-486 could be used as an unfavorable prognostic biomarker for NSCLC patients.

Keywords: miR-486; non-small cell lung cancer (NSCLC); prognosis; expression; tissue microarray (TMA)

Introduction

The morbidity and mortality of lung cancer, in which non-small cell lung cancer (NSCLC) accounts for approximately 85%, ranks first among all human malignant tumors in many countries (1,2). The morbidity of lung adenocarcinoma (LUAD) has gradually increased in the past decades. Nowadays, LUAD has replaced lung squamous cell carcinoma (LUSC), becoming the most common type of lung cancer in China (2). In recent years, although the surgical techniques, strategies of chemoradiotherapy and target therapy have been developed, the 5-year overall survival (OS) rate of lung cancer is still very low (3). It's well known that early detection, early diagnosis and early treatment are the most effective strategies to improve the survival rate of lung cancer patients (4). However, there is no efficient clinical biomarker for early diagnosis, treatment and prognosis prediction of lung cancer. Therefore, it's urgent to locate the diagnosis, prognosis markers and drug targets of lung cancer.

A microRNA (miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) that functions in RNA silencing and post-transcriptional regulation of gene expression (5). It is widely believed that miRNA

plays an important role in tumorigenesis. miR-486 located on chromosome Chr8 was first discovered in fetal liver tissue in 2005 (6). As a member of miRNAs, miR-486 also has been reported to be associated with many tumors. Previous studies have shown that the expression profile and mechanism of miR-486 in NSCLC were controversial (7-10). In present study, we mainly explore the expression profile and prognostic value of miR-486 in NSCLC.

Methods

Ethics

This study was reviewed and approved by the Research Ethics Committee of The affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University. Written informed consent was obtained from all the patients. All specimens were processed and anonymized according to the ethical and legal standards.

Tissue microarray (TMA)

A total of 150 tissue samples (140 NSCLC tissue samples and 10 normal lung tissue samples) were obtained from patients who were operated at the Thoracic Surgical Division in year 2005. Patients with NSCLC were staged and graded according to the international staging system or the 2004 World Health Organization criteria. Patients included in this study received no preoperative chemotherapy or radiotherapy and had no other malignant tumors within 5 years prior to diagnosis. According to the method described in our previous study (11), a TMA containing 150 tissue samples was constructed.

In situ hybridization (ISH)

A detection kit named miR-486 ISH Kit (Boster, Pleasanton, CA, USA) was used to detect miR-486 in TMA. Antisense miR-486 probes were as follows: 5'-CTCGGGGCAGCTCAGTACAGGA-3'. Briefly, the ISH was performed as follows (11): the sections were deparaffinized in xylene, rehydrated in graded ethanol, digested in pepsin solution and then postfixed in 1% paraformaldehyde (PFA) for 10 min. Hybridization was then carried out with a mixture containing target probes to miR-486 at 37 ℃ overnight. After wash with saline sodium citrate (SSC), the sections were incubated at 37 ℃ for 30 min with blocking buffer. The slides were visualized using DAB (3,3'-diaminobenzidine) and

counterstaining with hematoxylin.

ISH evaluation

Evaluation of miR-486 expression was semi-quantitatively performed by two independent investigators in a blinded fashion. The intensity of cytoplasmic staining was graded as absent, weak, moderate, or strong. The extent of cytoplasmic staining was expressed as the percentage of positive cancer cells from 0% to 100%. As previously described (12), tumors were scored (score 1–4) according to the intensity and extent of staining. In this study, tumors with score 3/4 were defined as positive miR-486 staining, whereas tumors with score 1/2 was defined as negative miR-486 staining.

Statistical analysis

The data were presented as mean ± standard deviation (SD). OS time was defined as the date of primary surgery to the date of death or latest follow-up. Student's t-test, χ^2 test, Kaplan-Meier survival analysis, and Cox regression analysis were performed. SPSS 23.0 software (IBM, NY, USA) was used to process the data and draw the graphs. P<0.05 was considered statistically significant.

Results

Characteristics of the patients

Table 1 shows the clinicopathological characteristics of the 140 NSCLC patients. The median age of the patients was 60 years (range from 26 to 79 years). The overwhelming majority of NSCLC patients are male. Fifty-seven percent of the tumors were LUSC and forty-two percent of the tumors were poorly differentiated. And, seventy-nine percent patients were in stage I or II. Clinical follow-up was recorded until July 2013. The median follow-up time was 45 months (range from 3–101 months). Ninety-one patients died at the end of follow-up.

Expression of miR-486 in NSCLC patients

The *in situ* miR-486 mRNA were stained and the representative images are shown in *Figure 1*. miR-486 was mainly expressed in the cytoplasm of lung cancer cells, but was less expressed in the cytoplasm of normal or para-tumor cells. The staining of miR-486 varied among tumors. Similar to lung cancer cells, miR-486 also mainly expressed

Table 1 Clinical and histologic features of 140 patients with NSCLC

Patient demographics	Number of patients	% of total
Age[a]	60 [26–79]	
Sex		
Male	112	80
Female	28	20
Location		
Left	64	46
Right	76	54
Pathological type		
Squamous cell carcinoma	80	57
Adenocarcinoma	46	33
Adenosquamous cell carcinoma	5	4
Bronchiolo-alveolar carcinoma	3	2
Sarcomatoid carcinoma	1	1
Neuroendocrine carcinoma	3	2
Mucoepidermoid carcinoma	2	1
Differentiation		
Poorly	59	42
Moderate	49	35
Well	32	23
T stage		
1	15	11
2	105	75
3	20	14
N stage		
0	82	59
1	31	22
2	27	19
NSCLC stage		
I	67	48
II	44	31
III	29	21
Follow-up period[a]	45 [3–101]	

[a], median (range). NSCLC, non-small cell lung cancer.

in the cytoplasm of renal clear cell carcinoma cells and breast cancer cells. According to the scoring criteria described above, positive miR-486 staining was observed in 51.43% cases (N=140, positive *vs.* negative: 51.43% *vs.* 48.57%).

miR-486 expression and clinicopathological characteristics

For investigating the correlation between miR-486 and the clinicopathological features, the staining score 2 was selected as the cut-off value. Then, the patients were divided into two groups: low miR-486 expression group (staining score ≤2) and high miR-486 expression group (staining score >2). As shown in *Table 2*, miR-486 expression was correlated with tumor differentiation (P=0.011). However, no statistically significant correlations were found between miR-486 and tumor size (P=0.683), lymph node status (P=0.640), tumor stage (P=0.425) or any other clinicopathological characteristics (all P>0.05) (*Table 2*).

miR-486 expression and patients' OS

The Kaplan-Meier survival curves were plotted to elaborate the correlation between miR-486 and the patients' OS. *Figure 2A* showed that in all the 140 patients (stage I–III), high expression of miR-486 was correlated strongly with shortened OS (P=0.001). Further, the survival of patients with stage I or II–III NSCLC was also analyzed. We discovered that the difference in survival between the low and high miR-486 groups was statistically significant in stage I patients (P=0.005, *Figure 2B*). However, miR-486 expression was not found to be correlated to OS in patients with stage II–III NSCLC (P=0.090, *Figure 2C*).

Finally, univariate and multivariate analysis were made. As shown in *Table 3*, miR-486 and lymph node status had an independent prognostic meaning (P=0.002 and 0.024, respectively) for the whole group of 140 patients for OS.

Discussion

NSCLC is a highly malignant and aggressive tumor type, with a poor 5-year OS rate (1,2). Similar to many other malignant tumors, NSCLC is initiated by either the activation of oncogenes or the inactivation of tumor suppressor genes (13). Thus, in the past decades, many attempts have been made to identify the key biomarker for diagnosis, treatment and prognosis prediction of NSCLC. Since the discovery of the first miRNA in 1993 (14), more
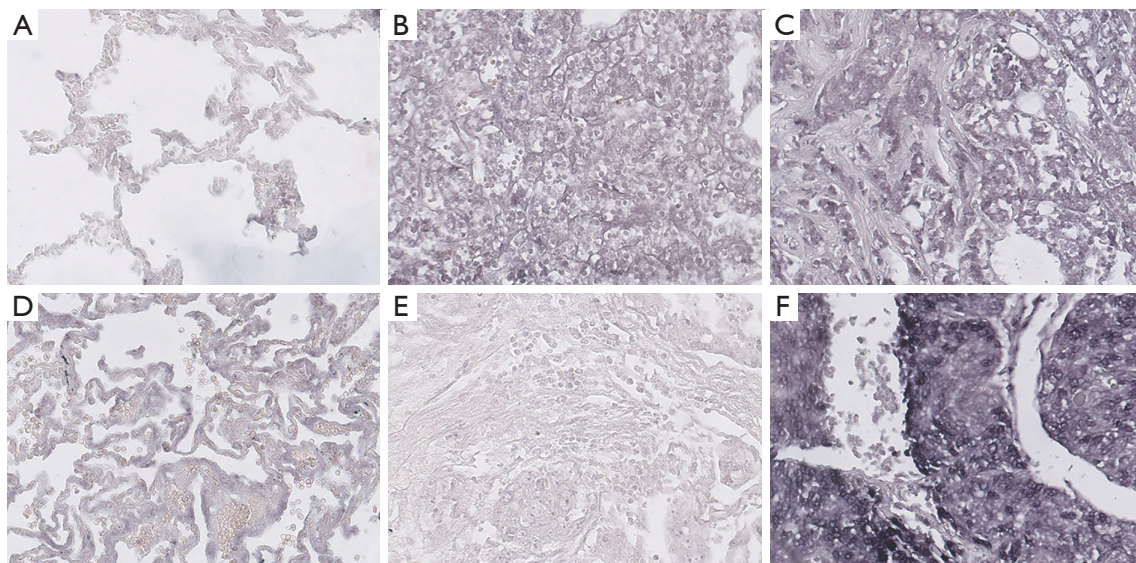
Figure 1 Expression of miR-486 in various tissues (200×). miR-486 is not expressed in normal (A) or para-tumor lung tissue (D). miR-486 is strong expressed in the cytoplasm of renal clear cell carcinoma tissue (B) or breast cancer tissue (C). Weak (E) or strong (F) cytoplasmic expression of miR-486 in lung tumor tissue.

Table 2 The relationship between miR-486-5p and clinicopathological characteristics

| Variable | miR-486-5p | | P value |
	Low	High	
Age	59.1±10.2	61.0±10.0	0.276
Sex			0.866
Male	54	58	
Female	14	14	
Location			0.084
Left	26	38	
Right	42	34	
Histological type[a]			0.770
Squamous	38	42	
Non squamous	30	30	
Differentiation			0.011*
Well	11	21	
Moderate	32	17	
Poorly	25	34	

Table 2 (*continued*)

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| Variable | miR-486-5p | | P value |
	Low	High	
pT			0.683
1	7	8	
2	53	52	
3	8	12	
pN			0.640
0	42	40	
1	15	16	
2	11	16	
TNM stage			0.425
I	35	32	
II	22	22	
III	11	18	

[a], some tumors such as adenocarcinoma, adenosquamous cell carcinoma, bronchiolo-alveolar carcinoma, sarcomatoid carcinoma, neuroendocrine carcinoma, and mucoepidermoid carcinoma were included in the non squamous cancer data; *, significant correlation.
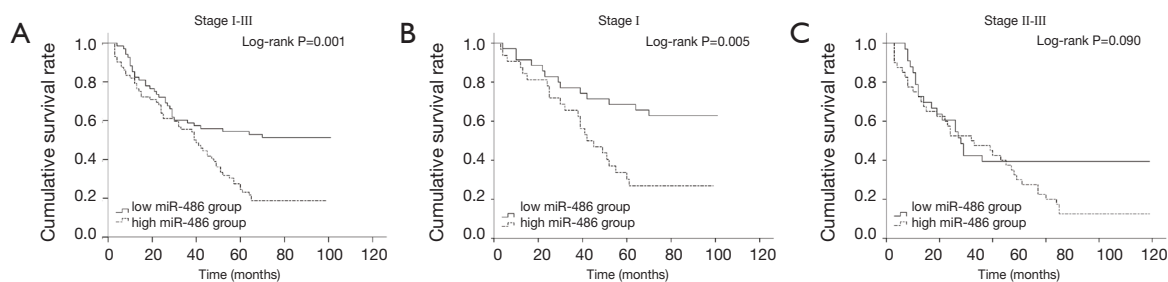
Figure 2 Survival curves for overall survival stratified by tumor miR-486 expression. miR-486 expression is associated with poorer prognosis in stage I–III patients (P=0.001) (A), especially in stage I patients (P=0.005) (B), but not in stage II-III patients (P=0.090) (C).

Table 3 Multivariate cox regression analysis for potential factors influencing overall survival

Parameters	B	SE	Wald	P value	Exp(B)	95% CI for Exp(B)	
						Lower	Upper
Univariate analysis							
Age (≤60 *vs.* >60)	0.423	0.213	3.936	0.047*	1.526	1.005	2.316
Sex (male *vs.* female)	−0.240	0.275	0.762	0.383	0.786	0.458	1.349
Location (left *vs.* right)	−0.216	0.210	1.059	0.303	0.806	0.534	1.216
Histological type (LUSC *vs.* non-LUSC)	−0.123	0.213	0.337	0.562	0.884	0.583	1.341
Differentiation (poorly *vs.* moderate *vs.* well)	−0.014	0.139	0.010	0.922	0.987	0.752	1.295
pT (T1 *vs.* T2–3)	0.839	0.423	3.940	0.047*	2.314	1.011	5.298
pN (N0 *vs.* N1–2)	0.825	0.212	15.166	0.000*	2.281	1.506	3.454
Stage (stage I *vs.* stage II–III)	0.655	0.215	9.275	0.002*	1.926	1.263	2.937
miR-486 (low *vs.* high)	0.713	0.220	10.526	0.001*	2.040	1.326	3.138
Multivariate analysis							
Age (≤60 *vs.* >60)	0.300	0.216	1.934	0.164	1.350	0.884	2.061
pT (T1 *vs.* T2–3)	0.695	0.430	2.609	0.106	2.004	0.862	4.656
pN (N0 *vs.* N1–2)	0.870	0.386	5.078	0.024*	2.388	1.120	5.091
Stage (stage I *vs.* stage II–III)	−0.193	0.396	0.236	0.627	0.825	0.380	1.793
miR-486 (low *vs.* high)	0.686	0.222	9.553	0.002*	1.986	1.285	3.069

*, significant correlation. LUSC, lung squamous cell carcinoma.

and more miRNAs have been discovered, and the role of miRNAs has been gradually revealed. The first miRNA found to be associated with NSCLC was let-7 (15). Since then, more miRNAs were found to be associated with NSCLC. Such as, Zheng *et al.* found that miR-155, miR-197 and miR-182 were significantly higher expressed in NSCLC, and the combined detection of the three miRNAs had a diagnostic value for lung cancer (16). A study, in which the CT scan and miRNA detection were combined, revealed that after a low-dose spiral CT scan, the detection of 34 miRNAs expression level in the serum could be used to identify benign pulmonary nodules, high-risk lung cancer and malignant tumors (17). Yet, another study discovered that miR-205 and miR-21 can be used to identify LUAD and LUSC (18). By using mouse NSCLC model, miR-34 and miR-7 were found could affect the tumor growth (19,20). It is reported that miR-192, miR-135a, miR-133b and miR-9 were associated with chemotherapy resistance

in NSCLC (21-23). miR-195, let-7, etc. were found to be associated with NSCLC prognosis (24,25).

miR-486 was first found and named in 2005 (6). In recent years, with the advancement of research, the roles of miR-486 played in tumors are becoming clear. A study found that miR-486 was lower expressed in esophageal cancer (EC) and inhibited growth and metastasis of EC cells by targeting CDK4/BCAS2 (26). It is reported that miR-486 was higher expressed and had a diagnosis value in pancreatic cancer (PC) and could promote the development of PC by targeting CD40 (27,28). The role miR-486 played in prostatic cancer was controversial. Yang *et al.* believed that miR-486 acted as an oncogene in prostatic cancer while Song *et al.* and Zhang *et al.* hold the opposite view (29-31). Similar to prostatic cancer, the role miR-486 played in NSCLC was also controversial. It was reported that miR-486 was down-regulated and its downregulation correlated to stages and lymph metastasis of patients with lung cancer (7,8). However, Hu *et al.* revealed that up-regulated miR-486 level in plasma was correlated with shorten OS (9). Sromek *et al.* reported that miR-486 was significantly high expressed in NSCLC patients' plasma (10). Wang *et al.* further reported that miR-486 was high expressed and had a diagnostic value in NSCLC patients (32).

In this study, we mainly investigated the prognostic value of miR-486. We found that miR-486 was high expressed in majority of NSCLC tissues (mainly expressed in the cytoplasm of tumor cells). Also, miR-486 expression was found to be correlated with tumor differentiation. Further, miR-486 was demonstrated to have a prognostic value in NSCLC patients, especially in stage I patients. And, miR-486 had an independent prognostic meaning for NSCLC patients. Combining our previous study results (26,33), we have more reasons to believe that miR-486 functions as an oncogene in NSCLC. However, this study still has some shortcomings, such as too few normal lung tissue samples.

In conclusion, miR-486 is an unfavorable prognostic biomarker for NSCLC patients. These findings provide additional evidence for clarifying the role of miR-486 in NSCLC.

Acknowledgments

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi. org/10.21037/tcr.2019.11.19). 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). This study was reviewed and approved by the Research Ethics Committee of The affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University. Written informed consent was obtained from all the patients.

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