

Original Article

Effect of miR-483-5p on apoptosis of lung cancer cells through targeting of RBM5

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Abstract: RBM5 has been reported to be a candidate tumor suppressor gene which plays an important role in the induction of apoptosis. In this study, we investigated the effect of miR-483-5p on apoptosis of lung cancer cells and the underlying mechanism. We found that the expression of miR-483-5p mRNA was significantly up-regulated in lung cancer compared with adjacent para-cancerous tissues by using real-time PCR. Silencing miR-483-5p promoted A549 cell apoptosis and enhanced caspase-3 activity by flow cytometry with annexin V-FITC/PI staining and caspase-3 activity report kit. Western blotting demonstrated that miR-483-5p mimicked down-regulated RBM5 protein expression and miR-483-5p inhibitor up-regulated RBM5 protein expression. With additional bioinformatics analysis, we confirmed that RBM5 is a target gene of miR-483-5p and is favored for treating NSCLC. The immunohistochemical pattern of RBM5 could be used to predict outcome for NSCLC. In conclusion, our results support that RBM5 expression can be regulated by miR-483-5p which is a prognostic marker for NSCLC patients. miR-483-5p inhibitor plays a role in lung cancer through targeting RBM5 to induce apoptosis.

Keywords: miR-483-5p, lung cancer, RBM5, A549, apoptosis

Introduction

Non-small cell lung cancer (NSCLC) is the most common malignant tumor of the respiratory system that is threatens human health with high morbidity and mortality [1]. MicroRNAs (miRNAs) are a class of endogenous non-coding single-stranded small RNA molecules that bind to the 3' untranslated region (3' UTR) of a target mRNA to inhibit or degrade the translation product and regulate target genes at the post-transcriptional level. miRNAs participate in the regulation of numerous genes in the body and are also involved in cell growth, proliferation and apoptosis [2]. Many studies have demonstrated that miRNAs play important roles in tumorigenesis and the progression of many malignant tumors, including lung cancer [3, 4]. Inducing tumor cell apoptosis has become one of the important anti-tumor approaches. Cancer cell apoptosis induced by miRNAs has received attention [5]. MiR-483-5p is confirmed to promote the proliferation and migration of lung cancer cells, but its effect

on lung cancer cell apoptosis and the associated mechanism remain unclear [6]. In addition, RBM5 is known to be an effective tumor suppressor gene of lung cancer cells and also can induce lung cancer cell apoptosis [7-9]. According to limited research results, RBM5 is believed to be a potential target of miR-483-5p [10]. However, the regulatory association between miR-483-5p and RBM5 protein expression in lung cancer cells has not been investigated to date. This study aims to elucidate the impact of miR-483-5p on lung cancer cell apoptosis and explore its possible mechanism.

Materials and methods

Cell line and patient samples

Human lung cancer cell line A549 was purchased from Shanghai Institute of Cell Biology. Lung cancer tissues and the corresponding paracancerous tissues (>3 cm from the cancer tumor) were collected from 40 pathologically

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confirmed NSCLC patients who were treated in the Second Affiliated Hospital of Harbin Medical University from January 2014 to January 2016, including 24 males and 16 females. Prior to surgery, none of the patients received radiotherapy or chemotherapy including molecular targeted therapy. Signed consent was obtained from all patients before operation (with the approval of Harbin Medical University Human Ethics Committee).

Cell culture

A549 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum with 100 IU/ml penicillin and 100 µg/ml streptomycin in an incubator at 37°C under 5% CO₂. Cells in logarithmic growth phase were collected for subsequent experiments.

Transient transfection

A549 cells were seeded in culture plates and randomly divided into three groups, including transfection group, non-target control (NC) group and non-transfected group, for transfection with a Hsa-miR-483-5p (5'-AAGACGGG-AGGAAAGAAGGGAG-3') mimic, miR-483-5p inhibitor, miR-NC (5'-UUUGUACUACACAAAAGUACUG-3') and RBM5 siRNA (Guangzhou RiboBio, CHINA) following the user's manuals of the X-treme GENE siRNA transfection kit (Roche, USA). RNA was mixed with Opti-MEM and incubated at room temperature for five minutes. The cells were cultured for 48 additional hours prior to collection.

Apoptosis assay

After the supernatant removed, the cells were trypsinized and collected to prepare single-cell suspensions. The cells were washed once with PBS and adjusted to a cell concentration of 1 × 10⁶ cells/mL followed by centrifugation at 2000 rpm for ten minutes. After Annexin V-FITC and PI staining (Thermo Fisher Scientific, USA), the cells were loaded by flow cytometer. The experimental data were analyzed by CytExpert.

Quantitative real-time PCR

Total RNA was extracted from lung cancer and the corresponding paracancerous tissues and A549 cells using the Trizol method. RNA was

reverse-transcribed into cDNA following the instructions of the Prime-Script™ Reverse Transcription Kit. U6 was used as the reference for miRNA, and GAPDH served as the reference for RBM5. The test was repeated three times for each group, and the data were analyzed using the 2^{-ΔΔCt} method. The upstream primer of miR-483-5p was 5'-AAGACGGGAG-GAAAGAAGGGAG-3', and the downstream primer was 5'-GTGCAGGGTCCGAGGTATTC-3'. The upstream primer of U6 was 5'-CGCTTCGGC-AGCACATATACTA-3', and the downstream primer was 5'-CGCTTCACGAATTTGCGTGTCA-3'. The upstream primer of RBM5 was 5'-GC-ACGACTATAGGCATGACAT-3', and the downstream primer was 5'-AGTCAAACCTGTCTGCTCCA-3'. The upstream primer of GAPDH was 5'-GAAGGTGAAGGTCGGAGTC-3', and the downstream primer was 5'-GAAGATGGTATGGGATTTC-3'.

Caspase-3 protease activity assay

A549 cells were trypsinized and collected. Then, lysis buffer was added and incubated on ice for 30 minutes. The cells were then centrifuged at 15000 rpm for 15 minutes at 4°C, and the supernatant was retained. The subsequent steps were performed in accordance with the instructions of the caspase-3 activity assay kit (KeyGEN Biotech, Nanjing). OD values were measured at 405 nm.

Western blotting

The cells in each group were collected and lysed on ice with RIPA lysis buffer for 30 minutes, then centrifuged at 15000 × g for 15 minutes. The supernatant was collected. Total protein was extracted and denatured at 95°C. After the protein was quantified using the BCA protein concentration assay kit, 50 µg of the protein was taken and subjected to 10% SDS-PAGE gel electrophoresis and membrane transfer. After blocking, the membrane was incubated with the primary antibody (RBM5, 1:500 dilution, Santa Cruz Biotechnology) overnight at 4°C. The membrane was washed three times with TBST and incubated with horseradish peroxidase-labeled secondary antibody (1:2000 dilution) for one hour at room temperature. β-actin served as reference. Odyssey image analysis software was employed to analyze optical density.

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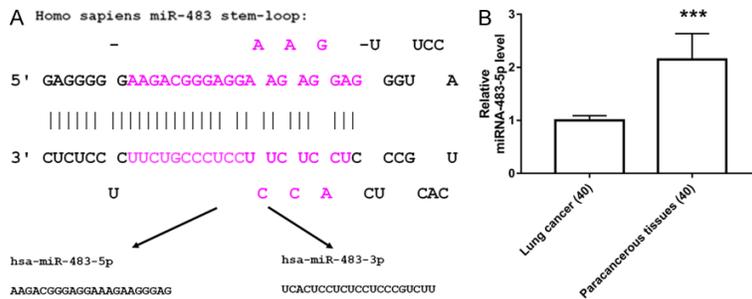


Figure 1. miR-483-5p in NSCLC patients. A: Precursor miRNA stem-loop structure of miR-483 and its two mature strands. B: miR-483-5p expression in 40 cases of NSCLC patients detected by quantitative real-time PCR. $^{***}P < 0.01$ vs. paracancerous tissue.

Atlas (HPA, <https://www.proteinatlas.org>) in TCGA NSCLC patients [12].

Statistical analysis

Data were expressed as the mean \pm SEM. The non-paired t-test was used to compare two groups, whereas ANOVA was utilized to compare multiple groups. Bonferroni-adjusted t-test was used for multiple paired comparisons. $P < 0.05$ was considered a significant difference. GraphPad

Prism 6.0 and SPSS 18.0 were used for data analysis and graphing. For overall survival analyses, the Kaplan-Meier method was used. NSCLC patients in the TCGA database were categorized into “high” or “low” expression group using the RBM5 cutoff value FKPM = 4.8.

Results

The structure and expression of miR-483-5p mRNA in lung cancer tissues

The precursor miRNA stem-loop structure of miRNA-483 with its two mature strands (miR-483-5p and miR-483-3p) is shown in **Figure 1A**. The expression levels of miR-483-5p mRNA in the lung cancer tissues and the adjacent paracancerous tissues from 40 lung cancer patients were examined using quantitative real-time PCR. In total, 92.5% (37/40) of the lung cancer patients exhibited significantly increased miR-483-5p mRNA expression in lung cancer tissues compared with the corresponding paracancerous tissues (**Figure 1B**). The patient clinical parameters are summarized in **Table 1**.

Effect of miR-483-5p on the apoptosis of A549 cells

To study the effect of miR-483-5p on A549 cell apoptosis, we examined the transfection efficiency of miR-483-5p using quantitative real-time PCR. Compared with the A549 group, the A549 cells transfected with miR-483-5p mimic exhibited remarkably increased miR-483-5p expression. A549 cells transfected with miR-483-5p inhibitor exhibited significantly reduced miR-483-5p miRNA expression (**Figure 2A**). Flow cytometry analysis revealed that the

Table 1. Patient characteristics

Characteristics	N (%)
Sex	
Male	22 (55.0%)
Female	18 (45.0%)
Median age	58.6
Tumor location	
Left Lung	25 (62.5%)
Right Lung	15 (37.5%)
Tumor size (cm)	
≤ 2	18 (45.0%)
$> 2, \leq 5$	20 (50.0%)
> 5	2 (5.0%)
Smoking habits	
Nonsmoker	13 (32.5%)
Smoker	27 (67.5%)
Histology	
Adenocarcinoma	26 (65%)
Squamous cell carcinoma	13 (32.5%)
Large-cell carcinoma	1 (2.5%)
Histological type	
Well differentiated	31 (77.5%)
Poorly differentiated	9 (22.5%)
TNM stage	
I-II	32 (80.0%)
III-IV	8 (20.0%)

TNM tumor, nodule, metastasis.

Public database mining

An online public database was used for prediction of miRNAs that had RBM5 as a binding target (MicroCosm Targets Version 5, <https://www.ebi.ac.uk/>) [11]. Survival and protein expression with immunohistochemical staining analysis were obtained from Human Protein

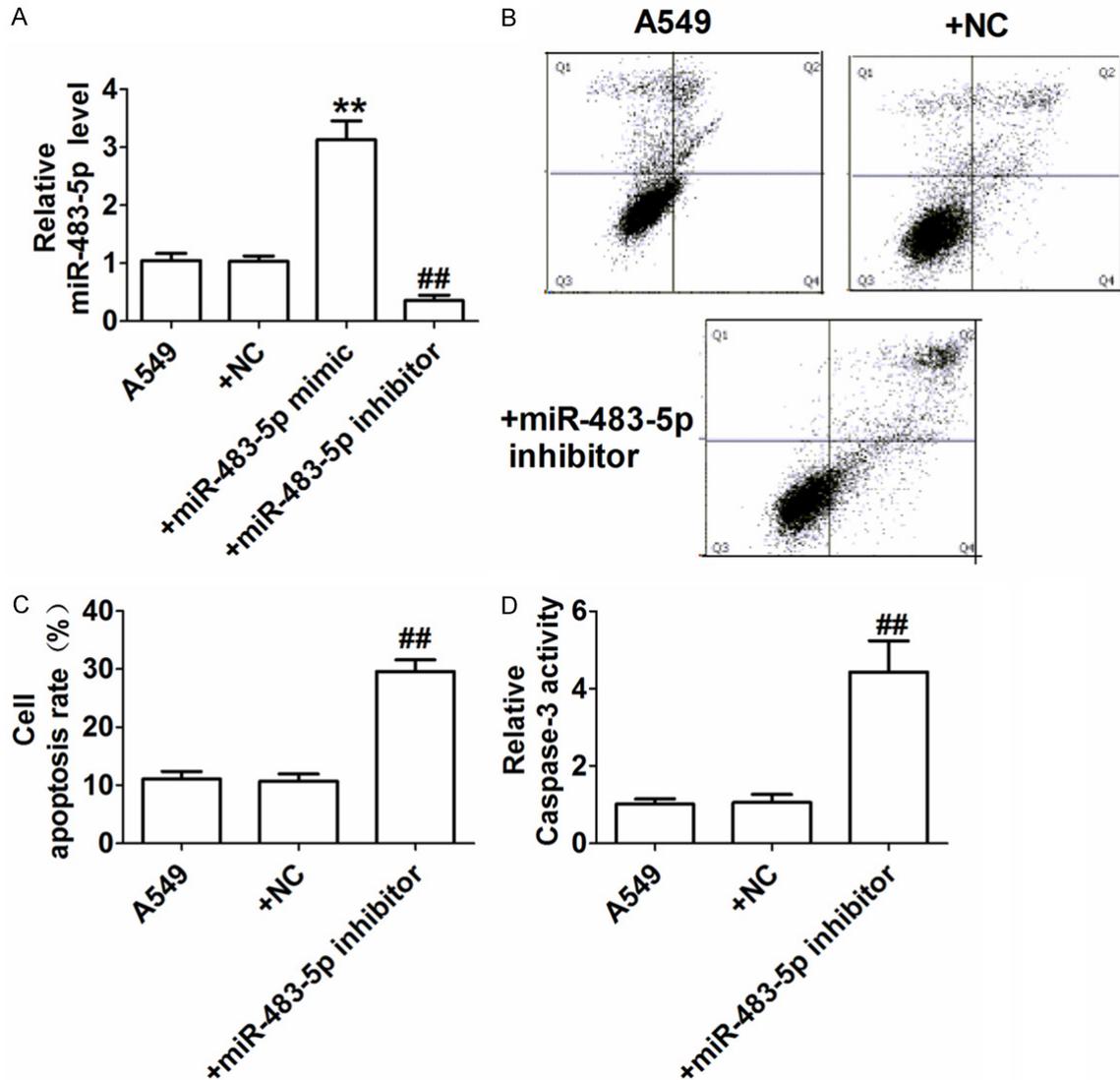


Figure 2. Effect of miR-483-5p on A549 cell apoptosis. A: Expression of miR-483-5p detected by quantitative real-time PCR; B: Representative scatter plot from apoptosis assay in A549 cells via flow cytometry; C: Apoptotic rate (%); D: Caspase-3 activity detected by colorimetric method. ** $P < 0.01$ and ## $P < 0.01$ vs. A549 NC group.

apoptosis rate of A549 cells was significantly increased after transfection with miR-483-5p inhibitor (Figure 2B, 2C). In addition, A549 cells showed significantly increased activity of the key protein kinase in the process of apoptosis, caspase-3 [13], after transfection with miR-483-5p inhibitor, which was consistent with the results from flow cytometry (Figure 2D). These results demonstrated that miR-483-5p inhibitor promoted A549 cell apoptosis.

Upregulation of RBM5 in A549 cells by miR-483-5p

RBM5 is a target gene of miR-483-5p [10]. However, whether miR-483-5p regulates RBM5 in A549 cells remains unclear. We used west-

ern blotting to detect the expression levels of RBM5 protein in A549 cells transfected with miR-483-5p mimic, miR-483-5p inhibitor, and miRNA NC. Compared with the A549 group, RBM5 protein expression was reduced in A549 cells transfected with miR-483-5p mimic and was increased in A549 cells transfected with miR-483-5p inhibitor (Figure 3). Those findings confirmed that RBM5 is a target of miR-483-5p.

Effect of miR-483-5p on apoptosis of A549 cells through targeting RBM5

To investigate the role of miR-483-5p in RBM5 regulation, we examined RBM5 mRNA expression in A549 cells transfected with RBM5

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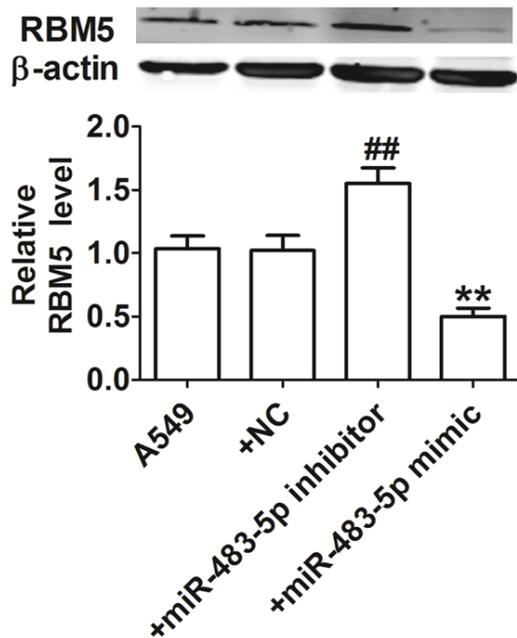


Figure 3. Effect of miR-483-5p on RBM5. β -actin serves as an internal control. The intensity of the band was assessed by ImageJ. ** $P < 0.01$ and ## $P < 0.01$ vs. A549 NC group.

siRNA or miR-483-5p inhibitor plus RBM5 siRNA via qRT-PCR. Compared with the A549 group, RBM5 mRNA was significantly decreased in A549 cells transfected with RBM5 siRNA or miR-483-5p inhibitor and RBM5 siRNA (Figure 4A). RBM5 induces apoptosis in lung cancer cells [7]. To investigate the effect of miR-483-5p on A549 cell apoptosis by targeting RBM5, we examined cell apoptosis in each group using flow cytometry and caspase-3 activity assays. A549 cells transfected with miR-483-5p inhibitor exhibited significantly reduced apoptosis rates, whereas the apoptosis rate of A549 cells transfected with miR-483-5p inhibitor and RBM5 siRNA was significantly reduced compared with cells transfected with miR-483-5p inhibitor alone (Figure 4B, 4C). The caspase-3 activity of A549 cells transfected with the miR-483-5p inhibitor was significantly increased, whereas the caspase-3 activity of A549 cells transfected with miR-483-5p inhibitor and RBM5 siRNA was significantly reduced compared with cells transfected with the miR-483-5p inhibitor alone (Figure 4D). The above results indicate that the miR-483-5p inhibitor promotes A549 cell apoptosis by targeting RBM5.

Identification of potential miRNAs targeting on RBM5 and RBM5-NSCLC patients' survival analysis (from TCGA Database)

We use MicroCosm Targets Version 5 (<https://www.ebi.ac.uk/>) to identify potential miRNAs which may have the target on RBM5. 61 miRNAs were found to have the match result including miR-483-5p (Figure 5A). The details of those miRNAs are shown in Supplementary Table 1. In addition, we found that RBM5 is a favored gene for NSCLC patients (TCGA database, total patient number = 994) with the cut-off FKPM value = 4.8 (Figure 5B, 5C).

Correlation between RBM5 and its IHC expression pattern in NSCLC patient from (HPA)

We obtained the IHC expression data from HPA for normal lung tissue and representative NSCLC patients. The IHC result shows us that in both normal lung tissue (Figure 6A) and NSCLC tumor tissue (lung adenocarcinoma and lung squamous cell carcinoma) the nuclear expression was the same. Also, the high and low expression level pattern in NSCLC patients could be used as a prognostic prediction biomarker (Figure 6B-E).

Discussion

Non-small cell lung cancer (NSCLC) is the most common lung cancer. Although the diagnosis and treatment of lung cancer continue to improve, the mortality rate remains high [1]. Therefore, it is of utmost importance to study the mechanism underlying the tumorigenesis and progression of lung cancer and identify gene targets for its diagnosis and treatment. In recent years, many studies have demonstrated that microRNAs are involved in the development and progression of lung cancer. A comprehensive analysis indicated that miR-21-5p and miR-30d-5p could be used as tumor markers of lung cancer. Upregulation of miR-483-5p is associated with the development and progression of lung cancer and promotes the metastasis and invasion of lung cancer cells through targeting RhoGDI1 and ALCAM [6]. In this study, we compared miR-483-5p mRNA expression in lung cancer tissues and corresponding paracancerous tissues from NSCLC patients and found significantly increased miR-483-5p mRNA in lung cancer. The above findings demonstrated that miR-483-5p is proba-

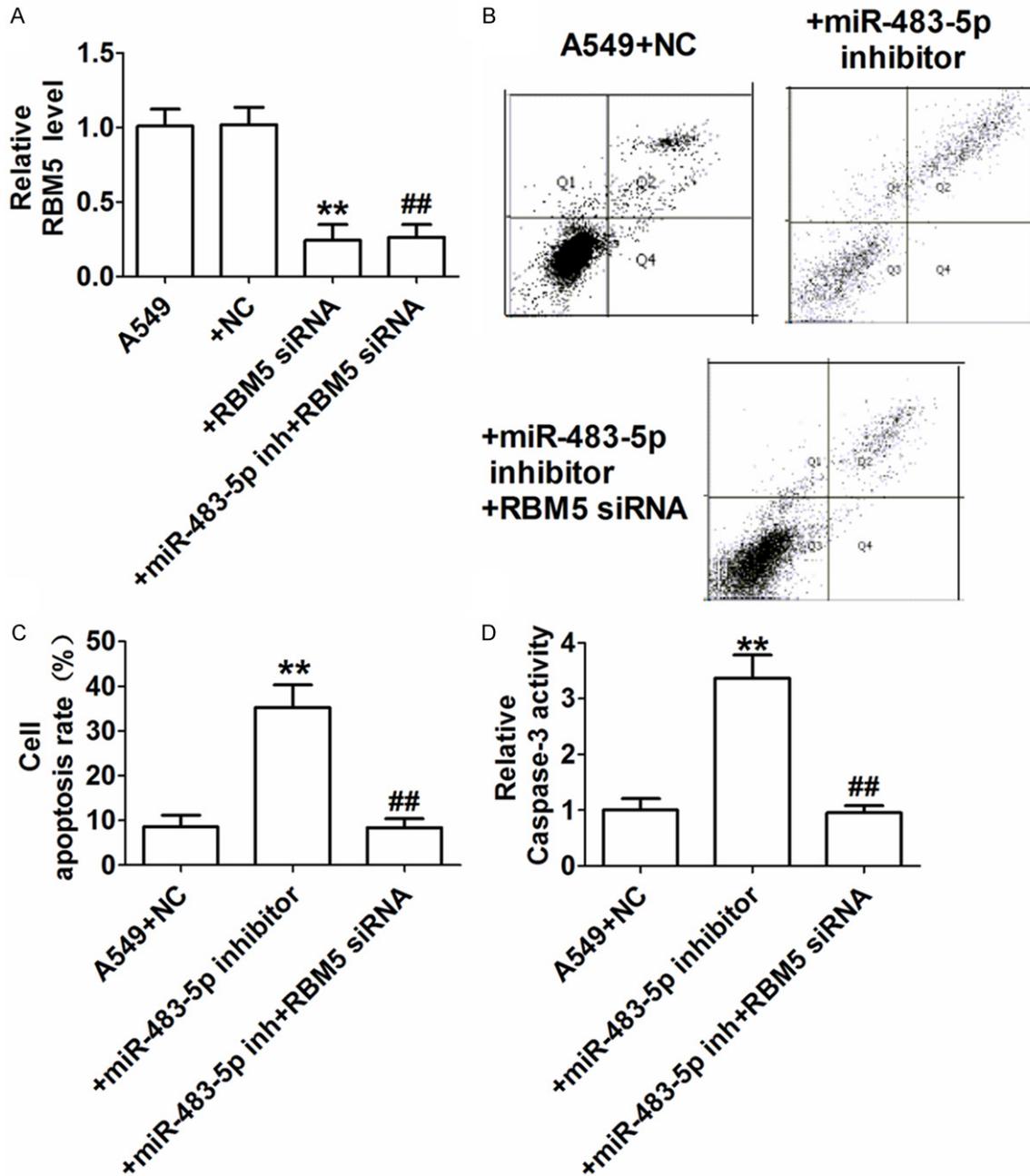


Figure 4. Effect of miR-483-5p on A549 cell apoptosis through targeting RBM5. A: RBM5 levels determined by qRT-PCR. **P<0.01 and ##P<0.01 compared with the A549 group. B: Representative scatter plot of the apoptosis of A549 cells from flow cytometry. C: Apoptosis rate (%). **P<0.01 vs. A549+NC group, ##P<0.01 vs. A549+miR-483-5p inhibitor group. D: Caspase-3 activity determined by colorimetric assay. **P<0.01 vs. A549+NC group, ##P<0.01 vs. A549+miR-483-5p inhibitor group.

bly involved in lung cancer tumorigenesis, and its specific mechanism awaits further studies.

Tumorigenesis results from imbalanced cell proliferation and apoptosis [14]. Inducing tumor cell apoptosis has become one of the important anti-tumor approaches [15]. Although

NSCLC is sensitive to certain chemotherapy and target therapy, the use of drugs has been limited due to their serious adverse reactions and high risk of refractory cancer [16]. miRNAs have received increasing attention given their anti-tumor activity by inducing tumor cell apoptosis [5]. MiR-107-5p promotes NSCLC cell

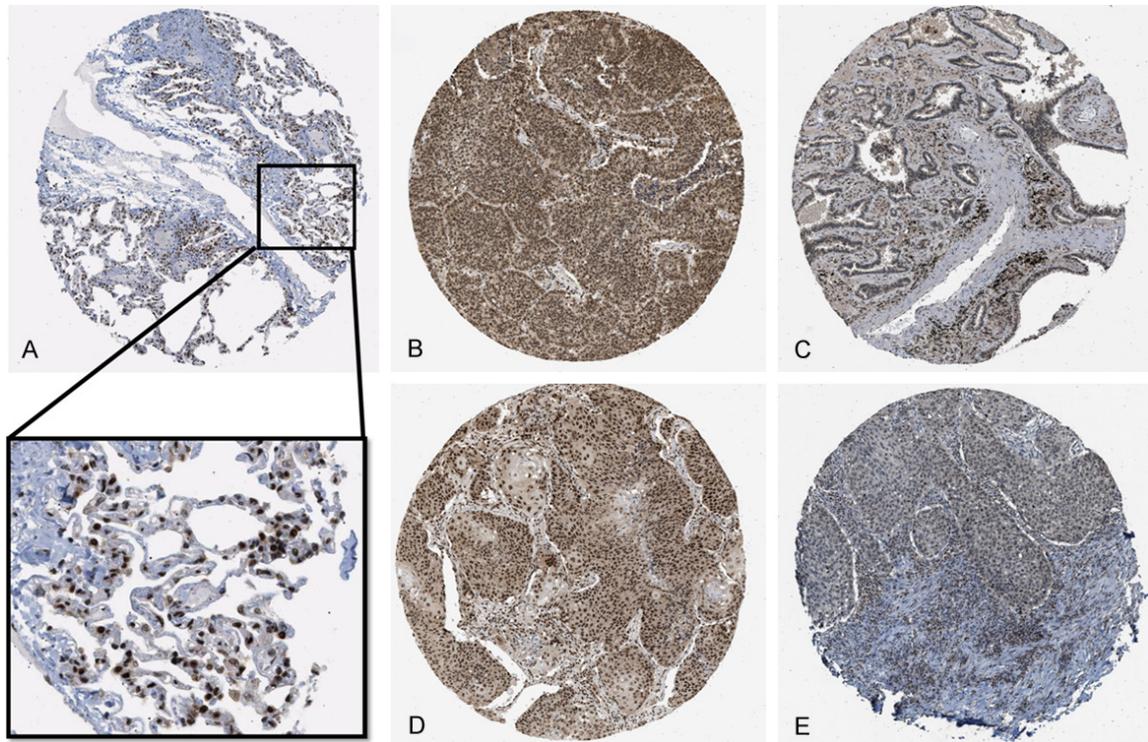


Figure 6. IHC expression pattern of RBM5 in normal lung tissue and NSCLC from HPA database. A: Normal lung tissue expression of RBM5: the magnified area shows a strong expression pattern in the nuclei of pneumocytes. B: Representative strong expression of RBM5 IHC in lung adenocarcinoma. C: Representative weak expression in lung adenocarcinoma. D: Representative strong expression in lung squamous cell carcinoma. E: Representative weak expression in lung squamous cell carcinoma.

will increase our patient numbers and obtain more survival-related data to analyze the correlations between prognosis and clinical parameters in the future. However, we hope our findings can provide a potential new target for diagnosis and treatment of NSCLC using miR-483-5p to influence RBM5 in the future.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Identify potential miRNAs target on RBM5 by MicroCosm Targets Version 5

Rfam ID	Score	Energy	Base P	Poisson P	Org P	Start	End
hsa-miR-708*	19.7566	-27.34	1.206870e-03	1.206140e-03	1.206140e-03	443	465
hsa-miR-30b	19.1088	-17.81	3.551940e-03	3.551940e-03	3.551940e-03	261	282
mmu-miR-743b-3p	19.1088	-16.89	6.585360e-03	6.585360e-03	8.905870e-04	371	392
hsa-miR-151-3p	18.8798	-19.4	1.671550e-03	1.670150e-03	7.352520e-04	442	462
hsa-miR-220c	18.7849	-25.33	6.739570e-03	6.716910e-03	2.321480e-03	390	411
hsa-miR-30c	18.3729	-17.24	6.535670e-03	4.544030e-03	4.544030e-03	260	282
hsa-miR-499-3p	18.2451	-19	5.360300e-03	5.345960e-03	5.345960e-03	367	388
hsa-miR-938	18.2451	-21.56	2.982260e-03	2.977820e-03	3.621500e-04	65	86
hsa-miR-140-5p	18.2451	-22.92	5.230800e-03	3.462110e-03	3.462110e-03	104	124
hsa-miR-578	18.1202	-24.04	8.773450e-03	8.735080e-03	7.653250e-03	19	39
mmu-miR-466e-5p	17.5974	-18.3	1.974680e-02	2.829100e-03	1.660680e-03	409	431
hsa-miR-7-1*	17.4894	-15.13	1.846800e-02	1.846800e-02	1.846800e-02	360	381
mmu-miR-705	17.4531	-26.73	5.971710e-02	5.796900e-02	2.559340e-02	131	150
hsa-miR-568	17.4531	-17.41	3.060590e-02	3.014230e-02	7.623440e-05	404	423
mmu-miR-694	17.4416	-13.94	3.674870e-02	3.608170e-02	8.266160e-04	440	457
mmu-miR-743a	17.3815	-12.28	2.395220e-02	2.366760e-02	8.905870e-04	371	392
mmu-miR-466a-5p	17.3815	-17.86	2.534490e-02	2.829100e-03	1.660680e-03	409	431
mmu-miR-466c-5p	17.2735	-17.79	2.654290e-02	2.829100e-03	1.660680e-03	409	431
mmu-miR-466b-5p	17.2735	-18.3	2.601580e-02	2.829100e-03	1.660680e-03	409	431
hsa-let-7a*	17.2522	-23.1	2.207760e-02	1.000000e-06	1.000000e-06	226	246
hsa-miR-641	17.2197	-13.47	2.429780e-02	2.400500e-02	2.237180e-03	368	391
hsa-miR-423-5p	17.191	-21.85	3.781790e-02	3.711170e-02	3.711170e-02	292	316
hsa-miR-30e	17.1655	-10.83	2.424060e-02	4.544030e-03	4.544030e-03	261	282
hsa-miR-30a	17.1655	-12.39	2.019310e-02	4.544030e-03	4.544030e-03	261	282
hsa-miR-323-3p	17.1437	-13.26	8.490540e-03	1.993120e-03	1.990160e-04	251	271
hsa-miR-206	17.0576	-18.51	2.777060e-02	2.738850e-02	2.738850e-02	303	323
hsa-miR-10b	16.8687	-21.32	1.207400e-02	1.207400e-02	1.207400e-02	117	138
hsa-miR-141*	16.8417	-13.99	3.023350e-02	2.978100e-02	1.561300e-02	431	452
hsa-miR-19a	16.7612	-13.16	3.106540e-02	3.106540e-02	2.556480e-04	400	422
hsa-miR-28-3p	16.7337	-14.97	1.524580e-02	1.513020e-02	1.872960e-03	442	464
hsa-miR-7-2*	16.6257	-12.43	3.089910e-02	3.042660e-02	3.042660e-02	360	381
hsa-miR-194	16.6257	-13.3	2.290410e-02	2.264380e-02	1.575790e-03	46	67
hsa-miR-371-5p	16.5804	-15.9	2.757080e-02	2.719420e-02	2.719420e-02	316	335
hsa-miR-574-5p	16.5463	-18.81	5.550450e-02	5.399220e-02	4.922530e-02	410	433
hsa-let-7f-1*	16.5178	-18.62	3.600690e-02	1.000000e-06	1.000000e-06	225	246
hsa-miR-125a-5p	16.471	-17.34	3.510410e-02	3.449510e-02	3.449510e-02	92	115
hsa-miR-182	16.471	-13.88	3.639680e-02	3.950610e-03	3.950610e-03	450	470
hsa-miR-221	16.4389	-14.82	3.075830e-02	3.029010e-02	3.029010e-02	252	274
hsa-miR-132	16.4098	-17.94	2.285870e-02	2.259940e-02	7.641820e-04	223	244
mmu-miR-290-5p	16.4098	-17.59	3.224690e-02	3.173250e-02	7.835090e-03	314	335
hsa-miR-30b	16.4098	-15.3	3.615520e-02	4.544030e-03	4.544030e-03	226	247
hsa-miR-15a*	16.4098	-18.09	3.991920e-02	3.913290e-02	3.913290e-02	170	193
hsa-miR-378	16.3842	-16.4	3.651900e-02	3.586020e-02	3.565170e-02	443	463
hsa-miR-30d	16.3019	-11.46	3.615340e-02	4.544030e-03	4.544030e-03	261	282
mmu-miR-467e	16.3019	-13.7	5.730700e-02	5.569590e-02	1.330220e-02	412	434
hsa-miR-489	16.3019	-20.56	2.473160e-02	2.442830e-02	2.442830e-02	368	390
hsa-miR-422a	16.3019	-16.54	3.935780e-02	3.859330e-02	3.859330e-02	442	463
mmu-miR-669b	16.3019	-16.47	7.163400e-02	6.912850e-02	1.063610e-02	421	442

miR-483-5p regulates NSCLC apoptosis through RBM5

hsa-miR-523	16.224	-19.33	7.510560e-03	7.482430e-03	7.482430e-03	257	279
hsa-miR-597	16.1939	-18.62	2.853030e-02	2.812720e-02	2.812720e-02	337	358
hsa-miR-483-5p	16.1939	-19.56	4.333250e-02	4.240710e-02	2.684530e-02	55	76
hsa-miR-30c	16.1166	-18.96	4.557700e-02	4.544030e-03	4.544030e-03	225	247
hsa-let-7f-2*	16.0859	-23.31	4.756710e-02	1.000000e-06	1.000000e-06	225	246
hsa-miR-139-5p	16.0859	-17.84	3.916460e-02	3.840760e-02	1.196310e-02	322	344
hsa-miR-122	16.0859	-19.92	5.868850e-02	5.699950e-02	7.693060e-03	228	252
hsa-miR-222	16.0586	-13.25	3.519270e-02	2.619020e-03	2.619020e-03	254	274
hsa-miR-103	16.0091	-13.45	6.562630e-02	6.351920e-02	5.060710e-03	320	342
hsa-miR-10a	16.0091	-20.38	3.347260e-02	3.291860e-02	3.291860e-02	117	138
hsa-miR-125b-2*	15.978	-20.19	4.404400e-02	1.431440e-03	1.431440e-03	75	96
hsa-let-7b*	15.978	-16.05	4.098600e-02	1.000000e-06	1.000000e-06	225	246
hsa-miR-448	15.978	-18.22	6.458340e-02	6.254210e-02	8.293750e-04	267	289
hsa-miR-581	15.9501	-17.93	5.561530e-02	5.409700e-02	3.804150e-02	17	37
hsa-miR-625	15.9501	-16.57	9.789560e-02	4.490230e-03	4.490230e-03	148	167
hsa-miR-625	15.9501	-17.75	9.789560e-02	4.490230e-03	4.490230e-03	124	144
hsa-miR-624	15.9501	-16.93	7.486800e-02	7.213400e-02	2.851940e-02	76	95
hsa-miR-19b	15.9017	-11.53	5.681900e-02	5.523490e-02	2.556480e-04	400	422
hsa-miR-125b-2*	15.7621	-24.53	5.448420e-02	1.431440e-03	1.431440e-03	196	216
hsa-miR-363	15.7621	-11.84	4.781370e-02	4.668860e-02	2.999670e-02	249	269
hsa-let-7a*	15.7331	-5	7.506170e-02	1.000000e-06	1.000000e-06	402	423
hsa-miR-573	15.7223	-11.62	9.268000e-02	8.851490e-02	3.088200e-02	105	127
hsa-let-7f-1*	15.6541	-12.02	7.291150e-02	1.000000e-06	1.000000e-06	391	413
hsa-miR-212	15.6246	-13.74	4.961960e-02	4.840870e-02	1.514810e-03	224	244
hsa-miR-182	15.5084	-15.05	9.163510e-02	3.950610e-03	3.950610e-03	378	401
hsa-miR-187*	15.4382	-20.81	4.466080e-02	4.367820e-02	4.367820e-02	282	303
hsa-miR-140-3p	15.4076	-18.86	8.561200e-02	3.462110e-03	3.462110e-03	283	303
mmu-miR-466f-5p	15.3302	-21.78	7.717470e-02	2.829100e-03	1.660680e-03	266	288
hsa-miR-222	15.2991	-19.54	7.418050e-02	2.619020e-03	2.619020e-03	457	477
hsa-miR-551b	15.2991	-18.81	4.878480e-02	4.878480e-02	1.371850e-02	158	178
hsa-miR-532-5p	15.2223	-18.9	9.010240e-02	8.616240e-02	1.220840e-02	69	88
hsa-let-7b*	15.1143	-4.91	8.572380e-02	1.000000e-06	1.000000e-06	402	423
hsa-miR-885-5p	15.1143	-13.88	6.352070e-02	6.154530e-02	1.529340e-03	250	271
hsa-miR-30d	15.1143	-15.21	9.850000e-02	4.544030e-03	4.544030e-03	228	247
hsa-miR-323-3p	15.0821	-13.78	6.450520e-02	1.993120e-03	1.990160e-04	367	388
hsa-miR-365	15.0064	-9.56	5.455920e-02	5.309750e-02	4.090480e-02	68	89
hsa-let-7d*	15.0064	-20.3	4.145360e-02	1.000000e-06	1.000000e-06	331	352
hsa-miR-100	15.0064	-16.99	3.207400e-02	3.156510e-02	7.920160e-04	154	176
mmu-miR-463	15.0064	-14.67	8.764440e-02	8.391340e-02	1.241210e-03	286	308
hsa-miR-551a	14.9736	-19.46	7.006370e-02	6.766560e-02	1.371850e-02	158	178
hsa-miR-369-5p	14.4666	-11.88	5.133330e-02	1.588800e-03	8.026500e-04	28	49
hsa-let-7d*	14.4666	-18.64	7.021790e-02	1.000000e-06	1.000000e-06	224	246
hsa-let-7i*	14.3586	-17.39	8.965440e-02	1.000000e-06	1.000000e-06	391	413
hsa-miR-369-5p	14.3586	-12.86	5.745760e-02	1.588800e-03	8.026500e-04	287	308
hsa-let-7i*	14.2506	-20.04	9.997960e-02	1.000000e-06	1.000000e-06	78	98
hsa-miR-99a	14.1427	-12.61	8.309590e-02	7.973710e-02	8.334790e-04	154	176

Hsa-miR-483-5p is highlighted in the Yellow background. * = Previous mature sequence ID.