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MiR-144 Inhibits Proliferation and Induces Apoptosis and Autophagy in Lung Cancer Cells by Targeting TIGAR

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Key Words
Lung cancer cell • MiR-144 • TIGAR • Proliferation • Apoptosis

Abstract
Background: MiRNAs are noncoding RNAs of 20–24 nucleotides that function as post-transcriptional negative regulators of gene expression. MiRNA genes are usually transcribed by RNA polymerase II in the nucleus. Their initial products are pre-miRNAs which have cap sequences and polyA tails. The p53-induced glycolysis and apoptosis regulator (TIGAR) was discovered through microarray analysis of gene expression following activation of p53. However, little is known about the effect of miR-144 on cell proliferation and apoptosis and how it interacts with TIGAR. ***Methods:*** We performed real-time PCR, western blotting, CCK8, colony formation, tumor growth, flow cytometry, Caspase3/7 activity, Hoechst 33342 staining, MDC staining of autophagic cells and luciferase reporter assays to detect the influence of miR-144 to lung cancer cells. ***Results:*** miR-144 targeted TIGAR, inhibited proliferation, enhanced apoptosis, and increased autophagy in A549 and H460 cells. ***Conclusions:*** Our study improves our understanding of the mechanisms underlying lung cancer pathogenesis and may promote the development of novel targeted therapies.

Introduction

MiRNAs are noncoding RNAs of 20–24 nucleotides that function as post-transcriptional negative regulators of gene expression [1]. It is now well known that miRNAs regulate the expression of multiple target genes and affect a variety of cellular pathways, specifically in cancer development and progression [2, 3]. MiR-144, sharing the same locus with miR-

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451[4], has been reported in many cancers. A report from Zhang et al. claimed that miR-144 promoted proliferation, migration, and invasion of nasopharyngeal carcinoma through repression of phosphatase and tensin homolog (PTEN) [5]. Sureban et al. also showed that knockdown of doublecortin and CaM kinase-like-1 (DCAMKL-1) increased miR-144 expression, which in turn inhibited epithelial-mesenchymal transition (EMT) of pancreatic cancer [6]. These data impelled us to further study the relative mechanism of miR-144 acting on lung cancer behavior.

Lung cancer is a major cause of cancer-related death worldwide. Although the diagnosis and therapy are in advances and the prognosis is encouraging, the number of cases and deaths related to lung cancer is still rising in many parts of the world. Lung cancer consisted of multiple sequential steps that are not completely understood to date, more investigation of this mechanism is urgently needed. Altered expression of miRNAs has been observed in lung cancer, suggesting that miRNA deregulation plays a role in lung carcinogenesis. For example, miR-145 inhibited lung cancer cell metastasis and EMT via targeting the Oct4 mediated Wnt/β-catenin signaling pathway [4]. Lan et al. elucidated that miRNA-15a/16 can enhance radiation sensitivity by regulating the TLR1/NF-κB signaling pathway and act as a potential therapeutic approach to overcome radioresistance for lung cancer treatment [7]. Data from Zha et al. have showed miR-144 exerted direct regulatory roles on Zinc finger X-chromosomal protein (ZFX) expression, which seems to be associated with tumorigenesis [8]. Our previously studies also found that the expression of miR-144 in these tissues was lower than in adjacent, paired non-tumor tissues by a miRNA chip-based expression analysis of lung cancer tissues.

Relevant bioinfomatics were analyzed and we hypothesized that miR-144 interacted with the p53-induced glycolysis and apoptosis regulator (TIGAR). TIGAR was discovered through microarray analysis of gene expression following the activation of p53 [9]. It is a p53-induced gene that can reduce the level of fructose2, 6-bisphosphate in cells, resulting in inhibition of glycolysis, and plays a crucial role in apoptosis [10, 11]. TIGAR overexpression has been described in several types of tumor. Its expression is increased in primary colon cancer and associated metastases [12], as well as in invasive breast cancer when compared to normal tissue [13]. TIGAR is also overexpressed in glioblastoma [14].

The effect of miR-144 on lung cancer cells and how it interacts with TIGAR remain unknown. In the present study, we observed miR-144 expression levels in tissues from 67 lung cancer patients, and investigated the function of miR-144 in lung cancer cells.

Materials and Methods

Patients and tissue specimens

A total of 67 cases of lung cancer cases were examined. All specimens were clinically and histologically diagnosed at the First Affiliated Hospital of Zhengzhou University between 2012 and 2013 and they were quick frozen in liquid nitrogen. This study was approved by the Ethics Committee of Zhengzhou University and written consent was obtained from all patients.

RNA extraction

For lung cancer tissues, if the proportion of lung cancer cells in a tissue section was >80% then the frozen block was subjected to RNA extraction. According to the manufacturer's protocol, total RNA was extracted from 67 pairs of snap-frozen cancer tissues and adjacent normal tissues using TRIzol reagent (Invitrogen, CA, USA). For lung cancer cell lines, total RNA was extracted with an RNA Extraction Kit (Qiagen). The integrity of the RNA was evaluated by a Nanodrop ND-1000 (Thermo Scientific, Worcester, USA). The value of OD_{260}/OD_{280} is around 1.8 as a criterion of acceptable purity.

Real-time fluorescence quantitative polymerase chain reaction analysis

Quantitative real-time PCR was performed to quantitate miR-144 expression, using SYBR Premix Ex TaqII (Tli RNaseH Plus) (TaKaRa, Dalian, China) in an ABI 7500 fast system (Applied Biosystems, Carlsbad,

CA, USA). U6 small nuclear RNA (snRNA) for miR-144 or GAPDH for TIGAR was used as an endogenous control. All samples were analyzed three times. The qRT-PCR results were expressed relative to miR-144 expression levels at the threshold cycle (Ct), which were then converted to fold changes ($2^{-\Delta\Delta Ct}$).

Cell lines and reagents

A549 and H460 human lung cancer cell lines were purchased from American Type Culture Collection (Manassas, VA, USA). Both cell lines were cultured with Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA), which contained 10% fetal bovine serum (FBS; Gibco, USA), 100 IU/ml penicillin and 100 IU/ml streptomycin, and the cells were incubated at 37°Cunder 5% CO_2 atmosphere.

Transfection with miRNAs

The miR-144 agomir (GMR-miRmicroRNA-144 agomir) and miR-144 scramble were synthesized by Shanghai GenePharma Co. Ltd. UntreatedA549 and H460 cells, growing exponentially, were plated at 2×10^7/well in 2.5 ml DMEM medium for 24 h on six-well plates. Once cells reached about 50% confluence, transfection was conducted. BTX ECM 2001(Nature gene, New Haven, CT, USA) was used in all transfection processes according to the manufacturer's instructions. Each cell line was separated into three groups: the non-transfected blank group (blank); scrambled miR-144 transfected negative control group (scramble); and the miR-144 agomir transfected group (miR-144).

Western blot analysis

The total protein content of cultured cells was extracted using RIPA buffer containing phenylmethanesulfonylfluoride. A BCA protein assay kit (Beyotime, Haimen, China) was used to determine the protein concentration. Proteins were subjected to SDS-PAGE and transferred onto polyvinylidenedifluoride membranes. After blocking with 5% skim milk for 1 h at 37˚C, the membrane was incubated with primary antibodies, polyclonal rabbit anti-TIGAR (1:300) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°Covernight. To detect autophagy, the markers of autophagic vacuoles, microtubule-associated protein 1 light chain 3 (LC3) and Beclin 1 were used, and so polyclonal rabbit anti-LC3 I and anti-LC3 II (1:200), polyclonal rabbit and polyclonal rabbit anti- Beclin-1(1:300) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were also incubated at 4°Covernight. The membranes were washed three times with Tris-buffered saline and Tween 20 (TBST) and incubated with diluted (1:3000) horseradish-peroxidase -conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology) for 1 h at room temperature. After three washes in TBST, the films of immunoreactive products were scanned using the Super Signal Substrate Western blotting detection system (Pierce, Rockford, IL, USA). An antibody against GAPDH (Santa Cruz Biotechnology) served as an endogenous reference.

Cell growth assay

Cell Counting Kit (CCK8; Dojindo, Japan) was used to measure cell proliferation. Cells were cultured in 96-well plates (100μl/well)in complete DMEM and were maintained in an incubator at 37°C and5% CO_2.CCK solution(10μl) was added to each well. Optical density (OD) value was measured daily over four consecutive days at 490 nm (OD_{490}) to estimate viable cell numbers. The assay was repeated in three independent experiments.

Colony formation assay

Cells were trypsinized, counted and propagated after transfection, and then cultured in six-well plates at 100 cells/well. The number of colonies was counted at 12daysafter seeding. The colonies formed were stained with crystal violet, and the colonies with >50 cells were scored as surviving colonies. This experiment had three replicates.

In vivo tumor growth assay

Tumor heterotransplantation was performed to investigate tumorigenic ability of miR-144 *in vivo*. A549 cells transfected with miR-144(miR-144 group) or with scrambled miRNA (scramble group) and A549 cells (blank group) were subcutaneously inoculated into the dorsal flank of 6-week-old female BALB/c nude mice that were purchased from The Laboratory Animal Unit of the University of Zhengzhou. Animal handling and experimental procedures were approved by the Animal Experimental Ethics Committee of the University of Zhengzhou. Each group contained five mice. Tumor size was measured every week for1 month. The mice were killed and tumors were removed, photographed and weighed. Tumor volumes were

calculated using the following equation: $(D \times d^2)/2$, where D means the longest diameter and d means the shortest diameter.

Flow cytometry assay

Cell cycle was synchronized for 24 h before collection and 20,000 cells were detected per group. The cells were suspended in 500 μl binding buffer and 10 μl AnnexinV-fluorescein isothiocyanate (FITC) and 5 μl propidium iodidewere added for 15 min. All the procedures were done according to the instructions of the FITC Annexin V Apoptosis Detection Kit I (BestBio, Shanghai, China).The cells were analyzed with a FACScan flow cytometer (BD Biosciences) equipped with Cell Quest software (BD Biosciences). Each treatment was carried out in triplicate.

Caspase3/7 activity assay

Cells from each treatment group were harvested at 48 h post-transfection and caspase activity was detected by Caspase-Glo 3/7 Assay kit (Promega, Shanghai, China). 100μl Caspase-Glo 3/7 Reagent was added to each well of the plates, followed by 1 min mixing using an IKA MTS4 plate shaker. Plates were incubated at room temperature for 2 h. Absorbance values were measured with a microplate reader at 405 nm (Infinite M200; Tecan, Mannedorf, Switzerland).

Hoechst 33342 staining assay

Cells in each group were seeded into a 96-well plate after transfection. Hoechst 33342 (Sigma, St Louis, MO, USA) was added to the culture medium at a final concentration of 5 μg/ml. The images were recorded on a computer with a digital camera attached to the microscope, and the images were merged by computer. For quantification of Hoechst 33342 staining, the percentage of Hoechst-positive nuclei per optical field (at least 50 fields) was counted. Apoptotic cells showed bright blue fluorescence.

MDC staining of autophagic cells

Each group of cells was plated on 24-well plates after transfection for 24 h. Exponentially growing cells were treated with 0.05 mM MDC (Sigma) in DMEM at 37°C for 10 min. After that, the cells were washed three times with PBS. The images were captured using a fluorescence microscope (Carl Zeiss, Jena, Germany) at an excitation wavelength of 380 nm and emission wavelength of 450 nm.

Bioinformatics analysis

miR-144 data of 67 pairs of primary tumors and normal lung tissues were downloaded from The Cancer Genome Atlas (TCGA) website () [15], and analyzed using Multi-Experiment Viewer (MeV) software (version 4.9, http://www.tm4.org/). The fold differences in miRNA expression were compared by paired t-test. The following online software was used to determine putative miR-144 targets: Target Scan 6.2 (http://targetscan.org) and miRanda (http://www.microrna.org/microrna/getGeneForm. do) [16, 17]. The function of TIGAR was investigated by DAVID bioinformatics (http://david.niaid.nih.gov).

Luciferase reporter assay

The human TIGAR 3′untranslated region (UTR) fragment containing putative binding sites for miR-144 were amplified by PCR from human genomic DNA. The mutant TIGAR 3′ UTRs were obtained by overlap extension PCR. The fragments were cloned into a pmirGLO reporter vector (Promega, Madison, WI, USA), downstream of the luciferase gene, to generate the recombinant vectors pmirGLO-WT and pmirGLO-MUT.

For the luciferase reporter assay, A539 cells were co-transfected with miRNA (miR-144 agomir or scrambled-miR-144 negative control) and reporter vectors (pmirGLO-WT reporter vectors or pmirGLO-MUT reporter vectors), using BTX ECM 2001 electroporator. Luciferase activities were measured with a Dual-Luciferase assay kit (Promega, Madison, WI, USA) according to manufacturer' s instructions at 24h post-transfection. Experiments were repeated three times in triplicate.

Statistical analysis

All data are expressed as means ± standard deviation. Statistical testing was analyzed using SPSS version 17.0 statistical software. The t-test and one-way analysis of variance were used to analyze data. $P< 0.05$ was the criterion for statistical significance.

Results

MiR-144 expression in lung cancer

To calculate relative miR-144 concentrations by fold changes ($2^{-\Delta\Delta Ct}$, ΔCt =Ct median miR-144 - Ct median GAPDH; ΔΔCt=ΔCt cancer -ΔCt normal), we found that miR-144 expression was significantly lower in lung cancer tissues than in paired adjacent normal tissues (**P* < 0.05). The clinicopathological characteristics of the 67 lung cancer cases are presented in Table 1. Of the 67 lung cancer patients, miR-144 expression was related to tumor size (<3cm: 0.41±0.124 vs. >3cm : 0.32±0.072, **P* < 0.01 ,Table 1). And miR-144 expression level showed significant differences in various TNM (**P* < 0.01, Table 1). MiR-144 expression was also lower in the lymph node metastasis-positive group than the negative group (0.31±0.069 vs. 0.42±0.116, respectively, **P* < 0.01, Table 1). While no significant differences were detected with CLPTM1L mRNA expression (*P* > 0.05, Table 1, Fig. 1F, J). No significant differences was observed between miR-144 expression and gender, age, smoking, differentiation or histology (*P* > 0.05, Table 1).

Table 1. Clinicopathological characteristics and miR-144 expression levels of 67 lung cancer patients *Indicated statistical significance (P < 0.05)

Clinicopathologic parameters	cases	miR-144 expression	*P* value
Gender			
Male	38	0.35±0.098	0.173
Female	29	0.39±0.126	
Age(years)			
＜50	24	0.37±0.093	0.853
＞50	43	0.36±0.122	
Smoking			
Smoker	24	0.37±0.107	0.697
Non-smoker	43	0.36±0.115	
Tumor size			
＜3cm	36	0.41±0.124	0.001*
＞3cm	31	0.32±0.072	
Differentiation			
well	28	0.38±0.116	0.651
moderate	25	0.37±0.122	
poor	14	0.34±0.083	
Histology			
Squamous carcinoma	24	0.35±0.121	0.463
Adinocarcinoma	36	0.38±0.109	
Others	7	0.33±0.094	
TNM			
I	25	0.44±0.132	0.000*
II&III	42	0.33±0.072	
Lymphnode status			
negative	35	0.42±0.116	0.000*
positive	32	0.31±0.069	

Upregulation of miR-144 inhibits proliferation of A549 and H460 cells

Expression of miRNA-144 and TIGAR after transfection in A549 and H460 cells is shown in Fig. 1A and 1B. Compared to the scramble group and blank group, miR-144 expression levels of miR-144 group were significantly higher in A549 and H460 cells, and TIGAR expression levels were significantly lower in the miR-144 group (**P* < 0.05, Fig.1A, 1B). Cell growth curves are presented in Fig. 1C. There were no significant differences in OD_{490} values between the blank and scramble groups (*P* > 0.05). Compared with the blank and scramble groups, the OD_{490} values for the miR-144 group at48, 72 and 96 h were all significantly decreased (**P* < 0.05) in A549 (Fig. 1C) and H460 (Fig. 1D) cells. Colony formation in the miR-144 group was lower than in the scramble and blank groups for A549 and H460 cells (**P* < 0.05, Fig. 1E, 1F). The result remains coordinate in tumor heterotransplantation experiment that the tumor volumes of miR-144 group cells were relatively lower than scramble group and blank group (**P* < 0.05, Fig. 1G, 1H).

Upregulation of miR-144 enhances apoptosis of A549and H460 cells

Flow cytometry indicated that the apoptosis rate in the miR-144 group was higher than in the scramble and blank groups for both A549 and H460 cells (**P* < 0.05, Fig. 2A, 2B). Caspase3/7 activity in miR-144 group was higher than in the scramble and blank groups for A549 and H460 cells, which also indicated that apoptosis rate in the miR-144 group was increased compared to the other groups (**P* < 0.05, Fig. 2C, 2D). In the Hoechst 33342 staining assay, similar results were observed by fluorescence microscopy for both A549 and H460 cells (**P* < 0.05, Fig. 2E, 2F).

Upregulation of miR-144 enhances autophagy of A549 and H460 cells

MDC staining was visualized by fluorescence microscopy. The number of autophagicvacuoles was increased in the miR-144 group compared to the scramble and

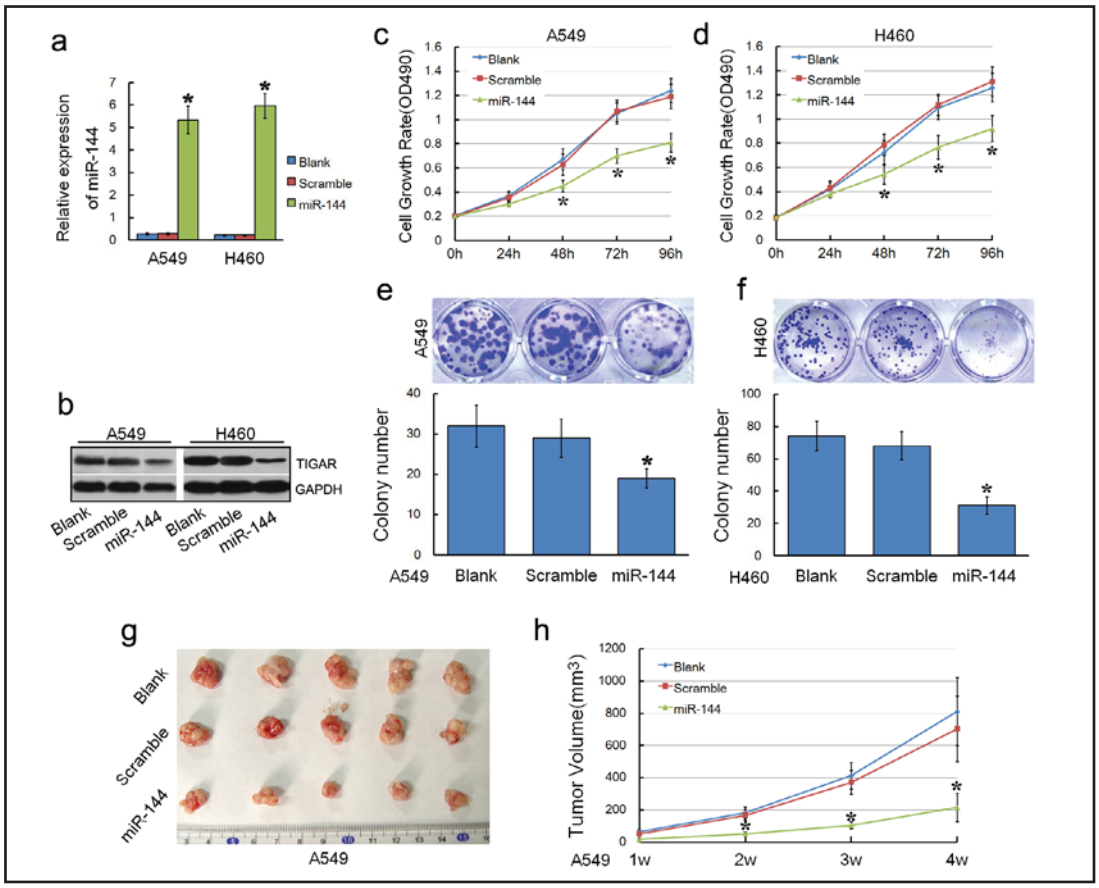

Fig. 1. miR-144 inhibits the proliferation level of A549 and H460 cells. (A, B) Compared to the scramble group and blank group in both A549 and H460 cells, the expression level of miR-144 was significantly higher and TIGAR was significantly lower in miR-144 group (*P < 0.05). (C, D) The cell growth rate (OD_{490}) of miR-144 group goes slower than scramble group and blank group both A549 and H460 cells. (E, F) After approximately 2 weeks of incubation, the colony formation number of miR-144 group cells came to lower colony than the scramble group and blank group in both A549 and H460 cells (*P < 0.05). (G, H) G: The tumor tissues were collected from athymic nude mouse after four weeks. H: The tumor volumes were measured every week. The tumor volumes of miR group cell were relatively lower than scramble group and blank group (*P < 0.05).

blank groups (*P < 0.05, Fig. 3A). In addition, LC3 I, LC3 II and Beclin 1, the markers of autophagic vacuoles, were detected by Western blotting. Compared to the scramble and blank groups, the relative expression levels of LC3 I protein in A549 and H460 cells was lower in the miR-144 group cells, and Beclin1 and LC3 II proteins were higher (*P < 0.05, Fig. 3B, 3C). These results indicated that upregulation of miR-144 can enhance autophagy of A549 and H460 cells.

TIGAR is a direct target of miR-144

Bioinformatics analysis predicted that the 3' UTRs of TIGAR contained binding sites for miR-144 (Fig. 4A). Both wild-type and mutant TIGAR 3' UTRs were shown in Fig. 4B. Cotransfection with miR-144 significantly suppressed luciferase activity of the reporter containing the wild-type 3' UTR (*P < 0.05, Fig. 4C). Our results indicate that miR-144 negatively regulates TIGAR expression by directly binding to putative binding sites in the 3' UTR.

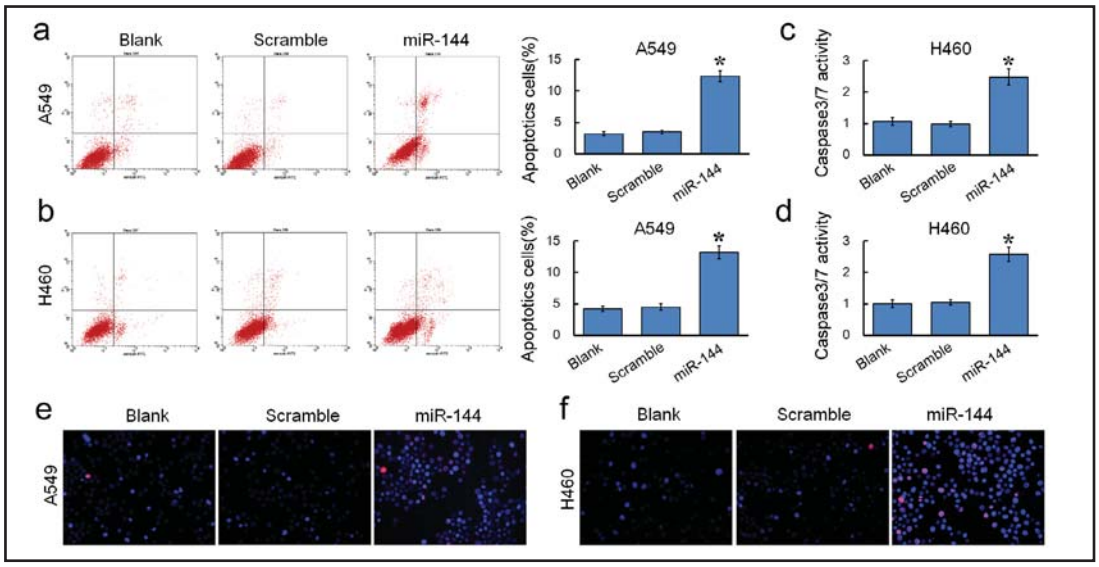

Fig. 2. miR-144 enhances the apoptosis rate of A549 cells and H460 cells. (A, B) Apoptosis rate in miR-144 group was higher than scramble group and blank group in A549 and H460 cells (*P < 0.05). (C, D) Caspase3/7 activity of miR-144 group went higher than that of scramble and blank group in A549 and H460 cells (*P < 0.05). (E, F) The hoechst 33342 staining assay come out that the apoptosis rate was higher in miR-144 group than the scramble group and blank group (*P < 0.05).

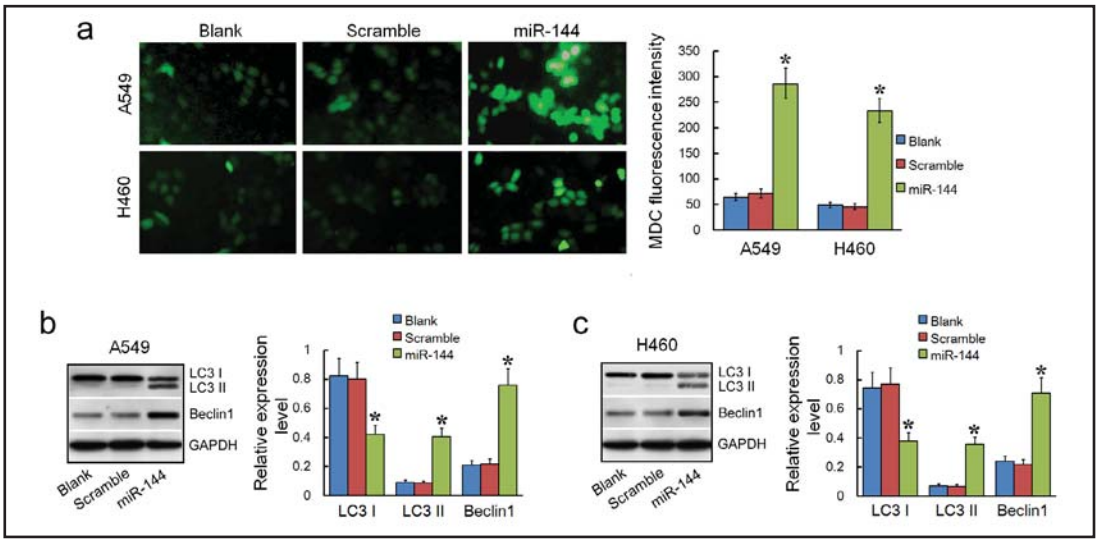

Fig. 3. miR-144 induces autophagy in both A549 and H460 cells. (A) Autophagic vacuoles of miR-144 group are much more than the scramble and blank group (*P < 0.05). (B, C) The expressions of autophagy-related proteins were detected by Western blotting. GAPDH was used as control. In both A549 and H460 cells, the expression levels of LC3-I was lower, and Beclin1 and LC3-II came out higher in miR-144 groups (*P < 0.05).

Function of miR-144 inA549 cells partially attributed to targeting TIGAR

We constructed si-TIGAR vector and transfected it into A549 cells (si-TIGAR group). By western blotting, we observed that expression of TIGAR protein in the si-TIGAR group was lower than in the miR-144 and blank group cells, and Beclin1 and LC3 II proteins were higher in A549 cells (Fig. 5A).The OD_{490} values of the si-TIGAR groupat48, 72 and 96 h were significantly decreased compared with the blank group cells, but increased compared with the miR-144 group cells (*P < 0.05,Fig. 5B).The number of colonies in the si-TIGAR group was significantly lower than in the blank group, but higher than in the miR-144 group (Fig.

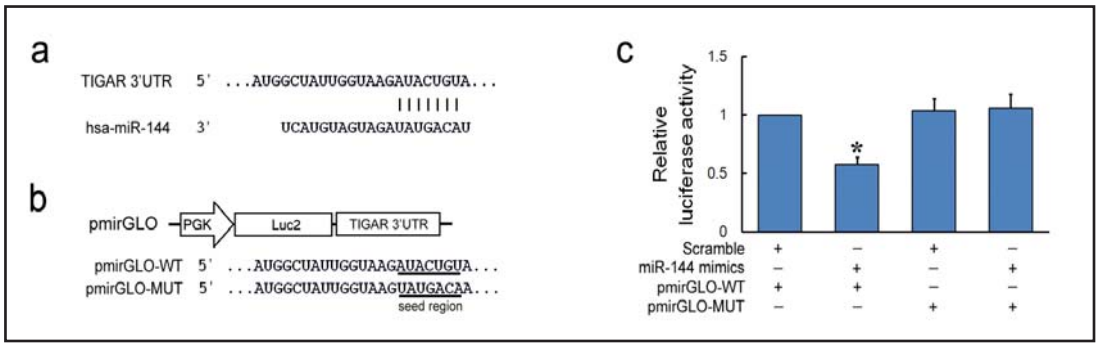

Fig. 4. miR-144 directly targets TIGAR. (A, B) Wild and mutant types of TIGAR 3'UTRs segments are showed. (C) Co-transfection with miR-144 significantly suppressed the luciferase activity of the reporter containing the wild-type 3' UTR (*P<0.05).

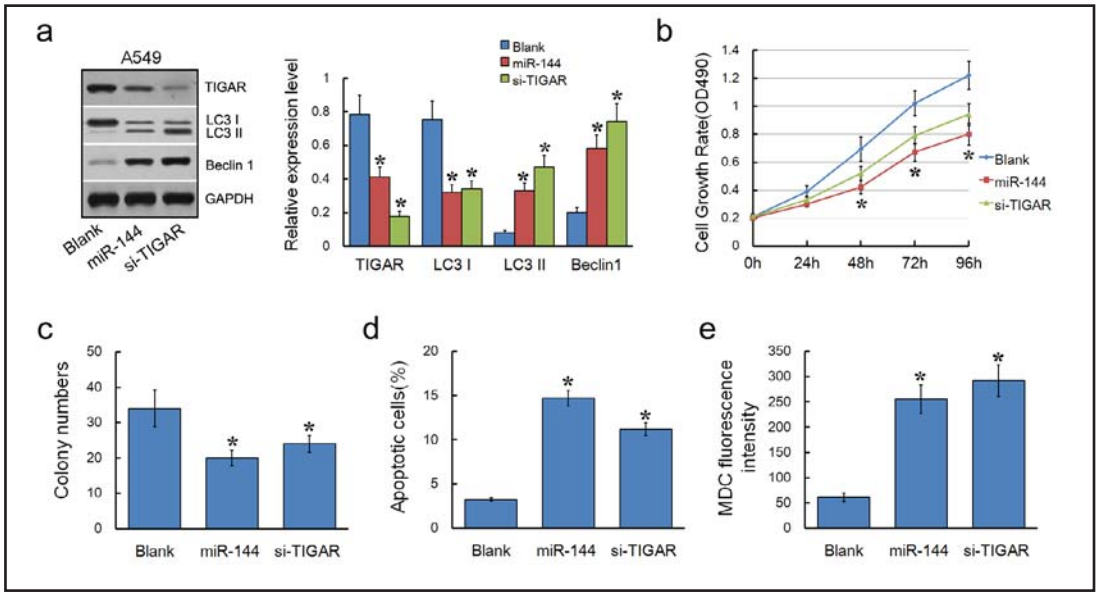

Fig. 5. Function of miR-144 partially attributes to targeting at TIGAR. (A) The expression of TIGAR protein of si-TIGAR group was lower than miR-144 and blank group cells, and Beclin1 and LC3 II proteins came out higher in A549 cell (*P < 0.05). (B) The OD_{490} values of si-TIGAR group on 48 h, 72 h and 96 h were significantly decreased than blank group cell, but increased than miR-144 group (*P < 0.05). (C) The colony numbers of si-TIGAR group was significantly decreased than blank group cell, but increased than miR-144 group (*P < 0.05). (D) Apoptotic cells of si-TIGAR group was significantly increased than blank group cell, but decreased than miR-144 group (*P < 0.05). (E) MDC fluorescence intensity of si-TIGAR group was higher than both blank and miR-144 group cells (*P < 0.05).

5C). The number of apoptotic cells in the si-TIGAR group was significantly higher than in the blank group, but lower than in the miR-144 group (*P < 0.05, Fig. 5D). MDC fluorescence intensity of the si-TIGAR group was higher than in the blank and miR-144 groups (*P < 0.05, Fig. 5E). While these results indicate that the functions of TIGAR and miR-144 in A549 cells are largely similar, their degree of function to A549 cell is somewhat inconsistent which supports that the function of miR-144 in A549 cells is partially attributed to targeting TIGAR.

Discussion

MiRNAs are a class of small noncoding single-stranded RNA molecules that are found in plants, animals, and some viruses, which function in RNA silencing and post-transcriptional

regulation of gene expression [18, 19]. Most miRNAs exist as single copies, multiple copies or clusters in the genome, and most of them have a hairpin structure. miRNAs function via base pairing with complementary sequences within mRNA molecules [20-23], cutting off the mRNA molecule on target genes, or inhibiting translation of target genes. Some research has indicated that miR-144 is downregulated in many cancers [24-31]. These previous findings coincide with our results, which identified that miR-144 is significantly downregulated in human lung cancer. We found that altered miR-144 expression levels were associated with tumor size, TNM stage and lymph node metastasis in human lung cancer. We also identified that in lung cancer cell lines (A549 and H460 cells), proliferation was inhibited, and apoptosis and autophagy were enhanced after transfection with miR-144 mimics. These results imply that miR-144 acts as an inhibitor of lung cancer, and that it contributes to the development, progression and metastasis of lung cancer.

Autophagy is a catabolic process that is correlated with various physiological procedures and pathological conditions including cancer. It refers to the degradation of unnecessary cells or dysfunctional organelles using lysosomes [32-35]. In the autophagic process, LC3 I, a soluble form in the cytoplasm, transforms into LC3 II which localizes on the autophagosome membrane [36]. Another protein with a known role in mediating autophagy is Beclin 1 [37]. Its increased expression has been found to promote autophagy as well as inhibit the cell's tumor forming potential. Here, we observed that the levels of both LC3 II and Beclin 1 increased in miR-144 groups ($P < 0.05$). This may imply that miR-144 can regulated the autophagy ability of A549 and H460 cells, thus concerned with the occurrence and development of lung cancer.

Bioinformatics analysis using TargetScan and miRanda revealed that one target of miR-144 was TIGAR (NM_020375), which lay within a cancer susceptibility locus on chromosome 12. TIGAR was discovered by microarray analysis of gene expression following activation of p53.TIGAR overexpression is observed in several types of tumor. Functions of TIGAR primarily manifest in lowering glycolysis, which results in the elimination of reactive oxygen species. All of these functions lead to less cell death, protection from apoptosis, and inhibition of autophagy. TIGAR affects the development of different types of tumors. For instance, in nasopharyngeal cancer cells, inhibition ofc-Met, a tyrosine kinase whose overexpression is associated with poor survival and metastasis [38], results in lower expression of TIGAR and increased cell death [39]. Also, knockdown of TIGAR by RNA interference induces apoptosis in HepG2 hepatocellular carcinoma cells; expression of TIGAR can affect the balance between cell growth and death; and TIGAR modulation influences apoptosis and autophagy in HepG2 cells [40]. To further confirm that TIGAR was one of the direct functional targets of miR-144, the 3′UTR region of TIGAR was amplified from human genomic DNA and inserted into the pmirGLO vector to construct a luciferase reporter plasmid, and qRT-PCR, western blotting, luciferase reporter and knockdown assays were performed. The CCK-8 assay, colony formation assay, flow cytometry assay, caspase3/7 activity assay and MDC staining results demonstrated that silencing TIGAR had similar functional effects as enhanced expression of miR-144. These results indicated that miR-144 might act as a metastasis suppressor by targeting TIGAR.

In conclusion, we showed that miR-144 expression is lower in human lung cancer. We also showed that in lung cancer cell lines (A549 and H460 cells), upregulation of miR-144 inhibited proliferation and promoted apoptosis and autophagy. Based on these findings, we propose that miR-144 might be used as a therapeutic agent against lung cancer.

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Disclosure Statement

None

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