MicroRNA-107 may regulate lung cancer cell proliferation and apoptosis by targeting TP53 regulated inhibitor of apoptosis 1

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Abstract. Lung cancer causes over 1.6 million mortalities worldwide annually. MicroRNAs (miRs) are involved in various types of cancer-associated processes. The present study investigated the possible mechanism of miR-107 in the development of lung cancer in order to identify novel targets for clinical treatment. The expression levels of miR-107 and its putative target gene TP53 regulated inhibitor of apoptosis 1 (TRIAP1) were measured in lung cancer tumor tissues and non-tumor adjacent tissues. Subsequently, the association between TRIAP1 and miR-107 was investigated using a dual-luciferase reporter assay. Following transfection, the effects of miR-107 and TRIAP1 on the proliferation and apoptosis of lung cancer cell lines *in vitro* were investigated using Cell Counting Kit-8 and flow cytometry assays, respectively. Furthermore, the regulatory effect of miR-107 on the expression levels of TRIAP1 and associated proteins was analyzed using a western blot assay. The results revealed lower expression levels of miR-107 and higher expression levels of TRIAP1 in lung cancer tumor tissues compared with non-tumor adjacent tissues. The dual-luciferase reporter assay demonstrated that TRIAP1 is a target gene of miR-107. Additionally, the results revealed that overexpression of miR-107 resulted in a lower proliferation rate and higher apoptosis rate of A549 cells, compared with the negative control (NC) and control groups (P<0.01). The variation of cell proliferation and apoptosis induced by miR-107 mimics was reversed by co-transfection with pcDNA3.1-TRIAP1. Furthermore, the expression levels of cyclin D1 and proliferating cell nuclear antigen were markedly decreased in the miR-107 mimics group compared with the NC group (P<0.01). The expression levels of BCL2 associated X apoptosis regulator, tumor protein p53 and caspase 3 were upregulated and the expression levels of TRIAP1 and BCL2 apoptosis regulator were significantly reduced in the miR-107 mimics group compared with the NC group (P<0.01). The results of the present study suggested that miR-107 regulates lung cancer cell proliferation and apoptosis by targeting TRIAP1.

Introduction

Lung cancer is a type of malignant tumor with unregulated and rapid proliferation that resulted in >1.6 million deaths worldwide in 2015 (1). Despite advances in clinical therapeutic options, the 5-year survival rate of patients with lung cancer remains <15% (2), which is markedly lower than that of patients with breast, colon or prostate cancer (3). Furthermore, advances in treatments for lung cancer, including surgical excision, medical treatment or radiotherapeutic intervention, have little effect on the long-term survival rate (4). Therefore, the identification of the underlying mechanisms of lung cancer tumorigenesis and progression may aid clinical diagnosis and treatment. Lung cancer tumorigenesis and development are closely associated with dysregulation of microRNAs (miRNAs/miRs) (5,6).

miRNAs are small non-coding RNAs consisting of 19-25 nucleotides (7), which modulate gene expression during cellular processes (8). An increasing number of studies suggest that miRNAs act as either tumor suppressors (9) or oncogenes (10) in the progression of various types of cancer, including lung cancer (11,12). A previous study revealed that c-Myc-activated long non-coding RNA H19 downregulated miR-107 and promoted the cell cycle progression of non-small-cell lung carcinoma (NSCLC) cell lines (13). Another study revealed that the expression of miR-107 was markedly reduced in pathological tissues obtained from patients with lung cancer (14). Furthermore, Takahashi *et al* (15) reported that the expression levels of miR-107 were decreased in lung tumor tissues compared with healthy tissues and that miR-107 induced cell cycle arrest in NSCLC cell lines *in vitro*. However, the underlying mechanism by which miR-107 functions in lung cancer progression and development remains largely unknown.

In the current study, the mechanism of miR-107 and its target gene TP53 regulated inhibitor of apoptosis 1 (TRIAP1) in lung cancer was investigated. The results obtained revealed that miR-107 decreased cell proliferation and induced cell apoptosis of lung cancer cell lines *in vitro*, providing a novel theoretical basis for the treatment of lung cancer.

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Materials and methods

Specimens. A total of 45 pairs of lung cancer tumor tissues and non-tumor adjacent lung tissues were obtained from Jingmen No. 2 People's Hospital (Jingmen, China) between July 2014 and April 2016. Among the patients that the tissues were obtained from, there were 31 males and 14 females, and the average age was 62.3 ± 6.8 years. All patients had been diagnosed with lung cancer and had not undergone any other therapy. The present study was approved by the Ethics Committee of Jingmen No. 2 People's Hospital and written consent was acquired from each patient.

Cell culture. The A549 human NSCLC cell line (cat no. SCSP-503; Type Culture Collection of the Chinese Academy of Sciences), BESA-2B cell line (cat no. CL-0496; Procell Life Science & Technology Co., Ltd.) and 293 cell line (cat no. GNHu43; Type Culture Collection of the Chinese Academy of Sciences) were cultured in RPMI-1640 (cat no. 11875093; Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (cat no. 10099-141; Invitrogen; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin. The cells were maintained in a humid atmosphere with 5% CO_2 at 37˚C.

Transfection efficiency. In order to determine the transfection efficiency, cells were divided into three groups as follows: i) Control group (untreated group); ii) microRNA-negative control (NC) mimics group (to eliminate non-sequence-specific effects); and iii) miR-107 mimics group (transfected with miR-107 mimics). The miR-107 mimics and NC oligonucleotides were purchased from Shanghai GenePharma Co., Ltd. The cells were seeded into 6-well plates at a density of 5×10^5 cells/well, and 50 nM miR-107 mimics or NC mimics were transfected into the cells using Lipofectamine® 2000 (cat no. 11668019; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Following transfection, cells were cultured for another 24 h before RT-qPCR assays were conducted. The primer sequences were as follows: miR-107 mimics forward, 5'-ATACCGCTCGAGTGCCAT GTGTCCACTGAAT-3'; miR-107 mimics reverse, 5'-ATA CCGCTCGAGTTCCATGCCTCAACTCCT-3'; miR-NC forward, 5'-ATAGCACAGCCTGGATAGCAACGTAC-3'; miR-NC reverse, 5'-CACCTTCTACAATGAGCTGCGTGT G-3'.

Dual-luciferase reporter assay. TRIAP1 was considered to be a predictive target gene of miR-107 by TargetScan online tool (www.targetscan.org/vert_72). Subsequently, the 293 cell line was transfected with the wild-type TRIAP1 3'untranslated region [UTR; (TRIAP1-3'UTR)] or mutant TRIAP1-3'UTR with either NC mimics or miR-107 mimics using Lipofectamine® RNAiMax Transfection reagent (cat no. 13778075; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Following transfection, the cells were incubated for 4 h in 5% CO_2 at 37˚C. Subsequently, the firefly and Renilla luciferase activities were detected using a dual-luciferase reporter kit (Beyotime Institute of Biotechnology). Firefly luciferase activity was normalized using Renilla luciferase activity.

Vector construction. In order to obtain pcDNA3.1-TRIAP1, a TRIAP1-expression vector was constructed. In brief, TRIAP1 cDNA was amplified by PCR (as will be described) from the cDNA of BEAS-2B cells (cat no. CL-0496; Procell Life Science & Technology Co., Ltd.). The TRIAP1 cDNA (50 nM) was subsequently inserted into pcDNA3.1 (cat no. VT8071; Yobio) to construct the pcDNA3.1-TRIAP1 expression vector. Subsequently, 50 nM pcDNA3.1 and pcDNA3.1-TRIAP1 plasmids were transfected into A549 cells using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.), following the manufacturer's protocol, and incubated at 37˚C for 24 h at 5% CO_2 for 2 h. The transfected cells were subsequently incubated at room temperature in 5% CO_2 at 37˚C for another 48 h, in order to determine cell proliferation and apoptosis. The sequences were described as follows: TRIAP1 forward, 5'-TATCTTGCA GGAACTGTGTGCTA-3', and TRIAP1 reverse 5'-AATTTA GGTTCTTCCTCCACAGC-3'.

Analysis of cell proliferation. In order to investigate the effect of miR-107 and TRIAP1 on the proliferation of A549 cells, cells were divided into four different groups as follows: i) control group; ii) microRNA-NC mimics group; iii) miR-107 mimics group; iv) miR-107 mimics + pcDNA3.1-TRIAP1 group. Briefly, the transfected cells were seeded onto a 96-well plate at a density of 1×10^4 cells with 100 μl/well of fresh RPMI-1640 medium. The cells were incubated in a humid atmosphere with 5% CO_2 at 37˚C for 48 h. Following 12, 24 or 48 h, 10 μl Cell Counting Kit-8 (CCK-8) solution (cat no. HY-P0093; MedChemExpress) was added to each well and the cells were incubated for a further hour at room temperature. The absorbance in each well was measured at a wavelength of 490 nm in order to quantify the proliferation.

Analysis of cell apoptosis. In order to investigate the effect of miR-107 and TRIAP1 on the apoptosis of A549 cells, cells were divided into four different groups: i) Control group; ii) microRNA-NC mimics group; iii) miR-107 mimics group; iv) miR-107 mimics + pcDNA3.1-TRIAP1 group. Briefly, the transfected cells in suspension were collected at a density of 1×10^6 cells/ml. The cells were washed with HEPES buffer solution (cat no. ACC0013A; Seebio Biotech, Inc.) for 5 min at room temperature and centrifuged for 5 min at 5,000 x g on ice. Subsequently, 5 μl Annexin V-fluorescein isothiocyanate (FITC) and 10 μl propidium iodide (BD Biosciences) were added to the cells of the four different groups and the cells were incubated for 10 min in the dark at room temperature. The apoptosis rate was then measured using a flow cytometer (BD Biosciences) and analyzed using FlowJo software (version 10; BD Biosciences).

RNA extraction and reverse transcription-quantitative (RT-q) PCR analysis. miRNA was isolated from the lung tumor and adjacent non-tumor tissues and A549 cells using the miRNeasy Mini kit (Qiagen, Inc.) according to the manufacturer's protocol. Following isolation, the One-Step PrimeScript miRNA cDNA Synthesis kit (Takara Biotechnology Co., Ltd.) was used to synthesize cDNA according to the manufacturer's instructions. The total RNA was extracted using TRIzol reagent (cat. no. 15596018; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Subsequently, 2 μl RNA was reverse transcribed into cDNA. qPCR analysis was performed using the TaqMan MicroRNA RT kit (cat no. 4366596; Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Briefly, 2 μl cDNA, 10 μl SYBRGreen RT-qPCR Master mix (cat. no. AB4106A; Applied Biosystems; Thermo Fisher Scientific, Inc.), 1 μl primers and nuclease-free water were combined during the PCR reaction. The primer sequences were as follows: miR-107 forward, 5'-AGCAGCATTGTACAG GGCTATCA-3'; and reverse, 5'-GCGAGCACAGAATTA ATACGAC-3'; U6 forward, 5'-AGAGCCTGTGGTGTCCG-3'; and reverse, 5'-CATCTTCAAAGCACTTCCCT-3'; TRIAP1 forward, 5'-AGGATTTCGCAAGTCCAGAA-3'; and reverse, 5'-GCTGATTCCACCCAAGTAT-3'; and GAPDH forward, 5'-AACGGATTTGGTCGTATTG-3'; and reverse, 5'-GGA AGATGGTGATGGGATT-3'. The PCR reaction conditions were as follows: i) Denaturing for 3 min at 95˚C; ii) denaturing for 30 sec at 94˚C, annealing for 30 sec at 56˚C and extension for 30 sec at 72˚C (35 cycles); and iii) extension for 10 min at 72˚C. The relative expression of miR-107 was normalized to that of U6 small nuclear RNA (Thermo Fisher Scientific, Inc.) and the expression levels of TRIAP1 mRNA were normalized to those of GAPDH according to the $2^{-\Delta\Delta Cq}$ method (16). U6 or GAPDH was used as the endogenous control.

Western blot analysis. Radioimmunoprecipitation assay buffer (cat. no. R0010; Beijing Solarbio Science & Technology Co., Ltd.) was used to extract the proteins from tissues and cells. Following centrifugation at 6,000 x g for 5 min at 4˚C, a protease inhibitor cocktail (cat. no. 78425; Thermo Fisher Scientific, Inc.) was added to the protein lysates. The Bradford method was used to quantify the concentration of proteins in the supernatant of the lysates. The whole protein lysates (20 μg) were separated by SDS-PAGE (8% gel; cat. no. LC26755; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Subsequently, 10 μg separated protein was transferred to a polyvinylidene fluoride membrane (Shanghai Ofluorine Chemical Technology Co., Ltd.) and the membrane was blocked with 50 ml 5% nonfat milk for 50 min at 37˚C. The membrane was then incubated with the following primary antibodies at 37˚C for 50 min: Rabbit anti-prolifer-ating cell nuclear antigen (PCNA; 1:1,000; cat. no. ab18197; Abcam); rabbit anti-cyclin D1 (1:100; cat. no. ab16663; Abcam); rabbit anti-TRIAP1 (1:1,000; cat. no. ABIN2970840; 4A Biotechnology Co., Ltd.), rabbit anti-BCL2 apoptosis regulator (BCL2; 1:1,000; cat. no. ab32124; Abcam), rabbit anti-BCL2 associated X apoptosis regulator (BAX; 1:1,000; cat. no. ab32503; Abcam), rabbit anti-tumor protein p53 (TP53; 1:1,000; cat. no. ab131442; Abcam), rabbit anti-caspase 3 (1:500; cat. no. ab13847; Abcam) and rabbit anti-GAPDH (1:2,500; cat. no. ab9485; Abcam). All anti-bodies were diluted in blocking buffer (concentration, 10x; cat. no. ab126587; Abcam). After washing, the membrane was then incubated for 1 h at 37˚C with goat anti-rabbit horseradish peroxidase (HRP) IgG H&L secondary antibody (1:1,000; cat. no. ab7090; Abcam). Subsequently, 200 μl chemilumi-nescent HRP substrate (cat. no. ab5801; Abcam) was added to the surface of the membranes. The signals were captured and exposed onto X-ray films. ImageJ software (version 1.49; National Institutes of Health) was used to quantify the relative
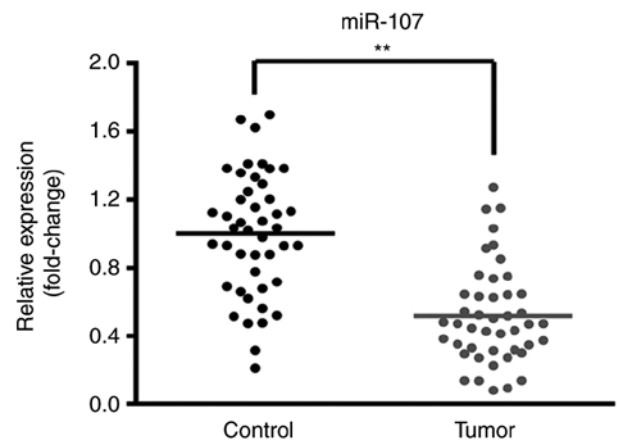

Figure 1. Comparison of the relative expression level of miR-107 between lung cancer tumor tissues and non-tumor adjacent tissues. The expression level of miR-107 was lower in the tumor tissues compared with the control non-tumor adjacent tissues (n=45). **P<0.01, as indicated. miR, microRNA.

expression levels of the proteins. GAPDH was used as the reference protein. The procedures above were conducted in triplicate.

Statistical analysis. SPSS (version 22.0; IBM SPSS) was used to analyze the results and the data are presented as the mean ± standard deviation. The Student's t-test was used to compare two groups. One-way analysis of variance followed by Newman-Keuls post hoc test was used to distinguish differ-ences among three and more groups. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-107 is downregulated in lung cancer tissues. In order to investigate the putative effects of miR-107 in lung cancer, the expression levels of miR-107 in lung cancer tumor tissues and adjacent non-tumor tissues from 45 patients were compared. As shown in Fig. 1, the expression levels of miR-107 were signifi-cantly reduced in the lung cancer tumor tissues compared with the adjacent non-tumor tissues (P<0.01).

TRIAP1 is a direct target of miR-107 in lung cancer. A dual-luciferase reporter assay was conducted in order to determine the association between miR-107 and TRIAP1. The 3'-UTR of the TRIAP1 gene was confirmed to contain binding sequences for miR-107 (Fig. 2A), suggesting that TRIAP1 may be a downstream target of miR-107. Furthermore, the results demonstrated that transfection of miR-107 mimics markedly reduced the luciferase activity in the wild-type TRIAP1-3'UTR plasmid-transfected cells; however, miR-107 had no significant influence on the mutant TRIAP1-3'UTR plasmid-transfected cells (P<0.01; Fig. 2B).

Expression levels of TRIAP1 in lung cancer tumor tissues and adjacent non-tumor tissues. In order to further investigate the potential function of TRIAP1 in lung cancer, the mRNA and protein expression levels of TRIAP1 in lung cancer tumor tissues and adjacent non-tumor tissues were quantified. As shown in Fig. 3, the mRNA and protein expression levels
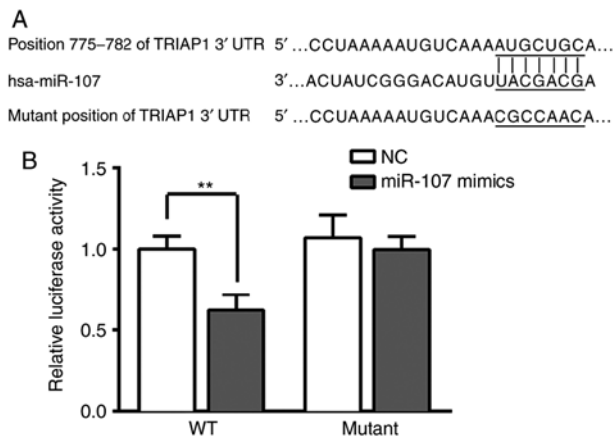
Figure 2. miR-107 directly targets TRIAP1. (A) Sequence alignment of the paired site of the 3'-UTR of miR-107 and TRIAP1. (B) The activity of the luciferases in different groups (n=3). **P<0.01, as indicated. miR, microRNA; TRIAP1, TP53 regulated inhibitor of apoptosis 1; UTR, untranslated region; WT, wild-type; NC, negative control.

of TRIAP1 were highly increased in the lung cancer tumor tissues compared with the adjacent non-tumor tissues (P<0.01).

miR-107 inhibits lung cancer cell proliferation. A549 cells were transfected with miR-107 mimics or NCs and the transfection efficiency was demonstrated using RT-qPCR analysis. As shown in Fig. 4A, there was no significant difference between the control group and NC group; however, compared with the NC group, the expression levels of miR-107 in the miR-107 mimics group were significantly increased (P<0.01). As demonstrated in Fig. 4B there was no significant difference in the proliferation of cells between the control group and the NC group throughout the whole experiment. As time increased, there was a significantly reduced proportion of proliferative cells in the miR-107 mimics group, in comparison with the control group (P<0.01). However, following transfection with pcDNA3.1 or pcDNA3.1-TRIAP1 (P<0.01; Fig. 5A), the miR-107 mimics-reduced A549 cell proliferation rate was reversed by co-transfection with pcDNA3.1-TRIAP1 (P<0.05; Fig. 5B).

miR-107 promotes lung cancer cell apoptosis. An Annexin V-FITC apoptosis kit was used to identify the apoptotic rate of A549 cells in order to investigate the effect of miR-107 on lung cancer cell apoptosis. The apoptotic rate of the cells in the miR-107 mimics group was almost four times higher than that of the cells in the control group or the NC group (P<0.01; Fig. 6A-D). There was no distinct difference in the apoptotic rate of the cells in the control group and the NC group. However, miR-107 mimics-induced A549 cell apoptosis was reduced by co-transfection with pcDNA3.1-TRIAP1 (P<0.01; Fig. 7A-C).

Effect of miR-107 on the expression levels of cyclin D1 and PCNA. In order to further demonstrate the effect of miR-107 on regulating A549 cell proliferation, the expression levels of cyclin D1 and PCNA were measured. As demonstrated in Fig. 8, the protein expression levels of cyclin D1 and PCNA were significantly decreased in the miR-107 mimics group, as compared with the NC group (P<0.01). However, there were no significant differences between the NC and control groups.
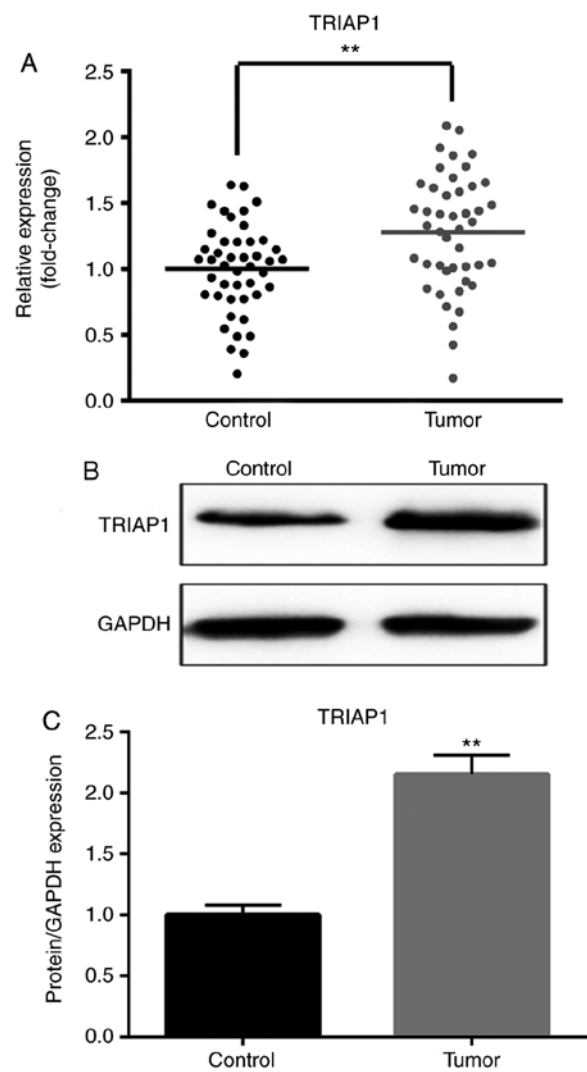

Figure 3. Comparison of relative mRNA and protein expression levels of TRIAP1 in lung cancer tumor tissues and adjacent non-tumor tissues. (A) The relative mRNA expression of TRIAP1 in lung cancer tumor tissues and adjacent tissues (n=45). **P<0.01, as indicated. The (B) protein expression and (C) quantification of TRIAP1 in lung cancer tumor tissues and adjacent non-tumor tissues (n=3). **P<0.01, as indicated. TRIAP1, TP53-regulated inhibitor of apoptosis 1; GAPDH, glyceraldehyde-3-phosphate hydrogenase.

Effect of miR-107 on the protein expression of BCL2, BAX, TP53 and caspase 3. In order to further investigate the association between miR-107 and its target gene TRIAP1, a western blot assay was performed to examine the protein expression levels of TRIAP1 and related apoptotic proteins among the different groups. As shown in Fig. 9, the protein expression levels of TRIAP1 and BCL2 were significantly reduced and the expression levels of BAX, TP53 and caspase 3 were significantly increased in the miR-107 mimics group, compared with those in the NC group (P<0.01). The protein expression levels of BCL2, BAX, TP53 and caspase 3 in the control group and the NC group were similar.

Discussion

A previous study revealed that miR-107 promoted the proliferation of hepatocellular carcinoma cells by targeting axin 2 (17). Furthermore, miR-107 promoted the proliferation and invasion
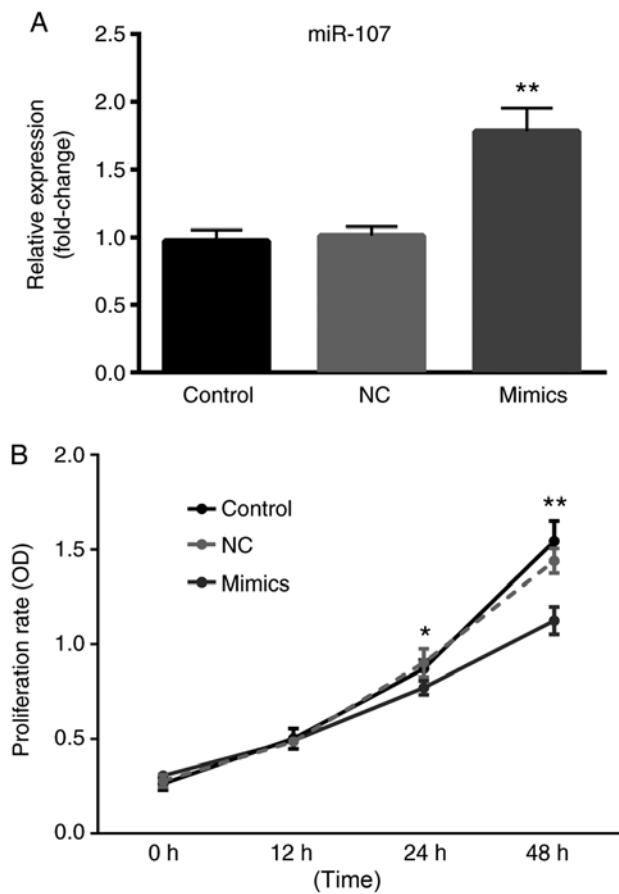
Figure 4. Effect of miR-107 overexpression on the proliferation of A549 cells. (A) Transfection efficiency of miR-107 determined using reverse transcription-quantitative polymerase chain reaction analysis in different groups (n=3). **$P<0.01$ vs. control untransfected cells and NC groups. (B) Effect of miR-107 overexpression on the proliferation of A549 cells (n=3). *$P<0.05$, mimics vs. NC at 24 h; **$P<0.01$, mimics vs. NC at 48 h. miR, microRNA; NC, negative control; OD, optical density.
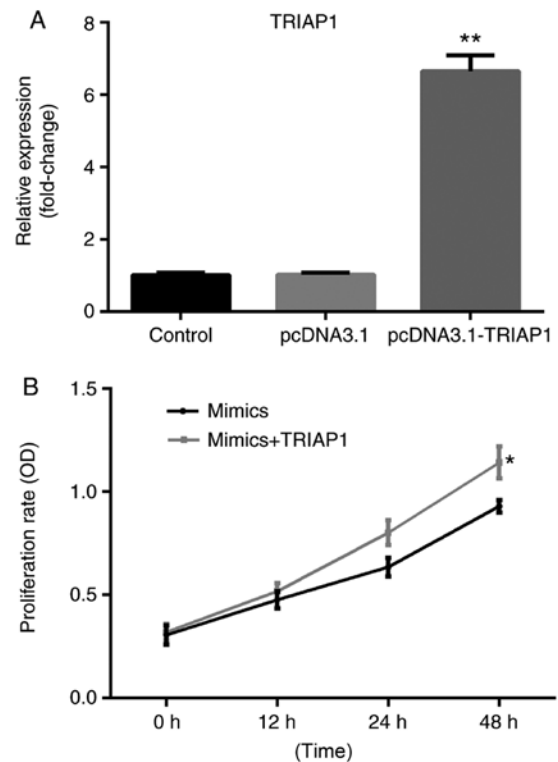

Figure 5. TRIAP1 reverses the miR-107-induced reduced proliferation of A549 cells. (A) Transfection efficiency of TRIAP1 was determined by reverse-transcription quantitative polymerase chain reaction analysis in different groups (n=3). **$P<0.01$, pcDNA3.1-TRIAP1 vs. pcDNA3.1. (B) The proliferation rate of A549 cells in different groups (n=3). *$P<0.05$, mimics + TRIAP1 vs. mimics. TRIAP1, TP53-regulated inhibitor of apoptosis 1; miR, microRNA; control, untransfected cells; mimics, miR-107 mimics-transfected cells; mimics + TRIAP1, cells co-transfected with miR-107 mimics and pcDNA3.1-TRIAP1.

of gastric adenocarcinoma cells through large tumor suppressor kinase 2 (18). However, miR-107 inhibited cell proliferation and metastasis in gastric cancer (19) and osteosarcoma (20). Therefore, miR-107 acts as a tumor suppressor or oncogene in different types of cancer. The present study demonstrated that miR-107 may function as a tumor suppressor in lung cancer, which corresponds with previous studies (17-20).

In the present study, the results of the CCK-8 and flow cytometry assays demonstrated that the overexpression of miR-107 reduced proliferation and promoted apoptosis in lung cancer cells in vitro. A previous study demonstrated that miR-107-5p suppressed NSCLC by directly targeting the oncogene epidermal growth factor receptor (21). Another study indicated that miR-107 inhibited tumor growth by targeting the brain derived neurotrophic factor-mediated PI3K/AKT signaling pathway in human NSCLC (14). The results of the present study were consistent with those of previous studies; however, a target gene of miR-107, which may provide novel approaches for clinical treatment, was also identified.

miRNAs regulate cell proliferation and apoptosis by targeting specific genes during cellular processes (22). According to previous studies, TRIAP1 was predicted to be a candidate oncogene in various types of cancer, including

ovarian cancer (23), nasopharyngeal carcinoma (24) and lung cancer (25). In the present study, TRIAP1 was revealed to be upregulated in lung cancer tumor tissues compared with adjacent non-tumor tissue. TRIAP1 was subsequently predicted to be a target gene of miR-107 using TargetScan and a dual-luciferase reporter assay. The CCK-8 and flow cytometry assays further demonstrated that the effects on A549 cell proliferation and apoptosis induced by miR-107 mimics were reversed by co-transfection with pcDNA3.1-TRIAP1. Collectively, miR-107 may inhibit cell proliferation and promote cell apoptosis in lung cancer by targeting TRIAP1. However, the association between miR-107 and TRIAP1 requires further investigation.

In order to demonstrate the function of miR-107 on cell proliferation of lung cancer, the expression levels of the proliferation-associated factors, cyclin D1 and PCNA were measured via a western blot assay. Previous studies reported that inhibitory expression of cyclin D1 decreased lung cancer cell proliferation (26,27). Furthermore, the decreased expression of PCNA may inhibit lung cancer cell proliferation (28,29). In the present study, the expression levels of cyclin D1 and PCNA were significantly decreased in the miR-107 mimics group compared with the miR-NC mimics group. Thus, it was hypothesized that the overexpression of miR-107 may inhibit lung cancer cell proliferation by reducing the expression levels of cyclin D1 and PCNA.
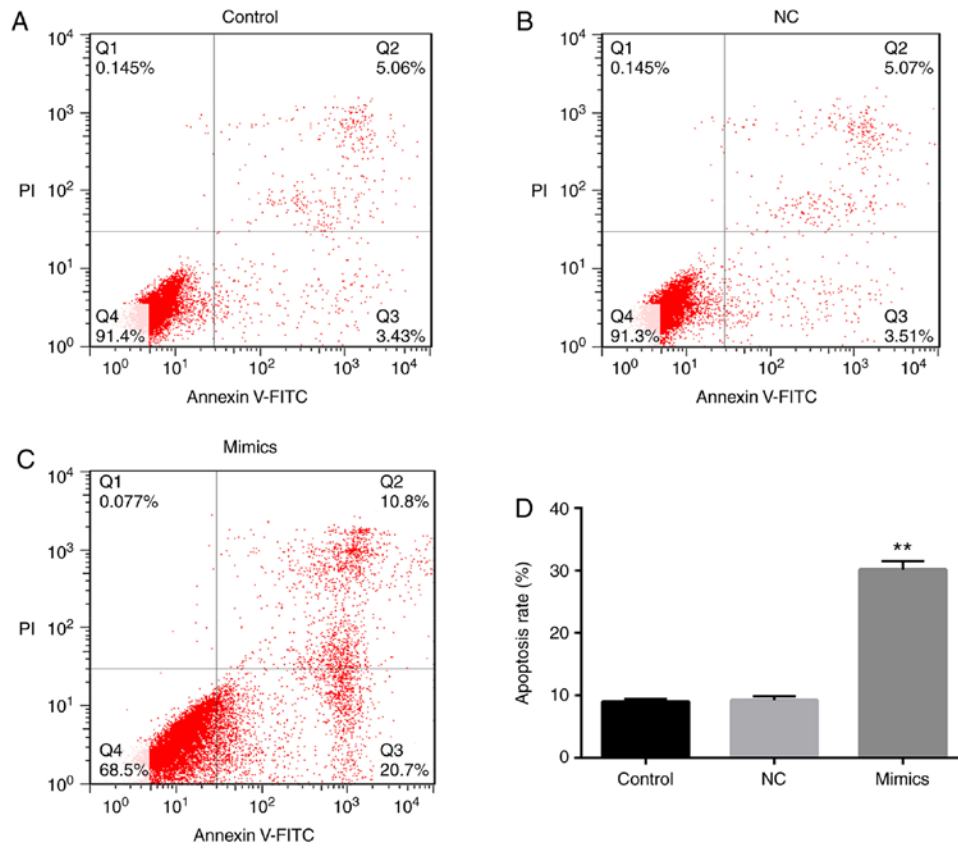
Figure 6. Effect of miR-107 on the apoptosis of A549 cells. (A) Control untransfected cells group (n=3). (B) miR-107 NC-transfected cells (n=3). (C) miR-107 mimics (n=3). (D) Quantified results of the flow cytometry analysis. **P<0.01 vs. the NC group. miR, microRNA; NC, negative control; FITC, fluorescein isothiocyanate; PI, propidium iodide.
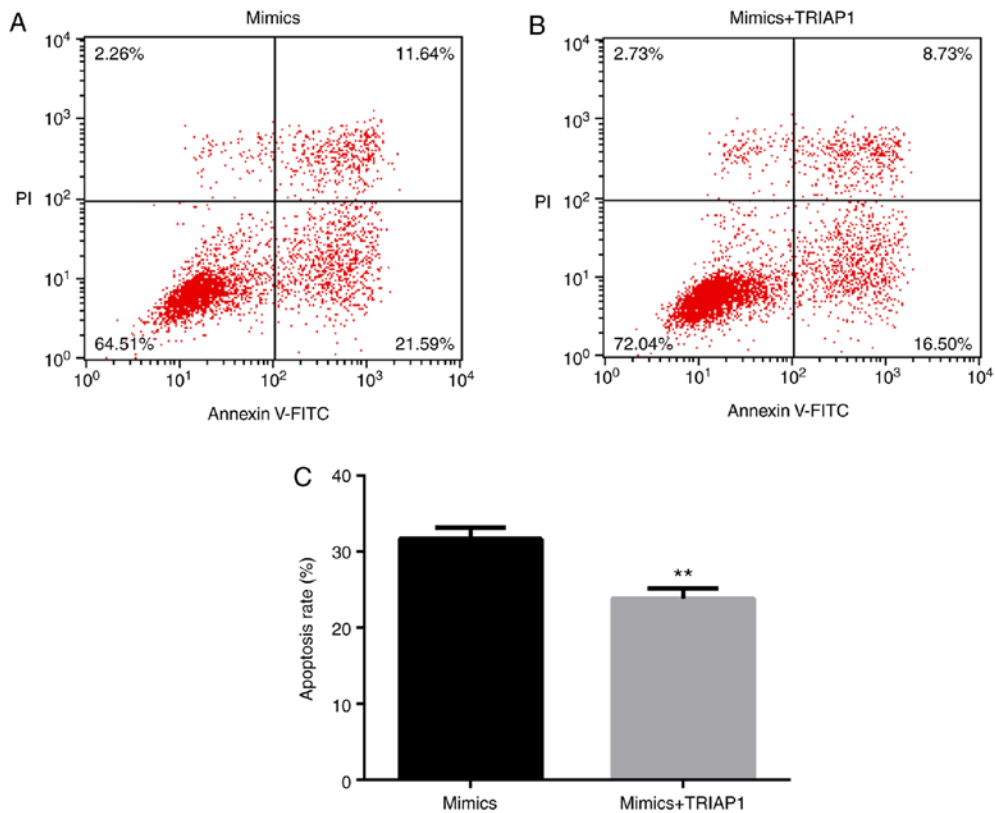

Figure 7. TRIAP1 reverses miR-107-induced apoptosis of A549 cells. (A) Mimics group (n=3). (B) Mimics + TRIAP1 group (n=3). (C) Quantified results of the flow cytometry analysis. **P<0.01 vs. the miR-107 mimics-transfected cells group. TRIAP1, TP53 regulated inhibitor of apoptosis 1; miR, microRNA; FITC, fluorescein isothiocyanate; PI, propidium iodide.
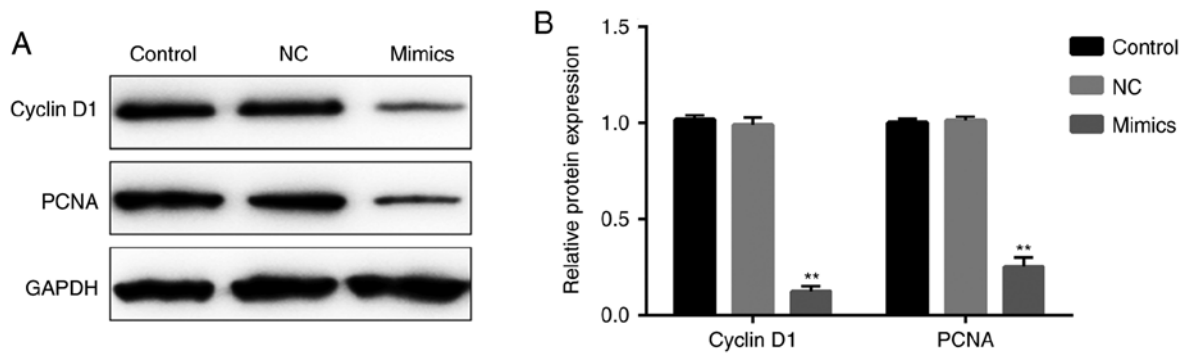
Figure 8. Effect of miR-107 on the expression of cyclin D1 and PCNA. (A) Protein expression levels of cyclin D1 and PCNA in different groups. (B) The quantified protein expression levels of cyclin D1 and PCNA in different groups (n=3). **P<0.01, vs. the miR-NC mimics-transfected cells. miR, microRNA; PCNA, proliferation cell nuclear antigen; GAPDH, glyceraldehyde-3-phosphate hydrogenase.
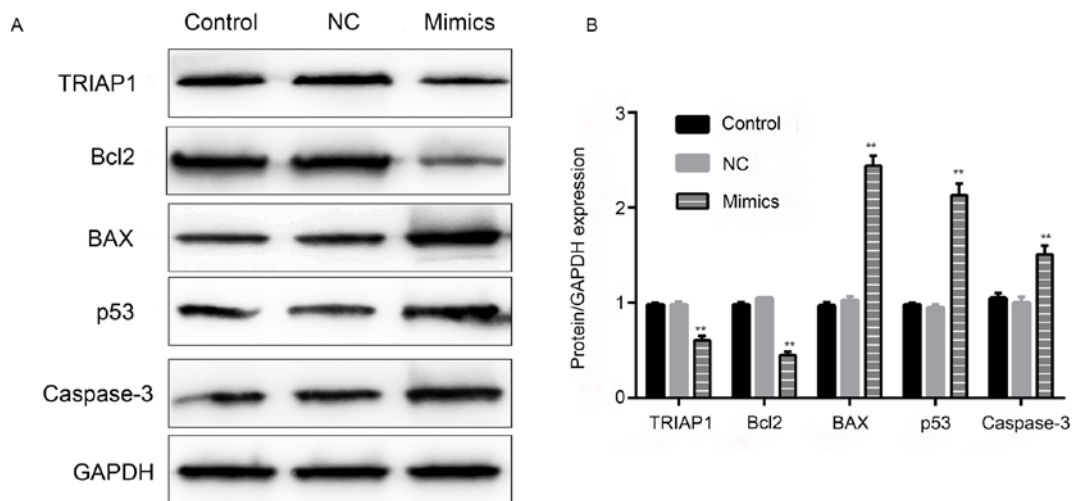

Figure 9. Effect of miR-107 on the expression of TRIAP1, BCL2, BAX, TP53 and caspase 3. (A) Protein expression level of TRIAP1, BCL2, BAX, TP53 and caspase 3 in different groups. (B) The quantified protein expression levels of TRIAP1, BCL2, BAX, TP53 and caspase 3 (n=3). **P<0.01 vs. the miR-107 NC-transfected cells. miR, microRNA; TRIAP1, TP53-regulated inhibitor of apoptosis 1; BCL2, BCL2 apoptosis regulator; BAX, BCL2 associated X, apoptosis regulator; TP53, tumor protein 53; NC, negative control; GAPDH, glyceraldehyde-3-phosphate hydrogenase.

In the current study, the expression levels of the apoptosis-associated factors BAX, TP53, caspase 3 and BCL2 were measured using a western blot assay. A previous study demonstrated that overexpression of caspase 3 may inhibit the apoptosis of lung cancer cells (30). Another study reported that activation of BAX contributed to the apoptosis of lung cancer cells (31). TP53 functions as a tumor suppressor and blocks cancer progression (32). Overexpression of miR-107 may increase the expression levels of caspase 3 (33), BAX (34) and TP53 (35); while the inhibition of TRIAP1 may increase the expression levels of TP53 and caspase 3 (36,37). In the present study, the protein expression levels of BAX, TP53 and caspase 3 were significantly increased, and those of TRIAP1 were decreased, in the miR-107 mimics group compared with the NC group. Therefore, it was hypothesized that miR-107 may induce the apoptosis of lung cancer cells by targeting TRIAP1 and increasing the expression levels of BAX, TP53 and caspase 3, which are three known tumor suppressors in lung cancer (32,33). Furthermore, previous studies have demonstrated that BCL2 knockdown decreased the viability and increased apoptosis of lung cancer cells (38), while overexpression of BCL2 decreased

apoptosis of human lung cancer cells (39). Therefore, BCL2 may act as a tumor promoter in lung cancer. In the present study, the expression levels of TRIAP1 and BCL2 were decreased in the miR-107 mimics group compared with the NC group. Therefore, the inhibition of TRIAP1 induced by the overexpression of miR-107 may reduce the expression of BCL2, a known tumor promoter in lung cancer (38,39), which contributes to apoptosis in the process of lung cancer. However, the underlying mechanisms involved in TRIAP1 and apoptosis-associated proteins require further investigation.

In conclusion, miR-107 may inhibit A549 lung cancer cell proliferation and promote apoptosis by targeting TRIAP1, indicating that miR-107 may be a novel target in lung cancer treatment. However, the current study had the following limitations: i) The association and interaction of miR-107 and its target TRIAP1 require further investigation; ii) the effects of miR-107 and other targets on regulating lung cancer cell proliferation and apoptosis requires further study; iii) the histopathological patterns of lung cancer tissues were not distinguished; and iv) further experiments on different lung cancer cell lines and in in vivo models are required.

Authors' contributions

PC collected lung cancer tumor and non-tumor adjacent tissues, analyzed and interpreted the patient data. JL, GC, BP, LY and BZ analyzed all the experimental data. YY performed all the cell experiments and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Jingmen No. 2 People's Hospital (Jingmen, China) and written consent was acquired from each patient.

Patients consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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