Conclusions: MiR-107 suppresses cell proliferation by targeting TRIAP1 in lung cancer. Our finding allows new insights into the mechanisms of lung cancer that is mediated by miR-107.

Keywords: miR-107, TRIAP1, proliferation, lung cancer

1 Introduction

Lung cancer is the principle cause of global cancer-associated mortality accounting for about 1.59 million deaths every year worldwide [1, 2]. Most of those cases have non-small-cell lung cancer (NSCLC) [3].

MicroRNAs (miRNAs) usually negatively modulate gene expression through mRNA cleavage or translational repression [4]. Normally, conserved miRNAs plays a part in many processes, such as cell proliferation, apoptosis, and metabolism. Numerous miRNAs play important roles in cancers as oncogenes or tumour suppressor genes [5-10]. MiR-107 is proven to be involved in many cancers, such as colon, breast, gastric, liver, and bladder [11-17]. It has been reported that miR-107 results in cell cycle arrest to suppress cell proliferation in lung adenocarcinoma [18]. Lowly-expressed miR-107 correlates with tumor development and patient survival in NSCLC [19]. However, it remains unclear how miR-107 works in lung cancer.

In the current study, we explored the effect of miR-107 and its novel target gene on proliferation of lung cancer cells. Our findings indicate that TRIAP1 serves as a novel target gene of miR-107 in lung cancer A549 cells. MiR-107 restrains the proliferation of lung cancer cells through regulating TRIAP1. Our finding takes a further step into the mechanism of miR-107-associated lung cancer.
2 Materials and Methods

2.1 Cell culture

A549 cells (a lung cancer cell line), were grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum (Gibco, USA), 100U/mL penicillin and 100U/mL streptomycin. The container works in 5% CO₂ at 37°C.

2.2 MiRNA and siRNA transfection

Cells were seeded into 6-well or 96-well plates 24h before transfection. Lipofectamine 2000 (Invitrogen, USA) was used to transfect miRNAs and siRNAs into cells. MiR-107 (5’-AGCAGCAUUGUAACAGGGCAUA-3’), miR-107 inhibitor (5’-UGAUAGCCCUAGCAUGCU-3’), miRNA control (Ctrl: 5’-UUUCUUAGGUGACGUGU-3’), siRNA control (siCtrl: 5’-AAUGGUAAGGCUAAUCC-3’) or siTRIAP1 (5’-AGGCAUGAGGCGAUGAAATT-3’) were synthesized by GenePharma (Shanghai, China).

2.3 Quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol (Invitrogen, USA) was utilized to extract total RNA of cells/patient samples as described previously [15]. We polyadenylated total RNA through poly (A) polymerase (Thermo Fisher Scientific, USA). Reverse Transcriptase came from TransGen Biotech (Beijing, China). The qRT-PCR was applied by TransStart Top Green PCR SuperMix (TransGen Biotech, China). The primers used were listed: U6 sense, 5’-CTCGTTCCGACAGCACA-3’, antisense, 5’-AACCTCTACTAATTTGCTG-3’; GAPDH sense, 5’-AACGATTGACGTATATGG-3’, antisense, 5’-GGAGATTTGATGGAGTT-3’; miR-107 sense, 5’-AGCAGCATTGTACAGGGCTATCA-3’, antisense, 5’-GGAGATTTGATGGAGTT-3’; miR-107 sense, 5’-AGCAGCATTGTACAGGGCTATCA-3’, antisense, 5’-GGAGATTTGATGGAGTT-3’; TRIAP1 sense, 5’-TATCTTGCAGGAACCTTTGCTG-3’, antisense, 5’-AATTTAGGTTCTCCTCCACAGC-3’.

2.4 Western blot

48 hours after transfection, cell lysis was prepared at 4°C using RIPA buffer (Biomed, China). Proteins were transferred to membranes from the electrophoresis gel and then were blocked for 2 h in 5% non-fat dried milk powder in 0.1% PBST. The membranes were detected with anti-TRIAP1 antibody (Santa Cruz Biotechnology, USA). Anti-β-actin (Sigma, USA) served as a protein loading control. Detection was visualized by ECL Western Blotting Substrate (Solarbio, Beijing, China). The experiments were repeated three times at least.

2.5 Cell growth measurement

For quantitative proliferation assays, one thousand A549 cells were seeded into 96-well plates for 12h. Methylthiazolyl tetrazolium (MTT) (Sigma, USA) assays were performed to evaluate cell proliferation. The absorbance value (OD) at 490 nm of each well was measured every 24h from 24h to 72h after transfection.

2.6 Colony formation assay

Forty-eight hours post-transfection, one thousand cells were plated in six-well plates. The cells have been grown in complete medium for 2 weeks until the colonies were visible. The colonies were stained with methylene blue. The experiments were repeated in triplicate.

2.7 Patient samples

Clinical lung tumor samples and corresponding peritumor samples (25 cases) were attained from Cancer Hospital Chinese Academy of Medical Sciences (Beijing, China) after surgical resection. The patients approved the use of their tissues for study. The information of the patients was listed in Table 1.

  **Informed consent:** Informed consent has been obtained from all individuals included in this study.

  **Ethical approval:** The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Research Ethics Committee at Cancer Hospital Chinese Academy of Medical Sciences.

2.8 Statistical analysis

The experiment was performed in triplicate. Statistical analysis was thought as significant by comparing mean values (standard deviation, SD) using a Student’s t test (*P < 0.05, **P < 0.01). The correlation between miR-107 and TRIAP1 in clinical lung cancer tissues was analysed using a Pearson’s correlation coefficient.
3 Results

3.1 MiR-107 depresses TRIAP1 expression in lung cancer cells

To elucidate the function of miR-107 in lung cancer, we predicted the target genes of miR-107 using Targetscan software (http://www.targetscan.org/). We found that oncogene TRIAP1, was a potential target gene of miR-107. To evaluate the effect of miR-107 on TRIAP1, miR-107 was transiently transfected into lung cancer A549 cells. Ectopic miR-107 expression decreased levels of TRIAP1 protein in A549 cells (Figure. 1A). Whereas, TRIAP1 was elevated when cellular miR-107 was inhibited by miR-107 inhibitor (Figure. 1B), implying that miR-107 can restrain TRIAP1 expression in cells. Overexpressed miR-107 or miR-107 inhibitor was confirmed by qRT-PCR assay (Figure. 1A and 1B). Overall, we find that miR-107 can inhibit TRIAP1 in lung cancer cells.

3.2 MiR-107 depresses lung cancer cell proliferation through TRIAP1

Based on that miR-107 could regulate oncogene TRIAP1, we further probed into the role of miR-107 targeting TRIAP1 in the development of lung cancer. We carried out MTT and colony formation assays in lung cancer A549 cells treated with miR-107 inhibitor. Our data demonstrated that A549 cell proliferation was increased after the cells were treated with miR-107 inhibitor, and then TRIAP1 silencing could abrogate miR-107 inhibitor-enhanced proliferation (Figure. 2A and 2B). We indicate that miR-107 inhibits cell proliferation by modulating TRIAP1 in lung cancer.

3.3 MiR-107 negatively correlates with TRIAP1 in clinical lung cancer patient samples

To validate the connection of miR-107 with TRIAP1 in clinical samples, we tested the TRIAP1 expression in 25 samples of human lung tumor tissues and peritumor tissues through qRT-PCR. Our data showed that TRIAP1 was very highly expressed in all the 25 lung tumor tissues when compared with their peritumor counterparts (Figure. 3A). Moreover, we observed a negative correlation between miR-107 and TRIAP1 (P <0.05, Pearson’s correlation coefficient, r=-0.7326) (Figure. 3B). Thus, we prove that miR-107 is negatively related to TRIAP1 in lung cancer tissues.

4 Discussion

Lung cancer remains the main cause of cancer-related mortality worldwide. Most cases are usually classified as non-small-cell lung cancer. Commonly, activated EGFR mutations are used as predictive biomarkers [20]. MiRNA networks are reported to be associated with cancers through various mechanisms. Evidence suggests that miR-107 is involved in many cancers [11-17].

In the present study, we discuss new target genes and the function of miR-107 in lung cancer. We observe that miR-107 could inhibit TRIAP1 expression in lung cancer cells. Our results suggest that TRIAP1 is a novel target gene of miR-107. Importantly, miR-107 inhibitor significantly promotes the proliferation of lung cancer cells in vitro. Conversely, knockdown of TRIAP1 could abrogate the miR-107 inhibitor-enhanced proliferation of lung cancer.

Table 1. Clinical characteristics of lung cancer samples

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MiR-107 inhibits proliferation of lung cancer cells through regulating TP53 regulated inhibitor of apoptosis 1.

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**Figure 1.** MiR-107 decreases TRIAP1 expression in lung cancer cells. (A) TRIAP1 level was analyzed in A549 cells with miR-107 treatment by using Western Blot analyses. A QRT-PCR assay was utilized to confirm miR-107 transfection. (B) TRIAP1 expression was tested in A549 cells with miR-107 inhibitor (anti-miR-107) through Western Blot analyses. QRT-PCR assay was utilized to confirm miR-107 inhibitor transfection. (* P < 0.05, ** P < 0.01; Student’s t test)

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**Figure 2.** MiR-107 restrains lung cancer cell proliferation via TRIAP1. (A, B) Cell proliferation of A549 cells by anti-miR-107 (or anti-miR-107/siTRIAP1) was evaluated by MTT and colony formation assays. (** P < 0.01; Student’s t test)

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**Figure 3.** MiR-107 is negatively related to TRIAP1 in clinical lung cancer samples. (A) TRIAP1 expression was investigated by qRT-PCR assay in 25 lung tumor samples and peritumor samples. (B) QRT-PCR analysis was applied to determine correlation of miR-107 with TRIAP1 in above tumor samples (Pearson’s correlation coefficient, r=-0.7326). (* P < 0.05, ** P < 0.01; Student’s t test)
cells. Clinically, TRIAP1 was remarkably augmented in clinical lung cancer tissues. Finally, we showed a significantly negative correlation of miR-107 with TRIAP1 in lung cancer tissues.

Different expression pattern of miR-107 is found in some signal pathways, such as cell cycle arrest, angiogenesis and hypoxia. A report has shown that miR-107 is closely related to survival in patients with NSCLC [19]. MiR-107 targeting cyclin dependent kinase 8 (CDK8) is involved in cisplatin chemosensitivity of NSCLC A549 cells [21]. Previous evidence indicates that IncRNA H19 activated by c-Myc can down-regulate miR-107 to promote cell cycle progression of NSCLC [22]. Recent findings suggest that miR-107 can suppress NSCLC metastasis by targeting BDNF [23]. Our findings further confirm these reports in which miR-107 serves as a tumor suppressor gene in cancers. Notably, we provide the first evidence that miR-107 targeting TRIAP1 participate in the lung cancer progression.

TRIAP1, also known as P53CSV, is firstly characterized as a p53-inducible gene and can inhibit apoptosis by interacting with the Hsp70/Apaf1 complex [24]. Some genes including TRIAP1 are reported to be up-regulated and play a crucial role in multiple myeloma [25, 26]. A genome-wide genetic screen in human cells indicates that TRIAP1 is a specific repressor of p21, one p53 target gene [27]. Evidence reveals that TRIAP1 is also involved in prostate cancer bone metastasis [28]. A report describes that TRIAP1 is up-regulated in drug-resistant breast cancer cells and altered expression of TRIAP1 changes cellular sensitivity to doxorubicin in breast cancer [29]. MiR-320b-inhibited TRIAP1 is involved in progression of nasopharyngeal carcinoma [30]. Our data take a further step to the function of TRIAP1 in lung cancer.

Taked together, we present a novel target gene of miR-107 in controlling proliferation of lung cancer cells. MiR-107 is capable of down-regulating TRIAP1 which suppresses cell proliferation in lung cancer. We validate the negative correlation of miR-107 and TRIAP1 in clinical lung cancer tissues. Thus, our finding takes a further step into the mechanism of the development of lung cancer mediated by miR-107. Therapeutically, the miR-107 or TRIAP1 genes may be potential targets for lung cancer treatments.

**Conflicts of interest:** Authors state no conflict of interest.

References

MiR-107 inhibits proliferation of lung cancer cells through regulating TP53 regulated inhibitor of apoptosis 1


