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ORIGINAL ARTICLE

Let-7a targets Rsf-1 to modulate radiotherapy response of non-small cell lung cancer cells through Ras-MAPK pathway

Zhenliang Shi[1,2], Jia Liu[3], Daqiang Sun[2]

[1]Graduate School, Tianjin Medical University, Tianjin, China. [2]Department of Thoracic Surgery, Tianjin Chest Hospital, Tianjin, China. [3]Department of Thoracic Surgery, Shaanxi Provincial Cancer Hospital, Xi'an Jiaotong University, Xi'an, China.

*Zhenliang Shi and Jia Liu contributed equally to this work

Summary

Purpose: *Radiotherapy is the most commonly selective medical treatment for non-small cell lung cancer (NSCLC) and the multiple underlying mechanisms are considered as the effectively theoretical foundation. Herein, we investigated the effects of let-7a targets Rsf-1 on modulating the radiotherapy response in NSCLC cells by Ras-MAPK pathway.*

Methods: *A549 cells were divided into different groups to investigate the role of let-7a and Rsf-1 on the radiotherapy response. The expression of let-7a and Rsf-1 were detected by RT-PCR. Bioinformatic analysis indicated that Rsf-1 is the target of let-7a. The binding site of let-7a in the Rsf-1 3'UTR was detected based on double luciferase reporter assay and Western blot. The cell variability and proliferation were assessed by MTT and colony formation assay. The expression levels of Ras-MARK signaling pathway related proteins were assessed by RT-PCR.*

Results: *RT-PCR results showed that radiotherapy could up-regulate the expression of let-7a, thereby reducing the expression of Rsf-1, and the correlation between the two factors was negatively correlated. At the same time, let-7a overexpression and Rsf-1 silencing could further reduce the activity of A549 cells after radiotherapy, have an inhibitory effect on cell proliferation and inhibit the expression of related proteins in the Ras-MAPK pathway.*

Conclusions: *Rsf-1 is the target of Let-7a. The present study provides evidence that let-7a targeting Rsf-1 can modulate radiotherapy response in NSCLC cells through Ras-MAPK pathway.*

Key words: *let-7a, non small cell lung cancer, radiotherapy, Ras-MARK, Rsf-1*

Introduction

Lung cancer is the major cause of cancer deaths in humans around the world. Small-cell lung cancers (SCLC) and non-small cell lung cancers (NSCLC) belong to two sorts of lung cancer, and they are identified according to how they appear under a microscope [1]. Especially, of all lung cancer cases, the patients with NSCLC account for about 85%. The existing medical treatment methods for NSCLC have some different types for these patients, such as surgery, chemotherapy, radiation therapy, immunotherapy and photodynamic therapy (PDT) [2]. Many new treatment plans have also been tested in clinical trials, for example, chemoprevention as well as radiosensitizers [3]. Radiation therapy is a traditional cancer treatment method that applies high-energy x-rays or other kinds of methods to prevent the growing of cancer [4]. The different ways for radiotherapy are depending on the stages and types of lung cancer [3,5].

Let-7 is the first identified tumor inhibiting microRNA (miR), which also includes 12 subtypes. These subtypes can regulate the oncogenes to in-

Corresponding author: Daqiang Sun, MD. Department of Thoracic Surgery, Tianjin Chest Hospital, 261 Taierzhuang South Rd, Jinnan District, Tianjin 300000, China.
Tel: +86 013034337758; Email: sdqmd@163.com;
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hibit the tumor progression through binding on the 3' UTR region of targeting mRNAs, for instance high-mobility group AT-hook 2 (HMGA2), c-Myc as well as Ras [6]. Beyond that, the expression of let-7 families has been discovered to decrease in many cancers, such as breast cancer [7]. Previous studies have discovered that the expression of let-7a is downregulated in lung cancer [8] and nasopharyngeal carcinoma [9], but upregulated in radiation therapy [10]. A good prognostic marker, and the role of let-7a in radiotherapy are not clear. A recent study has revealed that let-7a can target Rsf-1 to regulate cisplatin resistance in nasopharyngeal carcinoma [11]. Overexpression of let-7a can inhibit the growth of nasopharyngeal carcinoma and achieve therapeutic effect [12]. The high expression of Rsf-1 is a biomarker of NSCLC prognosis [13]. It is still unknown whether let-7a can improve the survival rate of NSCLC by adjusting Rsf-1 expression in radiotherapy. However, there is no enough evidence to reveal that let-7a targets Rsf-1 and provide a potential method for NSCLC treatment.

Ras proteins participate in multiple intracellular signaling pathways with reference to cell differentiation, proliferation and migration [14]. Among the Ras signaling pathways, the Ras-MAPK pathway was regarded as a therapeutic target according to the most extensive studies in human cancers [15]. Ras-MARK pathway was also discovered to affect the function in the radio-resistance of lung cancer.

In the present study performed were a series of experiments on A549 cells to investigate the underlying mechanism of let-7a on radiotherapy.

Methods

Cell culture and radiotherapy

The A549 NSCLC cell line was bought from BNCC (http://www.bncc.org.cn/A549), and were cultured in a RPMI 1640 medium (Solarbio, No. 31800, Beijing, China) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA, USA), http://www.excellbio.com/). All cells were incubated at 37°C, 5% CO_2, and saturated humidity (Thermo Fisher Scientific, Waltham, MA, USA), and then these cells were collected and tested during the logarithmic growth phase.

A549 cells were placed in a Petri dish with a 1.5 cm thick plexiglass plate on the surface at 24°C. Subsequently, they were exposed to a single dose of X-rays using linear accelerator (Elekta Compact, Bejing, China). The dose rate was 1.15 Gy/min and 160 kv X-ray energy [16].

Cell grouping and process

A549 cells were randomly grouped into: (1) Blank control group (Control): No treatment; (2) Radiotherapy group (Radiotherapy): A549 cells were treated with radiotherapy; (3) Silent let-7a group (Si-let-7a):A549 cells were first treated with radiotherapy and then transfected with let-7a siRNA vector; (4) Silent let-7a negative control group (NC1): A549 cells were first treated with radiotherapy and then transfected with let-7a siRNA negative control vector; (5) Over-expression of let-7a group (Over-let-7a): A549 cells were first treated with radiotherapy and then transfected with et-7a mimic vector; (6) Over-expression of let-7a negative control group (NC2): A549 cells were first treated with radiotherapy and then transfected with let-7a mimic negative control vector.

In order to verify whether target gene Rsf-1 effects on A549 radiotherapy, the experimental groups were designed as follows: (1) Blank control group (Control): No treatment; (2) Radiotherapy group (Radiotherapy): A549 cells were treated with radiotherapy; (3) Over- Rsf-1 group (over-Rsf-1): A549 cells were first treated with radiotherapy and then transfected with Rsf-1 mimic vector; (4) Rsf-1 negative control group (NC3): A549 cells were first treated with radiotherapy and then transfected with Rsf-1 mimic negative control vector; (5) Silent Rsf-1 group (si-Rsf-1): A549 cells were first treated with radiotherapy and then transfected with Rsf-1 siRNA vector; (6) Silent Rsf-1 negative control group (NC4): A549 cells were first treated with radiotherapy and then transfected with Rsf-1 siRNA negative control vector.

In order to verify the rescue effect of Let-7a on Rsf-1, the experimental groups were designed as follows: (1) Blank control group (Control): no treatment; (2) Radiotherapy group (Radiotherapy): A549 cells were treated with radiotherapy; (3) Over-expression of let-7a group (over-let-7a): A549 cells were first treated with radiotherapy and then transfected with let-7a mimic; (4) Rsf-1 siRNA group (si-Rsf-1): A549 cells were first treated with radiotherapy and then transfected with Rsf-1 siRNA vector; (5) Let-7a and Rsf-1 overexpression double transfection group (over-let-7a- Rsf-1): A549 cells were first treated with radiotherapy, and then transfected with let -7a mimic and Rsf-1 mimic vectors.

The liposome of Lipofectamine (TM) 2000 transfection reagent (Thermo Fisher Scientific, Waltham, MA, USA) vector carrying let-7a siRNA,Let-7a siRNA NC,let-7amimic, Let-7a mimic NC, Rsf-1 siRNA, Rsf1 siRNA NC and Rsf-1 mimic, Rsf1 mimic NC (Shanghai Gene Pharm Pharmaceutical Technology Co., Ltd, Shanghai, China) was transfected into A549 cells. After 72 hours, the cell transfection status was assessed by RT-PCR.

RT-PCR

TRIzol reagent (Takara, Dalian, China) was used to extract total RNA and then cDNA was synthesized using reverse transcription kits (Applied Biosystems, Waltham, MA, USA). Mastercycler® nexus X2 was utilized to perform RT-PCR. Conditions were as follows: 95°C 10 min, 95°C 15 s, 57°C 10 s (40 cycles). The relative expressions of let-7a, Rsf-1 were evaluated by using the $2^{-\Delta\Delta Ct}$ method, while U6 and β-actin mRNAs were used as the internal parameters, respectively. The following primers were as follows: Let-7a: F: 5'-UGAGGUAGUAGGUUGUAUAGUU-3'; R: 5'-CUAUACAACCUACUACCUCAUU-3';

U6: F: 5'-GAATTCCCCAGTGGAAAGACGC-3'; R: 5'-GGTGTTTCGTCCTTTCCACAAGATATATAAAGGG-3'; Rsf-1: F: 5'-TCCCAGATGGAGAATGGTTC-3'; R: 5'-AGTCTGGCTCTTGTGGAGGA-3'; β-actin: F: 5'-TGAGCGCGGCTACAGCTT-3'; R: 5'-TCCTTAATGTCACGCACGATTT-3'.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)

Trypsin was used to digest A549 cells and then cells were plated gently in 96-well plates at 1×10^4 cells per well. At 24 h, 48 h, 72 h, and 96 h, 200 μL of DMSO (Sigma-Aldrich, St. Louis, the MO, USA) was put to the plates, following with incubation for 4 h at 37°C. Finally, the optical density (OD) values were measured at 490 nm with a spectrophotometer to reveal the cell viability.

Colony formation assay

Cells were detached with 0.25% trypsin when they were in the logarithmic growth phase, following that the concentration was settled to 250 cells/mL. Subsequently, they were cultured in 6-well plates for 2 to 3 weeks in a humidified incubator at 37°C. The fresh RPMI1640 medium was replaced every 3 days. Hereafter, the plates were fixed in methanol. Then, each well was added with 1 mL of Giemsa stain solution (https://seebio.biomart.cn, Shanghai, China) and the cells were stained for 30 min. After the plates were washed, the records were imaged.

Western blot

First, A549 cells were lysed and centrifuged at 2000 rpm for 20 min, then the supernatant was removed. Bicinchoninic acid (BCA) kit (Solarbio, Beijing, China) was used to quantify the concentration of protein. The cell suspension was gently mixed with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to obtain a 1:1 dilution and then was heated for 5 min at 95°C. Next, proteins were transferred to polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA) for 0.5 h, and then blocked by 5% bovine serum albumin (BSA) for 1 h, and treated with the primary antibodies, including Rabbit anti-human anti-Rsf-1 antibody (1:1000, ab109002, Abcam, Cambridge, MA, USA), anti-Ras antibody (1:5000, ab52939, Abcam, Cambridge, MA, USA), anti-Raf1 antibody (1:1000, sc52827, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p-Raf1 antibody (1:1000, sc81513, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-MEK1 antibody (1:1000, #12671, Cell Signaling Technology, Danvers, MA, USA), anti-p-MEK1 antibody (1:1000, #9127, Cell Signaling Technology, Danvers, MA, USA) and β-actin (1:1000, ab8227, Abcam, Cambridge, MA, USA) polyclonal antibodies, goat anti-rabbit IgG (1:2000, ab6721, Abcam, Cambridge, MA, USA) for 1 h. After washing, the membrane was assessed with the chemiluminescence (ECL) system. β-actin was used to normalize the protein expression level and ImageJ software (NIH, Bethesda, MD, USA) was applied to calculate the grayscale images.

Double luciferase reporter assay

In this test, the wild-type and mutant 3' UTRs of A5499 cells were amplified from pGL3/luciferase vectors (Promega, Madison, WI, USA) and cloned into the down-

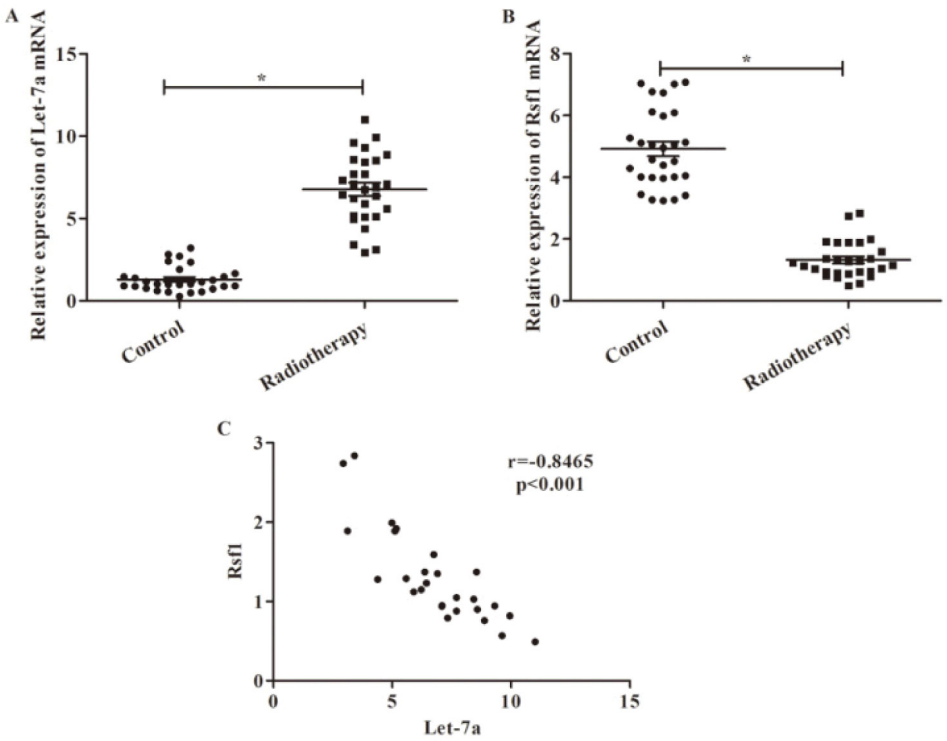

Figure 1. The radiotherapy effects on the expression of let-7a and Rsf-1 in A549 cell. RT-qPCR analysis of let-7a **(A)** and Rsf-1 **(B)** expressions in A549 cells undergoing radiotherapy before and after treatment. **(C)** Pearson correlation between let-7a and Rsf-1 in A549 cells undergoing radiotherapy before and after treatment. Compared with control group, *p<0.05.

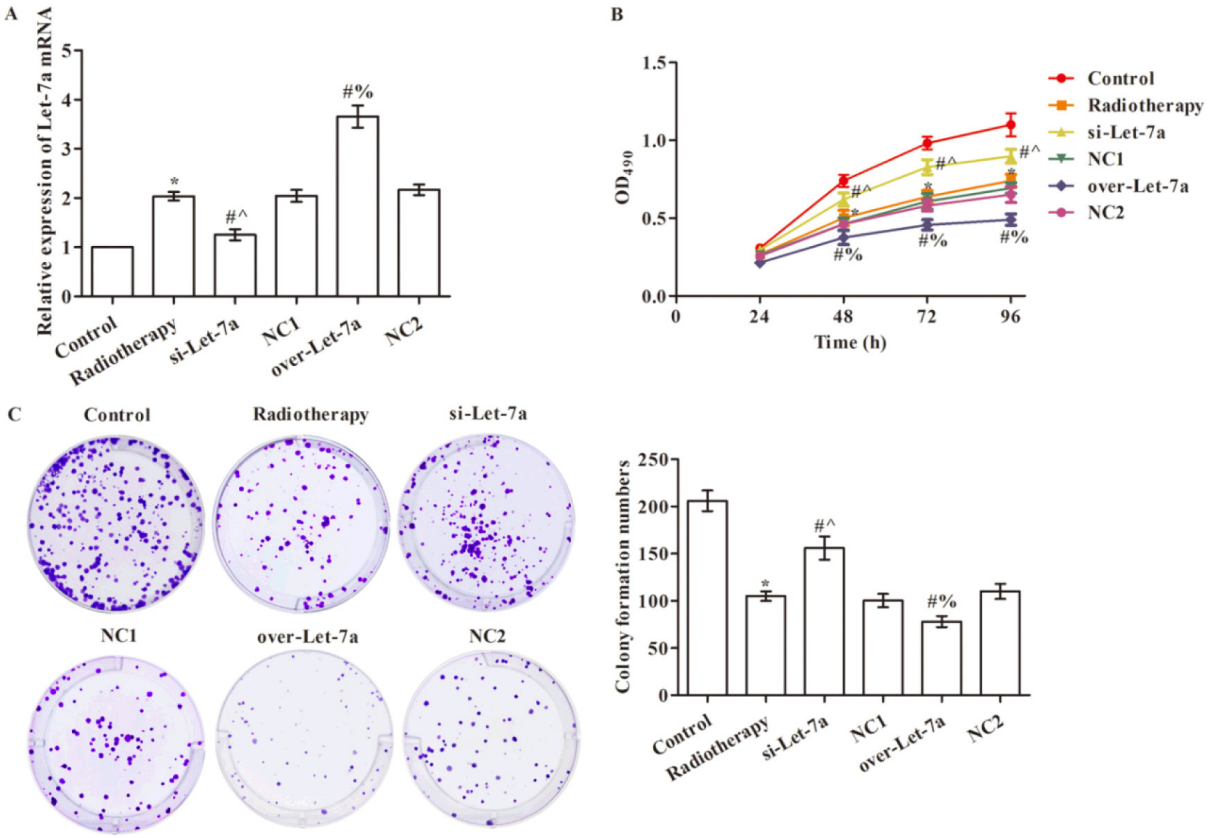

Figure 2. Effects of radiotherapy on cell proliferation in A549 cells. **A:** The expressions of let-7a were assessed by PT-PCR in A549 cells after transfected by let-7a siRNA and let-7a mimic. **B:** Cell viability determined based on MTT assay. **C:** Colony formation assay. Compared with control group, *p<0.05; Compared with radiotherapy group, #p<0.05. Compared with NC1 group, ^p<0.05. Compared with NC2 group, %p<0.05.

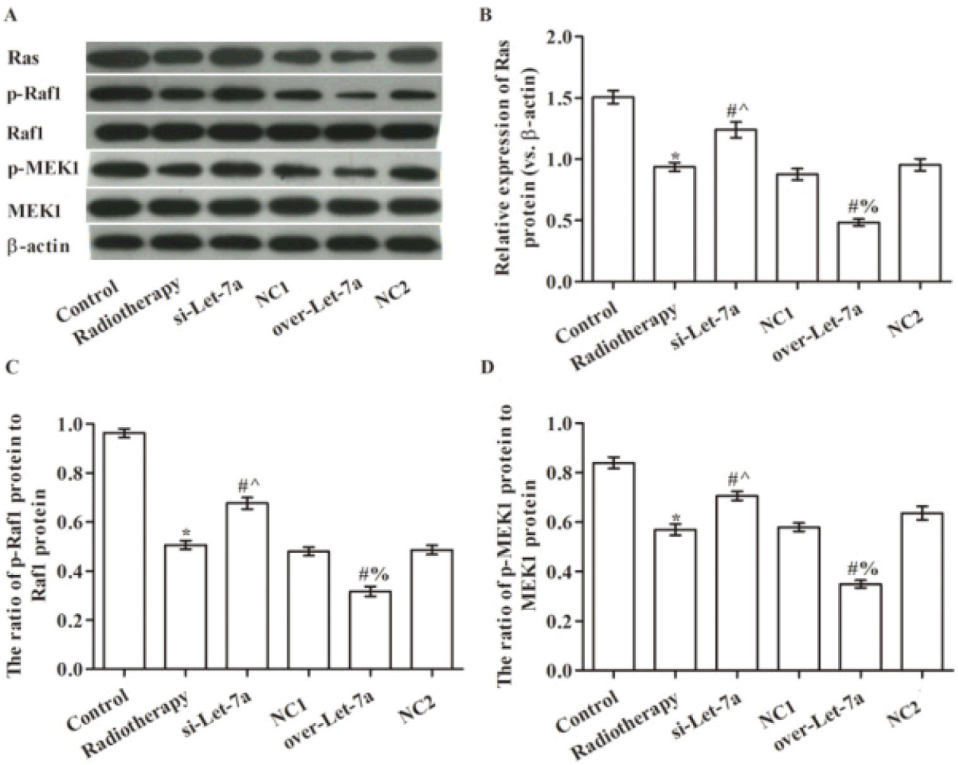

Figure 3. Radiotherapy effects on the expression levels of Ras-MAPK-related proteins in A549 cells. Compared with control group, *p<0.05; compared with radiotherapy group, #p<0.05; compared with NC1 group, ^p<0.05; compared with NC2 group, %p<0.05.

stream of luciferase gene. According to the instructions, the luciferase activity was exposed by a dual luciferase reporter system (Promega, Madison, WI, USA a) in 48 h after transfection.

Statistics

All data was processed by Prism 5.01 statistical software (La Jolla, CA, USA). Mean ± SD (standard deviation) was used to measure the categorical data. Differences between two groups were analyzed by using the Student's t-test. Comparison between multiple groups was done using One-way ANOVA test followed by *post hoc* test (least significant difference). Correlation analysis between let-7a and Rsf-1 was performed by Pearson method. P<0.05 was considered as statistically significant.

Results

Effects of radiotherapy on the expression of let-7a and Rsf-1 in A549 cells

To investigate the effect of radiotherapy for lung cancer treatment, RT-PCR assay was implemented to examine the expressions of let-7a and Rsf-1 inA549 cells. The results shown in Figure 1A revealed that let-7a had a higher expression in A549 cells after radiotherapy. Meanwhile, the downregulation of Rsf-1 was also shown in A549 cells (Figure 1B). To analyze whether the expression of let-7a can be related to the expression of Rsf-1, further correlation analysis showed that the relationship between let-7a and Rsf-1 was markedly negatively correlated (r=-0.8465, p<0.001, Figure 1C).

The effects of radiotherapy on cell proliferation in A549 cells

To reveal the effect of radiotherapy for cell proliferation, we performed RT-PCR method to detect the expression level of let-7a in Silent let-7a group (Si-let-7a). The results demonstrated that let-7a was lowly expressed in the radiotherapy group as compared to the control group (p<0.05, Figure 2A). However, the expression levels of let-7a were profoundly increased in A549 cells (p<0.05) after the transfection of let-7a mimic. The above results indicated that let-7a siRNA and let-7a mimic can successfully transfect A549 cells. As shown in Figure 2B, cell viability was reduced by radiotherapy

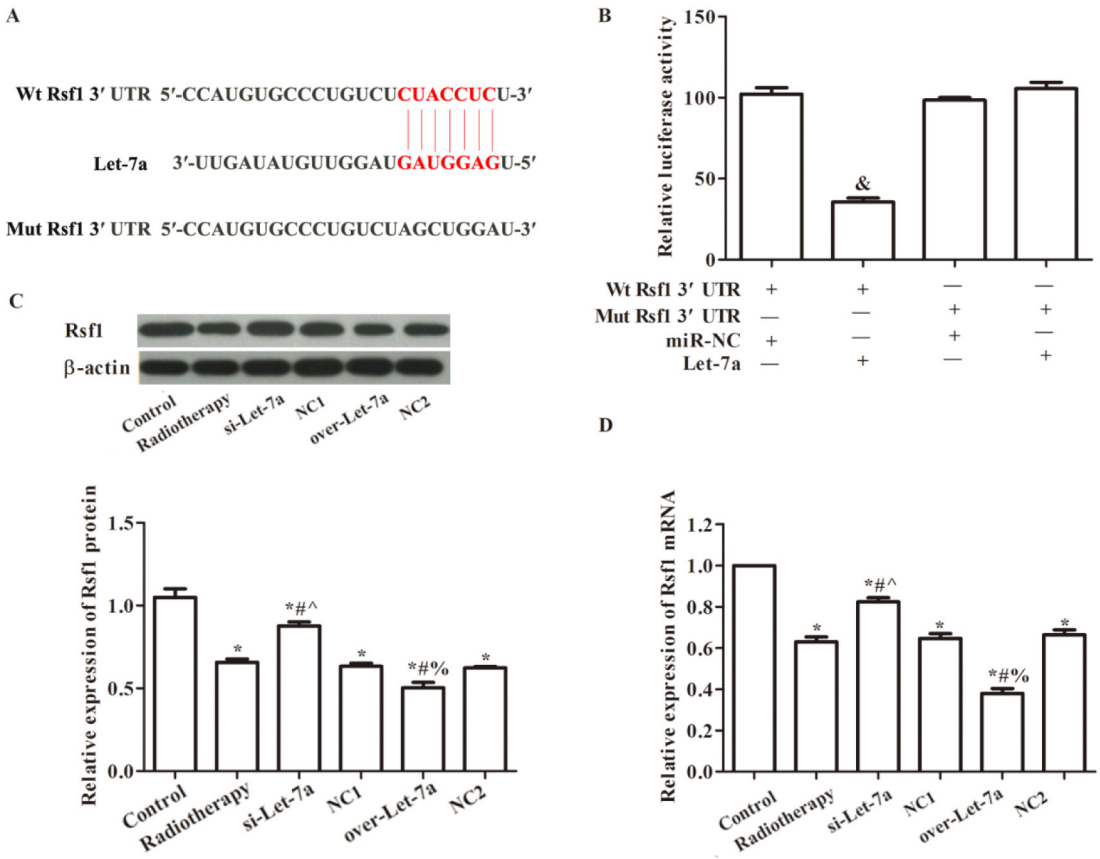

Figure 4. Rsf-1 is the target of Let-7a. **A:** The sequence predicted let-7a targeting site within Rsf-1 3'-UTR. **B:** Double luciferase reporting results for the recombinant vector of let-7a and the target gene Rsf-1.&p <0.05; **C:** The protein level of Rsf-1 was detected by Western blot. **D:** The mRNA expression of Rsf-1 was detected by RT-PCR. Compared with control group, *p<0.05; compared with radiotherapy group, #p<0.05; compared with NC1 group, ^p<0.05; compared with NC2 group, %p<0.05.

in A549 cells. On the basis of radiotherapy, the cell OD value was significantly decreased by let-7a overexpression than in the control and radiotherapy group ($p<0.05$), but the cell proliferation ability was enhanced after transfection with let-7a siRNA vector ($p<0.05$). Consistent with MTT experiments, the cloning assay results (Figure 2C) showed that radiotherapy significantly reduced the cell proliferation in A549 cells. All results indicated that the overexpression of let-7a played an inhibitory effect on A549 cells proliferation after radiotherapy.

The effects of radiotherapy on Ras-MAPK-related proteins' expression levels in A549 cells

Ras-MAPK pathway is related with the activation of many transcription factors, which leads to cancer cell growth. To validate the underlying mechanism of let-7a-regulated signaling pathways, Western blot was implemented to quantify the expressions of Ras, p-Raf1/Raf1, and p-MEK1/MEK1 in the Ras-MAPK pathway (Figure 3). After 540 cells were treated by radiotherapy the expression level of the above proteins were decreased ($p<0.05$). After cells were transfected with let-7a siRNA vector, the expression levels of the above proteins were markedly increased, but transfection with let-7a mimic vector the Ras-MAPK related proteins were significantly decreased in A549 cells ($p<0.05$). This indicated that Ras-MAPK signaling pathway was suppressed by let-7a.

Rsf-1 is the target of Let-7a

Bioinformatics analysis showed that the sequence of Rsf-1 contains a let-7a binding region (Figure 4A), so to further verify whether Rsf-1 is a target of let-7a, a segment of Rsf-1-3' UTR-WT containing the let-7a complementary site as well as a mutated fragment Rsf-1-3' UTR-Mut were cloned using a dual luciferase reporter system (Figure 4B). The result showed that let-7a significantly reduced the relative luciferase activity in the cells with Rsf-1-WR but not in the cells with Rsf-1-Mut. To further confirm that let-7a indeed impacts the protein and mRNA expression levels of Rsf-1 in A549 cells, we performed Western blot and RT-PCR to examine the above expression levels and the data demonstrated that overexpression of let-7a resulted in a decreased in Rsf-1 levels. Collectively, these results demonstrated that Rsf-1 is the target of let-7a and is downregulated by let-7a (Figure 4C,4D).

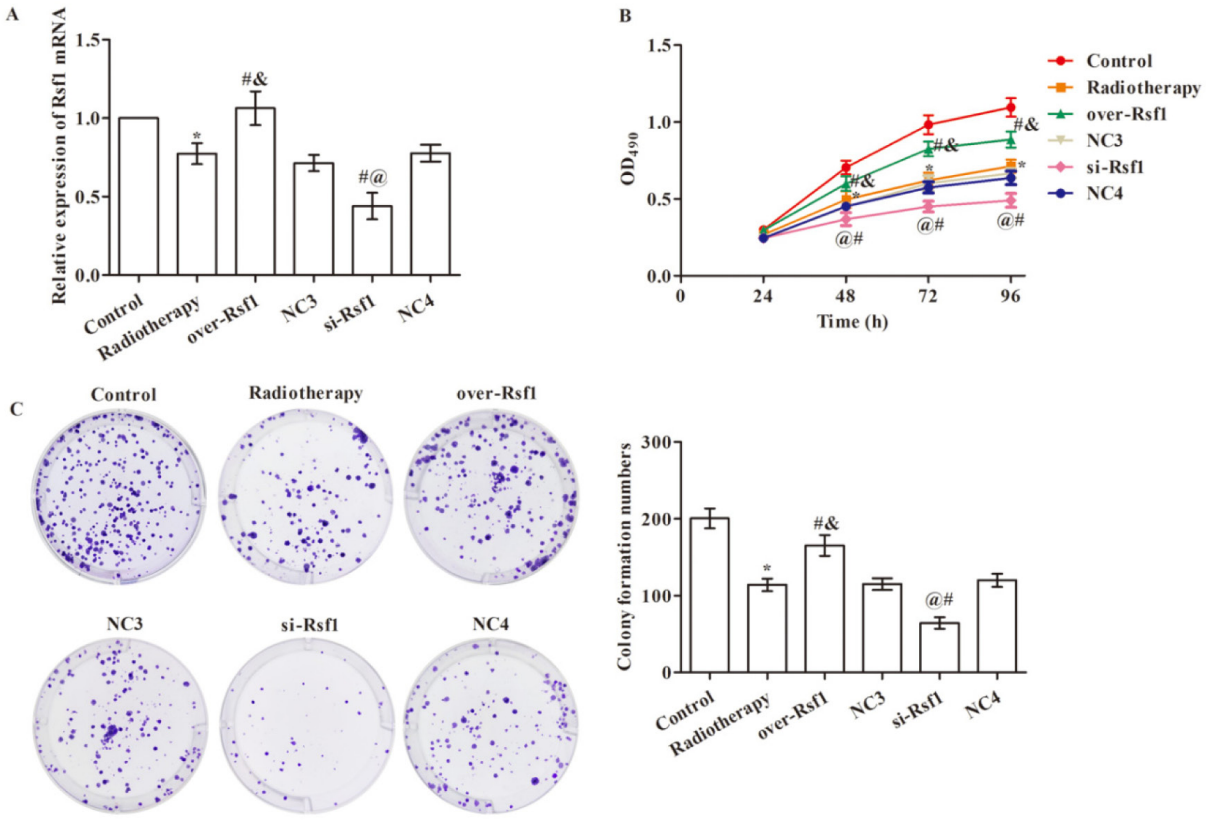

Figure 5. Effect of Rsf-1 on A549 cells' proliferation after radiotherapy. **A:** The expression of Rsf-1 after transfection with Rsf-1 siRNA and Rsf-1 mimic were assessed by PT-PCR in A549 cells. **B:** Cell viability was determined by MTT assay. **C:** Colony formation assay. Compared with control group, *$p<0.05$; compared with radiotherapy group, $^{\#}p<0.05$; compared with NC3 group, $^{\&}p<0.05$; compared with NC4 group, $^{@}p<0.05$.

Effect of Rsf-1 on A549 cell proliferation after radiotherapy

We used RT-PCR technology to detect Rsf-1 expression in A549 cells so as to investigate the effect of Rsf-1 on the cell proliferation. After transfection with Rsf-1 siRNA, the mRNA levels of Rsf-1 were significantly decreased in A549 cells compared with the radiotherapy group ($p<0.05$, Figure 5A). But the expression of Rsf-1 was markedly increased after transfection with Rsf-1 mimic ($p<0.05$, Figure 5A). These results indicated that Rsf-1 siRNA and Rsf-1 mimic successfully transfected A549 cells. Next, the role of Rsf-1 on cell variability and proliferation (Figure 5B) was investigated. Our results showed that the cell variability was reduced in A549 cells after radiotherapy ($p<0.05$). When transfected with Rsf-1 siRNA vector, the cells' OD value was markedly decreased in comparison with both the control and radiotherapy group ($p<0.05$). But, when transfected with Rsf-1 mimic overexpression vector, the cell proliferation was increased ($p<0.05$). Consistent with MTT results, the colony assay results also demonstrated that the cell proliferation of A549 cells was obviously decreased (Figure 5C). Silencing Rsf-1 could further reduce the activity of A549 cells after radiotherapy.

Effects of Rsf-1 on the expression levels of Ras-MAPK pathway related proteins in A549 cells after radiotherapy

Treatment with radiotherapy reduced the expression levels of p-Raf1 and p-MEK1 proteins in A549 cells (Figure 6). However, after transfection with Rsf-1 siRNA silencing vector, their expression levels were markedly increased, but after transfection with Rsf-1 mimic overexpression vector, the Ras-MAPK related proteins were significantly decreased in A549 cells ($p<0.05$). These results meant that Ras-MAPK signaling pathway was suppressed by Rsf-1.

Effect of Let-7a targeting Rsf-1 on A549 cells' proliferation after radiotherapy

As seen in Figure 7A, the cell viability of A549 cells was statistically significantly reduced after treatment with radiotherapy ($p<0.05$). When we further transfected with let-7a mimic overexpression vector and Rsf-1siRNA vector, the cell OD value became significantly lower than the control group and the radiotherapy group ($p<0.05$). But when transfected with let-7a mimic overexpression vector, the cell proliferation of A549 cells was significantly enhanced ($p<0.05$). And consistent with MTT results, the colony formation assay results also showed that Rsf-1 overexpression could reverse let-7a's inhibitory effect on A549 cell proliferation (Figure 7B).

Effect of Let-7a targeting Rsf-1 on the Ras-MAPK pathway related proteins' expression in A549 cells after radiotherapy

The above results showed that radiotherapy may inhibit the expression of Ras-MAPK pathway related proteins. As shown in Figure 8, after cells were transfected by Let-7a mimic overexpression vector and Rsf-1 siRNA vector, respectively, the related proteins in the Ras-MAPK pathway were further down-regulated. But when we first transfected with Rsf-1 over-expression vector and further transfected with let-7a transfection mimic vectors, the protein levels of Ras-MAPK were up-regulated. Our results also indicated that Rsf-1 reverses let-7a to inhibit the expression of Ras-MAPK pathway related proteins.

Discussion

Radiotherapy is a common treatment method for metastatic NSCLC [17] and radioresistance remains a major problem for cancer patients. There-

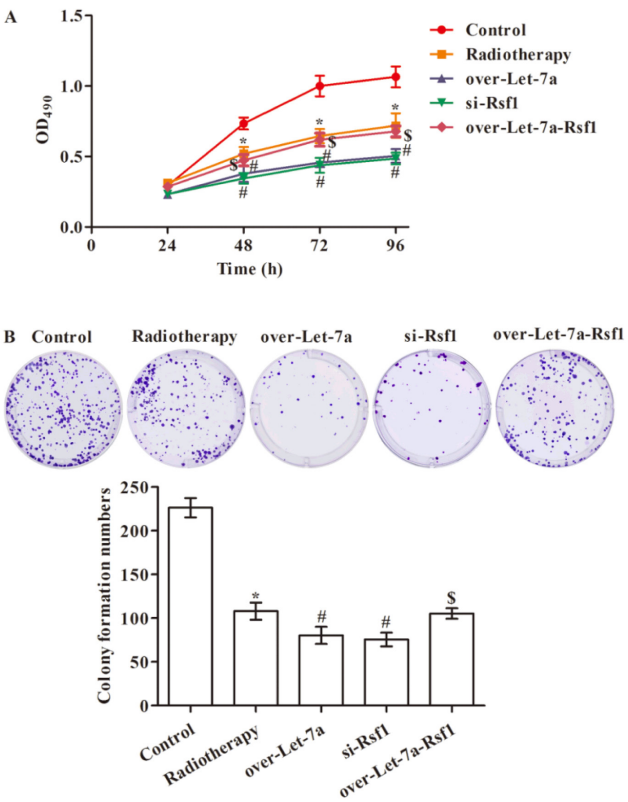

Figure 7. Effect of Let-7a targeting Rsf-1 on the proliferation of A549 cells after radiotherapy. **A:** Cell viability detected by MTT assay; **B:** Colony formation assay. Compared with control group, *$p<0.05$; compared with radiotherapy group, #$p<0.05$; compared with over-let-7a group, $$p<0.05$.

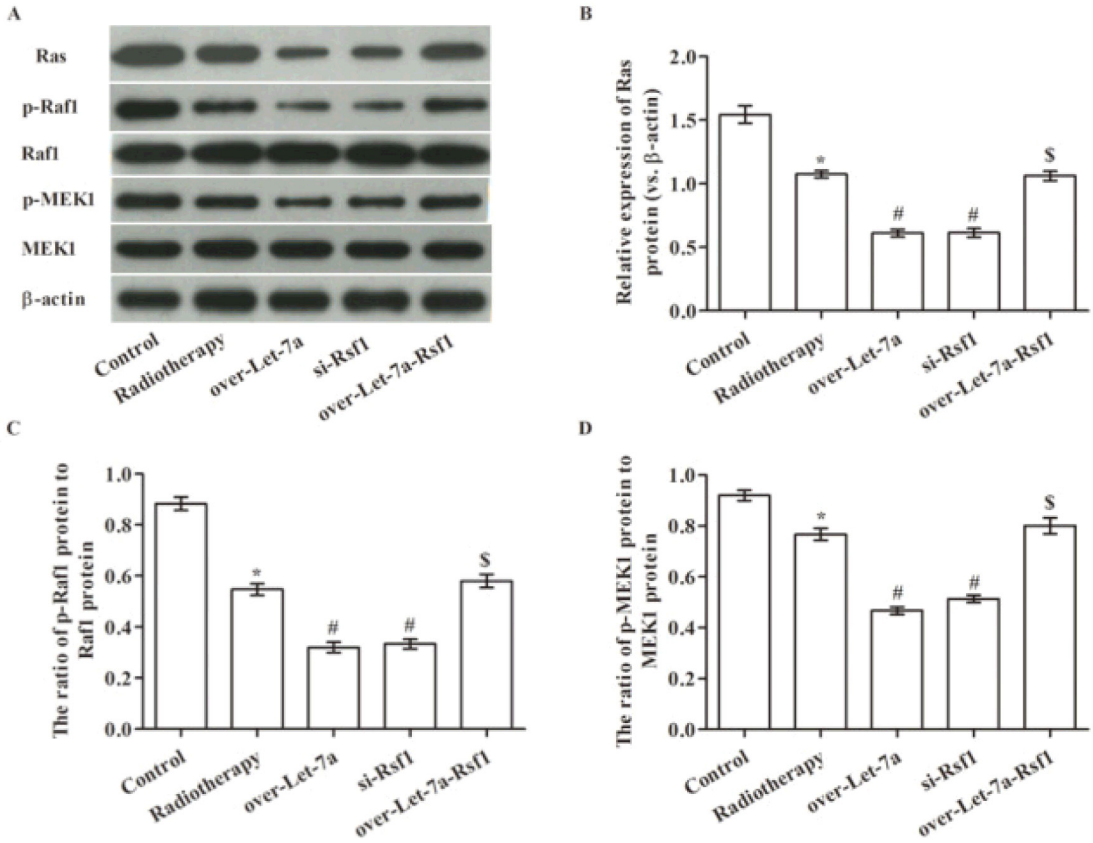

Figure 8. Let-7a effected on the expression of Ras-MAPK pathway related proteins in A549 cells after radiotherapy via targeting Rsf-1. **A:** The related proteins level of Ras-MAPK pathway was detected by western blot. **B:** Relative expression level of Ras protein. **C:** The ratio of p-Raf1 protein to Raf1 protein. **D:** The ratio of p-MEK1 protein to MEK1 protein. Compared with control group, *p<0.05; compared with radiotherapy group, #p<0.05; compared with over-let-7a group, $p <0.05.

fore, there is a great need to improve the effectiveness of radiotherapy for the medical treatment of NSCLC [18]. There are some previous studies that have revealed that microRNAs have a pivotal role in the radiotherapy response of various cancer types [7]. Cancer stem cell is an important material which has intrinsic resistance to radiation and possesses differentiation and self-renewal potential [19]. miR-214 and miR-29 were reported to regulate the viability and proliferation of cancer cells [19-21]. Increasing evidence has indicated that the let-7 miRNA family is linked to the cell differentiation and proliferation during cell development [8,13,22]. Let-7 miRNA has also been revealed to stop the cell cycle in the G1 phase by means of restricting the correlated genes. The expression of let-7 family was also shown to increase in the neural differentiation of embryonic stem cells. This evidence indicated that let-7 miRNA family has an essential role in senescence and aging in the pathogenesis of fibrogenic lung reactions. A recent research has reported that the expression of let-7a was downregulated in NSCLC samples [23]. In the present study, the expression of let-7a in NSCLC was also examined, and was consistent with former studies. Previous studies have speculated that let-7a miRNA targets epidermal growth factor receptor (EGFR) gene in NSCLC samples [24]. But the regulated mechanism of let-7a remained mysterious. Rsf-1 is a highly expressed mRNA in cancer cells located in chromosome 11p13.5-14. There are several reports demonstrating that Rsf-1 overexpression is a cancer marker which correlates with cancer cell proliferation, for instance, in prostate cancer and breast cancer as well as in colon cancer [12,25,26]. Additionally, the up-regulated Rsf-1 expression facilitated tumor radioresistance, aggressiveness and progression of cancer cells, which indicated worse survival and poor therapeutic response [11]. In our study, the expression of Rsf-1 was down-regulated in NSCLC cells and the correlation between Rsf-1 and let-7a showed a negative relationship. Furthermore, Rsf-1 was also confirmed as a target gene of let-7a based on the bioinformatic analysis in our study. Consequently, we concluded that the function of radiotherapy for NSCLC was improved due to let-7a targeting of Rsf-1.

Ras-mitogen activated protein kinase (Ras-MAPK) pathway is known as a key intracellular

signaling pathway, which comprises cell differentiation, proliferation and other cellular activities [27]. It has been published that it was a result of abnormal activation of receptor tyrosine kinases in human cancer or gain-of-function mutations mainly in the Ras genes [28,29]. Ras-MAPK pathway contains many inhibitors which can be taken as attractive therapeutic targets [30]. A large number of researches have been designed for assessing the prognostic importance of oncogenic Ras mutations in some cancers [31]. Now, there are also contradictory suggestions in NSCLC, as some studies are negative or equivocal [22]. In this study, we explored the role of let-7a via targeting Rsf the Ras-MARK pathway. The results shown in our study have indicated that Let-7a targets Rsf-1 to inhibit not only the cell proliferation of A549 cells but to also inhibit the Ras-MAPK pathway related proteins' expressions.

Conclusions

In general, our study concluded that let-7a was downregulated in NSCLC cells. Serial analysis suggested that let-7a could modulate the radiotherapy response of NSCLC cells via the Ras-MAPK pathway. Therefore, let-7a targets Rsf1 and might have essential therapeutic connotations for exceeding resistance responses in NSCLC.

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Conflict of interests

The authors declare no conflict of interests.

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