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ORIGINAL ARTICLE

Correlation between miR-564, TGF-β1, and radiation-induced lung injury*

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Abstract

Objective Our study aimed to analyze the expression of miR-564 and TGF- β1 in cancer tissues and the serum of patients with radiation-induced lung injury, and to investigate the relationship between them and radiation-induced lung injury.

Methods In situ hybridization and real-time fluorescence quantitative method were used to detect the expression of miR-564. Additionally, immunohistochemistry and enzyme-linked immunosorbent assay (ELISA) were performed to detect the expression of TGF- β1.

Results The overall incidence of acute radiation pneumonia was 55.9% (100/179). The incidence of ≥ grade 2 radioactive pneumonia was 24.0% (43/179) and that of grade 1 was 31.8% (57/179). The expression of miR-564 in grade ≥ 2 was slightly higher than that in patients without or with grade 1, but there was no statistical difference (P = 0.86). The serum level and ratio of miR-564 in patients with grade ≥ 2 were significantly higher than those without or with grade 1 (P = 0.005, P = 0.025, respectively). The expression of TGF- β1 in grade ≥ 2 was significantly higher than that of patients without or with grade 1 (P = 0.017). The serum levels of TGF- β1 in grade ≥ 2 were significantly higher than those in patients without or with grade 1 (P = 0.038). Although the ratio of TGF- β1 in radiation pneumonia of grade ≥ 2 was significantly higher than that of without or with grade 1, there was no significant difference (P = 0.24). Moreover, patients with higher expression of miR-564 and lower expression of TGF-β1 had better prognosis.

Conclusion MiR-564 and TGF-β1 are predictors of radiation-induced lung injury. Monitoring its changing trend can improve the accuracy of predicting radiation-induced lung injury. The levels and ratio of serum miR-564 and TGF- β1 in patients with radiation-induced lung injury are related to the severity of radiation-induced lung injury.

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Key words: radiation-induced lung injury; miR-564; TGF-β1

Radiation-induced lung injury is the most common side effect of chest cancer radiotherapy. It includes early inflammatory reaction and late fibrosis, which seriously affect the quality of life of patients and become the bottleneck of increasing the dose of radiotherapy; however, its underlying mechanism is unclear [1–2]. Exploring the mechanism of radiation-induced lung injury has become an interesting research topic at present. In recent years, studies have shown that radiation-induced lung injury results from the interaction of many kinds of cells, cytokines, and signaling pathways [3–4]. It has been proved that transforming growth factor-β1 (TGF- β1) is a predictor of radiation-induced lung injury, which can activate fibroblasts to differentiate into myofibroblasts,

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promote matrix synthesis, produce large amounts of collagen, and mediate radiation-induced lung injury [5]. Wang *et al*[6] found that with increase in radiation dose, the plasma level of TGF-β1 was consistently higher than the baseline level, and the level of TGF-β1 was closely related to grade 2 radiation-induced lung injury. However, only a few studies have been conducted to evaluate the relationship between microRNA (miRNA) and radiation lung injury, and limited studies have explored the combination of miRNA and TGF-β1 in radiation lung injury. It is not clear whether a correlation between them exists. This study is based on our previous findings that miR-564 serves as a negative regulatory gene in lung cancer [7]. However, it is not clear whether miR-564 and TGF-β1 are involved in the regulation of radiation-induced lung injury. In this study, we investigated the expression of miR-564 and TGF-β1 in tumor tissues and the blood of patients with radiation pneumonitis, and we explored the relationship between their expression levels and radiation-induced lung injury.

Materials and methods

Participants

From November 2014 to December 2016, patients with pathologically confirmed non-small cell lung cancer (NSCLC), who received intensity-modulated radiotherapy (IMRT) at the Hubei Cancer Hospital, and were assessed to have a KPS score > 70 and expected survival time of more than 6 months were enrolled in this study. There were 100 male and 81 female patients with a median age of 59 years (27, 87 years). Among the patients, there were 121 smokers and 60 non-smokers, 87 adenocarcinoma, 73 squamous cell carcinoma, 21 adenosquamous carcinoma, 97 stage III A, and 84 stage III B lung cancer patients (according to the 8th stage of lung cancer). In addition, 94 cases were central type, 87 were peripheral type, 165 subjects received chemotherapy (74 subjects received concurrent chemotherapy), and 16 subjects did not receive chemotherapy. The general information for the patients is shown in Table 1.

Table 1 The baseline characteristics of 181 patients with lung cancer

Clinical characteristics	No. of patients	%
Gender		
Male	100	55.2
Female	81	44.8
T staging		
T1	15	8.3
T2	38	21.0
T3	69	38.1
T4	59	32.6
N staging		
N0	8	4.4
N1	26	14.4
N2	66	36.5
N3	81	44.7
Pathological type		
Adenocarcinoma	87	48.1
Squamous cell carcinoma	73	40.3
Adenosquamous carcinoma	21	11.6
Clinical staging		
IIIA	97	53.6
IIIB	84	46.4
Gross type		
Central type	94	51.9
Peripheral type	87	48.1
Chemotherapy (yes/no)		
Yes	165	91.2
No	16	8.8
Curative effect evaluation		
CR	48	26.8
PR	107	59.8
SD	22	12.3
PD	2	1.1
Radiation pneumonia		
Grade 1	57	31.8
Grade 2 or above 2	43	24.0

Radiotherapy plan

All the patients were treated using Varian accelerator 23EX, with target dose of above 56 Gy, 1.8–2.0 Gy/F, administered once a day, and 5 times a week, and all patients were treated with intensity modulated radiotherapy (IMRT). To delineate the primary lung lesions at the pulmonary window (GTV) and the metastatic lymph nodes in the mediastinal fenestra (GTVnd), GTV exoduses 6 mm, 8 mm, and the corresponding regions of the metastatic lymph nodes CTV. CTV exoduses 3 mm as PTV; V20 ≤ 28%, V5 ≤ 60% in both lungs, D2 ≤ 40 Gy in spinal cord, V30 ≤ 40%, V40 ≤ 30% in heart.

Main reagent

The reagents used were RecoverALL Total Nucleic Acid Isolation Kit (Ambion) kit, Probe Mix (ABI Company), TaqMan probe (ABI), miScript SYBR Green PCR Kit (Qiagen Company), TGF- β 1 Antibody (Beijing Boosen Biotechnology Co., Ltd.), TGF- β 1 ELISA Kit (Xinbosheng Biotech Co., Ltd.), SP Kit (Beijing Zhongshan Jinqiao Biotechnology, Limited), and mirVanaTM PARISTM kit (American Applied Biosystem company product).

Serum collection

Before radiotherapy, 2 weeks, 4 weeks, 6 weeks, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 5 mL, and 4 °C cold storage of cubital venous blood were collected. A 4 °C low temperature centrifugation of –80 °C supernatant at 1000 g for 10 min was performed within 12 h.

RNA extraction from paraffin samples and serum samples

Paraffin slices were dewaxed using xylene, and xylene was removed using ethanol and dried at room temperature, and 200 μL was added to digest buffer. Digestive enzyme of 4 μL was heated at 50 °C for 15 min, and then heated again at 80 °C for 15 min. Next, nucleic acid separation additive of 240 μL was added, centrifuged rinsed with Wash1 and Wash2 respectively, and then digested and purified using nuclease. Serum sample of 400 μL per copy was extracted using the method described in the instruction manual of mirVanaTM PARISTM kit, and the total RNA was stored in a refrigerator at –80 °C for use.

Detection of miR-564 expression in cancer tissue by in situ hybridization

Paraffin sections were dry-heated in an oven at 68 °C for 30 min. Next, conventional xylene was dewaxed in water and the slices were treated with 0.5% H_2O_2 / methanol solution for 30 min, and inactivation of endogenous peroxidase occurred. Next, the slices were flushed with distilled water for 3 times. Pepsin was freshly diluted with 3% citric acid, digested at 37 °C for 25 min to expose miRNA fragments, and then flushed with 0.5 M PBS buffer for 3 times. Hybridization solution of 20 μL (probe concentration was 14 μg/mL) was added to each slice for overnight hybridization at 42 °C (about 16 h) each slice was then flushed with distilled water once for 5 min each time, and 20 μL hybridization solution (probe concentration 14 μmol) was added again to each slice for overnight hybridization (about 16 h). After hybridization, the slides were washed twice with 2 × SSC, preheated at 37 °C for 5 min each time, and then washed and sliced with 0. 5 × SSC and 0. 2 × SSC liquids, respectively. The slices were incubated at 37 °C for 30 min, then treated with biotinylated mouse anti-digoxin at 37 °C for 60 min, and washed with 0.5 M PBS for 3 times and 5 min each time. After incubating at SABC-POD, 37 °C for 30 min, the slices were washed with 0.5 M PBS for 4 times, 5 min each time, and the slides were incubated at 37 °C for 30 min with biotin peroxidase dripping, and then washed with 0.5 M PBS for 4 times, 5 min each time. Color with freshly prepared DAB solution, microscopically controlled coloration time, hematoxylin redying, 0.1% hydrochloric acid ethanol differentiation, distilled water turning blue, conventional alcohol gradient dehydration, dimethylbenzene transparent neutral gum sealing.

Real-time fluorescence quantitative PCR was used to detect the content of miR-564 in serum

The 2 × All-in-OneTMqPCR Mix in miRNA-qRT-PCR Detection Kit was melted at room temperature, and then mixed gently upside down and centrifuged briefly. In the process of the preparation, PCR mix was always stored in dark (operated on ice), and No Template Control (NTC), was designed as negative control of the experiment; therefore, other reagents of template cDNA, were replaced with water in the reaction. To determine whether the system was contaminated, PCR mix was quickly mixed and added to a 96-well plate. The 96-well plate was centrifuged briefly to ensure that all reaction fluids were at the bottom of the reaction hole.. The standard three-step procedure was used for the PCR. After the PCR was performed, the following procedure was used to analyze the melting curve: iQ5 software and SPSS 17.0 were used for data analysis, the relative expression rate (Relative Expression, RQ) of the target gene hsa-miR-564 of the sample was calculated using the difference multiple method (2 Ct), and the experiment was repeated three times.

The expression of TGF- β 1 was detected by immunohistochemistry

Paraffin sections of 4 μm in size were treated with xylene dewaxing, gradient ethanol (75%, 80%, 95%, and 100%) for dehydration, 3% H_2O_2 solution for incubation at 37 °C for 20 min, and PBS solution for washing thrice. The slices were placed in sodium citrate buffer at 100 °C, heated for 15 min, naturally cooled, and washed with PBS solution for 3 times. Goat serum was sealed and incubated at 37 °C for 20 min and TGF- β 1 (1:100) antibody diluent was infused into the serum. The negative control was replaced with PBS and incubated at 4 °C overnight. IgG, 37 was incubated at 30 min with PBS for 3 times, enzyme / streptavidin complex was incubated at 37 °C for 30 min, with PBS and washed 3 times, DAB color was developed, hematoxylin was redyed, and normal dehydration and transparent sealing were performed.

Detection of serum TGF- β 1 content through enzyme-linked immunosorbent assay (ELISA)

Venous blood coagulated naturally at room temperature for 20 min, and it was centrifuged at 3000 rpm for 20 min. The supernatant was collected and repacked with 500 pL number, and was transferred to –80 °C refrigerator for storage. The patients' serum TGF-β1 content was detected using ELISA kit (Xinbosheng Biotech Co., Ltd) within 2 h after melting at room temperature. The procedure was performed in strict accordance with the manufacturer's instructions.

Result judgment

Considering the number of positive cells in a single visual field / the total number of tumor cells × 100% as the evaluation criterion, according to the rate of positive cells in tumor cells, ≤ 1% was (–), 1%–5% was (+), and 5%–15% was (2+),15%–25% was (3+), and more than 25% was (4+). The positive cells were nucleoserous type (including

nucleoserous membrane, serosa, and membrane-positive) and karyotype (including nuclear and nucleocytoplasmic positive).

Assessment of radiation-induced lung injury

Acute radiation-induced lung injury was assessed weekly according to RTOG acute radiation-induced lung injury classification for a period of 3 months, from the beginning of radiotherapy to the end of radiotherapy [8].

Statistical analysis

SPSS 17.0 was used for statistical analysis, *t* test was used for mean comparison, and χ^2 and Fisher's precise probability method were used for rate comparison.

Results

Follow-up results

The shortest follow-up time was 7 months and the longest follow-up time was 31 months until June 30, 2017. The median follow-up period was 19 months and two cases were lost. The overall incidence of acute radiation pneumonitis was 55.9% (100 / 179), grade 2 and above (≥ grade 2) was 24.0% (43 / 179), and grade 1 was 31.8% (57 / 179). Efficacy evaluation: 48 cases of CR (26.8%), 107 cases of PR, 22 cases of 59.8%), SD (12.3%), 2 cases of PD (1.1%), Table 1.

Relationship between the expression of miR-564 in lung cancer tissues and the degree of radiation-induced lung injury

The expression of miR-564 in the cancer tissues of patients with ≥ grade 2 radiation pneumonitis was slightly higher than that of patients without or with grade 1. However, there was no statistical difference (P = 0.86; Fig. 1).

Relationship between the level of serum miR-564 and the severity of radiation-induced lung injury

The level of miR-564 in the serum of patients with ≥ grade 2 radiation pneumonitis increased gradually during radiotherapy, and reached the peak at the end of 4 weeks. Thereafter, it decreased gradually and was higher than that of patients without or with grade 1 radiation pneumonitis (P = 0.005) (Table 2). The ratio changes of miR-564 before and after radiotherapy were as follows: the ratio of patients with ≥ grade 2 radiation pneumonitis increased gradually before and after radiotherapy. It reached the peak at the 4th week after radiotherapy, and then decreased gradually, but was higher than that of patients without or with grade 1 radiation pneumonitis (P = 0.025) (Table 3).

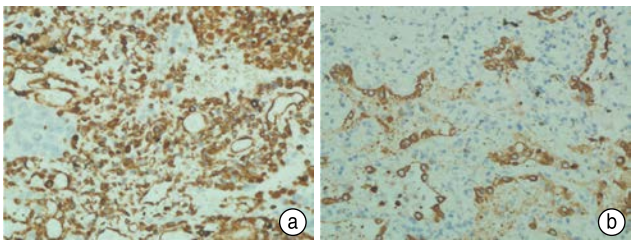

Fig. 1 Expression of miR-564 in cancer tissues of patients with different grades of radiation pneumonitis. (a) Grade 2 radiation pneumonitis patients; (b) Grade 1 radiation pneumonitis patients

Table 2 Relationship between serum miR-564 level and severity of radiation-induced lung injury in patients ($\bar{x} \pm s$, pg/mL)

Time	No or Grade 1 radiation pneumonia	Grade 2 or above 2 radiation pneumonia	*P*
Before radiotherapy	1.2 ± 0.3	1.5 ± 0.5	
2 weeks after radiotherapy	1.2 ± 0.8	1.5 ± 0.9	0.467
4 weeks after radiotherapy	1.1 ± 1.0	2.5 ± 1.0	0.005
6 weeks after radiotherapy	1.0 ± 0.7	2.7 ± 0.8	0.000
2 weeks end of radiotherapy	0.9 ± 0.4	3.0 ± 0.5	0.000
4 weeks end of radiotherapy	0.9 ± 0.5	3.3 ± 0.7	0.000
6 weeks end of radiotherapy	0.8 ± 0.6	2.7 ± 0.5	0.000
8 weeks end of radiotherapy	0.8 ± 0.4	2.4 ± 0.4	0.000

Table 3 Changes in miR-564 ratio before and after radiotherapy in two groups of patients

Time	No or Grade 1 radiation pneumonia	Grade 2 or above 2 radiation pneumonia
Before radiotherapy	1.2 ± 0.3	1.5 ± 0.5
2 weeks after radiotherapy	1.2 ± 0.8	1.5 ± 0.9
4 weeks after radiotherapy	1.1 ± 1.0	2.5 ± 1.0
6 weeks after radiotherapy	1.0 ± 0.7	2.7 ± 0.8
2 weeks end of radiotherapy	0.9 ± 0.4	3.0 ± 0.5
4 weeks end of radiotherapy	0.9 ± 0.5	3.3 ± 0.7
6 weeks end of radiotherapy	0.8 ± 0.6	2.7 ± 0.5
8 weeks end of radiotherapy	0.8 ± 0.4	2.4 ± 0.4

The relationship between the expression of TGF- β 1 in lung cancer tissues and the degree of radiation-induced lung injury

The expression of TGF-β1 in the cancer tissues of patients with ≥ grade 2 radiation pneumonitis was higher than that of patients without or with grade 1 radiation pneumonitis. The difference was statistically significant (P = 0.017) (Fig. 2).

Relationship between the level of TGF-β1 in the serum and severity of radiation-induced lung injury

The level of TGF-β1 in the serum of patients with ≥ grade 2 radiation pneumonitis increased gradually

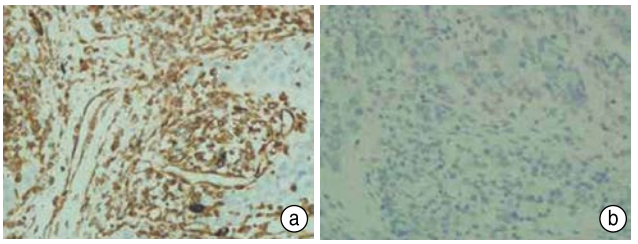

Fig. 2 Expression of TGF-β1 in cancer tissues of patients with different grades of radiation pneumonitis (a) Grade 2 radiation pneumonitis patients; (b) Grade 1 radiation pneumonitis patients

Table 4 Relationship between serum TGF-β1 level and severity of radiation-induced lung injury in patients ($\bar{x} \pm s$, pg/mL)

Time	No or Grade 1 radiation pneumonia	Grade 2 or above 2 radiation pneumonia	*P*
Before radiotherapy	2.4 ± 0.5	2.2 ± 0.3	
2 weeks after radiotherapy	2.4 ± 0.9	2.6 ± 0.5	0.087
4 weeks after radiotherapy	2.3 ± 1.0	2.9 ± 0.8	0.003
6 weeks after radiotherapy	2.5 ± 0.8	3.0 ± 0.9	0.000
2 weeks end of radiotherapy	2.4 ± 0.5	3.2 ± 1.0	0.000
4 weeks end of radiotherapy	2.2 ± 0.7	3.6 ± 0.7	0.000
6 weeks end of radiotherapy	2.4 ± 0.5	3.3 ± 0.8	0.000
8 weeks end of radiotherapy	2.2 ± 0.4	3.0 ± 0.6	0.000

during radiotherapy, and reached the peak at the end of 4 weeks. Thereafter, it decreased gradually and was higher than that in patients without or with grade 1 radiation pneumonitis (P = 0.038), and the rise of TGF-β1 was consistent with the rise of miR-564 (Table 4). The ratio of TGF-β1 in patients with ≥ grade 2 radiation pneumonitis increased gradually before and after radiotherapy, and then decreased, which was higher than that in patients without or with grade 1 radiation pneumonitis. However, there was no statistical difference (P = 0.24) (Table 5).

Relationship between miR-564, TGF-β1 expression, and patient prognosis

The prognosis of patients with high expression of miR-564 was better than that of patients with low expression of TGF-β1, while the prognosis of patients with low expression of TGF-β1 was better than that of patients with high expression of TGF-β1 (Fig. 3).

Discussion

miRNA is a group of 18-23 nucleotides long, endogenous non-coding single-stranded RNA, involved in a variety of important biological processes [9]. To date, it has been reported that numerous miRNAs exist in animals, plants, fungi, viruses, and other organisms, and are widely involved in the development of the body, cell proliferation and apoptosis, tumor formation, and other

Table 5 Changes in TGF-β1 ratio before and after radiotherapy in two groups of patients

Time	No or Grade 1 radiation pneumonia	Grade 2 or above 2 radiation pneumonia
Before radiotherapy		
2 weeks after radiotherapy	1.0 ± 0.2	1.1 ± 0.2
4 weeks after radiotherapy	0.9 ± 0.3	1.3 ± 0.4
6 weeks after radiotherapy	1.0 ± 0.3	1.4 ± 0.1
2 weeks end of radiotherapy	1.0 ± 0.1	1.4 ± 0.5
4 weeks end of radiotherapy	0.9 ± 0.1	1.6 ± 0.2
6 weeks end of radiotherapy	1.0 ± 0.1	1.5 ± 0.2
8 weeks end of radiotherapy	0.9 ± 0.1	1.4 ± 0.3

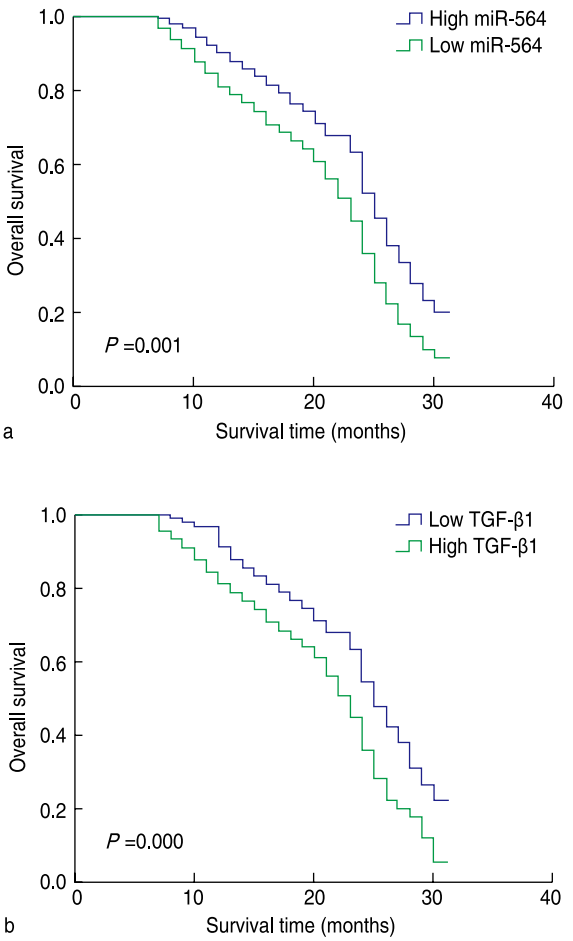

Fig. 3 (a) Relationship between the expression of miR-564 and prognosis; (b) Relationship between the expression of TGF-β1 and prognosis

physiological and pathological processes. In addition, it plays an important role in tumor angiogenesis [10]. miRNA spectra are their own characteristics in various tumors [11]. Our previous study found that miR-564 could inhibit the proliferation, cell cycle progression, migration, and infiltration of lung adenocarcinoma A549 cells, and was

significantly higher in the paracancerous tissues of NSCLC than in the cancerous tissues. Moreover, patients with high expression of miR-564 in NSCLC have better prognosis than those with low expression; it is a negative regulatory gene in lung cancer [7]. However, the relationship between miR-564 and radiation-induced lung injury has not been studied. We found that during radiotherapy, the serum levels of miR-564 in patients with ≥ grade 2 radiation pneumonitis increased gradually with increase in dose from the 2nd week, reached the peak at the end of the 4th week after radiotherapy, and then decreased gradually; however, it was always higher than that in patients without or with grade 1 radiation pneumonitis. The serum levels of miR-564 for patients without or with grade 1 radiation pneumonitis was decreased gradually all long. Further studies showed that miR-564 ratio of patients with ≥ grade 2 radiation pneumonia increased gradually after radiotherapy, reached the peak at the 4th week after radiotherapy, and then decreased gradually, while miR-564 ratio of patients without or with grade 1 radiation pneumonia decreased gradually after radiotherapy. It is suggested that patients with higher expression of miR-564 and ratio of serum miR-564 may have more severe radiation-induced lung injury, while patients with lower expression of miR-564 and ratio of serum miR-564 may have milder radiation-induced lung injury. It is suggested that the changes in miR-564 expressions miR-564 ratios are related to the occurrence of radiation-induced lung injury and provide a new idea for clinical prediction of radiation-induced lung injury. Studies have shown that miRNA affects apoptosis and autophagy by regulating TGF-β1, suggesting that TGF-β1 is a downstream gene of miRNA [12]. However, whether miR-564 can also regulate TGF-β1 is not clear; thus, we will continue to explore the relationship between the two in future studies.

TGF-β1 is a member of TGF-β superfamily. It is a polypeptidase growth inhibitor with many biological functions. It is involved in the signal transduction pathway, inhibiting the proliferation and activity of T cells and macrophages, and regulating the expression of many kinds of target cell genes. It is an immunomodulatory factor and an apoptosis-promoting factor in embryonic growth and development, cell differentiation, and cell proliferation [13]. TGF-β1 is not only a predictor of radiotherapy [14], but also an independent prognostic factor in non-small cell lung cancer [15]. We also found that patients with high expression of TGF-β1 in cancer tissues had poor prognosis. TGF-β1 is not only associated with the prognosis of lung cancer, but also with radiation-induced lung injury. Stenmark *et al* [16] found that the level of TGF-β1 in the plasma was correlated with the degree of radiation pulmonary fibrosis, and TGF-β1 could be used as an early predictor of radiation pulmonary fibrosis. Our study found that serum levels of TGF-β1 in patients with ≥ grade 2 radiation pneumonia during radiotherapy increased gradually after 2 weeks of radiotherapy, reached the peak at the 4th week after radiotherapy, and then decreased gradually. However, the serum levels were higher than that of patients without or with grade 1 radiation pneumonitis. A similar trend was not observed in the serum levels of TGF-β1 in patients without or with grade 1 radiation pneumonitis. It is suggested that monitoring the level of TGF-β1 may help predict the occurrence of radiation-induced lung injury and the radiation dose can be adjusted appropriately.

In conclusion, our study found that miR-564 and TGF-β1 were predictors of radiation-induced lung injury, and changes in miR-564 were observed early in radiation therapy. Therefore, monitoring their changing trends can improve the accuracy of radiation-induced lung injury prediction, and the levels and ratio of serum miR-564 in patients with radiation-induced lung injury are related to the severity of radiation-induced lung injury, which provides a new idea for clinical prediction of radiation-induced lung injury. Summarily, the factors affecting radiation-induced lung injury are complicated and need to be evaluated comprehensively to reduce the occurrence of radiation-induced lung injury and provide some guidance for clinical radiotherapy.

Conflicts of interest

The authors declare no potential conflicts of interest.

References

1. Love C, Palestro CJ. Radionuclide imaging of inflammation and infection in the acute care setting. Semin Nucl Med, 2013, 43: 102–113.
2. Xiong S, Pan X, Xu L, *et al*. Regulatory T cells promote β-catenin--mediated epithelium-to-mesenchyme transition during radiation-induced pulmonary fibrosis. Int J Radiat Oncol Biol Phys, 2015, 93: 425–435.
3. Ding NH, Li JJ, Sun LQ, *et al*. Molecular mechanisms and treatment of radiation-induced lung fibrosis. Curr Drug Targets, 2013, 14: 1347–1356.
4. Huang Y, Zhang W, Yu F, *et al*. The cellular and molecular mechanism of radiation-induced lung injury. Med Sci Monit, 2017, 23: 3446–3450.
5. Giridhar P, Mallick S, Rath GK, *et al*. Radiation induced lung injury: prediction, assessment and management. Aaian Pac J Cancer Prev, 2015, 16: 2613–2617.
6. Wang S, Campbell J, Stenmark MH, *et al*. Plasma levels of IL-8 and TGF-β1 predict radiation-induced lung toxicity in non-small cell lung cancer: a validation study. Int J Radiat Oncol Biol Phys, 2017, 98: 615–621.
7. Yang B, Jia L, Qiaojuan Guo, *et al*. MiR-564 functions as a tumor suppressor in hum an lung cancer by targeting ZIC3. Biochem Biophys Res Commun, 2015, 467: 690–696.
8. Yin WB, Yu ZH, Zhen XG, *et al*. Tumor radiotherapy. Beijing: China Union Medical University Press. 2008. 652.
9. Bounds KR, Chiasson VL, Pan LJ, *et al*. MicroRNAs: new players in the pathobiology of preeclampsia. Front Cardiovasc Med, 2017,

4: 60.
10. Kuninty PR, Schnittert J, Storm G, *et al*. MicroRNA targeting to modulate tumor microenvironment. Front Oncol, 2016, 6: 3.
11. Lu Jun, Getz Gad, Miska Eric A, *et al*. MicroRNA expression profiles classify human cancers. Nature, 2005, 435: 834–838.
12. Wang J, Zhang Y, Song W, *et al*. microRNA-590-5p targets transforming growth factor β1 to promote chondrocyte apoptosis and autophagy in response to mechanical pressure injury. J Cell Biochem, 2018, 119: 9931–9940.
13. Chabicovsky M, Wastl U, Taper H, *et al*. Induction of apoptosis in mouse liver adenoma and carcinoma in vivo by transforming growth factor-beta1. J Cancer Res Clin Oncol, 2003, 129: 536–542.
14. Gao F, Jia L, Han JJ, *et al*. Predictive value of serum levels of transforming growth factor beta 1 for the short-term effects of radiotherapy and chemotherapy in patients with esophageal cancer. Oncol Transl Med, 2018, 4: 1–5.
15. Togashi Y, Masago K, Fujita S, *et al*. Association of the transforming growth factor ß1 promoter polymorphism, C-509T, with smoking status and survival in advanced non-small cell lung cancer. Oncol Rep, 2011, 25: 377–382.
16. Stenmark MH, Cai XW, Shedden K, *et al*. Combining physical and biologic parameters to predict radiation-induced lung toxicity in patients with non-small-cell lung cancer treated with definitive radiation therapy. Int J Radiat Oncol Biol Phys, 2012, 84: e217–222.

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