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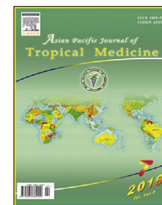

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Influence on radiosensitivity of lung glandular cancer cells when *ERCC1* gene silenced by targeted siRNA

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ABSTRACT

Objective: To identify the influence on radiosensitivity of lung glandular cancer cells when excisions repair cross-complementing group1 (*ERCC1*) gene was silenced by targeted siRNA.

Methods: siRNA which targeting to *ERCC1* and control siRNA was designed and synthesized. The human lung glandular cancer SPC-A-1 cells was transfected. A total of 56 nude mice were divided into two groups, and two kinds of SPC-A-1 cells were transplanted to armpit of right forelimb, to establish the nude mice subcutaneous xenotransplanted tumor model of human lung glandular cancer cells. After the tumor was developed, the nude mice were randomly divided into four groups and accepted different doses of X-Ray radiation, then the change of tumor volume, survival time of mice in every group were recorded and the average lifetime was calculated. Twenty-one days later of X-ray experiment, two mice were taken and killed in each group and the tumors organizations were stripped. The cell apoptosis rate and cell cycle distributions were obtained by FCM (flow cytometry).

Results: The volume of tumor which *ERCC1* gene was silenced was less than single irradiation group after X-ray irradiation, and the growth speed was slower and the lifetime of mice was lengthened as well ($P < 0.05$). The cells apoptosis rate and the rate of G_2/M cells which *ERCC1* gene was silenced were higher than the same dose control group and the rate of G_1 cells were lower, which indicated that the cells could be stopped at G_2/M point, the cell proliferation was inhibited, the cell apoptosis was promoted and the radiation sensitivity was improved after the *ERCC1* was silenced.

Conclusions: The radiation sensitivity of lung glandular tumor could be improved after the *ERCC1* gene was silenced by siRNA.

1. Introduction

Lung glandular cancer is a kind of non small cell lung cancer. In recent years, it has become a common subtype. Because of its unique pathological features, its recurrence rate of is higher than other types of lung cancer. It is difficult to obtain ideal clinic treatment effect [1,2]. Radiotherapy and chemotherapy are two kinds of treatment for this malignant tumor. The main mechanism is to disturb and destroy DNA transcription and replication of cancer cells by radiation or drug. However, it is hard to achieve ideal treatment effect because of drug resistance or antagonism. Among them, the main mechanism is the pathway of nucleotide excision repair (NER) [3].

Excision repair cross-complementing group1 (*ERCC1*), one of the DNA repair gene, plays an important role in the process of NER pathway and cell apoptosis [4]. Many studies have showed that high expression of the gene can cause cancer cells produce drug resistance and reduce the effect of radiotherapy. It has been proved that the generation, development and prognosis of tumors are related closely to the expression of this gene, including colorectal cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer, breast cancer, bile duct cancer *etc* [5–10]. Furthermore, some studies have indicated that the expression of *ERCC1* is related to the radiosensitivity of glioma and nasopharyngeal carcinoma [11,12]. In the study of

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lung glandular cancer, *ERCC1* is regarded as poor prognosis of lung glandular cancer patients [13], and its expression plays a certain negative effect on clinical drug resistance of cisplatin chemotherapeutics for curing lung glandular cancer [14,15]. But so far, few research study the relationship between the gene and radiosensitivity of lung glandular cancer. Therefore, the author intends to study the effect of *ERCC1* expression on the radiosensitivity of lung glandular cancer cells through the internal exposure transplant tumor model animal experiment, and to explore whether it can be gene targets to enhance the radiosensitivity of lung glandular cancer, so as to provide experimental and theoretical basis for the clinical treatment of lung glandular cancer.

2. Materials and methods

2.1. Materials

ERCC1 gene sequence is from GenBank, gene coding NM-001983. siRNA which targeting to *ERCC1* was designed according to the reference [16], and synthesized by QIAGEN. The sequence of designed siRNA-*ERCC1* is 5′-CAGGCGGCCC CTCAGACCTAC-3′; at the same time, a non specific siRNA target sequence 5′-GACTTCATAAGGCGCATGC-3′ was synthesized as negative control group. After Blast, the sequence has no homology with mRNA of human gene.

2.2. Cell culture and transfection

Human lung glandular cancer SPC-A-1 cells used in the study were from Shanghai Cell Bank of Chinese Academy of Sciences. After recover the cryopreserved SPC-A-1 cell line, it was conventionally cultured in RPMI solution containing 10% fetal bovine serum, 100 kU/L penicillin and 100 mg/L streptomycin (pH was adjusted to 7.2% with concentrated hydrochloric acid) at 37 °C, 5% CO_2 and 90% relative humidity condition in incubator. The cultured cells were divided into two groups (marked as the first group and the second group), and transfected *ERCC1*-siRNA and negative control group respectively. When transfected, SPC-A-1 cells in logarithmic phase were taken and routinely inoculated in 6-well plates, 2 mL per hole, density $(1.5–2.5) \times 10^5$/mL, then incubated for about 10 min in a constant-temperature incubator; 21×10^{-12} mol/L siRNA, serum-free medium and HiperFect Transfection Reagent were blended according to 4: 100: 6 in EP tube without RNA enzyme and then incubated for about (5–l0) min at room temperature to form complex. After that, every hole was added 110 μL mixture, shook it to mix well and cultured for 24 h at 37 °C, 5% CO_2 condition in incubator.

2.3. Nude mice xenotransplanted tumor model

The nude mice were purchased from the animal experimental center of Sun Yat-Sen University in China. The study was performed after the mice were allowed to acclimate for 1 week in the animal experimental center of Zhengzhou University. A total of 56 mice were (4–6) weeks old, weighting (15–20) g with half male and half female. Mice were randomly divided into two groups, 28 in each group, inoculated with cultured cells of the first group and the second group respectively. Armpit of right forelimb of mice which had been disinfected by alcohol were injected with 2×10^{-6}/mL cell suspension drew in 1 mL sterile syringes, each only 0.2 mL. Then observe the tumor growth in mice. Average 7 d later, tumor can be found in the mice subcutaneous tissues.

2.4. Radiation experiment

Fourteen days after completing the xenotransplanted tumor model experiment, radiation experiment started. The mice of the first group and the second group were divided into four groups (A1, B1, C1, D1 and A2, B2, C2, D2) and radiated according to different total radiation dose. The total radiation dose of A1, A2 group was 3 GY, the total radiation dose of B1, B2 group was 9 GY, the total radiation dose of C1, C2 was 15 GY, and the total radiation dose of D1, D2 group was 0 for receiving sham radiation. Radiation was operated three times, once a day for three days. The radiation source was 6 MV linear accelerator with 200 cGy/min dose rate and 10 cm × 15 cm radiation area. Before radiation, nude mice were anesthetized by 2% pentobarbital sodium injected in abdomens.

2.5. Tumor growth record

Before the radiation experiment, the tumor volume of mice was measured every 3 d. Specifically, tumor volume was measured with caliper including long diameter (a) and short diameter (b), then calculated according to the formula $V = 0.5 \times ab^{-2}$. The tumor growth curve was drawn from the volume change of mice in each group, and the tumor growth inhibition rate was calculated according to the formula: tumor growth inhibition rate = (tumor volume of the control group-tumor volume of the experimental group)/tumor volume of the control group × 100%. In this experiment, D2 group was the control group. Radiation sensitivity was calculated in accordance with the formula: radiation sensitivity = (the radiation effect of silenced *ERCC1*)/(Single radiation effect) × 100%. Among them, the effect referred to suppression (decrease) degree of tumor volume compared with the control group (D2), such as the radiation sensitivity under 3 GY radiation = $(V_{A2} - V_{A1})/(V_{A2} - V_{D2})$. Survival time of mice in every group was recorded and the average lifetime was calculated.

2.6. FCM experiment

Two mice in each group were killed 21 d after radiation experiment was performed by anesthetizing and dislocating the cervical vertebra; and the tumors organizations were stripped. The cell apoptosis rate and cell cycle distributions were obtained by FCM (model: EPICS-ELITE-ESP). The killed mice were not included in the cumulative survival curve. All data is statistical analyzed by SPSS19.0. $P < 0.05$ is considered to have significant difference.

2.7. Experimental ethics statement

All experiments in the study were conducted according to the guidelines on animal healthcare and use of animal experimental center of Zhengzhou University in Henan Province and approved by local ethics committee. Besides, experiments were conformed to the guidelines on healthcare and use of experimental animals made by National Institutes of Health (Publication No. 85-23, revised in 1985).

Table 1

Change of tumor volume of mice in each group (mm^{-3}).

Time (d)	Groups							
	A1	B1	C1	D1	A2	B2	C2	D2
14	202.69 ± 12.35	198.89 ± 10.23	204.36 ± 14.35	215.36 ± 10.68	246.86 ± 14.36	253.67 ± 17.34	247.68 ± 16.41	256.78 ± 15.20
17	384.51 ± 18.65*	331.52 ± 15.69*	295.89 ± 14.32*	436.86 ± 26.34*	476.85 ± 29.65	454.65 ± 24.62*	387.85 ± 19.51*	514.36 ± 31.65
20	553.67 ± 28.63*	476.34 ± 26.38*	346.37 ± 19.54*	741.62 ± 36.84*	704.35 ± 32.51*	684.51 ± 29.63*	519.35 ± 21.36*	829.65 ± 37.65
23	843.67 ± 46.35*	563.24 ± 58.61*	403.96 ± 25.36*	1 075.61 ± 59.62	1 044.42 ± 54.62*	938.64 ± 48.51*	631.67 ± 39.12*	1 194.32 ± 64.31
Growth inhibition ratio	29.36%	52.84%	66.18%	9.94%	12.55%	21.41%	47.11%	–
26	1 116.45 ± 52.34*	684.52 ± 33.65*	480.65 ± 21.63*	1 559.37 ± 79.65	1 423.21 ± 76.21*	1 167.84 ± 62.31*	799.51 ± 21.68*	1 751.63 ± 85.62
29	1 287.42 ± 70.26*	795.69 ± 35.12*	521.38 ± 26.74*	2 041.65 ± 106.31	1 684.35 ± 84.21*	1 456.38 ± 76.32*	850.42 ± 40.21*	2 201.63 ± 103.21
32	1 401.23 ± 79.21*	861.32 ± 48.53*	553.85 ± 29.31*	2 435.41 ± 118.31	1 863.42 ± 92.33*	1 597.34 ± 75.31*	922.64 ± 46.31*	2 563.25 ± 106.22
35	1 466.42 ± 79.65*	893.85 ± 52.31*	624.67 ± 36.54*	2 731.51 ± 125.61	2 007.53 ± 109.65*	1 657.83 ± 94.62*	1 014.54 ± 54.62*	2 815.32 ± 142.36
Growth inhibition ratio	47.91%	68.25%	77.81%	2.98%	28.69%	41.11%	63.96%	–

*Compared with the control group, $P < 0.05$; A1: siRNA-*ERCC1* + 3 GY irradiation; B1: siRNA-*ERCC1* + 9 GY irradiation; C1: siRNA-*ERCC1* + 15 GY irradiation; D1: siRNA-*ERCC1* + 0 GY irradiation; A2: single 3 GY irradiation; B2: single 9 GY irradiation; C2: single 15 GY irradiation; D2: blank control group.

3. Results

3.1. Tumor growth record

The volume change and the inhibition rate of tumor growth in each group were seen in Table 1, and the radiosensitivity of *ERCC1* gene which was silenced was shown in Table 2. From Table 1, we could see that after expression and irradiation of *ERCC1* gene which was silenced by targeted siRNA, the growth of tumor tissue has been greatly inhibited (It showed significant difference between tumors volume in each group and D2 the control group, $P < 0.01$), and the irradiation dose higher, inhibition effect was more obvious ($P_{A1B1} < 0.05$, $P_{A1C1} < 0.01$, $P_{B1C1} < 0.05$). After the *ERCC1* gene was silenced, the sensitivity of irradiation group was higher than single irradiation group, and the radiosensitivity effect was most obvious when the irradiation dose was 9 GY.

The average lifetime and cumulative survival time of mice in each group were shown in Table 3. From the cumulative survival time schedule, we could see after radiotherapy, the lifetime of mice in each group were extended in some degree compared to the control group ($P < 0.05$).

3.2. FCM experiment

The results of cell apoptosis rate obtained by FCM were as follows: A1: (23.21% + 4.32%); B1: (30.47% + 4.12%); C1: (36.60% + 5.21%); D1: (10.81% + 3.55%); A2: (13.86% +

Table 2

Radiosensitivity with different radiation doses.

Radiation dose	Time (d)							
	14	17	20	23	26	29	32	35
3 GY	0.17	0.18	0.18	0.17	0.18	0.18	0.18	0.19
9 GY	0.21	0.24	0.25	0.31	0.28	0.30	0.29	0.27
15 GY	0.17	0.18	0.21	0.19	0.18	0.15	0.14	0.14
0 GY	0.16	0.15	0.11	0.10	0.11	0.07	0.05	0.03

Table 4

The proportion of cell cycle distribution in each group (%).

Groups	G_1	S	G_2
A1	35.69	40.57	23.74
B1	26.35	39.44	34.21
C1	19.64	35.04	45.32
D1	54.21	32.23	13.56
A2	48.62	36.04	15.34
B2	42.29	39.08	18.63
C2	36.94	38.70	24.36
D2	71.61	21.16	7.23

Table 3

Change of survival mice numbers in each group.

Time (d)	Groups							
	A1	B1	C1	D1	A2	B2	C2	D2
30	5	5	5	5	5	5	5	5
32	5	5	5	5	5	5	5	5
34	5	5	5	5	5	5	5	5
36	5	5	5	5	5	5	5	4
38	5	5	5	4	5	5	5	4
40	5	5	5	4	4	5	5	3
42	4	5	5	3	4	4	5	2
44	4	4	4	2	2	3	4	1
46	3	4	4	1	2	2	3	0
48	2	3	3	0	1	2	3	0
50	1	3	2	0	0	1	2	0
Average lifetime	48.2 ± 4.3*	51.4 ± 6.1**	56.6 ± 4.7**	43.8 ± 3.5	44.8 ± 3.3*	47.2 ± 4.3*	49.6 ± 4.1**	42.6 ± 3.4

*Compared with the control group, $P < 0.05$; **Compared with the control group, $P < 0.01$.

2.33%); B2: (20.21% + 4.23%); C2: (26.82% + 4.11%); D2: (5.24% + 1.41%). The results of cell cycle distribution were shown in Table 4. We could see that after *ERCC1* gene was silenced by targeted siRNA (A2, A3 group), the apoptosis rate of them was higher than the control group, and the proportion of cells decreased at G_2/M point.

4. Discussion

The basic principle of radiotherapy is destructing nucleotide replication of tumor cells by ionizing radiation, and *ERCC1* is one of the most important genes involved in nucleotide damage repair. Many studies show that *ERCC1* can reduce the chemotherapy and radiotherapy effect of many tumors because of its impact on the nucleotide repair pathway [17]. In this study, the author inhibited the expression of *ERCC1* gene in lung glandular cancer cells by small interfering RNA technology, and then irradiated xenotransplanted tumor model of lung glandular cancer cells to study the relationship between *ERCC1* gene and the radiosensitivity of lung glandular cancer. From the experimental results, we could see that after transfection siRNA-*ERCC1* cells and independent sequence control group cells were transplanted into subcutaneous tissue of nude mice and radiated under different doses, the growth status had a significant statistical difference. The inhibition rate of tumor volume and growth of transfection siRNA-*ERCC1* was significantly lower than the control group, which meant siRNA-*ERCC1* could promote the apoptosis of lung glandular cancer cells under ray radiation, inhibits tumor growth and have certain radiosensitivity effect. When irradiation dose was 15 GY, radiosensitivity of it was lower than that of 9 GY radiation dose which proved that higher radiation dose didn't mean higher radiosensitivity. It provided new ideas and methods for the molecular targeted radiation of lung glandular cancer.

The reason why silenced *ERCC1* can improve the radiosensitivity of lung glandular cancer cells is worth exploring. Studies have shown that *ERCC1* can rapidly repair damaged DNA at G_2/M point and enter a state of normal proliferation [18]. Therefore, from Table 4, it can be seen that after silenced *ERCC1* expressed, cells were stopped at G_2/M point, could not be normally proliferated and induced apoptosis. The researchers believed that *ERCC1* played a dual role in tumor tissue. In some tumor tissues, when the cancer cells appeared, cell proliferation became abnormal. At this time, *ERCC1* played repair function of nucleotide to resist malignant proliferation of tumor [19]; On the other hand, in the course of treatment of tumors, platinum based chemotherapy drugs or radiation destroyed the replication of nucleotide in tumor cells, and repair function played by *ERCC1* also had an antagonistic effect [20,21]. That was because whether chemotherapy or radiotherapy, cancer cells were killed in non-normal means which caused harm to normal tissues and cells while inhibiting tumor growth. Physiological reactions not only determined organisms taking certain measures to repair these damages, but also produced drug resistance or radiation antagonism. When silenced *ERCC1* was expressed and radiation dose was 9 GY (not 15 GY), mice cumulative survival curve area was the largest, which might because high radiation dose increased the radiosensitivity, but damage the other organs of mice, thus the cumulative survival time of mice was reduced. Therefore, the detailed mechanism of *ERCC1* in different tumor tissues has not been clearly defined [22], so it still needs further experiment and research.

This study inhibited the expression of *ERCC1* gene in lung glandular cancer cells through small interfering RNA technology, establish mice xenotransplanted tumor model of lung glandular cancer cells, and evaluate the growth condition of xenotransplanted tumor model. After irradiation, the proliferation activity and apoptosis rate were measured and found that compared with the control group, siRNA-Bcl-2 cells could effectively improve the apoptosis rate of gastric cancer cells, which proved that the expression of silenced *Bcl-2* gene could enhance the radiosensitivity of gastric cancer cells.

Conflict of interest statement

We declare that we have no conflict of interest.

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