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Killing effect of EGFR−TKI combined with 125I seed implantation therapy on ⅢB−Ⅳ stage lung cancer tissue

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ABSTRACT

Objective: To analyze the killing effect of EGFR-TKI combined with 125I seed implantation therapy on ⅢB-Ⅳ stage lung cancer tissue. **Methods:** A total of 78 patients with ⅢB-Ⅳ stage lung cancer were randomly divided into observation group and control group (n=39), control group received EGFR-TKI treatment and observation group received EGFR-TKI combined with 125I seed implantation therapy. Differences in apoptosis gene, invasion gene and autophagy gene expression in lung tissue were compared between two groups after 1 month of treatment. **Results:** Apoptosis genes *PDCD5*, *bax* and *bcl−xS* mRNA expression levels in lung tissue of observation group after 1 month of treatment were higher than those of control group while *Bag−1*, *survivin* and *bcl−xL* mRNA expression levels were lower than those of control group; invasion genes *CD147*, *EGFR* and *DDX17* mRNA expression levels were lower than those of control group while *Bin1*, *E−cadherin* and *Ovol2* mRNA expression levels were higher than those of control group; autophagy genes *ARHI*, *Beclin1*, *Atg5*, *LC3B*, *pULK* and *PI3KC3* mRNA expression levels were higher than those of control group. **Conclusions:** EGFR-TKI combined with 125I seed implantation therapy can enhance the tumor killing effect on patients with ⅢB-Ⅳ stage lung cancer, and contribute to the optimization of overall condition and the extension of survival time.

1. Introduction

Patients with ⅢB-Ⅳ stage lung cancer need to accept the conservative treatment to prolong the survival time, it has been confirmed that epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIS) can inhibit the tumor malignancy in patients with terminal cancer, but after long-term application, the drug resistance rate is high and the long-term treatment outcome is not ideal[1]. 125I seed implantation therapy is the more popular way of conservative treatment at present, it implants radioactive seed within the tumor, and the seed continuously emits low-energy γ ray to disturb tumor cell cycle and kill tumor cells[2,3]. At present, there are still less domestic reports about the exact efficacy of EGFR-TKIS combined with 125I seed therapy, the therapy was applied in patients with ⅢB-Ⅳ stage lung cancer in our hospital in the study, and the expression of apoptosis genes, invasion genes and autophagy genes in tumor tissue were mainly observed after treatment, hereby reported as follows:

2. Materials and methods

2.1. General information

A total of 78 patients with advanced lung cancer treated in our hospital between December 2013 and December 2013 were selected, and the inclusion criteria were: (1) diagnosed by pathological biopsy; (2) with tumor ⅢB-Ⅳstage; (3) those who were with distant metastasis and could not accept surgery; (4) did not receive radiotherapy and chemotherapy one month before treatment; (5) with expected survival time > 6 months; (6) patients signed informed consent. Exclusion criteria were: (1) with recurrent

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lung cancer; (2) with metastatic lung cancer; (3) associated with severe liver and kidney dysfunction; (4) with interrupted treatment or incomplete clinical information. 78 patients conformed to the above criteria and were divided into observation group and control group (n=39) according to random number table. Control group included 21 male cases and 18 female cases, they were 48-71 years old and (62.83±7.14) years old in average, 12 cases were with squamous carcinoma and 27 cases were with adenocarcinoma; observation group included 20 male cases and 19 female cases, they were 45-72 years old and (63.76±7.59) years old in average, 13 cases were with squamous carcinoma and 26 cases were with adenocarcinoma. The two groups of patients showed no statistically significant difference in the distribution of gender, age and tumor type (P>0.05) and they were comparable.

2.2. Treatment methods

Two groups of patients received routine examination of blood coagulation function, blood routine, electrocardiogram, *etc* before treatment. The control group received the epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIS) treatment, and 2 weeks of oral administration was a course of treatment. Observation group received EGFR-TKIS + 125I seed implantation treatment, specifically as follows: after 2 weeks of EGFR-TKIS treatment, 125I seed (Seed Biological Pharmacy (Tianjin) Ltd., activity of 0.7-0.9 mCi, half-life of 59.6d and tissue penetration distance of 1.7 cm). Radioactive seed treatment planning system (TPS) was used to determine the total radiation dose, required particle number and so on according to the tumor size. CT scan was referred to determine the needle-inserting position, direction and depth. After disinfection, draping and local anesthesia, 125I seed was implanted every 0.5-1.0 cm. For places near the heart, great vessels, esophagus and other important tissues and organs, the seed implantation distance was > 1 cm. CT scan was conducted again after operation to confirm that there were no hemopneumothorax or other complications.

2.3. RT-PCR

After 1 month of treatment, biopsy was used to obtain lung cancer tissue from two groups of patients, grinded and homogenized to extract total RNA, the RNA purity and integrity were inspected, and then it was reverse-transcribed and synthesized into cDNA. cDNA was used as template to configure reaction system and design upstream and downstream primers. Reaction conditions: 95 ℃ for 10 min, then 95 ℃ 10 s, 55 ℃ 20 s, 72 ℃ 32 s, repeating for 40 cycles. Amplified target genes included: (1) apoptosis genes: *PDCD5, bax, Bag-1, survivin, bcl-xL* and *bcl-xS*; (2) invasion genes: *CD147, EGFR, Bin1, DDX17, E-cadherin* and *Ovol2*; (3) autophagy genes: *ARHI, Beclin1, Atg5, LC3B, pULK* and *PI3KC3*.

2.4.Statistical methods

Data in the study was input in software SPSS23.0, measurement data analysis between two groups was perfomed by t test and P<0.05 indicated statistical significant differences.

3. Results

3.1. Apoptosis genes

PDCD5, bax and *bcl-xS* mRNA expression levels in lung tissue of observation group were significantly higher than those of control group while *Bag-1, survivin* and *bcl-xL* mRNA expression levels were significantly lower than those of control group (P<0.05), shown in Table 1.

3.2. Invasion genes

CD147, EGFR and *DDX17* mRNA expression levels in lung tissue of observation group were significantly lower than those of control group while *Bin1, E-cadherin* and *Ovol2* mRNA expression levels were significantly higher than those of control group (P<0.05), shown in Table 2.

Table 1
Comparison of apoptosis gene expression levels in lung tissue between two groups after treatment.

Groups	Case No.	*PDCD5*	*bax*	*Bag-1*	*survivin*	*bcl-xL*	*bcl-xS*
Observation group	39	153.28±17.94	143.35±15.12	63.28±6.91	50.38±5.43	58.38±6.71	136.38±14.62
Control group	39	98.23±9.12	92.74±9.11	102.64±11.28	99.53±9.64	103.67±10.49	98.63±10.52
t		8.394	7.283	9.182	8.374	9.283	7.273
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2
Comparison of invasion gene expression levels in lung tissue between two groups after treatment.

Groups	Case No.	*CD147*	*EGFR*	*Bin1*	*DDX17*	*E-cadherin*	*Ovol2*
Observation group	39	50.37±5.83	59.83±6.12	142.74±16.09	53.82±5.93	139.74±15.28	150.38±16.49
Control group	39	96.27±10.13	94.58±9.63	102.09±9.82	101.64±10.85	96.35±9.16	105.68±12.47
t		8.932	7.945	8.192	10.384	9.283	9.843
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

3.3. Autophagy genes

ARHI, Beclin1, Atg5, LC3B, pULK and *PI3KC3* mRNA expression levels in lung tissue of observation group were significantly higher than those of control group (P<0.05), shown in Table 3.

4. Discussion

ⅢB-Ⅳ stage lung cancer belongs to terminal cancer, patients mostly miss operative change because of distant metastasis of tumor, and how to prolong the survival time and improve the quality of life of patients with advanced lung cancer is the focus of current clinical research. EGFR-TKIS is the first-line therapy for patients with advanced non-small cell lung cancer, has high selectivity, low toxicity and other advantages, and can inhibit the activation of EGFR effector so as to block the proliferation growth conduction pathway of cancer cells[4,5]. Current study has found that patients with advanced non-small cell lung cancer are with a greater incidence of EGFR-TKIS resistance rate during treatment, and the relevant statistical data shows that the best curative effect of EGFR-TKIS is less than 30%. For patients with advanced tumor who receive EGFR-TKIS, other therapies must be added to enhance the overall curative effect, and 125I seed implantation therapy is one of the most recognized ways at present. The γ rays released by 125I can damage the tumor DNA strand and make it lose proliferation ability, and 125I seed implanted in tumor can continuously release low-energy rays and intervene in the activity of tumor cells in different cell cycles[6,7]. In the study, 125I seed implantation therapy was added in the overall therapy for patients with ⅢB-Ⅳ stage lung cancer in our hospital, and the effect of different therapies on the expression of apoptosis, invasion, autophagy and other genes in lung tissue was mainly observed.

Tumor cell proliferation activity will directly decide the tumor development process, lung cancer biopsy tissues were obtained from two groups of patients after 1 month of treatment in the study, RT-PCR was used to detect apoptosis-related genes *PDCD5, bax, Bag-1, survivin, bcl-xL* and *bcl-xS* mRNA expression levels in them, and it was found that *PDCD5, bax* and *bcl-xS* mRNA expression levels in lung tissue of observation group were higher while *Bag-1, survivin* and *bcl-xL* mRNA expression levels were lower after treatment. *PDCD5, bax* and *bcl-xS* are all pro-apoptosis genes, *PDCD5* can both promote apoptosis and inhibit proliferation, *bax* can have coprecipitation with *bcl-xS* and is considered as the main effector of apoptosis, and studies have shown that when bax expression increases in cells, cell response to the death signal is enhanced[8,9]. *Bag-1* can form complexes with *Bcl-xL* and then strengthen the tumor cell proliferation activity and exert anti-apoptotic effect. Relevant study has confirmed that survivin has anti-apoptotic effect and is closely associated with the occurrence, development and prognosis of tumor[10]. The above results show that the EGFR-TKI combined with 125I seed implantation therapy can promote pro-apoptosis gene expression and inhibit anti-apoptosis gene expression in lung cancer tissue, and ultimately realize the accelerated death and inhibited proliferation of tumor cells.

The tumor cell invasion ability is closely related to the final treatment outcome of patients with tumor, tumor cells with strong invasion ability are prone to early distant metastasis, and therefore, inhibiting tumor cell invasion ability is one of the important mechanisms to treat the disease[11]. In the study, the invasion gene expression in tumor biopsy tissue of the two groups of patients was studied after treatment, and it was found that *CD147, EGFR* and *DDX17* mRNA expression levels in lung cancer tissue of observation group were lower while *Bin1, E-cadherin* and *Ovol2* mRNA expression levels were higher after treatment. CD147 is called the extracellular matrix metalloproteinase inducer and can degrade the extracellular matrix of tumor and promote tumor invasion and metastasis. EGFR is conformed to be directly involved in the tumor growth and differentiation, and can promote tumor cell metastasis[12,13]. DDX17 is involved in all aspects of the RNA splicing and metabolism, and has been found to be highly expressed in colon cancer. Bin1 is the ligand protein with tumor-suppressor function, and it is functional through combination with the N terminal of c-Myc. There is E-cadherin expression decrease and even deletion in gastric cancer, esophageal cancer and liver cancer, which directly leads to the decrease of the tumor cell adhesion ability, loss of cell polarity and enhancement of peripheral invasion ability[14,15]. Study has shown that the mice with Ovol2 knockout can die shortly, and there is lower Ovol2 expression in a wide variety of cancer cells. The above findings show that EGFR-TKI combined with 125I seed implantation therapy can reduce the tumor cell invasion and metastasis ability, which is the direct sign of reduced malignant degree of tumor.

Autophagy uses the lysosome to degrade the aging organelles and damaged materials in cells, and the energy from degradation is used to power the cell cycle. When cell autophagy process is in disorder and autophagy activity decreases, cell autophagy death

Table 3
Comparison of autophagy gene expression levels in lung tissue between two groups after treatment.

Groups	Case No.	*ARHI*	*Beclin1*	*Atg5*	*LC3B*	*pULK*	*PI3KC3*
Observation group	39	141.84±17.95	139.75±15.43	150.37±17.32	139.47±15.33	141.62±15.78	132.46±15.19
Control group	39	96.49±10.18	102.36±10.95	95.27±9.49	105.36±11.17	96.84±9.05	98.57±10.15
t		8.341	7.493	8.495	7.172	8.943	7.262
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

can appear[16,17]. At present, the relationship between autophagy and tumor has become the focus of clinical research, and the progressive decrease of autophagy activity has been found in a wide variety of tumor cells, indicating that autophagy gene expression decrease and even deletion is directly involved in the development of malignant tumors[18]. In the study, *ARHI*, *Beclin1*, *Atg5*, *LC3B*, *pULK* and *PI3KC3* mRNA expression levels in lung cancer tissue of observation group were higher after treatment, indicating that EGFR-TKI combined with 125I seed implantation therapy can enhance autophagy activity and increase the killing effect on tumor cells in patients with advanced lung cancer.

To sum up, it is concluded as follows: EGFR-TKI combined with 125I seed implantation therapy can enhance the tumor killing effect on patients with ⅢB-Ⅳ stage lung cancer and contribute to the optimization of overall condition and the extension of survival time, and it′s worth popularization and application in clinical practice in the future.

References

[1] Ying XH, Ji JS, Tu JF, et al. EGFR-TKIS combined with radioactive 125I seed implantation for the treatment of advanced non-small-cell lung cancer: analysis of clinical effect. *J Interventional Radiol* 2015; **24**(3): 2260229.

[2] Li JR, Sun Y, Liu L. Radioactive seed implantation and lobaplatin chemotherapy are safe and effective in treating patients with advanced lung cancer. *Asian Pac J Cancer Prev* 2015; **16**(9): 4003-4006.

[3] Li XN, Zhu GY. Clinical developments for the EGFR-TKI combined with radiotherapy in advanced non-small cell lung cancer. *Chinese J Lung Cancer* 2014; **17**(4): 357-360.

[4] Wu DW, Chen TC, Huang HS, et al. TC-N19, a novel dual inhibitor of EGFR and cMET, efficiently overcomes EGFR-TKI resistance in non-small-cell lung cancer cells. *Cell Death Dis* 2016; **7**(6): e2290.

[5] Yanwei Z, Bo J, Yuqing L, et al. Serum carcinoembryonicantigen levels predicts the efficacy of EGFR-TKI in non-small cell lung cancer harboring EGFR mutations. *J Cancer Res Ther* 2016; **12**(1):254-258.

[6] Zhang LB, Yin FX, Liu SY, et al. Efficacy of radioactive seeds combined with cisplatin in the treatment of advanced lung cancer. *J Harbin Med Univ* 2016; **50**(2): 124-127.

[7] Li JR, Sun Y, Liu L. Radioactive seed implantation and lobaplatin chemotherapy are safe and effective in treating patients with advanced lung cancer. *Asian Pac J Cancer Prev* 2015; **16**(9): 4003-4006.

[8] Matsumoto M, Nakajima W, Seike M, et al. Cisplatin-induced apoptosis in non-small-cell lung cancer cells is dependent on Bax- and Bak-induction pathway and synergistically activated by BH3-mimetic ABT-263 in p53 wild-type and mutant cells. *Biochem Biophys Res Commun* 2016; **473**(2): 490-496.

[9] Tian J, Hu L, Li X, et al. MicroRNA-130b promotes lung cancerprogression via PPAR γ /VEGF-A/BCL-2-mediated suppression of apoptosis. *J Exp Clin Cancer Res* 2016; **35**(1): 105.

[10]Song S, Jacobson KN, McDermott KM, et al. ATP promotes cell survival via regulation of cytosolic[Ca^{2+}] and Bcl-2/Bax ratio in lung cancer cells. *Am J Physiol Cell Physiol* 2016; **310**(2): C99-C114.

[11]Shen L, Yang M, Lin Q, et al. COL11A1 is overexpressed in recurrent non-small cell lung cancer and promotes cell proliferation, migration,invasion and drug resistance. *Oncol Rep* 2016; **36**(2): 877-885.

[12]Wang JL, Zhang XY, Deng J, et al. Bin1 gene inhibits non-small cell lung cancer A549 cell migration and invasion through the NF- κ B pathway. *Chinese J Cancer Biother* 2016; **23**(4): 481-484.

[13]Yi XH, Wang J. Expression of transcription factor Klf8 in lung cancer tissue and its biological effect on Klf8 expression in lung cancer cell lines. *J Hainan Med Univ* 2016; **22**(2): 112-115.

[14]Verma V, Lautenschlaeger T. MicroRNAs in non-small cell lung cancerinvasion and metastasis: from the perspective of the radiation oncologist. *Expert Rev Anticancer Ther* 2016; **16**(7): 767-774.

[15]Qin LY, Wang HX, Guo LC, et al. Expressions and significance of CD147, EGFR and HIF-1 in non-small lung cancer. *Jiangsu Med J* 2016; **42**(4): 423-426.

[16]Ma Y, Li M, Si J, et al. Blockade of Notch3 inhibits the stem-like property and is associated with ALDH1A1 and CD44 viaautophagy in non-small lung cancer. *Int J Oncol* 2016; **48**(6): 2349-2358.

[17]Wu N, Min HM, Qu HY, et al. Expression autophagy-related gene *pULK* and *PI3KC3* and their correlation with human non-small-cell carcinoma. *Tianjin Med J* 2015; **43**(6): 635-638.

[18]Wang Z, Du T, Dong X, et al. Autophagy inhibition facilitates erlotinibcytotoxicity in lung cancer cells through modulation of endoplasmic reticulumstress. *Int J Oncol* 2016; **48**(6): 2558-2566.