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Effect of cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy on esophageal cancer cell proliferation and invasion

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ABSTRACT

Objective: To study the effect of cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy on esophageal cancer cell proliferation and invasion. **Methods:** A total of 62 patients with esophageal cancer who were treated in the hospital between January 2015 and December 2016 were collected and divided into control group and observation group according to random number table, with 31 cases in each group. Control group of patients received paclitaxel + cisplatin neoadjuvant chemotherapy + surgery, and observation group of patients accepted cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy + surgery. The differences in proliferation and invasion gene expression in the tumor tissue were compared between two groups of patients before and after chemotherapy. **Results:** Before chemotherapy, differences in proliferation and invasion gene expression in tumor tissue were not statistically significant between two groups of patients. After chemotherapy, pro-proliferation genes FOXA1, ABCE1, USP39 and Nestin mRNA expression in tumor tissue of observation group were significantly lower than those of control group; anti-proliferation genes PETN, KLF4, TSLC1 and AnnexinA2 mRNA expression were significantly higher than those of control group; pro-invasion genes γ-synuclein, CXCR4 and Snail mRNA expression were significantly lower than those of control group; anti-invasion genes CIAPIN1, Fez and Lrig1 mRNA expression were significantly higher than that of control group. **Conclusions:** Cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy can effectively inhibit the malignant degree of esophageal cancer cells and inhibit its proliferation and invasion.

1. Introduction

Esophageal cancer is a common malignant tumor of the digestive system with high malignancy in clinical practice therefore, the early radical surgery is the ideal treatment for patients with esophageal cancer[1,2]. Some patients are with large tumor size or have already had micro metastases when diagnosed, which increases the difficulty in the implementation of surgery and the realization of final efficacy, so the neoadjuvant chemotherapy is applied in the clinical practice increasingly[3,4]. The paclitaxel + cisplatin neoadjuvant chemotherapy is commonly used for patients with esophageal cancer, and it has been confirmed to be effective in shrinking tumor volume and killing small metastases, nevertheless, tumor proliferation is still strong in some patients after neoadjuvant chemotherapy, for which many scholars recommend to add cetuximab to the auxiliary treatment. Cetuximab is a human-mouse chimeric monoclonal antibody, which is combined with the epidermal growth factor receptor (EGFR) on the surface of the tumor cell to play a series of anti-tumor roles[5,6]. In this study, cetuximab was introduced in the neoadjuvant chemotherapy for patients with esophageal cancer, and the value of adjuvant cetuximab therapy was analyzed from tumor proliferation and gene expression.

2. Materials and methods

2.1. Case information

A total of 62 patients with esophageal cancer who were treated in the hospital between January 2015 and December 2016 were enrolled as research subjects, and the patients themselves or their families signed informed consent. The enrolled patients were divided into control group and observation group according to random number table, with 31 cases in each group. Control group

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included 18 men and 13 women that were 43-78 years old,whose tumor stages were in Ⅰb-Ⅱa stage of 15 cases, in Ⅱb-Ⅲa stage of 13 cases and in Ⅲb-Ⅳ stage of 3 cases; while observation group included 17 men and 14 women that were 42-79 years old,whose tumor stage were in Ⅰb-Ⅱa stage of 15 cases, in Ⅱb-Ⅲa stage of 14 cases and in Ⅲb-Ⅳ stage of 2 cases. The differences in gender, age and esophageal cancer staging were not significant between the two groups of patients ($P>0.05$), and the research was approved by the hospital ethics committee.

2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) pathologically diagnosed with primary esophageal cancer; (2) diagnosed for the first time and not receiving systematic treatment before admission; (3) completing the systemic treatment and related inspection, and with complete clinical data. Exclusion criteria: (1) with history of esophageal surgery; (2) allergic to cetuximab, taxol and cisplatin; (3) combined with primary malignant tumor of other tissue organs; (4) combined with severe heart, liver and kidney insufficiency, and couldn't tolerate chemotherapy or surgical injuries.

2.3. Therapy

The patients in the control group received taxol + cisplatin neoadjuvant chemotherapy + surgical treatment, and the details were as follows: taxol (produced by Yangtze River Pharmaceutical Group Co., Ltd., Approval No. H20058719) 135 mg/m^2, by intravenous drip, on d1; cisplatin (produced by Jiangsu Hansoh Pharmaceutical Group Co., Ltd., Approval No. H20040813) 75 mg/m^2, by intravenous drip, on d1-d3, 21 d as 1 course of treatment, for continuous 2 courses of chemotherapy, followed by review and surgical treatment.

Observation group of patients accepted cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy + surgery, specifically as follows: cetuximab (produced by Boehringer Ingelheim Pharma GmbH Co KG, approval number S20050095), first load dose 400 mg/m^2, by intravenous drip in 120 min, followed by 250 mg/m^2 every week (intravenous drip in 60 min), and followed by paclitaxel + cisplatin neoadjuvant chemotherapy whose usage and dosage were the same as those of control group.

2.4. Gene expression

Esophageal cancer tissue samples were collected before chemotherapy by gastroscopy, intraoperative tumor tissue specimens were collected after chemotherapy, the cell splitting → total RNA precipitating → RNA washing and drying → RNA purity and concentration determining → sample cDNA synthesizing by reverse transcription → target gene amplifying by fluorescence quantitative PCR, etc., were conducted, and the mRNA of following genes were calculated: pro-proliferation genes: FOXA1, ABCE1, USP39 and Nestin; anti-proliferation genes: PETN, KLF4, TSLC1 and AnnexinA2; pro-invasion genes: γ-synuclein, CXCR4 and Snail; anti-invasion genes: CIAPIN1, Fez and Lrig1.

2.5. Statistical processing

Statistical software was SPSS 20.0, pro-proliferation genes, anti-proliferation genes, pro-invasion genes, anti-invasion genes and so on belong to measurement data and were in terms of mean ± standard deviation (Mean ± SD), and statistics $P<0.05$ was the standard of statistical significance in differences.

3. Results

3.1 Pro-proliferation gene expression

Comparison of pro-proliferation genes FOXA1, ABCE1, USP39 and Nestin mRNA expression in tumor tissue between two groups of patients before and after chemotherapy was as follows: before chemotherapy, differences in FOXA1, ABCE1, USP39 and Nestin mRNA expression in tumor tissue were not statistically significant between two groups of patients ($P>0.05$). After chemotherapy, FOXA1, ABCE1, USP39 and Nestin mRNA expression in tumor tissue of both groups were significantly lower than those before chemotherapy ($P<0.05$), FOXA1, ABCE1, USP39 and Nestin mRNA expression in tumor tissue of observation group were significantly lower than those of control group, and differences were statistically significant ($P<0.05$), shown in Table 1.

3.2. Anti-proliferation gene expression

Comparison of anti-proliferation genes PETN, KLF4, TSLC1 and AnnexinA2 mRNA expression in tumor tissue between two groups of patients before and after chemotherapy was as follows: before

Table 1
Comparison of pro-proliferation gene expression in tumor tissue between two groups of patients before and after treatment.

Groups	n	Time	FOXA1	ABCE1	USP39	Nestin
Control group	31	Before chemo	98.27±10.53	99.64±11.82	101.37±13.42	96.43±10.88
		After chemo	61.28±7.34*	75.28±8.47*	70.48±8.61*	65.29±7.18*
Observation group	31	Before chemo	99.63±10.27	100.36±12.15	98.25±10.38	98.71±10.64
		After chemo	29.64±4.51$^{*\#}$	46.17±5.88$^{*\#}$	43.27±5.62$^{*\#}$	40.76±5.37$^{*\#}$

Note: compared with the same group before chemotherapy, $^*P<0.05$; compared with control group after chemotherapy, $^\#P<0.05$.

Table 2
Comparison of anti-proliferation gene expression in tumor tissue between two groups of patients before and after treatment.

Groups	n	Time	PETN	KLF4	TSLC1	AnnexinA2
Control group	31	Before chemo	95.32±10.81	98.64±11.32	95.47±10.98	100.15±12.67
		After chemo	130.28±15.71*	121.57±14.38*	120.85±14.32*	118.64±13.59*
Observation group	31	Before chemo	96.71±10.62	99.85±10.61	97.38±10.54	99.76±10.38
		After chemo	156.39±18.23$^{*\#}$	140.66±15.83$^{*\#}$	137.48±15.92$^{*\#}$	135.27±19.51$^{*\#}$

Note: compared with the same group before chemotherapy, $^*P<0.05$; compared with control group after chemotherapy, $^\#P<0.05$.

Table 3.

Comparison of pro-invasion gene expression in tumor tissue between two groups of patients before and after treatment.

Groups	n	Time	γ-synuclein	CXCR4	Snail
Control group	31	Before chemo	98.61±10.48	95.73±11.48	100.18±12.35
		After chemo	70.17±8.54*	64.27±8.34*	72.55±8.17*
Observation group	31	Before chemo	98.53±11.42	97.64±10.37	99.76±11.24
		After chemo	40.82±5.61*#	43.85±5.62*#	38.49±5.41*#

Note: compared with the same group before chemotherapy, *$P<0.05$; compared with control group after chemotherapy, #$P<0.05$.

Table 4

Comparison of anti-invasion gene expression in tumor tissue between two groups of patients before and after treatment.

Groups	n	Time	CIAPIN1	Fez	Lrig1
Control group	31	Before chemo	96.47±10.39	100.14±12.75	98.42±10.17
		After chemo	120.85±14.29*	119.73±125.66*	132.58±15.29*
Observation group	31	Before chemo	95.88±9.84	99.63±11.36	99.85±10.53
		After chemo	143.27±15.63*#	135.19±15.67*#	154.29±17.52*#

Note: compared with the same group before chemotherapy, *$P<0.05$; compared with control group after chemotherapy, #$P<0.05$.

chemotherapy, differences in PETN, KLF4, TSLC1 and AnnexinA2 mRNA expression in tumor tissue were not statistically significant between two groups of patients ($P>0.05$). After chemotherapy, PETN, KLF4, TSLC1 and AnnexinA2 mRNA expression in tumor tissue of both groups were significantly higher than those before chemotherapy ($P<0.05$), PETN, KLF4, TSLC1 and AnnexinA2 mRNA expression in tumor tissue of observation group were significantly higher than those of control group, and differences were statistically significant ($P<0.05$), shown in Table 2.

3.3. Pro-invasion gene expression

Comparison of pro-invasion genes γ-synuclein, CXCR4 and Snail mRNA expression in tumor tissue between two groups of patients before and after chemotherapy was as follows: before chemotherapy, differences in γ-synuclein, CXCR4 and Snail mRNA expression in tumor tissue were not statistically significant between two groups of patients ($P>0.05$). After chemotherapy, γ-synuclein, CXCR4 and Snail mRNA expression in tumor tissue of both groups were significantly lower than those before chemotherapy ($P<0.05$), γ-synuclein, CXCR4 and Snail mRNA expression in tumor tissue of observation group were significantly lower than those of control group, and differences were statistically significant ($P<0.05$), shown in Table 3.

3.4 Anti-invasion gene expression

Comparison of anti-invasion genes CIAPIN1, Fez and Lrig1 mRNA expression in tumor tissue between two groups of patients before and after chemotherapy was as follows: before chemotherapy, differences in CIAPIN1, Fez and Lrig1 mRNA expression in tumor tissue were not statistically significant between two groups of patients ($P>0.05$). After chemotherapy, CIAPIN1, Fez and Lrig1 mRNA expression in tumor tissue of both groups were higher than that before chemotherapy, CIAPIN1, Fez and Lrig1 mRNA expression in tumor tissue of observation group were significantly higher than that of control group, and differences were statistically significant ($P<0.05$), shown in Table 4.

4. Discussion

Neoadjuvant chemotherapy is a reliable therapy for esophageal cancer that can effectively reduce the tumor malignancy and provide convenient conditions for follow-up radical operation, the latest research suggests that some patients do not have high sensitivity to conventional paclitaxel + cisplatin neoadjuvant chemotherapy, and many scholars recommend joining cetuximab for combination therapy. The anti-tumor mechanisms of cetuximab include the following aspects: (1) has a high affinity to EGFR, and directly inhibits EGFR activity and downgrades its endogenous expression; (2) inhibits the activity of tyrosine kinase, blocks cell division in G1 phase, inhibits tumor cell proliferation and induces its apoptosis; (3) reduces the expression of matrix metalloproteinases, vascular endothelial growth factor and transforming growth factor, and inhibits the tumor angiogenesis and tumor cell infiltration and metastasis[7-9]. There is not much research about the value of auxiliary cetuximab treatment of esophageal cancer at present, auxiliary cetuximab therapy was added at the same time of paclitaxel + cisplatin neoadjuvant chemotherapy in the study, and was discussed from two aspects of tumor cell proliferation and invasion.

The tumor malignancy mainly depends on tumor cell proliferation activity, pro-proliferation/anti-proliferation gene expression imbalance is the direct cause of the abnormal proliferation of tumor cells, and detecting the above gene expression can objectively reflect the tumor cell proliferation activity and drug treatment efficacy[10,11]. It is reported in different reports that FOXA1, ABCE1, USP39 and Nestin are the genes that promote tumor cell proliferation, the tumor cell proliferation activity decreases after miRNA targets and decreases their expression, and their expression are highly consistent with tumor malignancy[12,13]. PETN, KLF4, TSLC1 and AnnexinA2 are recognized as tumor suppressor genes, their expression reduction or even deletion is one of the important causes of a variety of malignant tumors in clinical practice, and to over-expressing them via genetic approach is the actively explored new therapy for malignant tumors at present[14,15]. In the study, pro-proliferation/anti-proliferation gene expression in tumor tissue were compared between two groups of patients, and it was found that compared with those before treatment, pro-proliferation genes FOXA1, ABCE1, USP39 and Nestin mRNA expression in tumor tissue of both groups of patients decreased while anti-proliferation genes PETN, KLF4, TSLC1 and AnnexinA2 mRNA expression increased after chemotherapy, indicating that both treatments can promote the pro-proliferation/anti-proliferation gene expression in esophageal tumor tissue in different degree; further compared with the control group, observation group were with lower pro-proliferation genes FOXA1, ABCE1, USP39 and Nestin mRNA expression, and higher anti-proliferation genes PETN, KLF4, TSLC1 and AnnexinA2 mRNA expression in tumor tissue after chemotherapy, confirming that cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy can more effectively inhibit tumor cell proliferation activity.

In addition to proliferation, invasion and metastasis of tumor cells is one of the main reasons leading to the increased tumor

malignancy and worse prognosis, and pro-invasion/anti-invasion gene expression imbalance is the direct cause of tumor cell invasion activity change, and can also directly reflect the clinical curative effect[16,17]. γ-synuclein, CXCR4 and Snail have been found to be highly expressed in various malignant tumor tissues, their expression are positively correlated with tumor malignancy, and they are also speculated to be directly related to the occurrence, infiltration and metastasis of esophageal cancer[18,19]. CIAPIN1, Fez and Lrig1 have been confirmed in different studies to be able to inhibit the invasion and metastasis of esophageal cancer cells, and inhibition of their expression can effectively reduce the invasion activity of tumor cells[20]. In the study, pro-invasion/anti-invasion gene expression in tumor tissue were compared between two groups of patients, and it was found that compared with those before treatment, pro-invasion genes γ-synuclein, CXCR4 and Snail mRNA expression decreased while anti-invasion genes CIAPIN1, Fez and Lrig1 expression increased in tumor tissue of both groups of patients after chemotherapy, verifying the effectiveness of both kinds of treatments; further compared with control group, the observation group of patients were with lower pro-invasion genes γ-synuclein, CXCR4 and Snail mRNA expression, and higher anti-invasion genes CIAPIN1, Fez and Lrig1 mRNA expression in tumor tissue after chemotherapy, confirming that cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy can more effectively inhibit the tumor cell invasion activity.

Cetuximab combined with paclitaxel + cisplatin neoadjuvant chemotherapy for patients with esophageal cancer can effectively inhibit the proliferation and invasion activity of tumor cells, is expected to improve patients′ treatment outcomes, and is worthy of popularization and application in clinical practice in the future.

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