Significance of IL-4 and IL-12 in Cervical Cancer and Relation to Taxol Allergy*

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ABSTRACT Objective: To investigate the expression of IL-4 and IL-12 in the cervical cancer and to investigate the relationships between the cervical carcinoma and Taxol allergy. Methods: Total RNA was extracted from paraffin-embedded tissues which were from 35 cases of cervical cancer and 15 cases of normal cervix. The results were analyzed by the quantitation access to densitometric scan of agarose gel electrophoresis photos. Results: 1.The expression of IL-4 in the cervical carcinoma was higher than that in normal cervical tissues while the expressions of IL-12p35 and IL-12p40 had the opposite tendency. 2. The expression of IL-4mRNA in allergic groups was higher than that in control groups during Taxol therapy with cervical cancer. IL-12p35mRNA and IL-12p40mRNA decreased. Conclusions: The increasing of IL-4 and (or) the dropping of IL-12 might promote cervical diseases and increase the risk of taxol allergy after the operation.

Key Words: IL-4; IL-12; Cervical cancer; Taxol; allergy;

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Introduction

IL-4 and IL-12, the regulatory protein that have important biological activities, have been showed to play an positive role in an immune response[1]. The various level of the correlate closely to immunity, tumor genesis and allergy occurrence. Recently, more and more attention to Taxol has treated cervical cancer, with its in-depth study. Taxol may result in allergic reactions duing to insoluble to water or other factors [2]. We have the experiment to test the expressions of IL-4mRNA and IL-12mRNA in the different teams of Taxol chemotherapy given in cervical cancer through Reverse Transcription Polymerase Chain Reaction (RT-PCR), and explore their influence of cerical cancer and taxol allergy.

1 Materials and Methods

1.1 Patients and tissue samples

Formalin-fixed, paraffin-embedded tissues from 35 cases of cervical cancer and 15 cases of normal cervix were collected from patients who underwent primary surgery at the Qing Dao University Medical College affiliated hospital between January in 2007 and June in 2009 following informed consent and approval by a local Human Research Ethics Committee. The cases of cervical cancer consisted 9 cases from Taxol allergy, 12 cases from no allergy and 14 cases without taxol therapy after operation. The mean age at the time that cervical cancer was suspected was 53.12 years (range: 36 57 years). All the specimens were not received radiotherapy or chemotherapy before operation,and histologically examined.

1.2 Methods

1.2.1 Total RNA Extraction Each paraffin embedded block was cut into 5 pieces of 8μ m in thickness and IL-4mRNA and IL-12mRNA were detected by the ultraviolet spectrophotometer. Total RNA was extracted by TaKaRa FFPE tRNA Isolation kit according to the instructions of the manufacturer (TAKARA Bio, Dalian, China.). The total tRNA was stored in -80℃.

1.2.2 RT-PCR Analysis cDNA was synthesized from RNA by TaKaRa primeScriptTM RT-PCR Kit (TAKARA Bio, Dalian, China.) according to the instructions of the manufacturer. The obtained cDNA was mixed with the primers (United Serge Bio, Shanghai). The sequences of the primers were shown in Supplemental Table 1. RT-PCR was performed using a EP384-Gradient RT-PCR System (Eppendorf, Germany.) according to the instructions of the manufacturer. The amplification conditions consisted of an initial incubation at 94℃ for 3 min, followed by cycles of denaturation at 94℃ for 30s, annealing at the indicated conditions in Supplemental Table 1 and elongation at 72℃ for 40s. The PCR products were evaluated by agarose gel electrophoresis. The reaction products were preservation by 4℃.

Table 1 Primer sequence and Annealing condition

Gene	NCBI	primer	Size	Tm	Cycle
IL-4	NM_000589.2	CTCCGGCAGTTCTACAGCCACTGTCGAGCCGTTTCAGGAA	110bp	56	30
IL-12p35	NM_000882.2	ATGCAGGCCCTGAATTTCAACCTGCCCGAATTCTGAAAGCA	121bp	55	32
IL-12p40	NM_002187.2	CATTCGCTCCTGCTGCTTCACTACTCCTTGTTGTCCCCTCTG	267bp	57	35
GAPDH	NM_002046.3	TCACTGCCACCCAGAAGACTTTCTAGACGGCAGGTCAGGT	209bp	57	31

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1.3 Statistical Analysis

Data were shown as the mean± standard deviation ($\overline{X}$± S). The statistical significance of differences between two means was examined by one-way analysis using chi-square test for analysis. P values of less than 0.05 were considered to indicate a significant difference.

2 Results

2.1 The expression of IL-4 and IL-12 in cervical cancer

The expression of IL-4mRNA in cervical cancer group was higher than that of the normal group, and the expression of IL-12 mRNA (p35and p40) decreased compared with the latter group, the differences were statistically significant ($P < 0.05$). (Fig.1, Table 2)

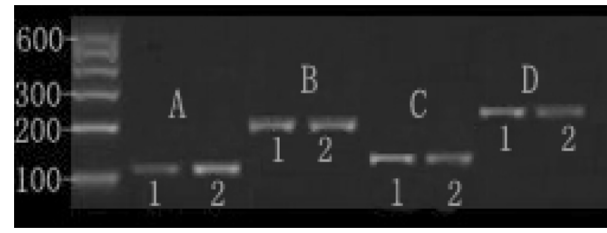

Fig.1 The expression of IL-4 and IL-12 in the cerical cancer :The vertical axis represents marker, used to sign the amplified product length, and the electrophoresis belt at the bottom and top means 100bp and 600bp; number 1 and 2 represent normal cervical group and cervical cancer group, respectively, A to D mean IL-4mRNA, GAPDH, IL-12p35mRNA and IL-12p40mRNA.

Table 2 Statistic data

Groups	IL-4	IL-12p35	IL-12p40
Normal group(n=15, mean age, 49.58 years)	0.415± 0.190	0.874± 0.245	0.627± 0.135
Cerical cancer group(n=35, mean age,53.12years)	0.804± 0.241	0.326± 0.124	0.316± 0.117
x^2	4.023	5.811	4.957

2.2 The expression of IL-4 and IL-12 with Taxol therapy in the cervical cancer

The result in electrophoresis shows that in Taxol allergy patients, the expression of IL-4mRNA was significantly higher than in control one ($P<0.05$), and the expressions of IL-12p35mRNA and IL-12p40mRNA were remarkable lower than the latter group. (Fig. 2, Table 3)

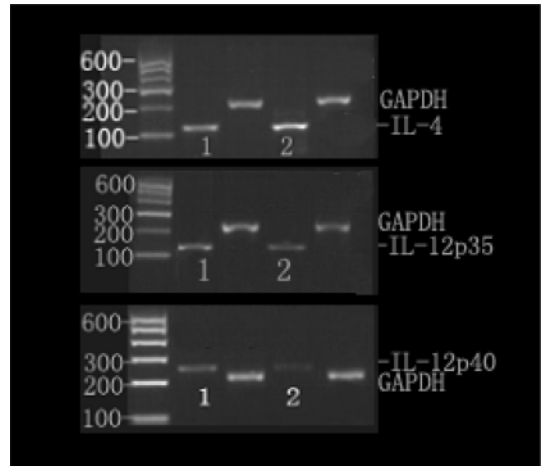

Fig.2 IL-4 and IL-12mRNA expression in Taxol groups :The vertical axis represents marker, used to sign the amplified product length, and the electrophoresis belt at the bottom and top means 100bp and 600bp; number 1and 2 represent respectively the allergy group and no allergy group with Taxol treated cerical cancer.

Table 3 Statistic data

Groups	IL-4	IL-12p35	IL-12p40
Taxol allergy group (n=9, mean age, 43.17 years)	1.045± 0.125	0.248± 0.341	0.251± 0.128
No allergy group (n=12, mean age, 42.12years)	0.783± 0.147	0.339± 0.194	0.327± 0.153
x^2	5.153	4.845	4.594

3 Discussion

Cervical cancer is one of the most common malignant tumor of women in the world[3]. Around 78% of the cases come from the developing countries and the mortality is second in the malignant female tumors [4]. It has been known that cervical carcinoma was

related to the abnormal immune function of human body [5-6]. The risks of cervical carcinoma may possibly be increased duing to Thl/Th2 cell ratio imbalance [7]. With the development of research on chemotherapy of cervical carcinomas in recent years, taxol chemotherapy has been gained increasingly interest. Taxol[8] with a natural drug and excellent antitumor activity, its unique anti-cancer mechanism, has been widely used in the clinical treatment. Due to its hydrophobic nature and other factors, the early study found [9-10] taxol had the higher incidence of allergies, associated with a variety of symptoms, for instance, flushing, urticaria, dyspnea, low blood pressure, blood vessels oedema, cardiovascular collapse and so on. Its allergy could be defined as IgE-mediated hypersensitivity reactions[11]. IgE antibodies, increased with serum tryptase, during an enzyme released on mast cell degranulation, following an anaphylactic reaction, are mainly dependent on IL-4 [12-13]. When Th2 cells undergo activation, as allergens stimulate, they can release a large number of cytokines, such as IL-4, and stimulate antigen-specific cells assembled to form plasmocyte to make effective IgE. In addition, the secretion of IL-4 further promotes Th2 cell differentiation, and produce more IL-4, IL-5 and IL-10. This case which make high levels of IgE with humoral immune response by Th2 main participation can lead to Thl/Th2 cells maladjusted, resulting in some autoimmune diseases and allergic diseases. In addition to making still B cells express Fcε R, IL-4 is able to boost B cells secreting more IgE or transform IgG to IgE, at the same time, to absolutely suppress IL-12 function which advance Th1 cells differentiation and cytokine derived from Th1 cells, and promote the opposite side to increase the occurrence of allergy with refraining from antineoplastics.

L-12 is the strongest enhancing biological activitical factors to activate NK cells and cytotoxic lymphocytes (CTL), and play important roles in the processes of antitumor immunity [14-15]. It can induce Th1 to promote anti-cancer cytokine (such as IL-2,TNF-β) and keep down IL-4 and IgE, when its two subunits (p35, p40) combine to the protein of heterodimer[16]. Some research found that IL-12 could inhibit various tumor growth and transfer in vivo [17-19]. Th2cytokines were connected with cervical lesions [20]. Therefore, the changing degrees of IL-4 and IL-12 could reflect the degree of the antitumor effects indirectly. This study found that the expression of IL-12 in cervical cancer was lower than that in the normal group, while IL-4 was higher. The circumstance with lower IL-12 and higher IL-4, will lead to the increasing of the imbalanced ratio between Thl and Th2, which means decrease the antineoplastic immune of organisms and promote the development of cervical disease. In this study, the the expression of IL-4 was significantly higher in Taxol allergy group than that in the control group, while IL-12 was adverse. The level of IL-4 and IL-12 could affect allergical reaction rate after operation for invasive cervical cancer. When immunity of cervical cancer patients is compromised, mainly for IL-4 higher or IL-12 lower, this case will be favorable to the increasing frequency of taxol allergy, due to excessive IL-4 further promoting to the function of IgE. As the study develops in depth, hoping, we can further understand the biological function and influence factors of IL-4 and IL-12, which contribute to shed light on mechanism of organisms antineoplastic immune process and taxol allergy. It will make advantage of anti-tumor perfecter with taxol allergy lower and have an important significance on the cervical cancer treatment.

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IL-4 和 IL-12 在宫颈癌中的表达及对紫杉醇过敏的影响

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摘要 目的:研究 IL-4, IL-12 在宫颈癌组织中的表达,探讨其对宫颈癌发生及术后对紫杉醇过敏的影响。**方法**:应用半定量逆反应 - 聚合酶链反应(RT-PCR)技术检测 IL-4mRNA, IL-12p35 以及 IL-12p40 mRNA 在正常宫颈组和宫颈癌组中的表达,并分析两者之间的相关性以对紫杉醇过敏的影响。**结果**:1. 宫颈癌组中 IL-4mRNA 表达水平高于正常宫颈组,而 IL-12p35 和 IL-12p40mRNA 表达低于正常宫颈组,差异有统计学意义 (P<0.05);2. 在术后给予紫杉醇治疗的宫颈癌患者中,过敏组中 IL-4mRNA 的表达高于不过敏组;IL-12p35 和 IL-12p40mRNA 则低于后者,差异有统计学意义(P<0.05)。**结论**:体内 IL-12 降低和(或) IL-4 升高可促进宫颈癌的发生发展增加紫杉醇过敏的发生率。

关键词:IL-4;IL-12;宫颈癌;紫杉醇;过敏

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