Original Article
Expression of tumor necrosis factor α induced protein-8 like-2 in allergic nasal mucosa of children and its relationship with inflammatory cells infiltration

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Abstract: To investigate the expression of tumor necrosis factor α induced protein-8 like-2 (TIPE2) mRNA and protein in nasal mucosa of allergic rhinitis children and its relationship with inflammatory cell infiltration. The nasal mucosal tissue in 76 cases of allergic rhinitis children in our hospital were selected as the research object, and 30 cases of healthy children were selected as the control group. The expressions of TIPE2 mRNA and protein were detected by real-time fluorescence quantitative PCR and immunohistochemistry; the inflammatory cells infiltration of nasal mucosa in two groups of children was analyzed using HE staining. And the relationship between TIPE2 and inflammatory cell infiltration was analyzed. Compared with the control group, the level of TIPE2 mRNA in nasal mucosa of children in the observation group was significantly decreased (P<0.05). The immunohistochemical staining showed that the expression of TIPE2 protein in nasal mucosa of children in the observation group was significantly lower than that of the control group (P<0.05). HE staining showed that there were a large number of eosinophils and mast cells infiltration in nasal mucosa of children in the observation group. The correlation analysis showed that the level of TIPE2 mRNA was negatively correlated with inflammatory cells infiltration. In conclusion, the TIPE2 protein was highly expressed in nasal mucosa of allergic rhinitis children, which was positively correlated with inflammatory cells infiltration.

Keywords: Tumor necrosis factor α induced protein-8 like-2 (TIPE2), allergic rhinitis, inflammatory cells

Introduction

Allergic rhinitis (AR) is a nasal mucosa allergic disease with the neurotransmitter release mediated by IgE, the involvement of many immunocompetent cells and cytokines after the atopic individual contacts allergen. Its pathogenesis may be related to genetics, immune, environment and other factors [1, 2]. Epidemiological studies have shown that the morbidity of allergic rhinitis is obviously increasing in children. It may lead to nasal obstruction, headache and other symptoms, which seriously affects the normal development, physical and psychological health in children [3, 4]. Therefore, to explore the pathogenesis of allergic rhinitis is important for the treatment of allergic rhinitis. Tumor necrosis factor α induced protein-8 is a member in the tumor necrosis factor family, including TIPE1, TIPE2, TIPE3 and TIPE4, which plays an important role in the maintenance of cellular immunity and humoral immunity [5-7]. TIPE2 may be a negative immunomodulatory protein, which plays a negative regulatory role in innate immunity and acquired immunity [8]. The present study showed that TIPE2 protein was abnormally expressed in meningitis, atherosclerosis, primary liver cancer and renal carcinoma [9-11]. In this study, the allergic rhinitis associated genes were screened. The result showed that TIPE2 was abnormally expressed. Therefore, the expressions of TIPE2 mRNA and protein in allergic rhinitis mucosa as well as its relationship with inflammation were further analyzed. The purpose was to provide a certain basis for the pathogenesis of allergic rhinitis.

Subjects and methods

General data

76 cases of allergic rhinitis children admitted in our hospital were selected as the research
Expression of TIPE2 in allergic rhinitis children

Object from June 2013 to July 2015. Inclusion criteria: ① All children conformed to AR diagnosis and treatment criteria formulated in Lanzhou conference [12]; ② AR skin prink test and serum specific IgE detection were positive. ③ All the children had no other allergic disease such as asthma and dermatitis; ④ All family members signed the informed consents. There were 45 cases of male and 31 cases of female; aged 2-7 years old, the average age was 5.1±2 years; including 21 cases of dust mite allergy, 37 cases of pollen allergy and 18 cases of mixed allergy. At the same time, 30 cases of children receiving physical examination in our hospital were selected as the control group, including 19 cases of males and 11 cases of females, aged 2-7 years old; the average age was 4.9±1.9 years. There was no statistical significance in age, gender or other indicators between two groups of children (P<0.05). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Huaihe Hospital of Henan University. Written informed consent was obtained from all participants and (or) their parents.

Specimen collection and processing

In the observation group, the nasal mucosa tissues were obtained from the children when they received the medical examination. Some was for RNA extraction. The other was embedded with paraffin for immunohistochemical analysis and HE staining. In the control group, the nasal mucosa were obtained from the children when they received the medical examination. Some was for RNA extraction. The other was embedded with paraffin for immunohistochemical analysis and HE staining.

Real-time fluorescence quantitative PCR

The nasal mucosal tissue in the control group and observation group were extracted using the total RNA extraction kit (TaKaRa, Dalian, China), and reversely transcribed into cDNA for real-time fluorescent quantitative PCR template using the reverse transcription kit (TaKaRa, Dalian, China). RNA extraction and reverse transcription were in strict accordance with the kit instruction. The primers were designed according to TIPE2 mRNA sequence as follows: TIPE2-F: 5’-GCCGGAATTCGTGCGTGAC-3’, TIPE2-R: 5’-GGTCAACTCTGCTCCTCA-3’. The β-actin primer was designed for internal reference: β-actin-F: 5’-GCGGGAAATCGTGCGTGAC-3’, β-actin-R: CGTCATACTCTGTGCTGTG-3’. After the primer was diluted, the specificity was optimized. The reaction mixture was prepared: 2*SYBR Green general qPCR Master Mix (TaKaRa, Dalian, China) 10 µl, upstream/downstream primers (10 µmol•L-1) 1 µl, cDNA 1 µl, the double distilled water was supplemented into 20 µl. The corresponding samples were prepared and added in the PCR board, 20 µl in each hole, centrifuged by 1500 rpm. The reaction mixture was thrown to the bottom of the tube. PCR was performed according to the following reaction: predenaturation at 95°C for 30 s, degeneration at 95°C for 3 s, annealing and extension at 60°C for 30 s; The solubility curve was constructed. Finally, the data were read directly from the real-time fluorescence quantitative PCR instrument (Applied Biosystems, Foster City, CA, USA).

Immunohistochemical analysis

The specimen tissue embedding with paraffin was cut into 5 µl slices, adhered on the slides, baked at 50°C oven for 1 h for dewaxing, hydrated with xylene and absolute ethyl alcohol with different concentrations successively, and then washed with the distilled water for 3 times. Then the slides were placed in the buffer solution containing sodium citrate, heated for 8min for antigen retrieval, washed with PBS for 3 times; 3% hydrogen peroxide (75% methanol preparation) was dropped on the slides for 30 min to remove the endogenous catalase, washed with PBS for 3 times, 5 min/each time. The mouse-anti-human TIPE2 monoclonal antibody (Santa-Cruz, CA, USA) was diluted according to 1:200, dropped on the slides, incubated at 37°C for 2 h, washed with PBST for 3 times, 5 min/each time; 10% sheep-anti-mouse labelled second antibody (ZSGB-BIO, Beijing, China) diluted with goat serum was dropped on the slides, incubated for 1 h, washed with PBST for 3 times, 5 min/each time; 50 µl DAB developing-coloring solution was dropped on the slides, flushed with tap water, dehydrated, dried, fixed with neutral resin and fixed under the microscope (Olympus, Tokyo, Japan).

HE staining

The conventional paraffin embedding was performed and cut into 4 µm pieces. The conventional dewaxing was performed with xylene,
Expression of TIPE2 in allergic rhinitis children

washed with ethyl alcohol with different concentrations: xylene (I) 5 min→xylene (II) 5 min→100% ethyl alcohol→95% ethyl alcohol→80% ethyl alcohol 1 min→75% ethyl alcohol 1 min→washed with distilled water for 2 min, stained with hematoxylin for 5 min, flushed with tap water, differentiated with hydrochloric acid alcohol for 30 s. Immersed with tap water for 15 min or warm water (about 50°C) for 5 min. The eosin solution was placed. The routine dehydration, transparency, mounting and neutral resin sealing were performed.

Statistical analysis

All data were analyzed using SPSS 13.0 software (SPSS Inc, Chicago, IL, USA). The measurement data were expressed by XS. The measurement data were compared with t test. The correlation was analyzed by Pearson. P<0.05 indicated that the difference was statistically significant.

Results

Comparison of TIPE2 mRNA levels in nasal mucosal tissue between two groups

In this study, the real-time fluorescence quantitative PCR system had higher specificity. The amplification curve repeatability was good without miscellaneous peak (Figure 1A). Quantitative PCR results showed that the level of TIPE2 mRNA in nasal mucosa tissue in the observation group was significantly decreased compared with the control group (Figure 1B).

Comparison of TIPE2 protein levels in nasal mucosal tissue between two groups

As shown in Figure 2, the TIPE2 protein was mainly located in the cytoplasm membrane. The TIPE2 protein was moderately expressed in nasal mucosa tissue in the control group, while the TIPE protein was lowly expressed in the observation group. Gray degree quantita-
Expression of TIPE2 in allergic rhinitis children

Quantitative analysis showed that the level of TIPE2 protein in nasal mucosa in the observation group was significantly lower than that of the control group, the difference was statistically significant (\(P<0.05\)).

Comparison of inflammatory cells infiltration in nasal mucosal tissue between two groups

There was very rare inflammatory cells infiltration in nasal mucosa in the control group, while there was a large number of inflammatory cells infiltrations in the observation group. Quantitative analysis showed that the proportion of inflammatory cells in nasal mucosa of the observation group was significantly higher than that of the control group, the difference was statistically significant (\(P<0.05\)) (Figure 3).

Relationship between TIPE2 mRNA level and inflammatory cells infiltration

The relationship between TIPE2 mRNA level and inflammatory cells infiltration showed that TIPE2 mRNA level was negatively correlated with inflammatory cells infiltration (\(P<0.05\)).

Discussion

Allergic rhinitis (AR) is a nasal mucosa allergic disease with the neurotransmitter release mediated by IgE, the involvement of many immunocompetent cells and cytokines after the atopic individual contacts allergen [13, 14]. Its essential conditions include [15]: ① specific antigen, namely the substance inducing the immunoreaction; ② atopic individual, namely individual difference and allergic constitution; ③ encounter of specific antigen and atopic individual. The occurrence of allergic rhinitis has a great relationship with genetic, body weight and environment. In recent years, the incidence of allergic rhinitis shows an obvious rising trend in children has become a common disease in pediatric department and otolaryngology department, which can not only affect the normal development of children, but also bring a lot of trouble for parents [16].

The pathogenesis of allergic rhinitis is still not completely clear. At present, many genes associated with allergic rhinitis have been found from the gene level, which provides a basis for clinical diagnosis and treatment. The earlier researches showed TIPE2 showed great difference between the normal nasal mucosa and allergic rhinitis mucosa through transcriptome sequencing analysis. TIPE2 is tumor necrosis factor induced protein, which was discovered in meningitis mice model. It plays an important role in the regulation of inflammatory response and is considered as an immunoregulatory negative protein [17, 18]. The recent studies showed that the level of TIPE2 in atherosclerosis was significantly increased and was positively correlated with tumor necrosis factor [19, 20]. In addition, the levels of TIPE2 protein were not consistent in different tumor tissues. For example, its expression was decreased in primary hepatocellular carcinoma, but its expression was increased in renal cell carcinoma [21]. The above results indicated that TIPE2 protein played different roles in different diseases. So we analyzed the expressions of TIPE2 protein in nasal mucosa of allergic rhinitis children.
Expression of TIPE2 in allergic rhinitis children

The TIPE2 expression in normal nasal mucosa and allergic rhinitis mucosa was analyzed by real-time fluorescence quantitative PCR. The results showed that TIPE2 mRNA level was significantly lower than that of normal nasal mucosa, indicating that TIPE expression was decreased in allergic rhinitis mucosa. The further immunohistochemical analysis showed that the expression of TIPE protein in allergic rhinitis mucosa was significantly lower than that of the normal mucosa, which was consistent with the change of mRNA level. This result suggested that TIPE2 protein might play a negative regulating role in the occurrence of allergic rhinitis. An important pathological change of allergic rhinitis is inflammatory cells infiltration, especially eosinophils and mast cells. Our study showed that the level of inflammatory cells infiltration in allergic rhinitis mucosa was significantly increased. The correlation analysis between the expression of TIPE2 mRNA and inflammatory cells infiltration was analyzed. The results showed that they were negatively correlated, suggesting that TIPE2 was an inflammation negative regulatory factor.

In conclusion, the expression of TIPE2 protein was lowest in allergic rhinitis mucosa, and it was negatively correlated with the degree of inflammatory cells infiltration.

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Disclosure of conflict of interest

None.

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References


Expression of TIPE2 in allergic rhinitis children


