

Expression of CCR3, SOX5 and LC3 in patients with elderly onset rheumatoid arthritis and the clinical significance

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Abstract. This study investigated the expression of C-C chemokine receptor type 3 (CCR3), transcription factor SOX5 (SOX5) and microtubule-associated protein 1 light chain 3 (LC3) in patients with elderly onset rheumatoid arthritis (EORA) and the clinical significance. Ninety patients with elderly onset rheumatoid arthritis were selected in our hospital from January to December in 2016 to serve as patient group. At the same time, 50 healthy people were selected as control group. Levels of CCR3, SOX5 and LC3 in serum of two groups were detected by enzyme-linked immunosorbent assay (ELISA). Expression levels of CCR3, SOX5 and LC3 mRNA in peripheral blood mononuclear cells (PBMCs) were detected by reverse transcription-PCR (RT-PCR). Expression level of CCR3 mRNA in patient group was 0.752 ± 0.054 , which was significantly higher than that in control group (0.287 ± 0.032 , $t=8.932$, $P<0.05$). Levels of CCR3, SOX5 and LC3 in serum of patients in patient group were significantly higher than those in control group ($P<0.05$). Positive correlations were found between serum levels of CCR3 and SOX5 ($r=0.613$, $P<0.05$), serum levels of CCR3 and LC3 ($r=0.637$, $P<0.05$), and serum levels of SOX5 and LC3 ($r=0.645$, $P<0.05$). CCR3, SOX5 and LC3 are highly expressed in PBMC and serum, which may be closely related to the occurrence and development of EORA. These indexes may be used as indicators of clinical diagnosis and prognosis of patients with EORA.

Introduction

Rheumatoid arthritis is a relatively common autoimmune disease, and this disease is usually complicated with pericarditis, lymphadenitis and other complications (1). Attentions should be paid to rheumatoid arthritis. Without treatment in time, rheumatoid arthritis can easily develop into irreversible

articular cartilage damage, causing serious adverse effects to patients (2). Early diagnosis of rheumatoid arthritis is beneficial to the clinical treatment and prognosis of this disease. Therefore, it would be of great clinical value to identify serum markers for the diagnosis and treatment of rheumatoid arthritis. C-C chemokine receptor type 3 (CCR3), transcription factor SOX5 (SOX5) and microtubule-associated protein 1 light chain 3 (LC3) are common serum markers. So far, only few reports on the functions of these serum markers in the development of elderly onset rheumatoid arthritis (EORA) have been reported. This study aimed to investigate the expression of CCR3, SOX5 and LC3 in patients with elderly onset rheumatoid arthritis and the clinical significance.

Materials and methods

General information. Ninety patients with elderly onset rheumatoid arthritis were selected in Huaihe Hospital from January to December in 2016 to serve as patient group. Inclusion criteria: i) meet the diagnostic criteria for rheumatoid arthritis (3), and age ≥ 60 years; ii) received no intervention treatment within 3 months before admission; iii) patients signed written informed consent. Exclusion criteria: i) patients combined with heart, lung and other important organ diseases; ii) patients with incomplete clinical data. In patient group, there were 50 males and 40 females, ages ranged from 61 to 74 years with an average age of 65.2 ± 2.3 years, and the course ranged from 1 to 6 years with an mean value of 4.5 ± 1.2 years. There was no significant difference in gender, age and general information between the two groups ($P>0.05$). This study was approved by the Ethics Committee of Henan University Huaihe Hospital.

Methods

Major reagents. Human CCR3 enzyme-linked immunosorbent assay (ELISA) kit (RunyuBio, Shanghai, China); human SOX5 ELISA kit (Shanghai Jiang Lai Biotechnology Co., Ltd., Shanghai, China); human LC3 ELISA kit (Shanghai Yanjin Biotechnology Co., Ltd., Shanghai, China); TRIZol (Bioss, Beijing, China); reverse transcription kit (Shenzhen Bao An Kang Biological Co., Ltd., Shenzhen, China), DNA polymerase (JiNingShiYe, Ltd., Shanghai, China).

Serum collection and processing. Fasting venous blood (5 ml) was collected in the morning, and heparin was added for anticoagulation. Blood was stored at -70°C before use.

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Key words: elderly onset rheumatoid arthritis, C-C chemokine receptor type 3, SOX5, light chain 3

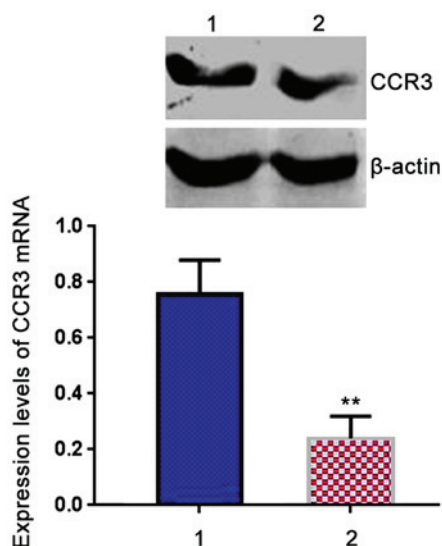


Figure 1. Comparison of expression levels of C-C chemokine receptor type 3 (CCR3) mRNA in peripheral blood mononuclear cells (PBMCs) between two groups. Lane 1, Patient group; lane 2, control group. RT-PCR results showed that expression level of CCR3 mRNA in patient group was significantly higher than that in control group. **P<0.05 compared with patient group.

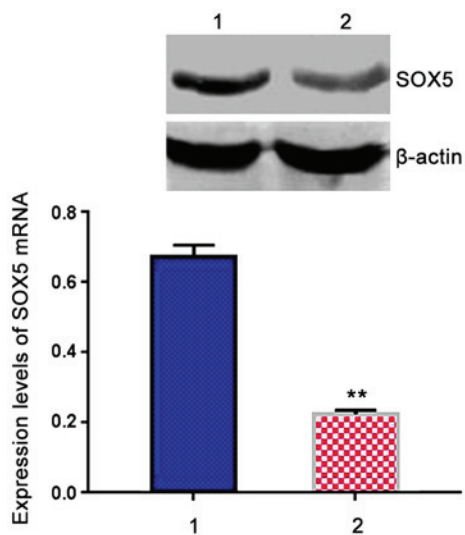


Figure 2. Comparison of expression levels of SOX5 mRNA in peripheral blood mononuclear cells (PBMCs) between two groups. Lane 1, Patient group; lane 2, control group. RT-PCR results showed that expression level of SOX5 mRNA in patient group was significantly higher than that in control group. **P<0.05 compared with patient group.

ELISA to detect CCR3, SOX5 and LC3 proteins in serum. Serum levels of CCR3, SOX5 and LC3 were measured by ELISA according to the instructions of the kits.

Reverse transcription PCR (RT-PCR) to detect the expression of CCR3, SOX5 and LC3 mRNAs in peripheral blood mononuclear cells (PBMCs). Appropriate amount of lymphocyte separation solution was added to serum samples. After centrifugation at 4°C, PBMC was isolated and washed with RPMI-1640. Total RNA was extracted using TRIzol reagent and reverse transcription was carried out in strict accordance with instructions of the kit. Reverse transcription conditions were: 42°C for 60 min, 95°C for 5 min and 5°C for 5 min. The

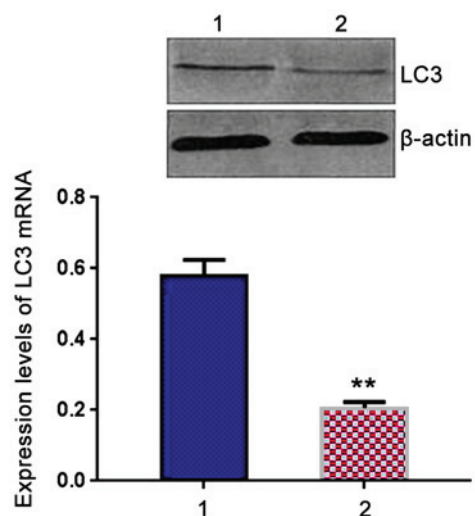


Figure 3. Comparison of expression levels of light chain 3 (LC3) mRNA in peripheral blood mononuclear cells (PBMCs) between two groups. Lane 1, Patient group; lane 2, control group. RT-PCR results showed that expression level of LC3 mRNA in patient group was significantly higher than that in control group. **P<0.05 compared with patient group.

product was stored at -20°C before PCR reaction. Primers used were: 5'-TGGCGGTTGGCGGTGTTTTTCATTTTC-3' (sense) and 5'-CCGGCTCTGCTGTGGAT-3' (antisense) for CCR3, the length of product was 315 bp; 5'-GTAGTGTTC CCTACCAACA-3' (sense) and 5'-ACAGCGTCTGGATGATTCTGA-3' (antisense) for SOX, the length of product was 502 bp; 5'-GAGTGGGAAGATGTC C GGTC-3' (sense) and 5'-CCAGGAGGAAGAAGGCTTGG-3' (antisense) for LC3, the length of product was 416 bp. Reaction conditions: 94°C for 5 min, followed by 38 cycles of 94°C for 30 sec; 59°C for 30 sec and 72°C for 60 sec, and 72°C for 5 min.

Ten microliters of PCR produce was subjected to 2% agarose gel electrophoresis and images were taken under ultraviolet light. Optical densities of the bands were measured and the relative expression levels of CCR3, SOX5 and LC3 mRNAs were normalized to the grey scale of β-actin.

Statistical analysis. Using SPSS 20.0 (IBM, Armonk, NY, USA) statistical software was used for statistical analysis, measurement data were expressed as mean ± SD and processed using t-test. P<0.05 was considered to be statistically significant.

Results

Comparison of expression levels of CCR3 mRNA in PBMCs between two groups. RT-PCR results showed that expression level of CCR3 mRNA in patient group was 0.752 ± 0.054 , which was significantly higher than that in control group (0.287 ± 0.032 , $t=8.932$, $P<0.05$) (Fig. 1).

Comparison of expression levels of SOX5 mRNA in PBMCs between two groups. Expression of SOX5 mRNA in patient group was 0.674 ± 0.032 , which was significantly higher than that in control group (0.223 ± 0.012 , $t=7.541$, $P<0.05$) (Fig. 2).

Comparison of expression levels of LC3 mRNA in PBMCs between two groups. Expression of LC3 mRNA in patient

Table I. Comparison of serum levels of CCR3, SOX5 and LC3 between two groups (ng/l).

Groups	n	CCR3	SOX5	LC3
Patient group	90	732.6±37.3	694.2±47.8	637.7±34.5
Control group	50	187.2±25.5	165.4±21.2	146.1±17.8
t-value		9.446	10.152	8.953
p-value		<0.05	<0.05	<0.05

CCR3, C-C chemokine receptor type 3; LC3, light chain 3.

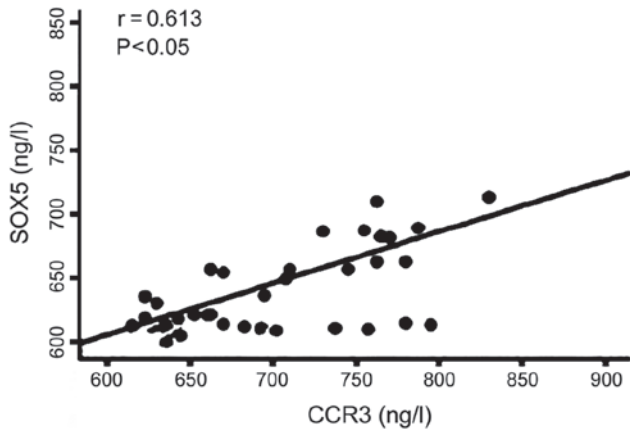


Figure 4. Serum level of C-C chemokine receptor type 3 (CCR3) was positively correlated with serum level of SOX5.

group was 0.674 ± 0.032 , which was significantly higher than that in control group (0.223 ± 0.012 , $t=7.541$, $P<0.05$) (Fig. 3).

Comparison of serum levels of CCR3, SOX5 and LC3 between two groups. Levels of CCR3, SOX5 and LC3 in serum of patient group were significantly higher than those in control group ($P<0.05$) (Table I).

Correlation analysis. There was a positive correlation between serum levels of CCR3 and SOX5 in patients ($r=0.613$, $P<0.05$) (Fig. 4). Serum level of CCR3 was positively correlated with serum level of LC3 ($r=0.637$, $P<0.05$) (Fig. 5). Serum level of SOX5 was positively correlated with serum level of LC3 ($r=0.645$, $P<0.05$) (Fig. 6).

Discussion

Immune deficiency is common in patients with rheumatoid arthritis, and immunosuppressive agents were usually used to treat this disease (4,5). In elderly patients, rheumatoid arthritis is usually combined with low fever, cough and other symptoms or even tuberculosis infection. The complex clinical symptoms of this disease bring difficulties to diagnose (6,7). The pathogenesis of EORA is still unclear. Rheumatoid arthritis is a genetic factor, infection and immune response mediated complex process (8-10).

Inflammatory response usually occurs in joints and the whole body of patients with EORA. A variety of cytokines

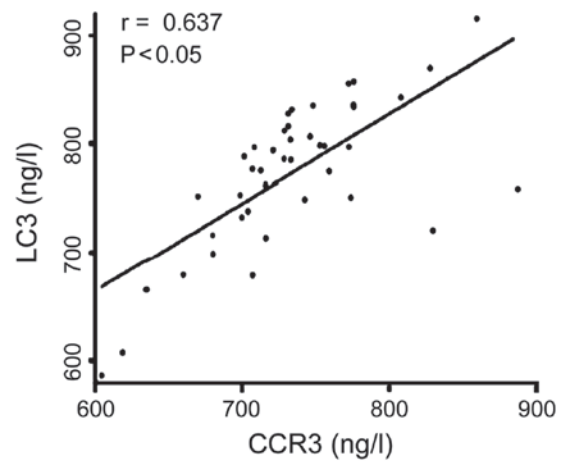


Figure 5. Serum level of C-C chemokine receptor type 3 (CCR3) was positively correlated with serum level of light chain 3 (LC3).

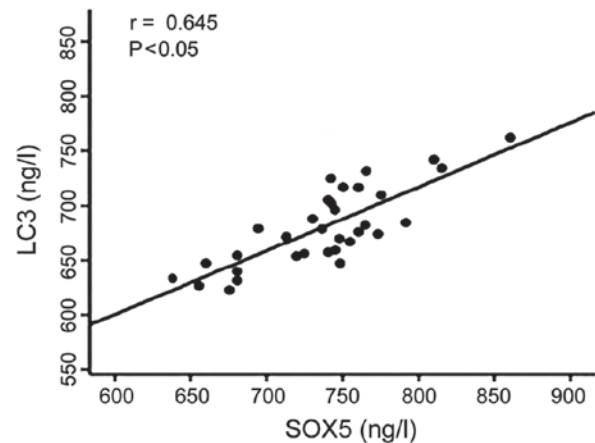


Figure 6. Serum level of SOX5 was positively correlated with serum level of light chain 3 (LC3).

will be produced during the development of disease, so those cytokines are actively involved in the progression of disease conditions and development of pulmonary fibrosis (11-14). Related studies have shown that (15,16), chemokines can promote the development of inflammatory factors, as well as the activation of white blood cells. Some studies have also shown that chemokine LKN-1 can induce the chemotactic reaction of endothelial cells through its receptor CCR1 and CCR3, which in turn promote the differentiation of endothelial cells and the formation of external blood vessels (17). It is worth mentioning that, as a G protein-coupled receptor, CCR3 can be expressed in both eosinophils and Th2 lymphocytes, and CCR3 can also mediate cell response to chemokines and promote a variety of biological activity (17). Related studies have shown that (18), CCR3 is closely related to asthma, allergies and other immune diseases. As a newly discovered important transcription factor, SOX5 plays an important role in cartilage formation and regulation, SOX5 also has important functions in regulating the proliferation and differentiation of chondrocyte (19). As an important indicator of autophagosomes, LC3 is mainly located in the cytoplasm and surface of autophagic membrane. LC3 can participate in the formation of autophagosome (20).

Results of this study showed that expression levels of CCR3, SOX5 and LC3 mRNAs in patient group were significantly higher than those in control group ($P < 0.05$). Levels of CCR3, SOX5 and LC3 in serum patient group were significantly higher than those in control group ($P < 0.05$). Positive correlations were found between serum levels of CCR3 and SOX5 ($r = 0.613$, $P < 0.05$), serum levels of CCR3 and LC3 ($r = 0.637$, $P < 0.05$), and serum levels of SOX5 and LC3 ($r = 0.645$, $P < 0.05$). Those results suggest that CCR3, SOX5 and LC3 are very likely to be involved in the pathogenesis of EORA, and have great clinical values in the diagnosis of this disease.

In conclusion, CCR3, SOX5 and LC3 are highly expressed in PBMC and serum, which may be closely related to the occurrence and development of EORA. These indexes may be used as indicators of clinical diagnosis and prognosis of patients with EORA.

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