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Expression of NF- κ B and osteopontin of synovial fluid of patients with knee osteoarthritis

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ABSTRACT

Objective: To explore the significance of osteopontin and nuclear factor κ B (NF- κ B) expression in patients with knee osteoarthritis. **Methods:** RT-PCR and enzyme-linked immunosorbent assay were used to measure the Osteopontin (OPN) and NF- κ B concentration of knee joint synovial fluid of patients with knee osteoarthritis and trauma fractures, and analyze the relationship between the expressions of them. **Results:** OPN and NF- κ B expression at the mRNA and protein levels of patients with knee osteoarthritis were significantly higher than the control group. the result showed statistical significance ($P < 0.05$). There was a positive correlation between the OPN levels in synovial fluid of patients with knee osteoarthritis and NF- κ B expression levels ($P < 0.05$). **Conclusions:** The high expression of OPN and NF- κ B are closely related to occurrence and development of knee osteoarthritis.

1. Introduction

Osteoarthritis (OA) is a common chronic joint disease. It will increase year by year with the age. The pathology features are primary or secondary degeneration of the articular cartilage, which specifically manifest as the degradation of the collagen network and the proteoglycan of articular cartilage and the destruction of the joint cartilage structure, and it can also lead to some degree of synovial inflammation[1]. The osteopontin (OPN) is a phosphorylated protein which widely distributed in human tissues with a

variety of functions[2]. Nuclear factor NF- κ B is a class of proteins that can specifically binding to a variety of gene promoter and enhancer and also promote the of gene transcription, through the regulation of the expression of a variety of chemokines, cytokines, growth factors, and adhesion molecule to participate in the collective immune response and inflammatory response. It plays a key role in cell proliferation, differentiation, and apoptosis. With the development of the research, it has proved that a variety of diseases were related to excessive activation of NF- κ B in recent years. This study used RT-PCR and ELISA to detect the OPN and NF- κ B expression in the synovial fluid of patients with knee osteoarthritis, to explore their relationship of the occurrence and development of knee osteoarthritis.

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2. Materials and methods

2.1. General information

A total of 42 cases with osteoarthritis were selected from April 2011 to June 2012 in our hospital. All the enrolled patients were in according with standard the diagnostic criteria of the American Rheumatism Association 1995 revised[3,4]. There were 26 males and 16 females in observation group, aged from 48 to 77, and the average age was (61.2 ± 8.5) years old. Forty cases of the normal control group had meniscus injury or lower extremity fracture surgery of our hospital at the same period, but without osteoarthritis. There were 24 males and 16 females in normal group, aged from 34 to 61, and the average age was 43.3 ± 7.6 . The enrolled patients with rheumatoid arthritis and gouty arthritis and secondary osteoarthritis were excluded.

2.2. Reagents and instruments

Mouse anti-human OPN monoclonal antibody, mouse anti-human NF- κ B monoclonal antibodies, human serum OPN and NF- κ B ELISA kit were all purchased from Wuhan Boster Biological Engineering Co., Ltd., Microporous 96-well microtiter plates, ELISA coupling liquid ,terminated liquid, TRIzol, DEPC, DNA the Marker (DL-2000) Takara, agarose, ethidium bromide (EB), chloroform, isoamyl alcohol, ethanol, isopropanol, and 20 times concentrated detergent solution, 5 times concentrated sample dilutions, medical distilled water, centrifuges, pipettes, pipette tips, timer ,processing pool, PCR amplification icycler type (Bio-Rad; United States), the DC2000 gel imaging analyzer (Bio-Rad; USA).

2.3. Experimental method

Synovial fluid were collected from all patients, conserved at 4 °C , centrifuged at 3 000 rpm for 20 min. The supernatant was collected and divided into two parts. Then it was placed at -20 °C refrigerator. RT-PCR and sandwich enzyme-linked immunosorbent assay (ELISA) were used to detect the expression of OPN and NF- κ B at mRNA and protein levels respectively. All measure operation steps were strictly followed.

2.4. Statistical analysis

The experimental data were analyzed with SPSS16.0 statistical software. Data are expressed as mean \pm SD values. The significantly difference between two groups were compared with *t* test. The trend was analyzed by using the Spearman rank correlation, and using Pearson method to analyze simple correlation. $P < 0.05$ was regarded as statistical significance.

3. Results

3.1. Expression levels of the OPN and NF- κ B

After a semi-quantitative analysis, the expression levels of OPN [(0.638 ± 0.082) ng/L vs. (0.492 ± 0.098) ng/L] and NF- κ B [(1.124 ± 0.146) ng/L vs. (0.943 ± 0.138) ng/L] of the observation group were both significantly higher than those in the control group. The result showed statistical significance ($P < 0.05$) (Figure 1, 2).

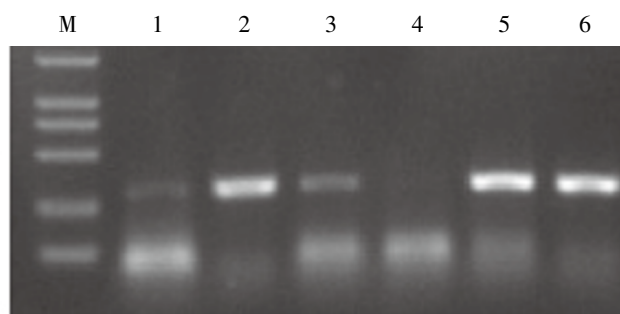


Figure 1. OPN mRNA expression levels.

1, 3, 5: observation group; 2, 4, 6: control group.

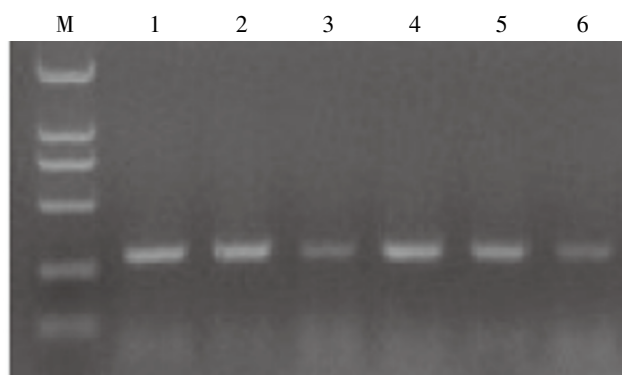


Figure 2. NF- κ B mRNA expression levels.

1, 3, 5: observation group; 2, 4, 6: control group.

3.2. Expression at protein levels of OPN and NF- κ B

The expression at protein levels of OPN [(3378.2 ± 419.5) ng/L vs. (892.5 ± 214.6) ng/L] and NF- κ B [(1756.4 ± 385.1) ng/L vs. (821.5 ± 323.4) ng/L] of the observation group were both significantly higher than those in the control group. The result showed statistical significance ($P < 0.05$).

3.3. Correlation between OPN levels and NF- κ B expression levels in synovial fluid of patients with knee osteoarthritis

Pearson correlation analysis showed that the OPN levels of NF- κ B level in synovial fluid of patients with knee osteoarthritis were positively correlated ($r = 0.876$, $P < 0.05$).

4. Discussion

Osteoarthritis is a common chronic joint disease, which is characterized by degeneration and destruction of articular cartilage and hyperostosis[5]. Osteoarthritis is also known as degenerative joint disease and hyperplastic osteoarthritis. Different names come from the pathological manifestations of joint disease, which is the cartilage degeneration accompanied with the formation of new bone formation. The incidence of the disease increased with age, and it is a common joint disease in the aged[6]. The treatment for osteoarthritis is often very difficult. Clinical treatment of osteoarthritis can not repair the articular cartilage, and it is also impossible to reverse the process of degeneration of the articular cartilage[7]. A molecular biology method to assess the degeneration of osteoarthritis is particularly important. In this study, we explored the expression of OPN and NF- κ B in patients with osteoarthritis.

Osteopontin, also known as OPN, is a highly phosphorylated glycoprotein rich in serine, glutamic acid and aspartic acid. OPN is distributed widely in the intercellular substance of the extracellular, inflammatory sites and the bone tissues. All of the osteoblasts, osteoclasts, smooth muscle cells, and vascular endothelial cells can synthesize and secrete OPN molecules[8]. OPN molecule can be combined with cell. It can combine with the extracellular matrix through an unknown substance to participate in cell adhesion, migration and proliferation. OPN is related to the osteoarthritis[9]. Studies have shown that OPN can stimulate the deposition of the calcium pyrophosphate dihydrate, which can lead the abnormal biomineralization in articular cartilage and degeneration of the joint cartilage. Gao's study[10] have showed that the expression levels of articular cartilage and the increase of the synovial fluid were closely related to the severity of the OA, which can be used as an effective indicator to assess the progression of knee OA. The results of this study showed that the semi-quantitative level of expression of OPN mRNA was 0.638 ± 0.082 , significantly higher than that in the control group 0.492 ± 0.098 , and the OPN of the observation group was (3378.2 ± 419.5) ng/L, significantly higher than that of the control group (892.5 ± 214.6) ng/L. The data indicated that the OPN expression of knee osteoarthritis patients was significantly increased. In the analysis of the correlation of osteoarthritis severity, this study showed that with the rise of the severity of knee osteoarthritis, the OPN expression of the observation group was also significantly increased, which showed there was a positive correlation between the OPN expression levels and the severity of osteoarthritis ($P < 0.05$), and suggested that OPN may played a role in the progress of osteoarthritis.

NF- κ B is a protein factor with multi-regulation function,

which plays a critical role in the cytokine-induced gene expression[11]. Through the regulation of kinds of gene expression, NF- κ B involved in inflammatory response, immune response, apoptosis, and the occurrence and development of the tumor. As the research is increasingly furthered and developed, it has been proved the overexpression of NF- κ B was associated with the occurrence of many diseases[12]. Many studies on NF- κ B as a target for disease treatment have been reported, and the correlation study of NF- κ B and OA has become popular. Bond *et al.*[13] showed that the expression and activation levels of NF- κ B in OA were significantly higher than in normal control group, and the IL-1 and MMP-9 transcription levels were also significantly higher than the normal control group, there was a positive correlation between IL-1 and MMP-9 expression levels and the NF- κ B activation levels. This study showed that the semi-quantitative level of NF- κ B mRNA expression in the observation group was 1.124 ± 0.146 , significantly higher than that in the control group 0.943 ± 0.138 ; NF- κ B protein expression level was (1756.4 ± 385.1) ng/L, significantly higher than the (821.5 ± 323.4) ng/L in the control group. The data show that the NF- κ B expression in patients with knee osteoarthritis was significantly higher. This study shows that the OPN, NF- κ B expression were both high in the knee osteoarthritis, and there was a positive correlation between their expression levels ($P < 0.05$), which suggests that OPN and NF- κ B were involved in the inflammatory response of knee osteoarthritis. In summary, OPN and NF- κ B play a certain role in the progress of osteoarthritis, but its specific pathogenesis needs further study.

Conflict of interest statement

We declare that we have no conflict of interest.

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