MIRNA-203 AFFECTED THE PROLIFERATION AND APOPTOSIS OF FIBROBLAST SYNOVIUM CELLS IN RHEUMATOID ARTHRITIS RATS BY REGULATING THE WNT/B-CATENIN SIGNALING PATHWAY

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ABSTRACT

Objective: *To investigate the effect of miRNA-203 on the proliferation and apoptosis of fibroblast synovium cells in rats with rheumatoid arthritis by regulating the Wnt/β-catenin signaling pathway.*

Methods: *60 healthy, male Sprague-Dawley (SD) rats of a clean grade were selected for the sample in this study. From these, 15 rats were randomly selected as the NC group and were each injected with 0.1ml /100g normal saline. The remaining 45 rats were injected with 0.1mL/100g Freund's complete adjuvant (FCA) intradermal to establish a rat model for rheumatoid arthritis. After successful modeling, the 45 rheumatoid arthritis rat models were randomly divided into a model group, a miR-203 mimics group, and a miR-203 inhibition group, with 15 rats in each group. The model group was not treated, while the miR-203 mimics group and the miR-203 inhibition group were transfected with miR-203 mimics and miR-203 inhibition, respectively. After feeding the rats for 4w, follow-up studies began. Rats in each group were taken for abdominal aortic bloodletting and then put to death; rat fibroblast synovial cells were isolated for culture and passage. The proliferation capacity (OD value), apoptosis capacity (apoptosis rate), adenomatous colon polyposis gene (APC), and Wnt/β-catenin signaling pathway-related proteins (β-catenin, C-myc) were compared.*

Results: *The OD values of the model group and the miR-203 mimics group were significantly higher than those of the NC group (P<0.05). There was no significant difference in the OD values from the model group and the miR-203 mimics group (P>0.05). The OD value from the miR-203 inhibition group was significantly lower than that of the model group (P<0.05). There was no significant difference in the OD values from the NC group and the miR-203 inhibition group (P>0.05). The apoptosis rates for the model group and the miR-203 mimics group were significantly lower than that of the NC group (P<0.05). There was no significant difference in the apoptosis rates of the model group and the miR-203 mimics group (P>0.05). The apoptosis rate of the miR-203 inhibition group was significantly higher than that of the model group (P<0.05). There was no significant difference in apoptosis rates between the NC group and the miR-203 inhibition group (P>0.05). The expression levels of the APC protein in the model group and the miR-203 mimics group were significantly lower than in the NC group, while the expression levels of β-catenin and the C-myc protein were significantly higher than in the NC group (P<0.05). There was no significant difference in the expression levels of APC0, β-catenin, and C-myc in the model group compared with the miR-203 mimics group (P>0.05). The expression level of the APC protein in the miR-203 inhibition group was significantly higher than that in the model group, while the expression levels of β-catenin and the C-myc protein in the model group were significantly lower than those in the model group (P<0.05). There was no statistically significant difference in the APC, β-catenin, and C-myc protein expression levels between the NC group and the miR-203 inhibition group (P>0.05).*

Conclusion: *Silencing the expression of miRNA-203 can improve the expression of APC in the fibroblast synovium cells of rats with rheumatoid arthritis, inhibiting the activation of the Wnt/β-catenin signaling pathway, which can significantly inhibit the proliferation of fibroblast synovium cells and promote apoptosis.*

Keywords: *miRNA-203, Wnt/β-catenin, rheumatoid arthritis, fibroblast synovium cells.*

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Introduction

Rheumatoid arthritis is a systemic disease with a pathogenesis that has not yet been elucidated. In the early stage of its onset, the disease mainly affects the synovium. As the disease progresses, the side joint is affected, and surrounding tissues may be damaged. At this stage, rheumatoid arthritis can become a potentially disabling disease and other system tissues may be affected[1]. At present, the global incidence of rheumatoid arthritis is about 1%. There are significant differences in its incidence among people of different ages[2]. As the disease is disabling and can seriously threaten the quality of life and, indeed, the life of a person who suffers from it, it is essential that the pathogenesis of rheumatoid

arthritis is further revealed and that targeted drugs are developed according to its pathogenesis, to improve the quality of life and prolong the survival of patients. Over the past decade, the findings of many studies have improved our knowledge of the pathogenesis and prognosis of rheumatoid arthritis; significant achievements have been made through in-depth studies on the pathophysiology of the disease[3]. However, the molecular mechanisms of synovial dysplasia, articular inflammation, and disease progression for patients with rheumatoid arthritis have not been confirmed. At present, the clinical treatment of rheumatoid arthritis can only improve its symptoms, while the potential is more restricted for curing the disease. Some scholars have started to apply fibroblast synovial cells in their research[4]. According to the related research and no known airfoil MMTV integration site family members (Wnt)/β-catenin protein (β-catenin) the characteristics of the signal path, we think of synovial cell in patients with rheumatoid arthritis fibroblasts Wnt/β-catenin signaling pathways activated in which involved in regulating it into a fiber synovial cell activation and participate in the onset of rheumatoid arthritis is reasonable this theory[5].

Clinical studies have shown that the expression of microRNA-203 in the fibroblast synovial cells of osteoarthritis patients is significantly higher than that of normal people and that this elevated expression level could induce an increase of matrix metalloproteinase-1 (MMP-1) and interleukin-1 (IL-1)[6]. Previous studies have found that miRNA-203 and the Wnt/β-catenin signaling pathway are associated with rheumatoid arthritis[7].

However, the specific role and mechanism of miRNA-203 are not yet clear. Therefore, this study aims to investigate the effect of miRNA-203 on the proliferation and apoptosis of fibroblast synovium cells in rats with rheumatoid arthritis, by regulating the Wnt/β-catenin signaling pathway and its mechanism of action.

Materials and methods

Experimental materials

60 healthy, male Sprague-Dawley (SD) rats of a clean grade were purchased from Jiangsu Synergetic Pharmaceutical Biological Engineering Co., Ltd. The body mass of the rats ranged from 200 to 240g. The rats were kept in cages with six in each cage; the temperature was set at ~ 22-26 °C and the humidity was set at ~50-55% every 12 hours.

Methods

• 15 rats were randomly selected as the NC group and injected with 0.1ml /100g normal saline. The remaining 45 rats were injected with 0.1mL/100g Freund's complete adjuvant (FCA) intradermal to establish a rat model for rheumatoid arthritis. After successful modeling, 45 rheumatoid arthritis rat models were randomly divided into a model group, a miR-203 mimics group, and a miR-203 inhibition group, with 15 rats in each group. The model group was not treated, while the miR-203 mimics group and the miR-203 inhibition group were transfected with miR-203 mimics and miR-203 inhibition, respectively. After feeding for 4w, follow-up studies commenced.

• After 4w, rats from each group were taken for abdominal aortic bleeding and then put to death. The knee joint and surrounding adipose fiber tissues of the rats were separated using surgical scissors. The synovium was removed and placed in Hank's reagent for cleaning. The synovium was later removed and cut into small pieces of about 2 mm2.

• The proliferation (OD value) and apoptosis (apoptosis rate) of the fibroblast synovium cells in each group were measured using CCK-8 and flow cytometry, respectively.

• The expression levels of adenomatous polyposis coli (APC) and Wnt/β-catenin signaling pathway-related proteins (β-catenin and C-myc) in the fibroblast synovium cells of the rats in each group were determined using a western blot.

Statistical data

The data that were measured for apoptosis rate, OD value, and APC, β-catenin, and C-myc protein expression levels, for the fibroblast-like synovial cells in each group, were expressed by ($x \pm s$). An independent sample t test was utilized for a comparison of the two groups, along with single-factor variance analysis. This study analyzed all data using SPSS 23.0 software, with $P<0.05$ as the difference that is considered to represent statistical significance.

Results

Effect of the upregulation of miRNA-203 on the proliferation of fibroblast synovial cells

The cell OD values for the model group and the miR-203 mimics group were significantly higher than those for the NC group ($P<0.05$). There was no significant difference in the OD values for the model

group and the miR-203 mimics group (P>0.05). The OD value for the miR-203 inhibition group was significantly lower than that for the model group (P<0.05). There was no significant difference in OD values for the NC group and the miR-203 inhibition group (P>0.05). These findings are shown in Table 1.

Group	n	OD value
NC group	15	0.28±0.09
Model group	15	0.58±0.03[a]
miR-203 mimics group	15	0.54±0.09[a]
miR-203 inhibition group	15	0.27±0.04[b]

Table 1: Effect of upregulated miRNA-203 expression on the proliferation of fibroblast synovium cells (x±s).
Note: Compared with the NC group, [a]P<0.05; compared with the model group, [b]P<0.05.

The effect of up-regulation of miRNA-203 expression on fibroblast synovium cell apoptosis

The apoptosis rates for the model group and the miR-203 mimics group were significantly lower than that for the NC group (P<0.05). There was no significant difference in apoptosis rate between the model group and the miR-203 mimics group (P>0.05). The apoptosis rate for the miR-203 inhibition group was significantly higher than that for the model group (P<0.05). There was no significant difference in apoptosis rate between the NC group and the miR-203 inhibition group (P>0.05). See Table 2.

Group	n	Apoptosis rate (%)
NC group	15	9.02±2.30
Model group	15	2.42±0.06[a]
miR-203 mimics group	15	2.33±0.09[a]
miR-203 inhibition group	15	11.23±1.19[b]

Table 2: Effect of upregulated miRNA-203 expression on the apoptosis of fibroblast synovium cells (x±s).
Note: Compared with the NC group, [a]P<0.05; compared with the model group, [b]P<0.05.

Upregulation of the expression level of miRNA-203 on the APC and Wnt/β-catenin signaling pathways in fibroblast synovium cells

The expression levels of the APC protein in the model group and the miR-203 mimics group were significantly lower than that in the NC group, while the expression levels of the β-catenin and C-myc proteins were significantly higher than that in the NC group (P<0.05). There was no significant difference in the expression levels of APC0, β-catenin, and C-myc in the model group compared with the miR-203 mimics group (P>0.05). The expression level of the APC protein in the miR-203 inhibition group was significantly higher than that in the model group, while the expression levels of the β-catenin and C-myc proteins in the model group were significantly lower than those in the model group (P<0.05).

There was no statistically significant difference in the APC, β-catenin, and C-myc protein expression levels between the NC group and the miR-203 Inhibition group (P>0.05). See Table 3 and Figure 1.

Group	n	APC	β-catenin	C-myc
NC group	15	0.85±0.21	0.61±0.11	0.41±0.11
Model group	15	0.57±0.07[a]	1.08±0.15[a]	0.89±0.15[a]
miR-203 mimics group	15	0.63±0.11[a]	1.12±0.13[a]	0.93±0.18[a]
miR-203 inhibition group	15	0.84±0.19[b]	0.65±0.13[a]	0.36±0.09[b]

Table 3: The effect of upregulation of the miRNA-203 expression level on APC and the Wnt/β-catenin signaling pathway in fibroblast synovium cells (x±s).
Note: Compared with the NC group, [a]P<0.05; compared with the model group, [b]P<0.05.

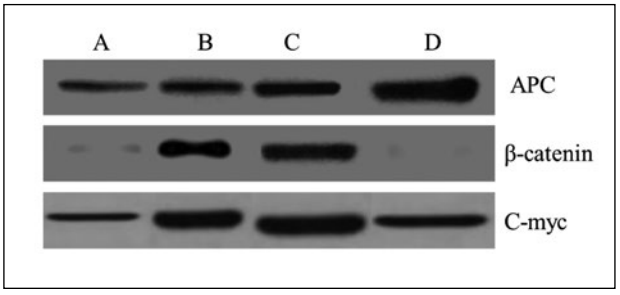

Figure 1: The effect of upregulation of the miRNA-203 expression level on APC and the Wnt/β-catenin signaling pathway in fibroblast synovium cells.
Note: A. NC team; B. model group; C. miR-203 mimics group; D. miR-203 inhibition group.

Discussion

Many reports have indicated that activated c plays a key role in the pathogenesis of rheumatoid arthritis and that the activated proliferation of this cell may promote the infiltration and retention of lymphocytes through the release of cytokines and chemokines[8]. Fibroblast synovium cells are known to upregulate proliferation factors, along with many endothelial growth factors, thereby accelerating the generation of blood vessels. In addition, fibroblast synovium cells in the state of activated hyperplasia can promote the degradation of continuous cartilage and thus form an antigen-stimulated immune response[9]. It has been reported that fibroblast

synovium cells with abnormal activation and hyperplasia are involved in the process of invasive vascular and joint destruction and are, in fact, key cells in the pathogenesis of rheumatoid arthritis[10]. However, no drugs have yet been found that can target the fibroblast synovium cells in patients with rheumatoid arthritis, as the underlying molecular mechanisms that trigger the activation of fibroblast synovium cells are not yet fully understood.

Many reports demonstrated how the Wnt/β-catenin signaling pathway can control the abnormal activation of fibroblast synovium cells. There are many proteins in the cells and these control the downstream signal-cascade reaction, to a certain extent. If there is no Wnt ligand, APC can jointly form a proteolytic complex with axin, glycogenase kinase-3, and collateral protein kinase-1; this complex can then control the degradation of wt-catenin, with a negative regulatory function[11]. When the Wnt signaling pathway is activated, the proteolytic complex consisting of PC, axin, glycogen synthase kinase-3, and Wnt kinase-1 will be disintegrated, leading to the aggregation of β-catenin in the cytoplasm and its transfer to the nucleus, where β-catenin can interact with transcription factors, leading to the transcriptional activation of the downstream target gene, C-myc[12]. MiRNAs are an essential part of many biological processes. Studies have shown that there can be abnormal expressions of miRNAs within the synovial fluid, chemical membrane tissues, and fibroblast synovial cells of patients with rheumatoid arthritis. While miR-203 is downregulated in many types of malignancies, its expression is high in rheumatoid arthritis[13]. The upregulation of miR-203 in the fibroblast synovial cells of patients with rheumatoid arthritis can induce an increased release of interleukin-6 and matrix metalloproteinase-1, suggesting that miR-203 may play a role in promoting inflammation and joint destruction in the pathogenesis of rheumatoid arthritis[14]. The tumor suppressor gene, APC, is a negative regulator that participates in, and plays a key role in, the Wnt/ β-catenin signaling pathway. Studies have established rat models of rheumatoid arthritis and have found a correlation between the APC expression level and the activation of the Wnt/ β-catenin signaling pathway, to a certain extent[15].

In this study, we have found OD values for the model group and the miR-203 mimics group that were significantly higher than those for the NC group ($P<0.05$). The OD value for the miR-203 inhibition group was significantly lower than that for the model group ($P<0.05$). The apoptosis rates for the model group and the miR-203 mimics group were significantly lower than that for the NC group ($P<0.05$). The apoptosis rate for the miR-203 inhibition group was significantly higher than that for the model group ($P<0.05$). It has been suggested that the upregulation of the miR-203 expression level in the fibroblast synovial cells of rats with rheumatoid arthritis could promote cell proliferation and inhibit apoptosis. The expression levels for the APC protein in the model group and the miR-203 mimics group were significantly lower than that for the NC group, while the expression levels for the β-catenin and C-myc proteins were significantly higher than those for the NC group ($P<0.05$). The expression level of the APC protein in the miR-203 inhibition group was significantly higher than that for the model group, while the expression levels of the β-catenin and C-myc proteins in the model group were significantly lower than those in the model group ($P<0.05$). These results suggest that elevated expression of miR-203 in the fibroblast synovium cells of rats with rheumatoid arthritis may inhibit the expression of the APC protein in the post-transcriptional level, thereby inhibiting the degradation of the β-catenin protein, thus activating the Wnt/β-catenin signaling pathway and participating in regulating the proliferation and apoptosis of fibroblast synovium cells.

To conclude, silencing the expression of miRNA-203 can increase the expression of APC in the fibroblast synovium cells of rats with rheumatoid arthritis and inhibit the activation of the Wnt/β-catenin signaling pathway, which can significantly inhibit the proliferation of fibroblast synovium cells as well as promoting apoptosis.

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