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*Original Article*

# LincRNA-p21 inhibits inflammatory response and angiogenesis through the NF-κB signaling pathway in rats with rheumatoid arthritis

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**Abstract:** Objective: The family of nuclear factor-kappaB (NF-κB) transcription factors is intimately involved in the regulation of expression of numerous genes in the setting of the inflammatory response in rheumatoid arthritis (RA). The purpose of the study is to determine interrelationships between the expression of long intergenic RNA-p21 (lincRNA-p21) and NF-κB activity and their effects on inflammatory response and angiogenesis in RA. Methods: Among 72 Sprague-Dawley (SD) rats, 60 rats were prepared for chicken collagen type II-induced RA modeling. In the further experiments, we altered the expression of lincRNA-p21 and the activity of NF-κB signaling pathway in RA models *in vivo*. The degree of joint swelling of rats was evaluated using paw volume. Fluorescence quantitative polymerase chain reaction (PCR) was performed to determine the expression level of lincRNA-p21. The protein levels of IκBα, p-IκBα, P65, and p-P65 were measured by western blot assay and serum levels of inflammatory factors (IL-1β, IL-6, tumor necrosis factor-α [TNF-α], and IL-10) and angiogenic factors (vascular endothelial growth factor [VEGF], basic fibroblast growth factor [bFGF], and hepatocyte growth factor [HGF]) by enzyme-linked immunosorbent assay (ELISA). Results: In contrast to the blank and NC groups, the RA group and the RA + si-NC group exhibited lower expression levels of lincRNA-p21, larger paw volume, severer joint swelling, higher levels of the NF-κB signaling pathway-related proteins and pro-inflammatory factors, lower serum level of the anti-inflammatory factor, and higher serum levels of angiogenic factors. Compared with the RA group, the RA + si-p21 group showed down-regulated expression levels of lincRNA-p21, enlarged paw volume, serious joint swelling, increased levels of the NF-κB signaling pathway-related proteins, dropped level of the anti-inflammatory factor, and elevated serum levels of pro-inflammatory factors and angiogenic factors. Conclusions: These findings indicate that lincRNA-p21 may alleviate inflammatory response and inhibit angiogenesis in rats with RA by inhibiting the activation of the NF-κB signaling pathway, suggesting a novel therapeutic strategy for RA.

**Keywords:** Long intergenic noncoding RNA-p21, rheumatoid arthritis, NF-κB signaling pathway, inflammatory response, angiogenesis

## Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease and primarily targets the cartilage, bone and synovial membrane, bone and cartilage [1]. RA affects 0.5%~1% adults in developed countries, with 5~50 new cases in 0.1 million individuals annually [2]. RA is often characterized by synovial inflammation, joint damage, and immunological abnormalities; more importantly, inflammation of the soft tissues around synovial joints is one of the common phenomenon occurring in RA [3, 4]. In RA patients, infiltration of macrophages, T cells, and B cells, proliferation of the lining cells, as well as production of such inflammatory cytokines as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) are found [5]. Many cell types, including T cells, B cells, and macrophages, are involved in the complex pathogenesis of RA; meanwhile, fibroblast-like synoviocytes, as key effector cells, play an important role in cartilage destruction by producing cytokines that perpetuate inflammation and proteases [6, 7]. Long intergenic noncoding RNAs (lincRNAs) regulate developmental gene expression via establishing chromatin domains in an allele- and cell-type specific manner [8].

Recently, a wealth of evidence reports that RA occurrence is attributed to lncRNA alternations in fibroblast-like synoviocytes [9].

LincRNA-p21, located on chromosome 17, regulates apoptosis in response to p53 signaling and to suppression of targeted gene translation [11, 12]. LincRNA-p21 is abnormally expressed in several types of cancer. Wang et al. shows that lincRNA-p21 decreases in colorectal cancer (CRC) cell lines and tissue samples, which is conducive to the elevation of β-catenin in CRC [13]. Furthermore, lincRNA-p21 is shown to be down-regulated in human prostate cancer, and low levels of lincRNA-p21 are associated with high disease stage and prediction of poor survival [14]. Interestingly, Tang et al. highlights that lincRNA-p21 is correlated with the development and progression of RA [15]. To our knowledge, lincRNA-p21 is of significance in regulating cellular responses to p53 that presents anti-inflammatory properties [16, 17]. In contrast, nuclear factor kappa B (NF-κB) is an important pro-survival and pro-inflammatory transcription factor, and a variety of stimulants, including cytokines and growth factors, can induce its activation [18]. Thus, p53 and NF-κB may be considered biologically antagonistic [19]. However it is unknown if lincRNA-p21 can regulate NF-κB activation and how this may occur mechanistically. Therefore, this study aims to investigate the effect of lincRNA-p21 on inflammatory response and angiogenesis by regulating the NF-κB signaling pathway in rats with RA.

## Materials and methods

### *Ethic statement*

The present study was approved by Ethics Committee of Nanfang Hospital, and all experiments were in strict accordance with relevant regulations in the Declaration of Helsinki [20].

### *Animal preparation and grouping*

A total of 72 healthy Sprague-Dawlay rats (36 males and 36 females) with a weight range of 270 ± 20 g were chosen, which were purchased (SCXK 2007-0005) from Laboratory Animal Center, Third Military Medical University (Chongqing, China). All SD rats were raised in rooms under specific pathogen-free (SPF) conditions to adapt to new environment for 1 week at room temperature of 22°C to 25°C, with free access to food and water, and a normal circadian rhythm. Totally 72 included rats were randomly assigned to 6 groups (12 rats in each group): blank group (normal rats without treatment), NC group (normal rats, injected with normal saline), RA group (RA rat models), RA + si-NC group (RA rat models, injected with siRNA negative control plasmid of lincRNA-p21), RA + si-p21 group (RA rat models, injected with siRNA plasmid of lincRNA-p21), and the RA + si-p21 + BMS-345541 group (RA rat models, injected with siRNA plasmid of lincRNA-p21 and BMS-345541, inhibitor of the NF-κB signaling pathway). si-p21 and si-NC plasmids were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China).

### *RA modeling*

Chicken type II collagen (CCII, Sigma, Saint Louis, MI, USA) was dissolved in 0.1 mol/L glacial acetic acid (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), and then fully emulsified with an equal volume of complete Freund's adjuvant (CFA, Sigma, Saint Louis, MI, USA) to a final concentration of 0.5 g/L. The rats were anesthetized with 3% pentobarbital sodium (30 mg/kg, Sigma, Saint Louis, MI, USA). Then they were conducted with multipoint subcutaneous injection of 1 ml type II collagen in the back of rats and rat tail. The same injection with the same dose was carried out once after a week. The rats in the NC group were injected with normal saline in the same way with the same volume. The joint swelling conditions of rats were observed in each group, and the changes in the volume of the rat toes in each group were measured by volume measuring device. Rats were sacrificed at 28 d, and Haematoxilin-eosin (HE) staining was used to observe the pathological changes of joint and synovial tissue.

### *Fluorescence quantitative polymerase chain reaction (PCR)*

The total RNA in the rat synovial tissues was extracted by single-step Trizol method (Invitrogen, Waltham, MA, USA) according to the instructions. The RNA purity and concentration were measured by ultra-violet (UV) analysis and formaldehyde gel electrophoresis. Reverse transcription of 1 ug RNA into cDNA was performed by using avian myeloblastosis virus

**Table 1.** The primer sequences of LincRNA-p21 and GAPDH for RT-qPCR

| Gene | Primer sequence |
|---|---|
| LncRNA-p21 | F: 5'-GCGAGCTCCGTCTCCAGTTCCTA-3' |
| | R: 5'-GCACGCGTGAGCATGAGACTCCTG-3' |
| GAPDH | F: 5'-CAAGTTCAACGGCACAGTCA-3' |
| | R: 5'-CCCCATTTGATGTTAGCGGG-3' |

Note: LincRNA-p21, long intergenic noncoding RNA-p21; RT-qPCR, reverse transcription quantitative polymerase chain reaction; F, forward; R, reverse.

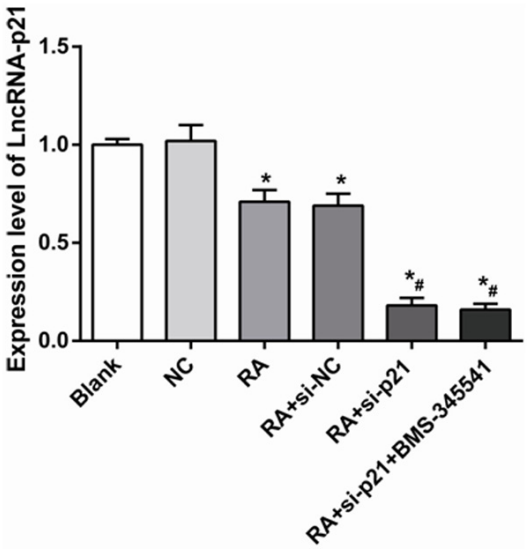


**Figure 1.** The expression levels of lincRNA-p21 in rat synovial tissues in blank, NC, RA, RA + si-NC, RA + si-cox-2, and RA + si-p21 + BMS-345541 groups, determined using RT-qPCR. *, $P < 0.05$ in comparison with the blank group; #, $P < 0.05$ in comparison with the RA group; RA, rheumatoid arthritis; NC, negative control; lincRNA-p21, long intergenic noncoding RNA-p21; RT-qPCR, reverse transcription quantitative polymerase chain reaction. The measurement data are compared among groups using one way analysis of variance (ANOVA).

(AMV) reverse transcriptase. The PCR primers were designed and synthesized by Invitrogen (Waltham, MA, USA), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference (**Table 1**). PCR amplification conditions were: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 40 s, annealing at 60°C for 40 s, extension at 72°C for 1 min, which run for 40 cycles and a final extension at 72°C for 10 min. The samples were electrophoresed in agarose gel, and the PCR results were analyzed using Opticon Monitor 3 software (Bio-Rad Laboratories, Hercules, CA, USA). The cycle threshold (Ct) of each reaction tube was the inflection point on the logarithmic amplification power curves, and the gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method: $\Delta\Delta Ct = (Ct_{target\ gene}-Ct_{reference\ gene})_{experimental\ group}-(Ct_{target\ gene}-Ct_{reference\ gene})_{control\ group}$. The experiment was repeated for three times and the data were averaged.

*Western blot assay*

The concentration of proteins extracted from synovial tissues of knee joints were determined by the bicinchoninic acid (BCA) Protein Assay Kit (Wuhan Boster Biological Technology, Ltd., Wuhan, Hubei, China) according to the manufacturer's instructions. Extracted proteins were mixed with the loading buffer, boiled for 10 min at 95°C, and then 30 μg protein was added to each well. The 10% polyacrylamide gel electrophoresis (Wuhan Boster Biological Technology, Ltd., Wuhan, Hubei, China) was performed to separate proteins with voltage transferred from 80 V to 120 V. Then the protein was transferred onto polyvinylidene fluoride (PVDF) membranes by wet transfer with transmembrane voltage of 100 mV for 45~70 min. The membrane was blocked with 5% bovine serum albumin (BSA) for 1 h and then incubated with the following primary antibodies (IκBα, p-IκBα, P65 and p-P65, 1:1000 dilution, Cell Signaling Technologies, Beverly, MA, USA) at 4°C for overnight. β-actin was set as the internal reference. Samples were then washed with tris-buffered saline with tween-20 (TBST) solution for 3 times (5 min per wash), and incubated with the corresponding second antibodies (Miao Tong Biological Technology Co., Ltd, Shanghai, China) for 1 h at room temperature. After membrane washing for 3 times (5 min per wash) and coloration, the samples were then developed using a Bio-Rad Gel Doc EZ Imager (Bio-Rad, Hercules, CA, USA). The gray values of the target bands were analyzed by the Image J software. The experiment was repeated for three times and the data were averaged.

*Enzyme-linked immunosorbent assay (ELISA)*

Rats of all the groups were anesthetized by intraperitoneal injection of 3% pentobarbital sodium (30 mg/kg, Sigma, Saint Louis, MI, USA) at 28 d of experiment. The blood samples of rats were obtained from abdominal aorta with heparin anticoagulation. Then the blood

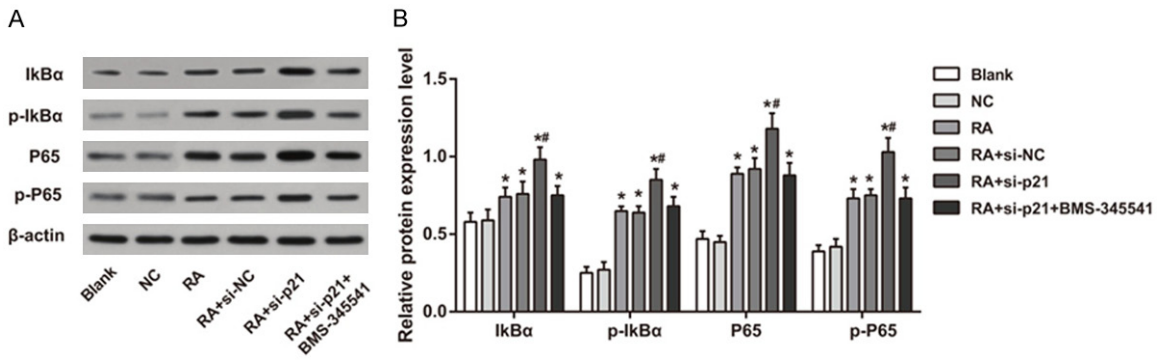


**Figure 2.** The protein levels of IκBα, p-IκBα, P65 and p-P65 in rat synovial tissues in blank, NC, RA, RA + si-NC, RA + si-cox-2, and RA + si-p21 + BMS-345541 groups, determined using western blot assay. (A) The grey value of IκBα, p-IκBα, P65 and p-P65 protein bands (B), the protein levels of IκBα, p-IκBα, P65 and p-P65; *, $P < 0.05$ in comparison with the blank group; #, $P < 0.05$ in comparison with the RA group; RA, rheumatoid arthritis; NC, negative control. The measurement data are compared among groups using one way analysis of variance (ANOVA).

was centrifugated with high speed at low temperature to separate plasma, and the supernatant was collected and stored at 4°C until use. ELISA was operated in accordance with kit instructions (Wuhan Boster Biological Technology, Ltd., Wuhan, Hubei, China) to detect the serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) respectively. The experiment was repeated for three times and the data were averaged.

*Statistical analysis*

The statistical analysis was conducted using the statistical package for the social sciences (SPSS) software 21.0 (SPSS Inc., Chicago, IL, USA). The measurement data were presented using the mean ± standard deviation (SD). The comparisons between two groups were analyzed with the least significant difference (LSD) method, and comparisons among multiple groups were conducted by one way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significantly different.

## Results

*RA rats exhibit low expression of lincRNA-p21 and BMS-345541 did not reverse lincRNA-p21 expression*

The results of PCR detection for lincRNA-p21 expression in rat synovial tissues of each group are exhibited in **Figure 1**. No significant difference between the blank group and the NC group was identified ($P > 0.05$). Compared with the blank group, expression levels of lincRNA-p21 in the RA group were remarkably decreased ($P < 0.05$), indicating that the construction of RA rat models significantly down-regulated the expression of lincRNA-p21. As compared with the RA group, no significant change was found in expression levels of lincRNA-p21 in the RA + si-NC group, while expression levels of lincRNA-p21 in the RA + si-p21 group and the RA + si-p21 + BMS-345541 group were reduced (both $P < 0.05$), which demonstrated that siRNA effectively silences lincRNA-p21 and the inhibition of the NF-κB signaling pathway exhibits no significant influence on lincRNA-p21 expression.

*LincRNA-p21 inhibits the activation of NF-κB signaling pathway*

The results of western blotting for protein expression of key cytokines of the NF-κB signaling pathway in rat synovial tissues are exhibited in **Figure 2**. Protein expression of IκBα, p-IκBα, P65, and p-P65 in the NC group was not significantly different from the blank group (all $P > 0.05$), while protein expression of IκBα, p-IκBα, P65, and p-P65 in the RA, RA + si-NC, and RA + si-p21 + BMS-345541 groups was up-regulated (all $P < 0.05$). As compared to the RA group, protein expression of IκBα, p-IκBα, P65, and p-P65 in the RA + si-p21 group was increased (all $P < 0.05$), suggesting that lincRNA-p21 modulates the NF-κB signaling pathway.

*Downregulation of lincRNA-p21 exacerbates RA rat joint swelling*

No changes of joint swelling were observed in the rats of the blank and NC groups at all stag-

**Table 2.** The paw volume in rats in blank, NC, RA, RA + si-NC, RA + si-cox-2, and RA + si-p21 + BMS-345541 groups at 0, 7, 14, and 28 days after treatment

| Group | 0 d | 7 d | 14 d | 28 d |
|---|---|---|---|---|
| Blank group | 1.08 ± 0.12 | 1.36 ± 0.14 | 1.42 ± 0.17 | 1.65 ± 0.18 |
| NC group | 1.06 ± 0.10 | 1.37 ± 0.13 | 1.39 ± 0.14 | 1.67 ± 0.17 |
| RA group | 1.11 ± 0.09 | 1.62 ± 0.20* | 2.03 ± 0.24* | 1.92 ± 0.25* |
| RA + si-NC group | 1.09 ± 0.12 | 1.64 ± 0.19* | 2.01 ± 0.22* | 1.94 ± 0.23* |
| RA + si-p21 group | 1.12 ± 0.14 | 1.97 ± 0.22*,# | 2.39 ± 0.28*,# | 2.08 ± 0.26*,# |
| RA + si-p21 + BMS-345541 group | 1.08 ± 0.11 | 1.61 ± 0.16* | 1.98 ± 0.21* | 1.93 ± 0.24* |

Note: RA, rheumatoid arthritis; NC, negative control; *, $P < 0.05$ in comparison with the blank group; #, $P < 0.05$ in comparison with the RA group. The data are analyzed among groups using two-way analysis of variance (ANOVA) Tukey's multiple comparison test.

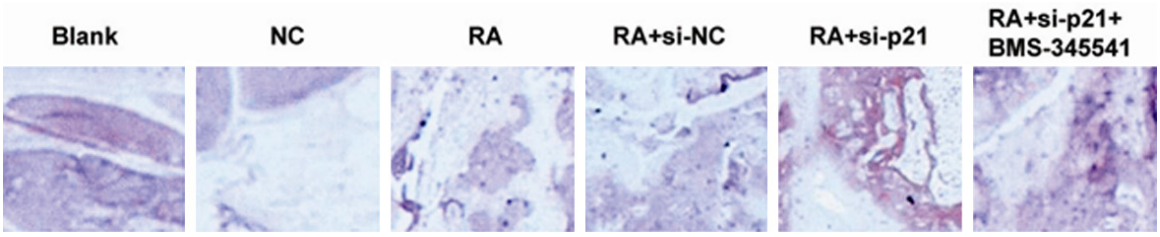


**Figure 3.** Pathological changes of rat synovial tissues in blank, NC, RA, RA + si-NC, RA + si-cox-2, and RA + si-p21 + BMS-345541 groups, evaluated by hematoxylin-eosin staining (× 200). RA, rheumatoid arthritis; NC, negative control.

es of the experiment. The toe volume of the rats in the RA, RA + si-NC, and RA + si-p21 + BMS-345541 groups reached the peak at 14 d. The swelling spread throughout the joints, local skin was ulcerated, and the rats even couldn't walk when bearing weight. The degree of swelling gradually reduced after 14 d. Joint swelling was observed in the rats in the RA + si-p21 group at 7 d, with bright and transparent joint and skin. The volume of the reaction joint and the degree of swelling were measured based on the toe volume, and the toe volume of rats in the RA + si-p21 group was obviously higher than that in the RA and the RA + si-NC groups at 14 d (all $P < 0.05$). The toe volume of rats in the RA, RA + si-NC, RA + si-p21, and RA + si-p21 + BMS-345541 groups were still significantly higher when compared with the blank and NC groups at 28 d (all $P < 0.05$) (**Table 2**).

*Pathological changes are more apparent in rat joints when lincRNA-p21 is downregulated*

As **Figure 3** shown, pathological changes are displayed after HE staining. In the RA, RA + si-NC, and RA + si-p21 + BMS-345541 groups, the joint space of rats was narrow, the middle of joint was completely filled with synovial tissues, and the articular cartilage and the articular surface were damaged severely with discontinuous articular surface. When compared with the RA, RA + si-NC, and RA + si-p21 + BMS-345541 groups, the rats in the RA + si-p21 group exhibited more serious condition with obvious joint inflammation and disappearance of joint space. While the articular surface of rats in the blank and NC groups was smooth and continuous, and no obvious soft tissue hyperplasia was found around the joint with clearly visible joint space.

*Severer inflammatory infiltration and more small vessels are found in rat knee joints when lincRNA-p21 is downregulated*

Histopathological changes of synovial tissues in rat knee joints were observed under an optical microscope (**Figure 4**). The synovial tissues of rat knee joints in the blank and NC groups were normal without inflammatory infiltration. In the RA + si-p21 group, much inflammatory infiltration was observed in the synovial tissues of rat knee joints, with a increase in the number of small vessels and in the thickness of arterial wall. In comparison with the synovial tissues of rat knee joints in the RA + si-p21 group, much

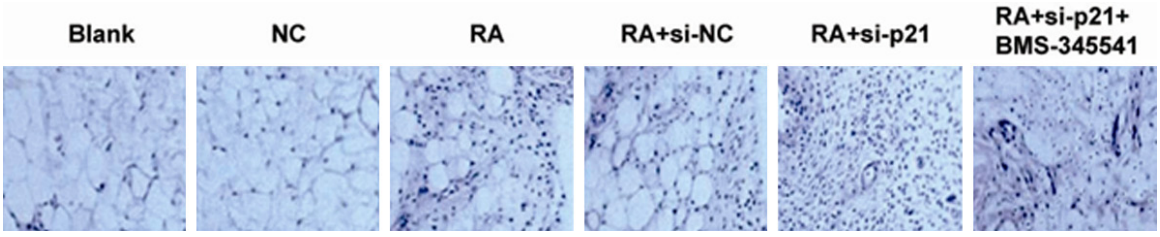


**Figure 4.** The changes of rat synovial tissues in blank, NC, RA, RA + si-NC, RA + si-cox-2, and RA + si-p21 + BMS-345541 groups under the light microscope (× 200). RA, rheumatoid arthritis; NC, negative control.

**Table 3.** The serum levels of pro- and anti-inflammatory factors in rats in blank, NC, RA, RA + si-NC, RA + si-cox-2, and RA + si-p21 + BMS-345541 groups, determined by ELISA (pg/ml)

| Group | IL-1β | IL-6 | IL-10 | TNF-α |
|---|---|---|---|---|
| Blank group | 30.18 ± 2.56 | 47.12 ± 3.05 | 39.26 ± 2.81 | 74.52 ± 5.88 |
| NC group | 31.24 ± 2.39 | 46.37 ± 3.12 | 40.63 ± 2.75 | 72.65 ± 5.71 |
| RA group | 49.52 ± 3.62* | 68.59 ± 5.61* | 28.34 ± 2.47* | 98.73 ± 6.82* |
| RA + si-NC group | 51.07 ± 3.28* | 69.34 ± 5.47* | 27.19 ± 2.26* | 101.26 ± 8.54* |
| RA + si-p21 group | 63.75 ± 5.13*,# | 89.75 ± 6.96*,# | 17.72 ± 1.39*,# | 139.75 ± 9.59*,# |
| RA + si-p21 + BMS-345541 group | 50.62 ± 3.36* | 70.56 ± 6.02* | 27.06 ± 2.32* | 101.34 ± 8.36* |

Note: RA, rheumatoid arthritis; NC, negative control; ELISA, enzyme-linked immunosorbent assay; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α; *, $P < 0.05$ in comparison with the blank group; #, $P < 0.05$ in comparison with the RA group. The data are compared among groups using one way analysis of variance (ANOVA).

**Table 4.** The serum levels of angiogenic factors in rats in blank, NC, RA, RA + si-NC, RA + si-cox-2, and RA + si-p21 + BMS-345541 groups, determined by ELISA (pg/ml)

| Group | VEGF | bFGF | HGF |
|---|---|---|---|
| Blank group | 529.38 ± 51.26 | 18.42 ± 1.38 | 619.03 ± 58.72 |
| NC group | 532.46 ± 52.03 | 17.69 ± 1.44 | 621.29 ± 58.15 |
| RA group | 702.39 ± 62.78* | 31.07 ± 2.26* | 804.46 ± 88.24* |
| RA + si-NC group | 698.72 ± 63.14* | 30.14 ± 2.58* | 802.35 ± 84.61* |
| RA + si-p21 group | 865.64 ± 73.53*,# | 55.63 ± 4.02*,# | 966.74 ± 98.16*,# |
| RA + si-p21 + BMS-345541 group | 699.12 ± 62.91* | 29.18 ± 2.47* | 807.36 ± 85.93* |

Note: RA, rheumatoid arthritis; NC, negative control; ELISA, enzyme-linked immunosorbent assay; VEGF, vascular endothelial cell growth factor; bFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; *, $P < 0.05$ in comparison with the blank group; #, $P < 0.05$ in comparison with the RA group. The data are compared among groups using one way analysis of variance (ANOVA).

inflammatory cell infiltration and the decrease of the number of inflammatory cells and small vessels were identified in the RA, RA + si-NC, and RA + si-p21 + BMS-345541 groups.

*Downregulation of lincRNA-p21 leads to release of inflammatory factors in RA rats*

The results of ELISA for serum levels of inflammatory cytokines are exhibited in **Table 3**. As compared with the blank group, the RA, RA + si-NC, and RA + si-p21 + BMS-345541 groups exhibited increased serum levels of IL-1β, IL-6 and TNF-α and decreased serum levels of IL-10 (all $P < 0.05$). When compared with the RA group, the serum levels of IL-1β, IL-6 and TNF-α were further increased, while the serum IL-10 levels were further decreased (all $P < 0.05$). No significant differences were found in the serum levels of IL-1β, IL-6, IL-10 and TNF-α between the blank and the NC groups, and also among the RA, RA + si-NC, and RA + si-p21 + BMS-345541 groups (all $P > 0.05$), illustrating that lincRNA-p21 reduces inflammatory responses in rats with RA by inhibiting the activation of the NF-κB signaling pathway.

*Downregulation of lincRNA-p21 promotes angiogenesis in RA rats*

The results of ELISA for serum levels of such angiogenic factors as VEGF, bFGF and HGF are exhibited in **Table 4**. Compared with the blank group, the serum levels of VEGF, bFGF and HGF in the NC group were not significantly different (all $P > 0.05$), while the serum levels of VEGF, bFGF and HGF in the RA group were remarkably increased (all $P < 0.05$), showing that RA promoted rat angiogenesis. Compared with the RA group, the serum levels of VEGF, bFGF and HGF in the RA + si-p21 group were elevated (all $P < 0.05$), which illustrated that interference on the expression of lincRNA-p21 may promote angiogenesis in rats with RA. While no significant difference was found in the serum levels of VEGF, bFGF and HGF between the RA group and the RA + si-p21 + BMS-345541 group, indicating that the inhibition of the NF-κB signaling pathway may reverse the promotion induced by siRNA against lincRNA-p21 on angiogenesis in rats with RA.

## Discussion

LincRNAs is newly emerged as pivotal mediators of gene regulation and cellular activities [21, 22]. Recent studies have demonstrated that lincRNA-p21 levels may significantly affect the biological properties of cells, including tumor cell proliferation, inflammatory responses and apoptosis [13, 23]. RA is a severe chronic inflammatory disease that causes increased mortality and significant morbidity [24], therefore, determining the role of lincRNA-p21 in the inflammatory response and angiogenesis by regulating the NF-κB signaling pathway in RA may provide a promising therapeutic target.

Initially, our study demonstrated down-regulation of lincRNA-p21 expression in rats with RA with the assistance of PCR detection, and inhibition of the NF-κB signaling pathway did not influence lincRNA-p21 expression. As for the under-expression of lincRNA-p21, multiple studies have documented that lincRNA-p21 levels are reduced in colorectal cancer, atherosclerosis and liver cirrhosis [25-27]. Consistently with our results, Spurlock et al. also finds that subjects with RA show decreased expression of lincRNA-p21 when compared with control subjects [28]. Wu et al. supports that lincRNA-p21 is in association with the progression of atherosclerosis by modulating cell proliferation and apoptosis through activation of p53 [26]. Moreover, a study reported by Tran et al. demonstrates that lincRNA-p21 induces cellular apoptosis in mouse models when ING1b and p53 signaling pathways converge [11]. Thus, it is safe to speculate that lincRNA-p21 may be involved in multiple cellular processes including cell proliferation, activation and apoptosis in the RA. In terms of the angiogenesis of RA rats, interfering lincRNA-p21 promoted it whereas inhibition of the NF-κB signaling pathway reversed this process. Previously, patients with non-small cell lung cancer were investigated for the impacts of lincRNA-p21 expression on treatment outcome, and it was a key risk factor with significant effects on angiogenesis [29]. In addition, lincRNAs have been identified with involvement in targeting different components of the NF-κB signaling pathway [30]. At the same time, NF-κB is a key regulator of inflammation, and its activity has been indicated to regulate the production of pro-inflammatory cytokines implicated in RA pathology [31].

Furthermore, we observed the relationship between the lincRNA-p21 expression and the activation of the NF-κB signaling pathway. The results showed that protein expression of such cytokines of the NF-κB signaling pathway as IκBα, p-IκBα, P65, and p-P65 in the RA + si-p21 group was increased as compared to the RA group, suggesting that lincRNA-p21 may regulate the activation of the NF-κB signaling pathway. Moreover, Compared with the RA group, the RA + si-p21 group showed increased toe volume, increased degree of joint swelling, increased protein expressions of the NF-κB signaling pathway-related proteins, increased serum levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), decreased serum levels of anti-inflammatory cytokines (IL-10), and increased serum levels of angiogenic factors (VEGF, bFGF, and HGF), which illustrates that lincRNA-p21 may reduce the inflammatory response and inhibit angiogenesis in rats with RA by inhibiting the activation of the NF-κB signaling pathway. As Liu et al. indicates, NKILA, a NF-κB-induced lincRNA, acts as a negative regulator of the NF-κB signaling to inhibit the NF-κB activities in breast cancer cell [32]. Similar findings have also been highlighted in a study of Charles et al., which proposes that lincRNA-p21 is a negative regulator of NF-κB activity that is a

major driver of the pro-inflammatory state of RA [28]. A possible mechanism involved in this regard pertains to a research by Pearson et al., which suggests that lincRNA-p21 has the ability to inhibit NF-kB signaling by sequestering RelA (the p65 subunit of NF-kB) in T cells in RA patients with methotrexate treatment [30]. It has been widely accepted that the expression and activity of NF-κB increased with the increase of inflammatory responses [33], thus we can conclude that the inhibition of NF-κB may decrease inflammatory response. According to the research of Roman-Blas et al., inhibition of NF-κB signaling has long been considered to be an attractive pathway for developing an effective therapy for chronic inflammatory disorders, and NF-kB inhibition is a rational target in the treatment of rheumatic diseases such as RA [31]. In the study of Xie et al., they find that suppression of the NF-κB signaling in glioblastoma cells inhibit the angiogenesis of glioblastoma cells in vitro and the formation of brain tumors in nude mice [31], which is consistent with our study.

Taken together, our study has provided evidence in relation to the notion that lincRNA-p21 may reduce inflammatory response and inhibit angiogenesis in rats with RA by inhibiting the activation of the NF-κB signaling pathway. The present study not only deepens our understanding in the regulation of lincRNA-p21 in NF-κB activity, but also further explores a therapeutic target for better treatment outcome of RA. Future studies will be required to determine the specific mechanisms underlying the protective role of lincRNA-p21 on RA rat models via the NF-κB signaling pathway. At the same time, it should also be explored how this hypothesis could be applied in clinical practices.

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## Disclosure of conflict of interest

None.

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