Anti-apoptotic effect of interleukin-22 on fibroblast-like synoviocytes in patients with rheumatoid arthritis is mediated via the signal transducer and activator of transcription 3 signaling pathway

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Abstract

Aim: Inadequate apoptosis of fibroblast-like synoviocytes (FLS) plays a crucial role in the immunopathogenesis of rheumatoid arthritis (RA). Interleukin-22 (IL-22) is a novel member of the cytokine network that has been found to be involved in the immunological process underlying RA. In this study, we investigated the effect of IL-22 on the survival of RA-FLS from RA patients and examined the possible mechanism to determine new therapeutic strategies for RA.

Methods: FLS obtained from patients with RA were cultured in vitro and treated with sodium nitroprussiate (SNP) to induce apoptosis in the presence or absence of IL-22. RA-FLS viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. RA-FLS apoptosis was analyzed by annexin V/propidium iodide staining (AV/PI). The levels of IL-22R1, pSTAT3-Y705, pSTAT3-S727, total STAT3, Bcl-xL and Bcl-2 were detected by Western blot analysis.

Results: IL-22R1 was expressed on RA-FLS. IL-22 pretreatment at concentrations ranging from 10 to 100 ng/mL increased RA-FLS viability and prevented SNP-induced apoptosis. Treatment with the STAT3 inhibitors, HO3867 or STA21, reversed the protective effect of IL-22 on SNP-induced apoptosis of RA-FLS. IL-22-induced phosphorylation of STAT3 (pSTAT3-Y705 and pSTAT3-S727) was increased in RA-FLS. Also IL-22 increased Bcl-2 expression in SNP-treated RA-FLS, and the effect was reversed by treatment with HO3867 or STA21.

Conclusion: IL-22 protects against SNP-induced apoptosis in RA-FLS by activating the STAT3 pathway and the downstream target gene, Bcl-2. Therefore, therapeutic strategies that target the IL-22/STAT3 pathway are implicated as candidates for RA treatment.

Key words: apoptosis, IL-22, RA-FLS, STAT3.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder that is characterized by progressive and destructive polyarticular joint disease. Approximately 0.5–1.0% of the global population are affected by this disease, which is associated with functional disability and severely reduced quality of life in affected individuals.¹,² Although the exact mechanisms underlying RA remain to be fully elucidated, the main pathological characteristics of RA are known to be related to abnormal inflammatory cytokine secretion in the synovial tissue and abnormal
proliferation of synovial cells in the joint. Fibroblast-like synoviocytes (FLS) are the major cell-type involved in the pathogenesis of RA, contributing to hyperplasia of the synovium and formation of the vascular pannus. RA-FLS are resistant to apoptosis, a characteristic that is pivotal to the development of RA. Although the molecular mechanisms by which apoptosis is regulated in RA-FLS remain to be fully elucidated, the nitric oxide (NO) system is implicated in this process. Elevated levels of NO have been detected in the serum, synovial fluid and synovial membrane of RA patients, due to activation of inducible NO synthase (iNOS) in inflammatory arthritis. Therefore, in this study, we investigated apoptosis in RA-FLS induced by the NO donor sodium nitroprusside (SNP) as a model.

Interleukin-22 (IL-22) is a cytokine that is structurally related to IL-10 and was originally identified as a gene induced by IL-9 in murine T lymphocytes; human IL-22 was subsequently identified. IL-22 plays an important role in the pathogenesis of immune-mediated diseases, such as psoriasis, Sjögren’s syndrome and systemic lupus erythematosus. RA patients have significantly higher serum IL-22 levels and elevated IL-22+ CD4+ T cell frequencies in the peripheral blood mononuclear cells compared with control subjects. Ikeuchi demonstrated that IL-22 increased RA-FLS proliferation. A previous study demonstrated that IL-22 promotes RA-FLS proliferation via signal transducer and activator of transcription 3 (STAT3) signaling. However, the mechanisms underlying the anti-apoptotic properties of IL-22 remain to be completely elucidated.

In this study, we explored the potential effects of IL-22 on apoptosis in RA-FLS induced by the NO donor SNP, and the possible molecular mechanisms underlying these effects.

**MATERIALS AND METHODS**

**Cell culture**

Isolation, culture and identification of RA-FLS

This study protocol approved by, and ethics approval provided by, the First Affiliated Hospital of Dalian Medical University, and informed consent was obtained from each patient. Synovial tissues were obtained from four patients with RA during joint replacement surgery. RA was diagnosed according to RA classification criteria established by the American Rheumatism Association in 1987. The clinical characteristics of these patients are listed in Table 1. FLS isolation from synovial tissue was performed according to a previously described method.

<table>
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<td>MTX, LFN, SSZ, NSAID</td>
<td>ETN, MTX, SSZ, NSAID, Pd</td>
</tr>
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</table>

Normal ranges: ESR 0–20 mm/h, CRP 0–8 mg/L, RF 0–20 IU/mL, CCP <5 U/mL, CCP, cyclic citrullinated peptide antibody; CRP, C-reactive protein; DAS28, Disease Activity Score of 28 joints; ESR, erythrocyte sedimentation rate; ETN, etanercept; HCQ, hydroxychloroquine; LFN, leflunomide; MTX, methotrexate; NSAID, nonsteroidal anti-inflammatory drug; Pd, prednisolone; RF, rheumatoid factor; SSZ, sulfasalazine.
Synoviocytes were isolated by enzymatic digestion of synovial tissue specimens obtained from patients with RA undergoing total joint replacement surgery. The tissue samples were cut into 2-3 mm pieces and treated for 4 h with 1 mg/mL of type I collagenase (Sigma-Aldrich, St. Louis, MO, USA) in Dulbecco’s modified Eagle’s medium (DMEM; GIBCO-BRL, Grand Island, NY, USA) at 37°C under 5% CO₂. Dissociated cells were then centrifuged at 500× g, resuspended in DMEM supplemented with 10% fetal calf serum (FCS; Gibco), 100 IU/mL penicillin, and 100 µg/mL streptomycin, and plated in 75-cm² flasks. After overnight culture, non-adherent cells were removed, and adherent cells were cultivated in DMEM supplemented with 10% FCS. The cultures were maintained at 37°C under 5% CO₂, and the medium was replaced every 3 days. FLS from passages 3–6 were used in experiments.

RA-FLS were identified by flow cytometric analysis of cells after staining with fluorescein isothiocyanate (FITC)-anti-CD14 and FITC-CD90 antibodies (Santa Cruz Technology, Santa Cruz, CA, USA) to distinguish macrophage-like and fibroblast-like synovial cells, respectively. Immunoglobulin G1 (IgG1) was used as a negative control.

**Culture of HepG2 cells**

The human liver carcinoma cell line HepG2 was obtained from the American Type Culture Collection (Manassas, VA, USA) and used as a control. HepG2 cells were maintained in DMEM (GIBCO-BRL) medium supplemented with 10% FBS (GIBCO-BRL) and 1% antibiotics/antimycotics (GIBCO-BRL). Cells were cultured in a humidified incubator at 37°C under 5% CO₂. Cell viability studies were performed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, RA-FLSs were seeded into 24-well culture plates (2 × 10⁴/well in a volume of 200 μL) and treated according to the experimental design with SNP (Sigma, St. Louis, MO, USA) and/or hIL-22 (PeproTech, Rocky Hill, NJ, USA). At the end of the treatment, 20 μL MTT (Sigma) was added to each of the wells, and the cells were incubated for 4 h at 37°C. The reaction was terminated by the removal of MTT. Thereafter, 150 μL dimethyl sulfoxide (Sigma) was added to dissolve the formazan crystals. The plates were then subjected to 10 min of gentle shaking to ensure complete dissolution of the crystals, and absorbance values at 490 nm were measured using a Microplate Reader BioTek Instruments, Inc., Winooski, VT.

**Annexin V-FITC/PI flow cytometric analysis of cell apoptosis**

RA-FLS pretreated with IL-22 (from 1 ng/mL to 100 ng/mL) and SNP were washed with phosphate-buffered saline and then stained with Annexin-FITC (KeyGen, Nanking, China) and propidium iodide (PI) (KeyGen) for 15 min at room temperature. The cells were then subjected to flow cytometric analysis using FACS Calibur (BD Biosciences, New York, USA) according to the manufacturer’s protocol. Apoptotic cells were defined as PI−Annexin V+ and PI+Annexin V+ cells.

RA-FLS was pretreated with HO-3867 (10 μmol/L; Selleckchem) or STA21 (25 μmol/L; Santa Cruz Biotechnology, Santa Cruz, USA) 2 h before the addition of IL-22 (100 ng/mL). Cells were then cultured for 30 min prior to the addition of SNP (1.33 mmol/L) and incubation for an additional 24 h. Apoptosis was assessed by flow cytometry using PI and Annexin V as described above. Apoptotic cells were defined as PI−Annexin V+ and PI+Annexin V+ cells.

**Western blot analysis**

**Detection of IL-22R1**

Whole cell lysates were prepared by homogenization of approximately 2 × 10⁵ cells in lysis buffer. The lysate was then centrifuged at 8000× g for 15 min. The protein concentration in the supernatant was determined using the Bicinchoninic Acid (BCA) Protein Assay Kit (Sigma). Protein samples (40 μg) were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Millipore, Billerica, MA, USA). The membranes were probed with primary antibodies for the detection of IL-22R1 (ProSci, Poway, CA, USA) 2 h before the addition of IL-22 (100 ng/mL). Cells were then cultured for 30 min prior to the addition of SNP (1.33 mmol/L) and incubation for an additional 24 h. Apoptosis was assessed by flow cytometry using PI and Annexin V as described above. Apoptotic cells were defined as PI−Annexin V+ and PI+Annexin V+ cells.

**Detection of proteins in RA-FLS treated with IL-22 or STAT3 inhibitors**

RA-FLS were pretreated with HO-3867 (10 μmol/L; Selleckchem) or STA21 (25 μmol/L; Santa Cruz Biotechnology) 2 h before the addition of IL-22 (100 ng/mL).
Cells were then cultured for 30 min prior to the addition of SNP (1.33 mmol/L) and incubation for an additional 24 h. Whole cell lysates were prepared by homogenization of approximately 2 × 10^6 cells in lysis buffer. The lysate was then centrifuged at 8000 × g for 15 min. The protein concentration in the supernatant was determined using the BCA method (Sigma). Protein samples (40 μg) were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Millipore). The membranes were probed with primary antibodies for the detection of p-STAT3 Y705 (Cell Signaling Technology, Danvers, MA, USA, 1:1000), p-STAT3 S727 (Cell Signaling Technology, 1:1000), Bcl-2 (Cell Signaling Technology, 1:500), Bcl-xl (Cell Signaling Technology, 1:500) and β-actin (Sigma-Aldrich, 1:5000). All antibodies were diluted in TBST containing 5% non-fat milk. Membranes were incubated overnight at 4°C and then incubated with the secondary detection antibody (horseradish peroxidase-conjugated anti-rabbit IgG) at room temperature for 1 h. After washing with TBST, the hybridized bands were visualized using an ECL detection kit and Hyperfilm-ECL (Millipore). The band intensity was determined using ImageJ software (version 10.2).

Statistical analysis
All statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA). Data are represented as the mean ± standard deviation (SD) of three independent experiments. The differences between groups were analyzed with one-way analysis of variance or Student’s t-test. P-values < 0.05 were considered to indicate statistical significance.

RESULTS
Morphological features of RA-FLS
RA-FLS (passages 3–6) were morphologically homogeneous, with a typical bipolar configuration visible under inverse microscopy (Fig. 1a). CD90-positive RA-FLS were distinguished from CD14-positive macrophage-like synovial cells by flow cytometry (Fig. 1b), which showed that an isolated population contained 97.36% RA-FLS.

IL-22R1 expression in RA-FLS
To confirm IL-22R1 expression at the protein level, Western blotting was performed. RA-FLS expressed higher levels of IL-22R1 compared with Hep-G2, an IL-22-responsive human hepatoma cell line, was used as a positive control (P > 0.05; Fig. 1c), as in previous studies.23,27

IL-22 preserves cell viability in the presence of SNP
To evaluate the protective effects of IL-22 on RA-FLS, cell viability was measured in the presence or absence of SNP. RA-FLS were treated with SNP at the predetermined inhibitor concentration at 50% (IC₅₀) (1.33 mmol/L; Fig. S1) and cell viability was determined by MTT assay. IL-22 treatment (10 μg/mL) alone did not influence the viability of RA-FLS (P > 0.05). However, cell viability was significantly decreased when cells were treated with SNP (P < 0.01). Low concentrations of IL-22 (1 ng/mL) had no effect on the SNP-mediated reduction in RA-FLS viability (P > 0.05), while higher concentrations of IL-22 (10 and 100 ng/mL) significantly inhibited the SNP-mediated reduction in RA-FLS viability (both P < 0.01). These results showed that IL-22 has a protective effect on SNP-mediated reduction in RA-FLS viability (Fig. 2a).

Apoptotic cells were defined as PI-Annexin V+ and PI+Annexin V+ cells (Fig. 2b upper panel). RA-FLS in the control group demonstrated low rates of spontaneous apoptosis. IL-22 (10 ng/mL) had no significant effect on the rates of RA-FLS apoptosis (P > 0.05 vs. control). The rates of RA-FLS apoptosis increased significantly after treatment with SNP for 24 h (P < 0.01). Low levels of IL-22 (1 ng/mL) did not inhibit SNP-mediated RA-FLS apoptosis (P > 0.05). In contrast, higher levels of IL-22 (10 and 100 ng/mL) significantly inhibited SNP-induced RA-FLS apoptosis (both P < 0.01), with a greater effect mediated by IL-22 at 100 ng/mL (Fig. 2b, lower panel).

Anti-apoptotic effects of IL-22 are mediated via the STAT3 signaling pathway
Based on previous observations that IL-22 protects cells from apoptosis via the STAT3 signaling pathway, we investigated the role of the STAT3 signaling pathway in the protective effects of IL-22 on SNP-induced apoptosis in RA-FLS. First, we demonstrated the IL-22-induced (100 ng/mL) activation of the STAT3 signaling pathway over time in RA-FLS (Fig. 3a). Western blot analysis showed that IL-22 (100 ng/mL) significantly increased the phosphorylation of STAT3 at Tyr705 and Ser727 (pSTAT3-705 and pSTAT3-727) in RA-FLS in a time-dependent manner over 120 min (P < 0.01).

We then used the STAT3-specific inhibitors HO3867 and STA21 to explore whether STAT3 signaling contributes to the anti-apoptotic effect of IL-22. The efficacies of the two inhibitors were examined to determine...
the appropriate concentrations for use in these studies (Fig. S2). IL-22 treatment (100 ng/mL) significantly inhibited SNP-induced apoptosis in RA-FLS cells (P < 0.05 vs. SNP). Neither STA21 nor HO3867 treatment alone had any significant effect on RA-FLS apoptosis (P > 0.05 vs. control). However, after pretreatment with STA21 (10 μmol/L) or HO3867 (25 μmol/L) for 2 h, the protective effects of IL-22 on SNP-induced apoptosis were significantly reduced (Fig. 3b). These findings indicated that the protective effects of IL-22 against RA-FLS apoptosis are mediated via the STAT3 signaling pathway.

Anti-apoptotic effects of IL-22 are mediated by upregulation of the anti-apoptotic proteins Bcl-2 and Bcl-xL.

To determine if these genes are crucial downstream factors in the protective role of IL-22 against SNP-induced apoptosis in RA-FLS, we examined the ability of IL-22 to induce expression of Bcl-2 and Bcl-xL in SNP-treated RA-FLSs (Fig. 4a,b, respectively). SNP treatment significantly downregulated the expressions of Bcl-2 and Bcl-xL (both P < 0.05 vs. SNP). Furthermore, neither STA21 nor HO3867 treatment alone had any significant effect on the expression of Bcl-2 (P > 0.05 vs. control), although the expression of Bcl-xL was significantly reduced (P < 0.05). However, IL-22 treatment significantly protected against the SNP-induced downregulation of the expressions of Bcl-2 and Bcl-xL (both P < 0.05 vs. SNP); this effect was significantly reduced by pretreatment with STA21 or HO3867 (both P < 0.05 vs. SNP+ IL-22). These observations indicated that IL-22 induces the expression of Bcl-2 and Bcl-xL via the STAT3 signaling pathway in SNP-induced RA-FLS.

Fig. 1 Morphology of fibroblast-like synoviocytes established from rheumatoid arthritis (RA) patients (RA-FLS) and interleukin (IL)-22R1 protein expression. a. Inverted microscope image of the structure of the cultured human RA-FLS. In the third passage, most RA-FLS showed typical bipolar configuration (black arrow) (original magnification ×100). b. RA-FLS were identified by flow cytometric analysis of cells after staining with fluorescein isothiocyanate (FITC)-anti-CD14 and FITC-CD90 antibodies to distinguish macrophage-like and fibroblast-like synovial cells, respectively. Immunoglobulin G1 (IgG1) was used as a negative control. c. Western blot analysis of IL-22R1 expression in RA-FLS; Hep-G2 cells were used as a positive control. A representative blot is shown in the left panel. Protein expression was quantified by densitometric analysis (right panel). Data represent the mean ± SD of three independent experiments. Each value is expressed as the ratio of the measured protein level to that of β-actin. P > 0.05.
DISCUSSION

In this study, we investigated the effect of IL-22 on the survival of RA-FLS and its possible mechanism. We showed that IL-22 protects against RA-FLS apoptosis induced by the NO donor, SNP, through activation of the STAT3 pathway and the downstream target gene, Bcl-2.

Fig. 2 Interleukin (IL)-22 pre-treatment preserves cell viability and protects against apoptosis in presence of sodium nitroprussiate (SNP). Cells were pretreated with IL-22 at the indicated concentrations for 24 h. SNP (1.33 mmol/L) was then added and the cells were incubated for a further 24 h. a. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Data represent the mean ± SD of three independent experiments. #P < 0.01, *P > 0.05 versus control. %P > 0.05, &P < 0.01 versus SNP. b. Apoptosis was assessed by flow cytometry using propidium iodide (PI) and Annexin V. Apoptotic cells were defined as PI-Annexin V+ and PI+ Annexin V+ cells. &P < 0.01, *P > 0.05 versus control, %P > 0.05, #P < 0.01 versus SNP.
Interleukin (IL)-22 protects against sodium nitroprussiate (SNP)-induced apoptosis in fibroblast-like synoviocytes established from rheumatoid arthritis (RA) patients (RA-FLS) via the signal transducer and activator of transcription 3 (STAT3) signaling pathway. Effect of IL-22 on STAT3 phosphorylation in RA-FLS. RA-FLS were stimulated with IL-22 (100 ng/mL) for the indicated periods of time. a. Western blot analysis showed that IL-22 treatment increased pSTAT3-727 and pSTAT3-705 in a time-dependent manner. A representative Western blot is shown in the left panel. Expression of pSTAT3-727 and pSTAT3-705 was quantified by densitometric analysis (right panel). Data represent the mean ± SD of three independent experiments. Each value is expressed as the ratio of the measured protein level to that of β-actin. *P < 0.01 versus control. b. Effects of the STAT3 inhibitor HO-3867 and STA21 on the anti-apoptotic activity of IL-22. Cells were pretreated with HO-3867 (10 μmol/L) or STA21 (25 μmol/L) 2 h before the addition of IL-22 (100 ng/mL). Cells were then cultured for 30 min prior to the addition of SNP (1.33 mmol/L) and incubation for an additional 24 h. Apoptosis was assessed by flow cytometry using propidium iodide (PI) and Annexin V. Apoptotic cells were defined as Annexin V+ PI- and Annexin V+ PI+ cells. Data represent the mean ± SD of three independent experiments. *P < 0.05, #P > 0.05 versus control, $P < 0.05 versus SNP, &P < 0.05 versus SNP + IL-22.
Research in humans and mice has implicated IL-22 in the pathogenesis of RA. In humans, IL-22 levels are significantly higher in RA patients than those in healthy controls and the levels correlate positively with Disease Activity Score of 28 joints (DAS28).29,30 IL-22−/− mice exhibited decreased incidence of collagen-induced arthritis (CIA) and decreased pannus formation than their wild-type counterparts, indicating that IL-22 leads to increased susceptibility to RA. Moreover, after onset of arthritis in a CIA mouse model, IL-22 antibody injection reduced arthritis activity and improved arthritis tissue pathological changes.31 Synoviocytes, which are the major cell population in the inflamed synovial tissue in RA patients, are a critical component of RA development. IL-22 and IL-22R1 are highly expressed in the rheumatoid synovium.23 In the present study, we demonstrated IL-22R1 expression in RA-FLS (Fig. 2). The effects of IL-22 on synoviocytes have been studied. IL-22 promotes proliferation and monocyte chemoattractant protein-1 production in RA-FLS.23,32 Furthermore, treatment of RA synovial fibroblasts with rhIL-22 increases receptor activator of nuclear factor kappa-B ligand (RANKL) expression at both the messenger RNA and protein levels in a dose-dependent manner. IL-22 also upregulates RANKL expression in RA-FLS and induces osteoclastogenesis in synoviocytes and osteoblasts, which contributes to the bone destruction that is closely related to functional disability in RA patients.33,34 However, in our study, although IL-22 alone did not increase RA-FLS viability, IL-22 preserved RA-FLS viability in the presence of SNP (Fig. 2a). Until now, few reports have described the effect of IL-22 on the survival of RA-FLS. Our study showed that hIL-22 treatment reduced SNP-induced RA-FLS apoptosis in a dose-dependent manner, with the most conspicuous effect observed following treatment with IL-22 at 100 ng/mL in our study (Fig. 2b). As shown in Figure 2a, IL-22 alone did not influence the viability or proliferation of RA-FLS. This is in...
Conflict with the results of a previous study demonstrating that IL-22 promotes RA-FLS proliferation via STAT3 signaling. This discrepancy might be accounted for by the effects of IL-22 at 10 ng/mL in the present study, while concentrations over 50 ng/mL were used in the study by Zhu et al.

STAT3 is activated by phosphorylation of a critical tyrosine residue (Tyr 705) that induces STAT3 dimerization through phosphoryrosin-SH2 domain interaction. Activated STAT3 dimers translocate to the nucleus, where they bind DNA to regulate the expression of target genes. Phosphorylation of STAT3 at the serine 727 residue enhances its transcriptional activity. STAT3 influences cell apoptosis by upregulating the expression of the pro-survival Bcl-2, Bcl-xL, Mcl-1, Bcl-w and Survivin transcripts in a cancer microenvironment. Cytokines activate the STAT3 pathway in various cancer and autoimmune cells. IL-22 has been shown to protect cancer cells from apoptosis by activating STAT3 and upregulating the expression of the downstream anti-apoptotic genes, Bcl-2 and Bcl-xL. Although few studies have focused on the IL-22/STAT3 pathway in RA, it has been reported that IL-22 may enhance RA-FLS proliferation by activating STAT3. Indeed, one study has shown that survival of rheumatoid synoviocytes is STAT3-dependent. In this study, IL-22 upregulated RA-FLS expression of pSTAT3-Y705 and pSTAT3-S727, both of which are required for full STAT3 activation (Fig. 3a). This effect was observed as early as 30 min after IL-22 treatment and increased further for at least 2 h. Thus, it can be speculated that IL-22 contributes to apoptotic resistance via STAT3 activation and that the IL-22/STAT3 pathway may be associated with pathogenesis of RA, with a particular influence on RA-FLS survival.

In the current study, we investigated the role of the STAT3 signaling pathway in the mechanism by which IL-22 protects RA-FLS against SNP-induced apoptosis using the selective STAT3 inhibitors, STA21 and HO3867. These curcumin analogs exert potent STAT3-inhibiting activity by impeding STAT3 dimerization, DNA binding and transcription of target genes. STA21 inhibits breast cancer cells that express constitutively active STAT3 and has been used in previous RA-FLS studies as a STAT3 inhibitor. HO3867 is more novel curcumin analog, which induces apoptosis in ovarian cancer cells by decreasing pSTAT3 (Tyr705 and Ser727) and its downstream targets, including Bcl-xL and Bcl-2, with minimal toxic effects on non-cancerous cells and tissues. In the present study, we showed that the protective effect of IL-22 on SNP-induced apoptosis in RA-FLS was inhibited in the presence of STA21 (25 μmol/l) or HO3867 (10 μmol/l), confirming that the protective effects of IL-22 are mediated by activating STAT3 (Fig. 3b). To further verify the effects of IL-22 on the downstream target genes of STAT3 that are associated with RA-FLS survival, we investigated the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL. IL-22 inhibited the decrease in expression of both Bcl-2 and Bcl-xL observed in RA-FLS undergoing SNP-induced apoptosis (Fig. 4). STA21 or HO3867 alone had no effect on Bcl-2 expression in RA-FLS, and weakened the effect of IL-22 on Bcl-2 expression. However, Figure 4b shows that treatment with either STA21 or HO3867 alone had inhibitory effects on Bcl-xL expression. The IL-22-induced upregulation of Bcl-xL expression in RA-FLS undergoing SNP-induced apoptosis was inhibited by both STA21 and HO3867, indicating that this effect might be mediated by STAT3. However, the direct role of STAT3 activation in the anti-apoptotic effects of IL-22 on SNP-treated RA-FLS remain to be confirmed in studies using other specific STAT3 inhibitors that do not influence Bcl-xL expression. However, our results demonstrate that IL-22 induces Bcl-2 expression in RA-FLS undergoing SNP-induced apoptosis via STAT3.

CONCLUSION

Our study demonstrates that IL-22 protects RA-FLS against SNP-induced apoptosis by activating STAT3 and upregulating the expression of the downstream target gene, Bcl-2. These findings indicate that the IL-22/STAT3 pathway is a potential candidate for therapeutic modulation of RA.

DISCLOSURES

Zhao Min, Li Yishuo and Xiao Weiguo have no conflicts of interests.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Induction of apoptosis of fibroblast-like synoviocytes established from rheumatoid arthritis (RA) patients (RA-FLS) by sodium nitroprussiate (SNP).

Figure S2 The effective inhibition concentration of HO3867 and STA21 on the STAT3 signaling pathway.
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