Interleukin-22 Promotes Proliferation of Rheumatoid Arthritis Fibroblast-like Synoviocytes Postprint

Authors: Junqing Zhu, Wang Ran, Chen Shixian, Zhao Di, Li Juan

Date: 2018-06-15T00:00:00+00:00

Abstract

Objective: To elucidate the mechanism by which interleukin-22 (IL-22) promotes the proliferation of rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) through IL-22 intervention.

Methods: RA synovial tissues obtained under aseptic conditions were used to culture RA-FLS via the tissue block isolation method. Cells were passaged in DMEM medium containing 10% FBS, and the 4th-5th generation FLS were identified by immunohistochemistry using vimentin/CD68. Following intervention with different concentrations of IL-22 for 24, 48, and 72 h, cell proliferation was assessed by MTT assay. FLS were treated with IL-22 and/or AG490, and total cellular proteins were extracted on ice using cell lysis buffer containing protease inhibitors. After sonication, centrifugation, and protein quantification, Western blot analysis was performed to detect the expression of STAT3, ERK1/2, P38 proteins and their phosphorylated counterparts. Statistical analysis was conducted using SPSS 20.0 software, with $P<0.05$ considered statistically significant.

Results: Proliferation of FLS following intervention with different concentrations of IL-22 showed a dose-dependent increase compared with the control group, with statistically significant differences ($P<0.05$). With prolonged IL-22 intervention time, no statistically significant difference was observed in the overall level of relative gray values for total STAT3 protein expression ($P=0.68$); however, the difference in overall levels of relative gray values for phosphorylated protein expression was statistically significant ($P<0.001$). At various intervention time points, relative expression of phosphorylated STAT3 protein was significantly elevated compared with baseline at 0 h, with statistically significant differences ($P<0.001$). No statistically significant differences were found in the relative gray values of total and phosphorylated ERK1/2 and P38 protein expression following IL-22 intervention ($P>0.05$). After combined treatment with

50 ng/mL IL-22 and 100 mol/L AG490 for different durations, cell proliferation was significantly reduced compared with IL-22 intervention alone (P<0.01).

Conclusion: IL-22 promotes cell proliferation in RA fibroblast-like synoviocytes through STAT3 protein phosphorylation in a concentration-dependent manner, rather than via signaling pathways mediated by ERK1/2 and P38 proteins.

Full Text

Abstract

Objective: To elucidate the mechanism by which interleukin-22 (IL-22) promotes the proliferation of fibroblast-like synoviocytes (FLS) from patients with rheumatoid arthritis (RA). **Methods:** FLS were isolated from RA synovial tissue using an explant culture method and subcultured in DMEM medium containing 10% FBS. Cells from passages 4–5 were identified by immunohistochemistry for vimentin/CD68. FLS were treated with various concentrations of IL-22 for 24, 48, or 72 hours, and cell proliferation was assessed using the MTT assay. FLS were also treated with IL-22 and/or AG490, after which total cellular proteins were extracted on ice using lysis buffer containing protease inhibitors. Following sonication, centrifugation, and protein quantification, Western blotting was performed to detect the expression of STAT3, ERK1/2, and P38 proteins and their phosphorylated forms. Statistical analysis was conducted using SPSS 20.0 software, with P<0.05 considered statistically significant. **Results:** IL-22 intervention at different concentrations significantly increased FLS proliferation in a dose-dependent manner compared with the control group (P<0.05). With prolonged IL-22 treatment, the overall level of total STAT3 protein expression showed no significant difference in relative gray values (P=0.68), whereas the expression of phosphorylated STAT3 protein demonstrated statistically significant differences (P<0.001). At each intervention time point, the relative expression of phosphorylated STAT3 protein was significantly elevated compared with baseline (0 h) (P<0.001). No statistically significant differences were observed in the expression of total or phosphorylated ERK1/2 and P38 proteins following IL-22 intervention (P>0.05). Combined treatment with 50 ng/mL IL-22 and 100 mol/L AG490 significantly reduced cell proliferation compared with IL-22 alone at all time points (P<0.01). **Conclusion:** IL-22 promotes RA-FLS proliferation in a concentration-dependent manner through STAT3 protein phosphorylation, but not via ERK1/2 or P38-mediated signaling pathways.

Keywords: rheumatoid arthritis; fibroblast-like synoviocytes; interleukin-22; signaling pathway

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease characterized by progressive, symmetric inflammation of peripheral small joints, leading to bone and cartilage destruction, joint deformity, and functional

impairment. While the exact pathogenesis of RA remains unclear, the prevailing view holds that cytokines, growth and differentiation factors, and other immune proteins mediate complex regulatory networks among dendritic cells, T/B lymphocytes, fibroblast-like synoviocytes (FLS), osteoblasts/osteoclasts, and other immune cells through intracellular signal transduction, thereby participating in RA onset and progression.

Interleukin-22 (IL-22), a member of the IL-10 cytokine family, is primarily produced by activated helper T lymphocytes and natural killer cells, exerting pathogenic effects through its action on target cells. Previous studies in collagen-induced arthritis mouse models demonstrated that IL-22-deficient mice exhibited significantly reduced susceptibility to arthritis compared with wild-type mice. In RA patients, IL-22 receptor 1 was found to be highly expressed in vimentin-positive FLS in the synovial lining and sublining layers, suggesting IL-22 involvement in synovial pathogenesis. Our previous research also identified that natural killer cell subpopulations expressing NKp44 could produce IL-22 and tumor necrosis factor- (TNF), and their culture supernatants promoted FLS proliferation to some extent. However, the precise mechanism by which IL-22 contributes to RA-FLS proliferation and activation remains incompletely understood. Therefore, this study investigated IL-22 intervention in isolated RA-FLS, examining cell proliferation and related signaling pathway protein expression to clarify the mechanism underlying IL-22-mediated FLS proliferation.

Methods

1.1 Study Subjects

Synovial tissue samples were obtained from three RA patients undergoing arthroscopic surgery or joint replacement at the Department of Rheumatology and Joint Surgery, Nanfang Hospital, Southern Medical University, between February 2012 and December 2013. All patients fulfilled the 1987 revised American College of Rheumatology classification criteria for RA or the 2010 ACR/EULAR classification criteria. The study adhered to the principles of the Declaration of Helsinki, and all human tissue acquisitions were approved by the Medical Ethics Review Committee of Nanfang Hospital, Southern Medical University (NO.NFEC-20120201) with informed consent obtained from all participants.

1.2 Major Instruments and Reagents

Equipment included an automated microplate reader (BIOTEK), Mini-PROTEAN Tetra Cell electrophoresis apparatus, Trans-Blot SD semi-dry transfer system, and Gel Doc 2000 gel imaging system (Bio-Rad). Reagents comprised the BCA protein assay kit (Sangon Biotech), RIPA Lysis Buffer I, ECL Plus kit (Amersham Pharmacia), immunoCruz mouse LSAB staining kit (Santa Cruz), recombinant human IL-22 (R&D Systems), mouse IgG1 anti-human vimentin, mouse IgG1 anti-human CD68, mouse IgG1 isotype control antibody, -cyano-(3,4-dihydroxy)N-benzylcinnamamide (AG490) (Santa

Cruz), anti-signal transducer and activator of transcription 3 (STAT3) mouse monoclonal antibody, anti-phosphorylated STAT3 (p-STAT3) mouse monoclonal antibody, anti-extracellular signal-regulated kinase 1/2 (ERK1/2) rabbit monoclonal antibody, anti-phosphorylated ERK1/2 (p-ERK1/2) rabbit monoclonal antibody, anti-P38 rabbit monoclonal antibody, anti-phosphorylated P38 (p-P38) rabbit monoclonal antibody, anti-GAPDH rabbit monoclonal antibody (Cell Signaling), Peroxidase AffiniPure goat anti-mouse IgG, Peroxidase AffiniPure goat anti-rabbit IgG (Jackson ImmunoResearch), fetal bovine serum (FBS), DMEM medium, 0.25% trypsin (GIBCO), methylthiazolyldiphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and Triton X-100 (Sigma).

1.3 FLS Isolation, Culture, and Identification

FLS isolation and culture: RA-FLS were isolated using the tissue explant method. Under aseptic conditions, synovial tissue was washed with PBS and minced into 1 mm^3 pieces using surgical scissors. Tissue fragments were placed at the bottom of culture flasks with 1 mL DMEM containing 20% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin. Flasks were inverted and incubated at 37°C with 5% CO for 2 hours, after which 3 mL of the same medium was added to cover the tissue pieces. Flasks were then returned to normal orientation for continued culture, with medium replacement every 2–3 days. Once abundant FLS outgrowth was observed, adherent tissue fragments were removed. Cells were passaged when they reached 80% confluence.

FLS identification: Passages 4–5 were identified by immunohistochemistry. FLS were resuspended in DMEM with 10% FBS, counted, and adjusted to 2×10 cells/mL. Cells were seeded at 0.5 mL/well in 48-well plates and cultured for 24, 48, or 72 hours. After fixation with 4% paraformaldehyde for 20 minutes at room temperature, cells were processed according to the immunohistochemistry kit protocol for primary and secondary antibody incubation. Following nuclear counterstaining and mounting, cells were observed and photographed under a fluorescence inverted microscope.

1.4 Cell Intervention Groups

IL-22 proliferation groups: FLS were treated with 1, 10, 50, or 100 ng/mL IL-22, or normal culture medium (control) for 24, 48, or 72 hours before MTT assay.

IL-22 signaling pathway groups: FLS were treated with 50 ng/mL IL-22 for 0, 0.25, 0.5, 1, 1.5, 2, 4, or 8 hours, followed by Western blot detection of total and phosphorylated STAT3, ERK1/2, P38, and internal control proteins.

AG490 inhibition groups: FLS were treated with 50 ng/mL IL-22, 50 ng/mL IL-22 + 100 mol/L AG490, 100 mol/L AG490 alone, or normal culture medium (control) for 24, 48, or 72 hours before MTT assay.

1.5 MTT Assay for FLS Proliferation

Passages 4–5 FLS were prepared as single-cell suspensions at 3×10 cells/mL and seeded at 100 L/well in 96-well plates (triplicate wells). After 4-hour incubation at 37°C with 5% CO to allow attachment, intervention media were added at 0, 24, and 48 hours post-seeding. At 72 hours after initial seeding, medium was removed and replaced with 90 L DMEM containing 10% FBS plus 10 L MTT solution (5 mg/mL in PBS). Following 4-hour incubation, 120 L DMSO was added to each well to dissolve formazan crystals. Absorbance at 490 nm was measured using a microplate reader after low-speed agitation.

1.6 Western Blot Detection of Signaling Pathway Proteins

Passages 4–5 FLS were seeded at 1×10 cells/mL (1 mL/well) in 6-well plates with 2 mL DMEM containing 10% FBS. After 24-hour culture, intervention media were added. At the end of treatment, cells were lysed on ice with RIPA Lysis Buffer I containing protease inhibitors. Following sonication, centrifugation, and protein extraction, protein concentrations were determined using the BCA protein assay kit. Protein samples (30 g) were denatured by boiling, separated by SDS-PAGE (100 V for 20 minutes in stacking gel, 200 V for 40 minutes in separating gel), and transferred to PVDF membranes via semi-dry transfer. Membranes were blocked for 1 hour, incubated with primary antibodies overnight at 4°C, and with secondary antibodies for 1 hour at room temperature. After ECL Plus kit development, images were captured using a gel imaging system and band densities were analyzed using Gel-Pro Analyzer 4.0.

1.7 Statistical Analysis

Data were analyzed using SPSS 20.0 software. Continuous variables were expressed as mean ± standard deviation. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance using Levene' s test (=0.1). Multiple group comparisons of normally distributed data were performed using one-way ANOVA, with post-hoc Bonferroni (equal variances) or Tamhane' s T2 (unequal variances) tests. Non-normally distributed data were analyzed using the Kruskal-Wallis test. All tests were two-tailed, with $P<0.05$ considered statistically significant.

Results

2.1 FLS Culture and Identification In Vitro

RA-derived FLS exhibited a spindle-shaped morphology with "whorl-like," adherent growth patterns [Figure 1: see original paper]A–C. Immunohistochemical identification of passages 4–5 demonstrated vimentin expression with brownish-yellow cytoplasmic staining, while CD68 staining was negative [Figure 1: see original paper]D–F.

2.2 IL-22 Promotes FLS Proliferation in a Dose-Dependent Manner

MTT assay results following IL-22 treatment for 24, 48, and 72 hours are presented in . Significant differences in overall FLS proliferation were observed across all time points (F=12.81, 17.36, 14.16; P<0.001). Post-hoc comparisons revealed that at 24 and 48 hours, all concentrations except 1 ng/mL IL-22 showed gradient increases in proliferation compared with controls (P<0.05). At 72 hours, all IL-22 concentrations induced concentration-dependent proliferation (P<0.05).

2.3 IL-22 Promotes FLS Proliferation via STAT3 Phosphorylation

Western blot analysis after 50 ng/mL IL-22 treatment showed increased phosphorylated STAT3 expression over time, while total STAT3 and GAPDH remained unchanged [Figure 2: see original paper]. Semi-quantitative analysis revealed no significant difference in total STAT3 expression (STAT3/GAPDH) across time points (F=4.87; P=0.68), whereas phosphorylated STAT3 (p-STAT3/STAT3) showed significant variation (F=4918.43; P<0.001). Post-hoc comparisons confirmed significantly elevated p-STAT3 at all time points versus baseline (0 h) (P<0.001).

2.4 IL-22-Induced FLS Proliferation Is Independent of ERK1/2 and P38 Phosphorylation

Western blot analysis after 50 ng/mL IL-22 treatment showed no apparent changes in total or phosphorylated ERK1/2 and P38 proteins, or GAPDH, across different time points [Figure 3: see original paper]. Semi-quantitative analysis confirmed no significant differences in ERK1/2/GAPDH, p-ERK1/2/ERK1/2, P38/GAPDH, or p-P38/P38 ratios over time.

2.5 Blocking STAT3 Phosphorylation Inhibits FLS Proliferation

MTT proliferation assays after combined treatment with 50 ng/mL IL-22 and 100 mol/L AG490 for 24, 48, and 72 hours are shown in . Significant differences in overall proliferation were observed at all time points (F=47.20, 58.04, 32.90; P<0.001). IL-22 alone significantly increased proliferation versus controls (P=0.001, 0.008, 0.005). Combined IL-22 + AG490 treatment significantly reduced proliferation compared with IL-22 alone (P<0.01). AG490 alone also significantly decreased proliferation versus controls at all time points (P<0.01).

Discussion

The synovial membrane represents the primary site of inflammation in RA, and the hyperproliferation of FLS, which invade tissues in a tumor-like manner, constitutes a critical mechanism underlying synovitis and subsequent bone destruction. FLS achieve this hyperproliferative and activated state through

autocrine or paracrine stimulation by various cytokines and growth factors, including TNF , IL-1, IL-17, IL-22, and TGF- , thereby migrating to effector sites and exerting important biological functions. Our in vitro FLS cultures displayed characteristic spindle-shaped, whorl-like, adherent growth patterns with vimentin expression in passages 4–5, confirming their identity and supporting their use for investigating upstream immunoregulatory mechanisms in RA.

IL-22 is secreted primarily by CD4 T cells, natural killer T cells, innate lymphoid cells, and TH22 cells, acting on various cell types including keratinocytes, epithelial cells, hepatocytes, and fibroblasts to promote innate immunity, reduce tissue damage, and accelerate regeneration. Previous studies demonstrated that IL-22-deficient mice exhibited reduced incidence and severity of collagen-induced arthritis with decreased expression of TNF , IL-6, IL-1 , and matrix metalloproteinases. Allogeneic mesenchymal stem cell transplantation has been shown to ameliorate arthritis in rats by reducing IL-22 expression and suppressing matrix metalloproteinase-3. Systematic analyses in humans have revealed elevated IL-22 levels in synovial fluid of both psoriatic arthritis and RA patients compared with osteoarthritis. Given that IL-22 receptors are highly expressed on vimentin-positive FLS in RA synovial tissue, we hypothesized that IL-22 directly participates in RA-FLS pathogenesis. Our findings that IL-22 promotes FLS proliferation in a concentration-dependent manner corroborate our previous observation that NKp44 natural killer cells from RA patients secrete IL-22 and TNF , and their supernatants enhance FLS proliferation.

RA-FLS activation and function depend on specific signaling pathways, including the mitogen-activated protein kinase pathway (JNK/ERK/P38), which shows elevated phosphorylation in RA synovium and participates in proliferation and inflammatory mediator production; the JAK/STAT pathway, where STAT3 serves as a critical signaling hub for cell survival, proliferation, and differentiation; the spleen tyrosine kinase pathway, which regulates immune receptor signaling and FLS activation; and the NF- B pathway, which promotes transcription of IL-6, IL-8, matrix metalloproteinases, and adhesion molecules upon inflammatory stimulation. These pathways exhibit stimulus-specific activation patterns and distinct biological effects. Our previous work demonstrated that NK-22 cell supernatants upregulate STAT3 mRNA and p-STAT3 expression in FLS, and that IL-22 protects against nitroprusside-induced FLS apoptosis via STAT3 phosphorylation. However, whether IL-22 affects total STAT3 protein or specifically its phosphorylated form remained unclear. Furthermore, while IL-22-preconditioned RA-FLS can promote osteoclast differentiation from monocytes via p38 MAPK/NF- B or JAK2/STAT3 pathways, the specific signaling mechanisms underlying IL-22-induced FLS proliferation required investigation.

Our Western blot analysis revealed that 50 ng/mL IL-22 enhanced STAT3 phosphorylation in a time-dependent manner without affecting total STAT3, ERK1/2, or P38 protein levels or their phosphorylation status. Moreover, AG490-mediated blockade of STAT3 phosphorylation significantly attenuated IL-22-induced FLS proliferation, demonstrating that this process specifically

depends on STAT3 activation rather than ERK1/2 or P38 pathways.

In summary, our study demonstrates that IL-22 promotes RA-FLS proliferation in a concentration-dependent manner through STAT3 protein phosphorylation, without significantly affecting ERK1/2 or P38 pathways. Further investigation into the interactions between elevated IL-22 and FLS or other immune cells in RA will provide important theoretical insights and clinical value for understanding RA pathogenesis and developing targeted therapeutics.

References

[1] Smolen JS, Aletaha D, Mcinnes IB. Rheumatoid arthritis. Lancet, 2016, 388(10055): 2023-38.

[2] Valesini G, Barone F, Bompane D, et al. Advances in immunology and rheumatoid arthritis pathogenesis. Reumatismo, 2004, 56(1 Suppl 1): 9-20.

[3] Mcinnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med, 2011, 365(23): 2205-19.

[4] Bellucci E, Terenzi R, La Paglia G, et al. One year in review 2016: pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol, 2016, 34(5): 793-801.

[5] Shabgah AG, Navashenaq JG, Shabgah OG, et al. Interleukin-22 in human inflammatory diseases and viral infections. Autoimmun Rev, 2017, 16(12): 1209-18.

[6] Geboes L, Dumoutier L, Kelchtermans H, et al. Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. Arthritis Rheum, 2009, 60(2): 390-5.

[7] Ikeuchi H, Kuroiwa T, Hiramatsu N, et al. Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. Arthritis Rheum, 2005, 52(4): 1037-46.

[8] Ren J, Feng Z, Lv Z, et al. Natural killer-22 cells in the synovial fluid of patients with rheumatoid arthritis are an innate source of interleukin 22 and tumor necrosis factor- . J Rheumatol, 2011, 38(10): 2112-8.

[9] Zhu J, Jia E, Zhou Y, et al. Interleukin-22 secreted by NKp44+ natural killer cells promotes proliferation of Fibroblast-Like synoviocytes in rheumatoid arthritis. Medicine (Baltimore), 2015, 94(52): e2137.

[10] Arnett FC, Edworthy SM, Bloch DA, et al. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum, 1988, 31(3): 315-24.

[11] Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis, 2010, 69(9): 1580-8.

[12] Ota F, Maeshima A, Yamashita S, et al. Activin a induces cell proliferation of fibroblast-like synoviocytes in rheumatoid arthritis. Arthritis Rheum, 2003, 48(9): 2442-9.

[13] Neidhart M, Seemayer CA, Hummel KM, et al. Functional characterization of adherent synovial fluid cells in rheumatoid arthritis: destructive potential in vitro and in vivo. Arthritis Rheum, 2003, 48(7): 1873-80.

[14] Meinecke I, Rutkauskaite E, Gay S, et al. The role of synovial fibroblasts in mediating joint destruction in rheumatoid arthritis. Curr Pharm Des, 2005, 11(5): 563-8.

[15] Guiducci S, Del Rosso A, Cinelli M, et al. Rheumatoid synovial fibroblasts constitutively express the fibrinolytic pattern of invasive tumor-like cells. Clin Exp Rheumatol, 2005, 23(3): 364-72.

[16] Pierer M, Brentano F, Rethage J, et al. The TNF superfamily member LIGHT contributes to survival and activation of synovial fibroblasts in rheumatoid arthritis. Rheumatology (Oxford), 2007, 46(7): 1063-70.

[17] Xue C, Hasunuma T, Asahara H, et al. Transcriptional regulation of the HOX4C gene by basic fibroblast growth factor on rheumatoid synovial fibroblasts. Arthritis Rheum, 1997, 40(9): 1628-35.

[18] Stanford SM, Aleman Muench GR, Bartok B, et al. TGF responsive tyrosine phosphatase promotes rheumatoid synovial fibroblast invasiveness. Ann Rheum Dis, 2016, 75(1): 295-302.

[19] Xu S, Xiao Y, Zeng S, et al. Piperlongumine inhibits the proliferation, migration and invasion of fibroblast-like synoviocytes from patients with rheumatoid arthritis. Inflamm Res, 2017, 8: doi: 10.1007/s00011-017-1112-9. [Epub ahead of print].

[20] Zhen J, Yuan J, Fu YW, et al. IL-22 promotes Fas expression in oligodendrocytes and inhibits FOXP3 expression in T cells by activating the NF-kappa B pathway in multiple sclerosis. Mol Immunol, 2017, 82(5): 84-93.

[21] Liu GY, Bian S, Li F, et al. Effect of allogenic mesenchymal stem cells transplantation on the expression of interleukin-22 and matrix metalloproteinase-3 in rats with collagen induced arthritis. Zhonghua Yi Xue Za Zhi, 2017, 97(9): 698-702.

[22] Altobelli E, Angeletti PM, Piccolo D, et al. Synovial fluid and serum concentrations of inflammatory markers in rheumatoid arthritis, psoriatic arthritis and osteoarthritis: a systematic review. Curr Rheumatol Rev, 2017, [Epub ahead of print].

[23] Thalhamer T, Mcgrath MA, Harnett MM. MAPKs and their relevance to arthritis and inflammation. Rheumatology, 2008, 47(4): 409-14.

[24] Okamoto H, Kobayashi A. Tyrosine kinases in rheumatoid arthritis. J Inflamm, 2011, 8(1): 21.

[25] Krause A, Scaletta N, Ji JD, et al. Rheumatoid arthritis synoviocyte survival is dependent on Stat3. J Immunol, 2002, 169(11): 6610-6.

[26] Cha HS, Boyle DL, Inoue T, et al. A novel spleen tyrosine kinase inhibitor blocks c-Jun N-terminal kinase-mediated gene expression in synoviocytes. J Pharmacol Exp Ther, 2006, 317(2): 571-8.

[27] Aupperle KR, Bennett BL, Han ZN, et al. NF-kappa B regulation by I kappa B kinase-2 in rheumatoid arthritis synoviocytes. J Immunol, 2001, 166(4): 2705-11.

[28] Ren J, Zhou Y, Wu H, et al. Role of NK-22 cells and interleukin-22-related molecules in proliferation of fibroblast-like synoviocytes in patients with rheumatoid arthritis. Nan Fang Yi Ke Da Xue Xue Bao, 2014, 34(1): 20-4.

[29] Zhao M, Li Y, Xiao W. 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. Int J Rheum Dis, 2017, 20(2): 214-24.

[30] Kim KW, Kim HR, Park JY, et al. Interleukin-22 promotes osteoclastogenesis in rheumatoid arthritis through induction of RANKL in human synovial fibroblasts. Arthritis Rheum, 2012, 64(4): 1015-23.

Note: Figure translations are in progress. See original paper for figures.