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Original Article

Role of lentivirus-mediated overexpression of SOCS3 in proliferation and apoptosis of fibroblasts-like synoviocytes in Lewis rats with adjuvant-induced rheumatoid arthritis

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Abstract: The purpose of this study is to explore the effects of lentivirus-mediated overexpression of the SOCS3 gene on proliferation and apoptosis of fibroblasts-like synoviocytes (FLSs) in rheumatoid arthritis (RA). A total of 20 Lewis rats were randomly assigned into experimental and normal groups. Rats in the experimental group were modeled with adjuvant arthritisand the normal group was given no treatment. After culture for 28 days, rats in the experimental group were sacrificed, and the third-generation FLSs were collected and randomly allocated into SOCS3 group, control group, and blank group. MTT assay was used for detecting cell viability, flow cytometry was used for analysis ofcell apoptosis, and enzyme-linked immunosorbent assay (ELISA) was used to determine levels of inflammatory factors (interleukin [IL]-2, interferon [IFN-γ] and tumor necrosis factor [TNF-α]). MTT assay showed that the optical density of the SOCS3 group was significantly higher than that of the control and blank groups. Flow cytometry showed that the apoptosis rate of FLSs in the SOCS3 group was significantly lower than that in the control and blank groups. The results of ELISA assay showed that the levels of IL-2, IFN-γ and TNF-α in the SOCS3 group were higher than those in the control and blank groups. Our study demonstrates that over-expression of SOCS3 promotes proliferation and inhibits apoptosis of FLSs in RA.

Keywords: SOCS3, fibroblasts-like synoviocytes, rheumatoid arthritis, lentiviral vector, proliferation, apoptosis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease and autoimmune disorder that often leads to pain, swelling, and ultimately destruction of bones and joints, as well as immune cell infiltration of the synovia [1, 2]. RA affects over 1% of the worldwide population [3], and is related to high morbidity and mortality rate of cardiovascular [4]. Patients with RA have higher risk of malignancies than general population [5]. RA is triggered by a complicated interaction between genetics and the environment [6]. Smoking is a one of the most preventable environmental factors for the development and severity of RA [7]. Inflammation results in progressive injury to joints and bones and is a sign of RA [8]. Interstitial lung disease is also a common pulmonary reflection of RA and a major source of increased incidence and death in RA [9, 10]. No therapeutic agent has been proven to be generally and consistently effective in all RA patients [11]. Therefore, there is an urgent need to find new diagnostic and therapeutic treatments for RA patients.

Suppressor of cytokine signaling 3 (SOCS3) can inhibit cell growth and migration by blocking the activation of Janus-activated kinase/signal transducers and activators of transcription (JAK/STAT) in oncogenesis [12]. SOCS3 is a negative feedback regulator of the JAK/STAT signaling pathway, leukemia inhibitory factor, IL-6, and IL-11, and plays an essential role in the pathogenesis of various cancers [13, 14]. SOCS3 deficiency has far-reaching effects on placental development, inflammation, and insulin sensitivity [13]. Furthermore, the role of

SOCS-3 over-expression in arthritis has been reported [15]. Fibroblast-like synoviocytes (FLSs), key effector cells in the inflamed joints, show a high proliferation rate, constitutive expression of cytokines, and anchorage-independent cell growth [16]. As the mesenchymal cells of synovial joints, activation of FLSs in RA helps to activate cells of the immune system and resident joints, stimulating inflammation and joint damage [17]. FLSs have been recognized to play an increasingly important role in the development of RA by increasing joint inflammation and destruction [18]. Lentiviral vectors have functions in gene delivery with the ability to enter and integrate their genetic material into non-dividing cells [19], so they are useful in transplantation when pro-inflammatory factors are unavailable [20]. Recently, a large number of lentivirus-based vectors with unique characteristics available for gene therapy have been identified, and some recombinant lentivirus vectors also exhibit an anti-tumor function [21, 22]. Therefore, we carried out an experiment to explore whether and how lentivirus-mediated SOCS3 would affect FLSs in RA.

Material and methods

Ethical statement

This study was performed with the approval of ethics committee for laboratory animals of China-Japan Union Hospital of Jilin University. All experimental procedures were done strictly in accordance with the regulations of the protection and use of animals of the International Association for the Study of Pain [23].

Study subjects

A total of 20 female Lewis rats (4~5 weeks old, weighing 100~120 g) were selected randomly. Rats were all healthy, free of other diseases, and specific pathogens, and were provided by the Animal Experimental Center of the Hubei Academy of Medical Sciences (Certification No: SCXK11-00-0010). The rats were fed for one week with controlled temperature, humidity and ventilation so as to ensure the good health of the rats.

Construction of recombinant lentiviral over-expression vector of SOCS3 gene

Primers were designed according to the mRNA sequences of SOCS3 gene, and the primers were as follows, rSOCS3 forward primer: 5'-CTCACGA-AGCTAGCGTTGA-3' (spanning the hEFla and CMV promoters), reverse primer: 5'-CTCGACGCTCA-GTGTGAAGA-3' (in rSOCS3). The restriction enzyme site Xba I was inserted in forward primer, and GGG was inserted in the reverse primer to form the blunt end of the restriction site of Sam I (Restriction enzymes were purchased from Takara Holdings Inc., Dalian, China). FLSs cDNA of Lewis rats served as the template and gene fragments were obtained by polymerase chain reaction (PCR) amplification. pHIV-H2BmRFP vector (Takara Holdings Inc., Dalian, China) was digested with Xba I and Sam I enzymes for linearization. After enzyme digestion, 2 μL of digested DNA vector and 6 μL digested SOCS3 fragments were selected for ligation overnight using T4 DNA ligase. The ligation product was transformed into DH5α competent cells (Takara Holdings Inc., Dalian, China). The positive clones were screened out using PCR amplification and sequence identification.

The 293FT cells (Takara Holdings Inc., Dalian, China) in logarithmic growth phase were chosen for lentivirus packaging. In a proportion of 1:3, the cells were cultured and seeded in a 6 cm plate. When the cells reached confluence of 85%, transfection was done for 72 h and the cell supernatant was collected, centrifuged at 3000 r/min for 20 min, filtered through a 0.45 μm filter and stored at -80°C. One day before the determination of virus titer, the recombinant virus was digested, counted and diluted into 5×10^4 cells/mL. The recombinant virus was added into a 96-well plate with 100 μL/well, and six duplicated wells were prepared for each virus. The supernatant of 10 μL virus was taken and diluted for $10\sim10^6$ times, then 100 μL of supernatant was added into the cell culture wells successively and incubated in a 5% CO_2 incubator at 37°C. Twenty-four hours later, 100 μL of fresh culture medium was added to each well. Seventy-two hours later, the titer was testedand calculated according to the following formula: virus titer (TU/mL) = The green-fluorescent protein (GFP) × dilution times/virus volume.

Rat model establishment

The rats were randomly assigned into 2 groups: experimental group and normal group, with 10 rats in each group. The rat model was induced

by adjuvant arthritis (AA) modeling [24]. Lanolin and paraffin oil (North China Pharmaceutical CO., Ltd, Shijiazhuang, Hebei, China) were heated and mixed evenly at the ratio of 1:2. After autoclaving, the mixtures were preserved at 2°C, and 12 g/L of bacille calmette-guerin (BCG) vaccine (North China Pharmaceutical Co., Ltd, Shijiazhuang, Hebei, China) was added to prepare Freund's complete adjuvant (CFA). The tail root of rats in the experimental group was sterilized by iodine. Along the midline of the rat spinal column, a subcutaneous injection of 50 μL of adjuvant was performed. Rats in the normal group were injected with 50 μL of normal saline. After modeling, rats were raised with normal diet and activity, and the body weight, ankle joint thickness, and growth status were observed every day.

Isolation and culture of FLSs in RA rats

The animals were sacrificed at the 28th day after modeling, and cells in the joint cavity of rats in the normal and experimental groups were observed and pathological analysis was carried out. The posterior ankle joint with severe swelling was isolated, and the incrassated synovial tissues of the posterior ankle joint were stripped on the clean operating table. The tissues were cultured in serum-free serum Dulbecco's Modified Eagle's Medium (DMEM) (North China Pharmaceutical Co., Ltd, Shijiazhuang, Hebei, China) containing type I collagenase (2.5 mg/mL) (Sigma-Aldrich Chemical Company, St Louis MO, USA), then crushed into small fragments and placed in a culture dish for digestion and culture. On the second day, the culture dish was taken out and filtered to remove impurity, and the filtrate was collected in a centrifuge tube. The filtrate was centrifuged at 1200 r/min for 10 min with the supernatant removed. DMEM (100 U/mL) containing 20% fetal bovine serum (FBS), penicillin and streptomycin was added to re-suspend cells. Cells (1×10^6 cells/mL) were seeded in a 25 cm^2 culture bottle overnight. The bottom of the culture bottle was washed by phosphate buffer saline (PBS) the next day. The non-adherent cells were removed and the adherent cells were FLSs.

FLSs transfected with lentivirus-mediated SOCS3 gene

The primary FLSs were cultured to the third generation, and cells in the third generation were seeded in a 6-well plate at 5×10^5 cells/well, and 4 mL of DEME containing 10% FBS was added into each well. When cell confluence reached 75%~80%, FLSs were allocated randomlyinto SOCS3, control, and blank groups, with added lentivirus-mediated SOCS3 gene, blank carrier solution, and PBS respectively. Cells were then transfected and the culture plate was gently shaken every 15~25 min to promote adequate infection. After the cells were cultured for 48 hours, the supernatant was collected for further use.

3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

Cells infected with lentivirus-mediated SOCS3 gene for 48 h were taken and seeded in a 96-well plate with 2×10^4 cells/well and 100 μL per well. Four duplicated wells were set up for each group. Cell viability was detected at 0, 24 h, and 48 h respectively. Each group was added with 5 mg/mL MTT (20 μL) (Sigma-Aldrich Chemical Company, St Louis, MO, USA), and cells were further cultured for 4 h after gently shake. Then the supernatant was removed, and each well was added with 200 μL dimethyl sulphoxide (DMSO) solution (Sigma-Aldrich Chemical Company, St Louis, MO, USA), gently shake for 10 min to make Formazan fully dissolved. The optical density (OD) values at 490 nm were measured and recorded with a microplate reader (Omega Bio-TekInc., Norcross, GA, USA). The experiment was repeated 3 times.

Flow cytometry

Apoptosis assay was carried out according to the operator's instructions. The cells of each group were digested and collected into the centrifuge tube. The cells were washed 3 times with PBS and then suspended with 400 μL binding buffer (Sigma-Aldrich Chemical Company, St Louis, MO, USA). V-fluorescein isothiocyanate (FITC) (Sigma-Aldrich Chemical Company, St Louis, MO, USA) (5 μL) was then added, mixed and reacted in the dark for 20 min. 10 μL of propidium iodide (PI) (Sigma-Aldrich Chemical Company, St Louis, MO, USA) was then added, mixed and reacted in thedark for 5 min. Flow cytometry was usedto detect cell apoptosis. As the scatter diagram showed, the upper left quadrants represented the dead cells, the lower left included thelive cells, the upper right were late apoptotic cells, and the lower right were early apoptotic cells.

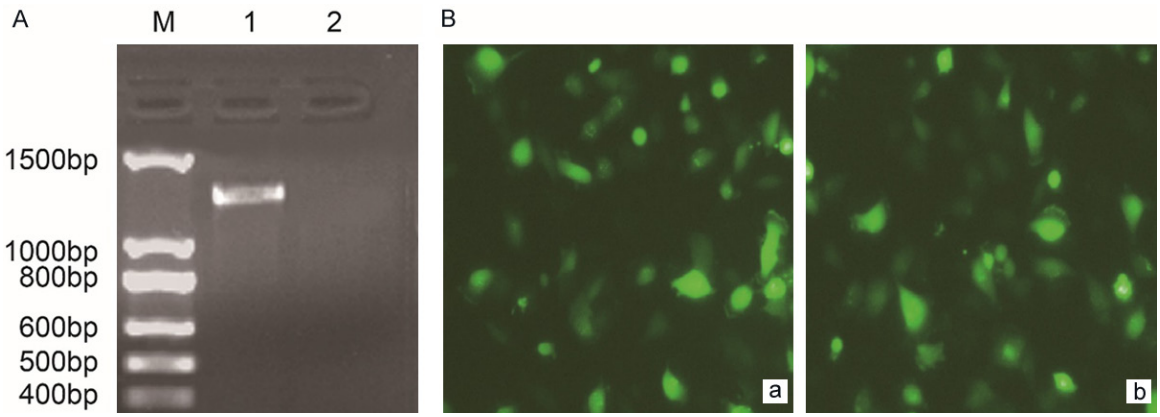

Figure 1. Identification of recombinant lentiviral vector by enzyme digestion and infection. Note: A, Electrophoretic strip of the empty vectors of pHIV-SOCS3 and pHIV; B, Recombinant lentiviral vector fluorescence; M, DNA marker; 1, recombinant lentiviral vector of pHIV-SOCS3; 2, lentiviral blank vector; a, pYrAd-RSOCS3 infection effect, b, yRrAd-GFP infection effect; SOCS3, suppressor of cytokine signaling 3.

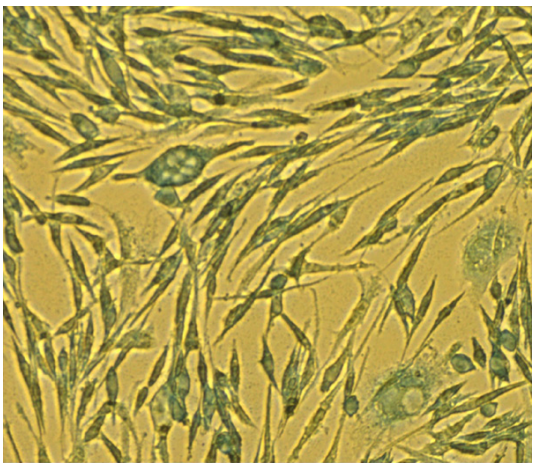

Figure 2. FLSs in the third generation under the microscope. Note: FLSs, fibroblasts-like synoviocytes.

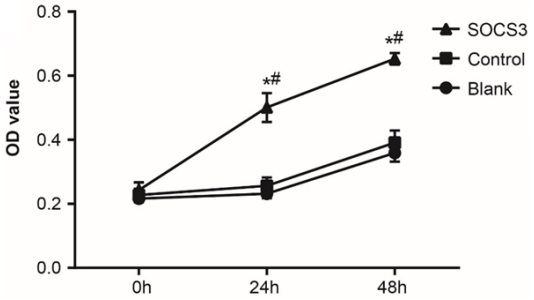

Figure 3. Viability of FLSs in the SOCS3, blank and control groups detected by MTT assay. Note: *, compared with the blank group, $P < 0.05$; #, compared with the control group, $P < 0.05$; FLSs, fibroblasts-like synoviocytes; MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; SOCS3, suppressor of cytokine signaling 3.

Enzyme-linked immunosorbent assay (ELISA)

The supernatants were collected as samples, and the levels of interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) in the 3 groups were detected by ELISA Kit (Perlin-Elmer, Waltham, MA, USA). The supernatants of the experimental group and cells in the control group were added to the corresponding wells (100 μL/well) with the reaction wells sealed and incubated in a 37°C incubator for 90 min. The plate was washed 5 times, and biotinylated antibody working solution was added to each well (100 μL/well) (except the blank wells). The reaction wells were sealed and the samples were incubated in a 37°C incubator for 60 min. The plate was washed 5 times, and the enzyme conjugate working solution was added to each well (100 μL/well) (except the blank wells). The reaction wells were sealed by gummed paper and the samples were incubated in a 37°C incubator for 30 min in the dark. The plate was washed 5 times, and chromogenic substrate was added to each well (100 μL/well) (including the blank wells). The reaction wells were sealed and the samples were incubated in a 37°C incubator for 10~15 min in the dark. The stop buffer was added to each well (100 μL/well) (including the blank wells), and the OD value was immediately measured at 450 nm within 10 min after mixing.

Statistical analysis

SPSS 20.0 statistical software (IBM Corp, Armonk, NY, USA) was used to process the data. Measurement data were presented as

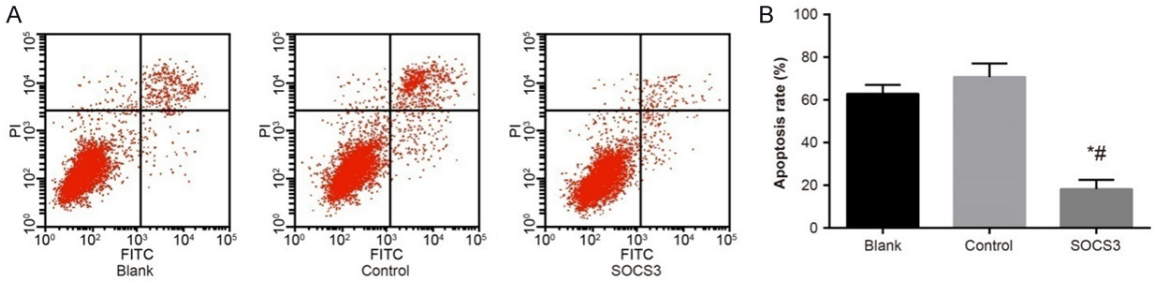

Figure 4. Cell apoptosis of FLSs in the SOCS3, blank, and control groups detected by flow cytometry. Note: *, compared with the blank group, $P < 0.05$; #, compared with the control group, $P < 0.05$; FLSs, fibroblasts-like synoviocytes; SOCS3, suppressor of cytokine signaling 3.

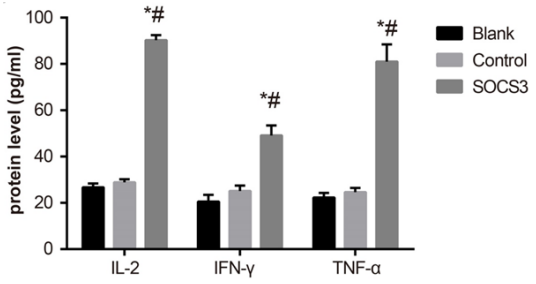

Figure 5. Levels of inflammatory cytokines (IL-2, IFN-γ and TNF-α) in supernatant were detected by ELISA. Note: *, compared with the blank group, $P < 0.05$; #, compared with the control group, $P < 0.05$; ELISA, Enzyme-linked immunosorbent assay; IL-2, interleukin-2; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor.

mean ± standard deviation. The comparisons between two groups were done by the *t* test and the comparisons among multiple groups were conducted by the one-factor analysis of variance (ANOVA). Enumeration data were expressed as percentages and rates, and checked by chi-square test. A probability value of $P < 0.05$ indicated the difference was statistically significant.

Results

The lentiviral vector was successfully transfected

After the double enzyme digestion of the empty vectors of pHIV-SOCS3 and pHIV, the positive clones were digested by double enzymes, and a DNA strip around 1400 bp was formed by gel electrophoresis. The same method was used after negative clone, but there was no band at the same location (**Figure 1A**). After enzyme digestion, the positive clones were screened to ensure the inserted sequences were correct. After lentiviral vector was transfected with 293FT cells for 24 h, the green fluorescence of cells was observed under a fluorescence microscopy (**Figure 1B**), indicating that the lentiviral vector was successfully transfected. The titer of lentivirus was determined by hole-by-dilution titer assay, showing that the titers of therecombinant lentiviral over-expression vector SOCS3 and lentivirus empty vector were 4.5 × 10^6 TU/mL and 2 × 10^7 TU/mL respectively.

FLSs in the third generation were used as experimental cells

The FLSs were isolated from the posterior ankle joint of rats in the SOCS3 group and cultured by collagenase digestion. After enlargement culture of the primary FLSs, FLSs in the third generation were used as experimental cells (**Figure 2**).

Lentivirus-mediated overexpression of SOCS3 promotes viability of FLSs

The results of MTT assay showed that the number of FLSs increased gradually with time, and showed a temporal dependence, as is shown in **Figure 3**. The OD values in the blank group and the control group gradually increased with time. Their data were similar without significant differences ($P > 0.05$) at 0 h, however compared with the control and blank groups, the SOCS3 group had a similar OD value and there was no significant difference ($P > 0.05$). At 24 h and 48 h, compared with the control and blank groups, the SOCS3 group had a higher OD value ($P < 0.05$). The results show that lentivirus-mediated overexpression of SOCS3 promotes the viability of FLSs.

Lentivirus-mediated overexpression of SOCS3 inhibits apoptosis of FLSs

The cells in the 3 groups were stained by an apoptosis kit and detected by flow cytometry.

The result is shown in **Figure 4**. The cell distribution in the control group and the blank group was similar, and the difference was not statistically significant ($P > 0.05$). Compared with the blank and control groups, the early apoptotic cells and the late apoptotic cells in the SOCS3 group were significantly decreased ($P < 0.05$).

Lentivirus-mediated SOCS3 inducesIL-2, IFN-γ and TNF-α

The expression of inflammatory factors (IL-2, IFN-γ and TNF-α) was detected by ELISA assay (**Figure 5**). As can be seen from the figure, the levels of IL-2, IFN-γ and TNF-α were similar and there was no significant difference in the blank group and the control group ($P > 0.05$). The levels of IL-2, IFN-γ and TNF-α in the control group and the blank group were lower than that in the SOCS3 group ($P < 0.05$).

Discussion

RA patients have higher mortality rate than the general population, and RA affects about 1% of the population and over 3% of the population aged over 65 years [25, 26]. A priorstudy indicated that better understanding of how FLSs are triggered and how they corporate with other cells in the synovium tissues of RA may offer insights to develop new targets for RA therapy [17]. The role of induction or inactivation of SOCS3 has been already reported in RA in a former study mainly conducted by Croker BA [27]. In this study, we investigated the role of lentivirus-mediated SOCS3 in FLSs in RA. Our experimental results indicate that lentivirus-mediated overexpression of SOCS3 promotes proliferation and inhibited apoptosis of FLSs in RA.

Initially, our study showed that compared with the control and blank groups, rats in the SOCS3 group had higher OD value, i.e. increased proliferation of FLSs, and reduced apoptosis of FLSs. A recent study demonstrated that FLS proliferation is important forthe development of RA [28]. Previous studies have reported that SOCS3 is a classic inhibitor of the JAK/STAT signaling pathway [29], and regulates the PI3K/AKT signaling pathway [30]. The activation of nuclear factor kappa B (NF-κB) is a critical factor binding inflammation and tumor formation, and its activation leads to the proliferation of FLSs [31, 32]. The p-AKT (also known as protein kinase B) signaling pathway is also crucial in regulating cell proliferation in RA [28]. The increased cell proliferation of FLSs in RA can also be caused by the PI3K/AKT signaling pathway through the over-expression of microRNA-126 [33]. The protein arginine methyltransferase 5 (PRMT5) mediated cell proliferation of FLSs, can bealtered by the NF-κB and AKT signaling pathways [34]. The JAK/STAT signaling pathway promotes activation of NF-κB [35]. Over-expression of SOCS3 was reported to induce a marked increase in apoptosis of chronic myeloid leukemia cells [36]. A former study revealed that SOCS3 suppressed cytokine-induced apoptosis in keratinocytes [37]. Overexpression of SOCS3 was also found to reduce tumor necrosis factor-related apoptosis inresveratrol-treated DU145 cells in prostate cancer [38]. These results were all similar to our result, therefore, we conclude that lentivirus-mediated overexpression of SOCS3 promoted proliferation and inhibited apoptosis of FLSs.

Furthermore, compared with the blank and control groups, the levels of IL-2, IFN-γ, and TNF-α of rats in the SOCS3 group were increased. IFN-γ is a pro-inflammatory cytokine and produced by T-helper 1 (Th1) cells and involved in Th1-mediated autoimmune processes [39]. Itplays a pivotal role in the progression of RA [40]. IFN-γ activates macrophages for inflammatory response through the activation of the JAK/STAT1 signaling pathway, and is negatively regulated by SOCS [41]. IL-2 has also been considered to play an important role in the pathogenesis of RA [42]. TNF-α is a pivotal inflammatory cytokine which exerts great importance between inflammation and cancer, and plays an important role in tumor proliferation and angiogenesis [43]. TNF-α, a key inflammatory factor in the pathogenesis of RA, facilitates development of RA symptoms [44]. SOCS3 is a negative regulator associated with IFN-γ and TNF-α [45]. In addition, SOCS3 is produced by TNF-α and is a negative regulator of the NF-κB-mediated signal pathway [46]. Previous studies have figured out that anti-TNF-α therapy canachieve considerable improvement in RA therapy, and it has been the most effective therapy for RA patients [47, 48]. The Th1 differentiated cells induce IL-2, IFN-γ and TNF-α [49]. A previous study mainly performed by Toh ML demonstrated that RA patients have systemic low accumulation of Th1 cells correlated with inflammation [50].

The results showed that over-expression of lentivirus-mediated SOCS3 promoted the levels of IL-2, IFN-γ and TNF-α.

Conclusions

In summary, our data indicated that over-expression of lentivirus-mediated SOCS3 increased proliferation and suppressed apoptosis of FLSs in RA. This is a preliminary experimentand more complicated mechanisms should be further studied to find effective therapeutic regimens for RA patients.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Tobon GJ, Youinou P and Saraux A. The environment, geo-epidemiology, and autoimmune disease: rheumatoid arthritis. J Autoimmun 2010; 35: 10-14.

[2] Conigliaro P, Perricone C, Benson RA, Garside P, Brewer JM, Perricone R and Valesini G. The type I IFN system in rheumatoid arthritis. Autoimmunity 2010; 43: 220-225.

[3] Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, Li Y, Kurreeman FA, Zhernakova A, Hinks A, Guiducci C, Chen R, Alfredsson L, Amos CI, Ardlie KG; BIRAC Consortium, Barton A, Bowes J, Brouwer E, Burtt NP, Catanese JJ, Coblyn J, Coenen MJ, Costenbader KH, Criswell LA, Crusius JB, Cui J, de Bakker PI, De Jager PL, Ding B, Emery P, Flynn E, Harrison P, Hocking LJ, Huizinga TW, Kastner DL, Ke X, Lee AT, Liu X, Martin P, Morgan AW, Padyukov L, Posthumus MD, Radstake TR, Reid DM, Seielstad M, Seldin MF, Shadick NA, Steer S, Tak PP, Thomson W, van der Helm-van Mil AH, van der Horst-Bruinsma IE, van der Schoot CE, van Riel PL, Weinblatt ME, Wilson AG, Wolbink GJ, Wordsworth BP; YEAR Consortium, Wijmenga C, Karlson EW, Toes RE, de Vries N, Begovich AB, Worthington J, Siminovitch KA, Gregersen PK, Klareskog L and Plenge RM. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010; 42: 508-514.

[4] Panoulas VF, Douglas KM, Stavropoulos-Kalinoglou A, Metsios GS, Nightingale P, Kita MD, Elisaf MS and Kitas GD. Long-term exposure to medium-dose glucocorticoid therapy associates with hypertension in patients with rheumatoid arthritis. Rheumatology (Oxford) 2008; 47: 72-75.

[5] Simon TA, Thompson A, Gandhi KK, Hochberg MC and Suissa S. Incidence of malignancy in adult patients with rheumatoid arthritis: a meta-analysis. Arthritis Res Ther 2015; 17: 212.

[6] Essouma M and Noubiap JJ. Is air pollution a risk factor for rheumatoid arthritis? J Inflamm (Lond) 2015; 12: 48.

[7] Kallberg H, Ding B, Padyukov L, Bengtsson C, Ronnelid J, Klareskog L, Alfredsson L; EIRA Study Group. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. Ann Rheum Dis 2011; 70: 508-511.

[8] Chung KC, Burns PB, Kim HM, Burke FD, Wilgis EF and Fox DA. Long-term followup for rheumatoid arthritis patients in a multicenter outcomes study of silicone metacarpophalangeal joint arthroplasty. Arthritis Care Res (Hoboken) 2012; 64: 1292-1300.

[9] Solomon JJ, Chung JH, Cosgrove GP, Demoruelle MK, Fernandez-Perez ER, Fischer A, Frankel SK, Hobbs SB, Huie TJ, Ketzer J, Mannina A, Olson AL, Russell G, Tsuchiya Y, Yunt ZX, Zelarney PT, Brown KK and Swigris JJ. Predictors of mortality in rheumatoid arthritis-associated interstitial lung disease. Eur Respir J 2016; 47: 588-596.

[10] Antin-Ozerkis D, Evans J, Rubinowitz A, Homer RJ and Matthay RA. Pulmonary manifestations of rheumatoid arthritis. Clin Chest Med 2010; 31: 451-478.

[11] Burmester GR, Blanco R, Charles-Schoeman C, Wollenhaupt J, Zerbini C, Benda B, GrubenD, Wallenstein G, Krishnaswami S, Zwillich SH, Koncz T, Soma K, Bradley J, Mebus C; ORAL Step investigators. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inade-

quate response to tumour necrosis factor inhibitors: a randomised phase 3 trial. Lancet 2013; 381: 451-460.
[12] Deng J, Jiao X, Liu H, Wu L, Zhang R, Wang B, Pan Y, Hao X and Liang H. Lymph node metastasis is mediated by suppressor of cytokine signaling-3 in gastric cancer. Tumour Biol 2013; 34: 3627-3636.
[13] Babon JJ and Nicola NA. The biology and mechanism of action of suppressor of cytokine signaling 3. Growth Factors 2012; 30: 207-219.
[14] Puhr M, Santer FR, Neuwirt H, Susani M, Nemeth JA, Hobisch A, Kenner L and Culig Z. Down-regulation of suppressor of cytokine signaling-3 causes prostate cancer cell death through activation of the extrinsic and intrinsic apoptosis pathways. Cancer Res 2009; 69: 7375-7384.
[15] Sun LP, Ma XL, Liu HX, Wang YS and Li XF. No association of polymorphisms in the suppressor of cytokine signaling (SOCS)-3 with rheumatoid arthritis in the Chinese Han population. Genet Mol Res 2010; 9: 1518-1524.
[16] Wang M, Wu J, Guo Y, Chang X and Cheng T. The tripartite motif-containing protein 3 on the proliferation and cytokine secretion of rheumatoid arthritis fibroblast-like synoviocytes. Mol Med Rep 2017; 15: 1607-1612.
[17] Noss EH and Brenner MB. The role and therapeutic implications of fibroblast-like synoviocytes in inflammation and cartilage erosion in rheumatoid arthritis. Immunol Rev 2008; 223: 252-270.
[18] Li Z, Cai J and Cao X. MiR-19 suppresses fibroblast-like synoviocytes cytokine release by targeting toll like receptor 2 in rheumatoid arthritis. Am J Transl Res 2016; 8: 5512-5518.
[19] Matrai J, Chuah MK and VandenDriessche T. Recent advances in lentiviral vector development and applications. Mol Ther 2010; 18: 477-490.
[20] Zhao J and Lever AM. Lentivirus-mediated gene expression. Methods Mol Biol 2007; 366: 343-355.
[21] Philippe S, Sarkis C, Barkats M, Mammeri H, Ladroue C, Petit C, Mallet J and Serguera C. Lentiviral vectors with a defective integrase allow efficient and sustained transgene expression in vitro and in vivo. Proc Natl Acad Sci U S A 2006; 103: 17684-17689.
[22] Song LP, Li YP, Qiu SD and Wang N. [Recombinant lentivirus-mediated gene transfer of NT4-p53(N15)-Ant inhibits the growth of hepatocellular carcinoma cells in vitro]. Zhonghua Zhong Liu Za Zhi 2010; 32: 10-16.
[23] Orlans FB. Ethical decision making about animal experiments. Ethics Behav 1997; 7: 163-171.
[24] Guo W, Yu D, Wang X, Luo C, Chen Y, Lei W, Wang C, Ge Y, Xue W, Tian Q, Gao X and Yao W. Anti-inflammatory effects of interleukin-23 receptor cytokine-binding homology region rebalance T cell distribution in rodent collagen-induced arthritis. Oncotarget 2016; 7: 31800-31813.
[25] Pieringer H and Pichler M. Cardiovascular morbidity and mortality in patients with rheumatoid arthritis: vascular alterations and possible clinical implications. QJM 2011; 104: 13-26.
[26] da Silva E, Doran MF, Crowson CS, O'Fallon WM and Matteson EL. Declining use of orthopedic surgery in patients with rheumatoid arthritis? Results of a long-term, population-based assessment. Arthritis Rheum 2003; 49: 216-220.
[27] Croker BA, Kiu H, Pellegrini M, Toe J, Preston S, Metcalf D, O'Donnell JA, Cengia LH, McArthur K, Nicola NA, Alexander WS and Roberts AW. IL-6 promotes acute and chronic inflammatory disease in the absence of SOCS3. Immunol Cell Biol 2012; 90: 124-129.
[28] Zeng S, Wang K, Huang M, Qiu Q, Xiao Y, Shi M, Zou Y, Yang X, Xu H and Liang L. Halofuginone inhibits TNF-alpha-induced the migration and proliferation of fibroblast-like synoviocytes from rheumatoid arthritis patients. Int Immunopharmacol 2017; 43: 187-194.
[29] Qin H, Yeh WI, De Sarno P, Holdbrooks AT, Liu Y, Muldowney MT, Reynolds SL, Yanagisawa LL, Fox TH 3rd, Park K, Harrington LE, Raman C and Benveniste EN. Signal transducer and activator of transcription-3/suppressor of cytokine signaling-3 (STAT3/SOCS3) axis in myeloid cells regulates neuroinflammation. Proc Natl Acad Sci U S A 2012; 109: 5004-5009.
[30] Wan J, Che Y, Kang N and Wu W. SOCS3 blocks HIF-1alpha expression to inhibit proliferation and angiogenesis of human small cell lung cancer by downregulating activation of Akt, but not STAT3. Mol Med Rep 2015; 12: 83-92.
[31] Ding J, Huang S, Wang Y, Tian Q, Zha R, Shi H, Wang Q, Ge C, Chen T, Zhao Y, Liang L, Li J and He X. Genome-wide screening reveals that miR-195 targets the TNF-alpha/NF-kappaB pathway by down-regulating IkappaB kinase alpha and TAB3 in hepatocellular carcinoma. Hepatology 2013; 58: 654-666.
[32] Qi L, Zhang X and Wang X. Heparin inhibits the inflammation and proliferation of human rheumatoid arthritis fibroblastlike synoviocytes through the NFkappaB pathway. Mol Med Rep 2016; 14: 3743-3748.
[33] Gao J, Zhou XL, Kong RN, Ji LM, He LL and Zhao DB. microRNA-126 targeting PIK3R2 promotes rheumatoid arthritis synovial fibroblasts proliferation and resistance to apoptosis by regulating PI3K/AKT pathway. Exp Mol Pathol 2016; 100: 192-198.

[34] Chen D, Zeng S, Huang M, Xu H, Liang L and Yang X. Role of protein arginine methyltransferase 5 in inflammation and migration of fibroblast-like synoviocytes in rheumatoid arthritis. J Cell Mol Med 2017; 21: 781-790.
[35] Zhang S, Liu X, Goldstein S, Li Y, Ge J, He B, Fei X, Wang Z and Ruiz G. Role of the JAK/STAT signaling pathway in the pathogenesis of acute myocardial infarction in rats and its effect on NF-kappaB expression. Mol Med Rep 2013; 7: 93-98.
[36] Liu YX, Wang L, Liu WJ, Zhang HT, Xue JH, Zhang ZW and Gao CJ. MiR-124-3p/B4GALT1 axis plays an important role in SOCS3-regulated growth and chemo-sensitivity of CML. J Hematol Oncol 2016; 9: 69.
[37] Madonna S, Scarponi C, Pallotta S, Cavani A and Albanesi C. Anti-apoptotic effects of suppressor of cytokine signaling 3 and 1 in psoriasis. Cell Death Dis 2012; 3: e334.
[38] Horndasch M and Culig Z. SOCS-3 antagonizes pro-apoptotic effects of TRAIL and resveratrol in prostate cancer cells. Prostate 2011; 71: 1357-1366.
[39] Kelchtermans H, Billiau A and Matthys P. How interferon-gamma keeps autoimmune diseases in check. Trends Immunol 2008; 29: 479-486.
[40] Sigidin YA, Loukina GV, Skurkovich B and Skurkovich S. Randomized, double-blind trial of anti-interferon-gamma antibodies in rheumatoid arthritis. Scand J Rheumatol 2001; 30: 203-207.
[41] Yu T, Zuo Y, Cai R, Huang X, Wu S, Zhang C, Chin YE, Li D, Zhang Z, Xia N, Wang Q, Shen H, Yao X, Zhang ZY, Xue S, Shen L and Cheng J. SENP1 regulates IFN-gamma-STAT1 signaling through STAT3-SOCS3 negative feedback loop. J Mol Cell Biol 2017; 9: 144-153.
[42] Oncul O, Top C, Ozkan S, Cavuslu S and Danaci M. Serum interleukin 2 levels in patients with rheumatoid arthritis and correlation with insulin sensitivity. J Int Med Res 2002; 30: 386-390.
[43] Wu Y and Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. Br J Cancer 2010; 102: 639-644.
[44] Gu X, Gu B, Lv X, Yu Z, Wang R, Zhou X, Qiao W, Mao Z, Zuo G, Li Q, Miao D and Jin J. 1,25-dihydroxy-vitamin D3 with tumor necrosis factor-alpha protects against rheumatoid arthritis by promoting p53 acetylation-mediated apoptosis via Sirt1 in synoviocytes. Cell Death Dis 2016; 7: e2423.
[45] Liu Z, Gan L, Zhou Z, Jin W and Sun C. SOCS3 promotes inflammation and apoptosis via inhibiting JAK2/STAT3 signaling pathway in 3T3-L1 adipocyte. Immunobiology 2015; 220: 947-953.
[46] Collins AS, Ahmed S, Napoletano S, Schroeder M, Johnston JA, Hegarty JE, O'Farrelly C and Stevenson NJ. Hepatitis C virus (HCV)-induced suppressor of cytokine signaling (SOCS) 3 regulates proinflammatory TNF-alpha responses. J Leukoc Biol 2014; 96: 255-263.
[47] Hyrich KL, Watson KD, Silman AJ, Symmons DP; British Society for Rheumatology Biologics Register. Predictors of response to anti-TNF-alpha therapy among patients with rheumatoid arthritis: results from the British society for rheumatology biologics register. Rheumatology (Oxford) 2006; 45: 1558-1565.
[48] Lee YH, Rho YH, Choi SJ, Ji JD and Song GG. Association of TNF-alpha-308 G/A polymorphism with responsiveness to TNF-alpha-blockers in rheumatoid arthritis: a meta-analysis. Rheumatol Int 2006; 27: 157-161.
[49] Pandey A, Bani S, Dutt P and Suri KA. Modulation of Th1/Th2 cytokines and inflammatory mediators by hydroxychavicol in adjuvant induced arthritic tissues. Cytokine 2010; 49: 114-121.
[50] Toh ML, Kawashima M, Hot A, Miossec P and Miossec P. Role of IL-17 in the Th1 systemic defects in rheumatoid arthritis through selective IL-12Rbeta2 inhibition. Ann Rheum Dis 2010; 69: 1562-1567.