

MiR-222-3p Aggravates the Inflammatory Response by Targeting SOCS1 to Activate STAT3 Signaling in Ulcerative Colitis

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ABSTRACT

Background: Ulcerative colitis is characterized by relapsing inflammation in the gastrointestinal tract with limited treatment options. The aim of the present study was to assess the anti-inflammatory effect of Suppressor of cytokine signaling (SOCS1) on lipopolysaccharide-stimulated RAW264.7 cells and to investigate its potential mechanisms.

Methods: The in vitro ulcerative colitis model was established by using lipopolysaccharide-stimulated RAW264.7 cells. Western blotting was used to detect the protein expression levels of SOCS1, JAK2, STAT3, and VDR. Reverse transcription-quantitative polymerase chain reaction was used to measure the mRNA expression of SOCS1, miR-222-3p, and VDR. An enzyme-linked immunosorbent assay was performed to measure the levels of inflammatory cytokines. A luciferase assay assessed the binding of SOCS1 to miR-222-3p. A total of 15 patients with ulcerative colitis and 18 healthy controls were recruited. The expression levels of SOCS1 and miR-222-3p in the colonic mucosa tissues of patients with ulcerative colitis and healthy controls were determined by reverse transcription-quantitative polymerase chain reaction.

Results: SOCS1 upregulation inhibited the lipopolysaccharide-induced inflammation in RAW264.7 cells. SOCS1 was confirmed to be targeted by miR-222-3p. Silencing SOCS1 significantly abolished the inhibitory effects of miR-222-3p downregulation on inflammation. MiR-222-3p activated STAT3 signaling and reduced VDR expression by targeting SOCS1 in lipopolysaccharide-treated RAW264.7 cells. Additionally, miR-222-3p expression was upregulated in ulcerative colitis patients ($P = 5.16E-10$), while SOCS1 ($P = 2.75E-10$) and VDR ($P = 52.5E-9$) expression was downregulated in ulcerative colitis patients. Endoscopic scores (UCEIS) revealed significant positive correlation with miR-222-3p and negative correlation with SOCS1 and VDR.

Conclusion: MiR-222-3p targets SOCS1 to aggravate the inflammatory response by suppressing VDR and activating STAT3 signaling in ulcerative colitis.

Keywords: miR-222-3p, SOCS1, ulcerative colitis, VDR

INTRODUCTION

Inflammatory bowel disease (IBD) is an intestinal inflammatory disorder encompassing ulcerative colitis (UC) and Crohn's disease (CD).¹ The diagnosis rate and hospitalization rate of IBD patients worldwide have been increasing annually over the past decades.^{2,3} Ulcerative colitis is characterized by rectal bleeding, mucosal damage, and ulceration.⁴ Multiple pathogenic factors have been documented, including genetics, immune response, inflammatory factors, bacterial infection, and environmental factors, among which environmental and immunological factors play important roles in UC.⁵ Highly activated monocytes and macrophages in UC patients have been reported.⁶ Activated monocyte/macrophage releases pro-inflammatory cytokines, leading to epithelial barrier disruption and intestinal inflammation.^{7,8} The severity and

duration of diffuse mucosal inflammation of the colon are closely related to the risk of colorectal cancer formation.⁹ Therefore, more effective diagnostic and therapeutic targets are needed for early clinical risk assessment and treatment.

Suppressors of cytokine signaling 1 (SOCS1) is a key negative regulator of inflammatory processes.¹⁰ Depletion of SOCS1 in helper T cells suppresses Th17 differentiation by enhancing the antagonistic effect of IFN- γ .¹¹ The pro-inflammatory genetic background owing to SOCS1 deficiency causes dysbiosis of the gut microbiota, thereby generating a pro-colitogenic environment.¹² SOCS1 relieves dextran sulfate sodium¹³-induced colitis in mice by blocking IFN- γ /STAT1 pathway.¹⁴ SOCS1 plays a key role in attenuating murine colitis by inhibiting cytokine signaling

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and controlling intestinal T cell activation.¹⁵ These findings demonstrate the important role of *SOCS1* in the pathogenesis of colitis. Additionally, *SOCS1* is a critical negative modulator of JAK/STAT pathway and functions in inhibiting systemic autoimmunity mediated by dendritic cells.¹⁶ Vitamin D receptor (VDR) belongs to the steroid receptor family and its protective effects against colitis have been reported.^{17,18} It has been reported that STAT3 signaling contributes to downregulation of VDR.¹⁹

MicroRNAs (miRNAs) are short non-coding RNAs, which control gene expression by binding to the 3'UTR of mRNAs.^{20,21} Emerging reports have indicated that miRNAs are associated with the procession of UC, such as miR-129-5p, miR-21, and miR-200, suggesting that miRNAs could be therapeutic targets for UC.²²⁻²⁴ As previously reported, miR-222-3p expression is upregulated in doxorubicin-resistant colon cancer cells and induces doxorubicin resistance.²⁵ Additionally, miR-222-3p depletion alleviates liver inflammatory damage by elevating *SOCS1* expression.²⁶ However, the regulatory mechanisms of the miR-222-3p/*SOCS1* in UC are unclear.

In this study, we aimed to investigate the anti-inflammatory effect of *SOCS1* on UC using Raw264.7 cells inflammatory model induced by LPS. We also investigated its possible mechanism involving the regulation of JAK/STAT3 signaling and the involvement of miR-222-3p. This study might provide some new potential research targets for molecular mechanisms of UC development.

MATERIALS AND METHODS

Tissue Samples

Colonic mucosa tissues were collected from the sigmoid colon of 15 patients with active UC and 18 healthy controls undergoing screening colonoscopies between June 2015 and July 2016 at The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, Jiangsu, China. Informed consent was obtained from all study

participants. The inclusion criteria included: active stage of UC, but no prior treatment at the time of serum sampling. Exclusion criteria included: history of autoimmune diseases and previous treatment (immunomodulators, steroids, antibiotics, 5-aminosalicylic acid, blood cell apheresis, biologics, or surgery) at the time of serum sampling. The biopsies were immediately snap-frozen and stored at -80°C after pinching. The study was approved by the Ethics Committee of The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.

The endoscopic index was graded following the UCEIS.²⁷ The UCEIS includes bleeding, erosions, visible vascular patterns, and ulcers. The UCEIS is stratified into 4 categories: remission (UCEIS 0-1), mild (UCEIS 2-4), moderate (UCEIS 5-6), and severe (UCEIS 7-8).

Cells

The mouse macrophage RAW264.7 and HEK293T cells (ATCC; Manassas, VA, USA) were cultured in Dulbecco's modified eagle medium (Gibco, Rockville, USA) containing 10% fetal bovine serum, 100 mg/mL streptomycin, and 100 U/mL penicillin at 37°C with 5% CO_2 . Cells were plated in 96-well plates at $1 \times 10^5/\text{mL}$ at 37°C for 24 hours. Next, cells were cultured with 1 $\mu\text{g}/\text{mL}$ LPS (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 6, 12, and 24 hours to establish an UC model in vitro.

Transfection

Small interfering RNA targeting *SOCS1* (Si-*SOCS1*) and Si-NC were constructed by Ribobio (Guangzhou, China). Anti-miR-222-3p and miR-NC were also obtained from Ribobio. Full-length *SOCS1* was amplified and then digested with XbaI to obtain nucleotides of *SOCS1* cDNA sequence and inserted into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA) between EcoRI and EcoRV cleavage sites as the backbone. LPS-cultured cells were transfected with the above vectors using Lipofectamine 2000 (Invitrogen). After 24 hours, the transfection was tested by reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Reverse Transcription-Quantitative Polymerase Chain Reaction

Total RNA was extracted from LPS-cultured RAW264.7 cells or specimens utilizing TRIzol (Invitrogen). The extracted RNA was kept at -80°C for later use. Next, cDNA was obtained using a High Capacity cDNA Reverse Transcription kit (Takara, Japan). The primers

Main Points

- *SOCS1* upregulation inhibits the lipopolysaccharide-induced inflammatory response in RAW264.7 macrophages.
- *SOCS1* is targeted by miR-222-3p.
- miR-222-3p activates STAT3 signaling and downregulates VDR by targeting *SOCS1*.
- miR-222-3p targets *SOCS1* to aggravate the inflammatory response by activating STAT3 signaling.

were synthesized by GenePharma (Shanghai, China), and U6 or GAPDH was as an internal reference. Reverse transcription-quantitative polymerase chain reaction was conducted using a TaqMan MicroRNA Assay kit (Applied Biosystems, Foster City, CA, USA) for miR2223p and a Power SYBR Green qPCR Master Mix (Takara) for SOCS1, VDR, TNF- α , IL-6, and IL-8 following the manufacturer's instructions. The expression was evaluated using the $2^{-\Delta\Delta C_t}$. The mouse primers used are shown in Table 1.

Western Blotting

Total proteins were collected from specimens in ice-cold RIPA lysis buffer (Beyotime, China). Next, a bicinchoninic acid kit (Soliba, China) was utilized to determine the protein concentration. Total proteins (40 μ g) were separated by 12% SDS-PAGE and blotted onto nitrocellulose membranes. The membranes were blocked in Tris-buffered saline and 5% nonfat milk for 1 hour, and then incubated overnight at 4°C with antibodies including SOCS1 (ab280886, Abcam, Cambridge, UK), P-JAK2 (ab32101), JAK2 (ab32101), p-STAT3 (ab76315), STAT3 (ab68153), VDR (ab109234), and GAPDH (ab9485), and further probed with peroxidase-labeled goat anti-rabbit (Santa Cruz Biotechnology, CA, USA). The membranes were washed before detection and observed utilizing

an enhanced chemiluminescence method (Amersham Biosciences, Arlington Heights, IL, USA). Signal quantification was performed with ImageJ software.

Enzyme-Linked Immunosorbent Assay

The inflammatory cytokine (TNF- α , IL-6, and IL-8) levels in the supernatant were determined using commercial mouse-specific enzyme-linked immunosorbent assay (ELISA) kits (Abcam). Culture supernatant was obtained from the transfected RAW264.7 following LPS treatment at the indicated time. The absorbance was measured using a Universal Microplate Spectrophotometer (BioTek Instruments, Winooski, VT, USA). The obtained results were expressed as picograms per milliliters of protein.

Luciferase Reporter Assay

The binding site of miR-222-3p in SOCS1 3'UTR was predicted at the TargetScan website. The SOCS1 3'UTR fragment at the miR-222-3p binding site was synthesized to generate SOCS1-Wt plasmids. SOCS1-Mut plasmids were constructed by mutating the miR-222-3p binding site in SOCS1-Wt. 293T cells were seeded in 96-well plates until reached 70%, and Lipofectamine 2000 was used for transfection. The SOCS1-Wt/Mut plasmids were co-transfected into 293T cells with miR-NC (75 nM) or Anti-miR-222-3p (75 nM). After 48 hours, cells were lysed for analyses using Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Statistical Analysis

Each assay was conducted at least 3 times to prevent errors. GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analyses. Values are expressed as mean $\pm$ standard deviation. The Student's *t*-test was used for data analysis of pairwise comparison. Comparison between 3 or more groups was analyzed by one-way ANOVA. Correlations between the UCEIS and gene expression were analyzed using non-parametric Spearman's correlation. *P* < .05 was considered statistically significant.

RESULTS

Lipopolysaccharide Induces Inflammatory Response and Downregulates SOCS1

We established inflammatory response models in RAW264.7 cells using LPS. The ELISA results showed that LPS notably elevated the concentrations of TNF- α (at 24 hours: 188 ± 20.01 ; *P* = 7.55E-6), IL-6 (at 24 hours: 333 ± 35.37 ; *P* = 4.98E-6), and IL-8 (at 24 hours:

Table 1. Primer sequences used for RT-qPCR

Target	Primer sequences (5'-3')
SOCS1	Forward, CTTCTGTAGGATGGTAGCACAC
SOCS1	Reverse, AGGAAGAGGAGGAAGTTCT
miR-222-3p	Forward, GGGGAGCTACATCTGGCT
miR-222-3p	Reverse, TCGCT GTCGTGGAGTC
IL-6	Forward, TCCAGTTGCCTTCTTGGGACTG
IL-6	Reverse, AGCCTCCGACTTGTGAAGTGGT
IL-8	Forward, ATGACTTCCAAGCTGGCCGT
IL-8	Reverse, TTACATAATTTCTGTGTTGGC
TNF- α	Forward, GACCCTCACACTCAGATCATCT
TNF- α	Reverse, CCTCCACTTGGTGGTTTGCT
VDR	Forward, GAATGTGCCTCGGATCTGTGG
VDR	Reverse, ATGCGGCAATCTCCATTGAAG
GAPDH	Forward, AGGTCGGTGTGAACGGATTTG
GAPDH	Reverse, GGGGTCGTTGATGGCAACA
U6	Forward, CTCGCTTCGGCAGCACA
U6	Reverse, AACGCTTCACGAATTTGCGT

RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

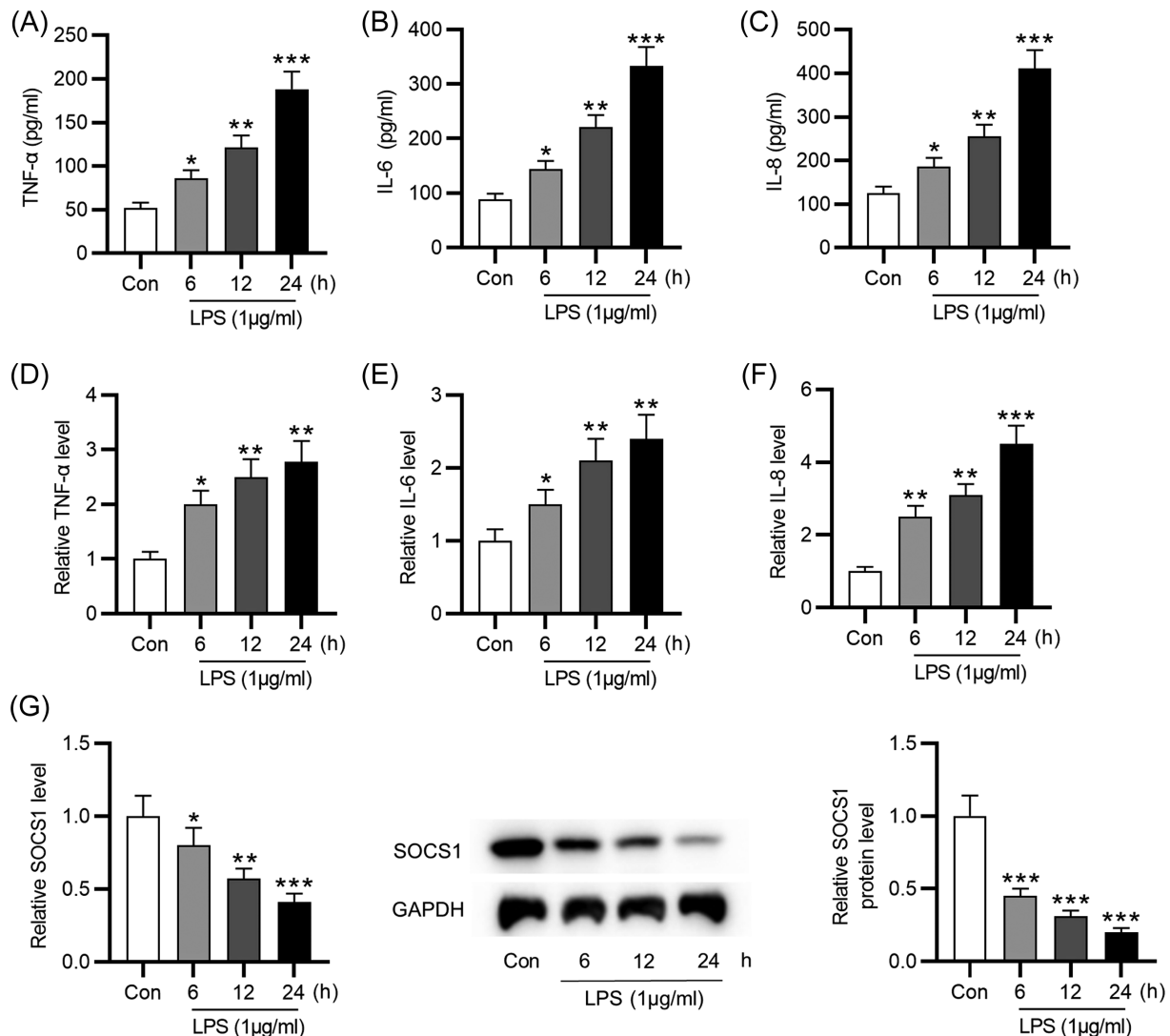


Figure 1. LPS induces inflammatory response and downregulates SOCS1. The concentrations of TNF-α (A), IL-6 (B), and IL-8 (C) in LPS-treated RAW264.7 cells were detected by ELISA. The mRNA expression of TNF-α (D), IL-6 (E), and IL-8 (F) was detected by RT-qPCR. (G) SOCS1 mRNA and protein expression was measured by RT-qPCR and western blotting. * $P < .05$, ** $P < .01$, *** $P < .001$. LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay.

411 ± 42.04 ; $P = 6.8E-6$) in RAW264.7 cells in a time-dependent manner (Figure 1A-C). Their mRNA levels were detected by RT-qPCR. The data indicated that the mRNA levels of TNF-α (at 24 hours: 2.78 ± 0.33 ; $P = 2.05E-4$), IL-6 (at 24 hours: 2.4 ± 0.33 ; $P = .001$), and IL-8 (at 24 hours: 4.5 ± 0.5 ; $P = 5.74E-6$) in RAW264.7 cells were upregulated after LPS treatment (Figure 1D-F). Subsequently, the SOCS1 level in LPS-cultured RAW264.7 cells was evaluated by RT-qPCR and western blotting. The findings revealed that SOCS1 mRNA (at 24 hours: 0.41 ± 0.06 ; $P = .001$) and protein (at 24 hours: 0.2 ± 0.03 ; $P = 3.44E-6$) expression was decreased after LPS treatment (Figure 1G).

Effects of SOCS1 on Inflammation of Macrophages

We further tested the role of SOCS1 in UC. In Figure 2A, the SOCS1 mRNA (10.4 ± 1.18 ; $P = 1.65E-4$) and protein (3.87 ± 0.4 ; $P = 3.0E-4$) expression in RAW264.7 cells was upregulated after transfection with SOCS1, indicating the successful transfection. Then, the levels of cytokines in LPS-treated RAW264.7 cells were detected after SOCS1 was overexpressed. SOCS1 upregulation significantly downregulated the mRNA levels of TNF-α (0.41 ± 0.05 ; $P = .002$), IL-6 (0.48 ± 0.09 ; $P = .004$), and IL-8 (0.38 ± 0.06 ; $P = .001$) (Figure 2B-D). Similarly, the supernatant levels of TNF-α (121 ± 12.6 ; $P = .004$),

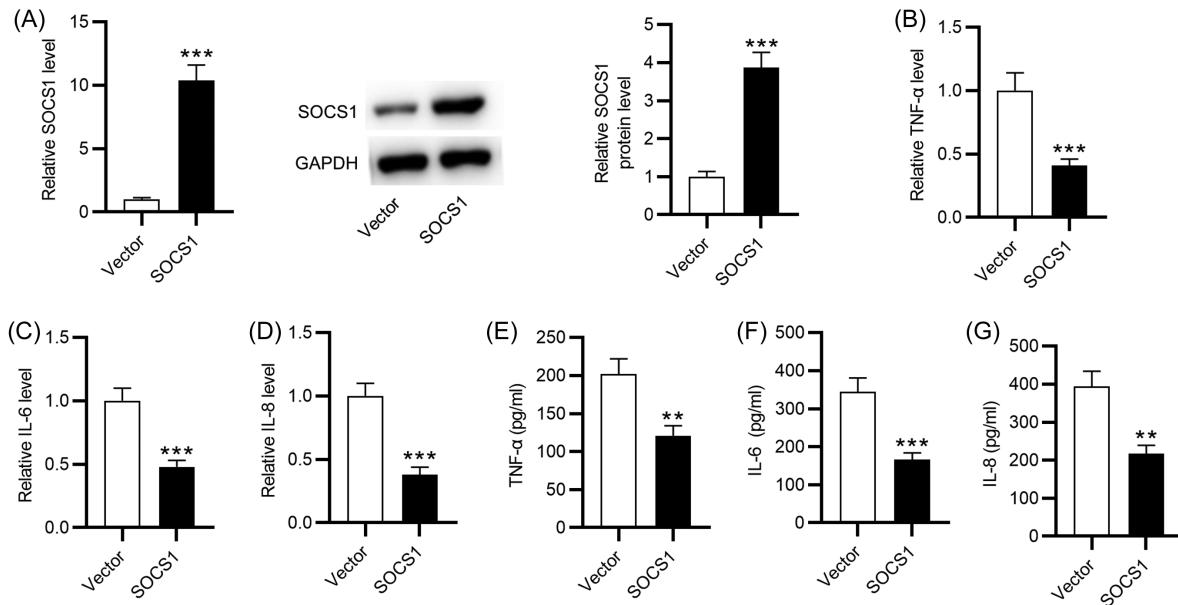


Figure 2. Effects of SOCS1 on inflammation of macrophages. (A) SOCS1 mRNA and protein expression in LPS-treated RAW264.7 cells transfected with SOCS1 or vector was measured by RT-qPCR and western blotting. The mRNA expression of TNF- α (B), IL-6 (C), and IL-8 (D) after transfection with SOCS1 or vector was detected by RT-qPCR. The concentrations of TNF- α (E), IL-6 (F), and IL-8 (G) in LPS-treated RAW264.7 cells transfected with SOCS1 or vector were detected by ELISA. * $P < .01$, *** $P < .001$. LPS, lipopolysaccharide, RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

IL-6 (166 ± 17.84 ; $P = .002$), and IL-8 (217.09 ± 22 ; $P = .003$) were reduced after overexpressing SOCS1 in RAW264.7 cells (Figure 2E-G). Overall, SOCS1 upregulation inhibited the LPS-induced inflammatory response.

SOCS1 is Targeted by miR-222-3p

TargetScan was utilized to identify the miRNAs binding to SOCS1. The prediction results show 9 miRNAs that have conserved sites for SOCS1 (Figure 3A), among which miR-155-5p, miR-142-5p, and miR-98-5p were reported in IBD. Therefore, we measured the levels of the other 6 miRNAs in LPS-treated RAW264.7 and uncovered that miR-222-3p was significantly upregulated (2.23 ± 0.24 ; $P = .002$) (Figure 3B), showing the potential role of miR-222-3p in UC. Next, RAW264.7 cells were transfected with Anti-miR-222-3p. As revealed in Figure 3C, miR-222-3p was downregulated (0.21 ± 0.04 ; $P = 4.34E-4$) and SOCS1 was upregulated (2.44 ± 0.33 ; $P = .002$) after transfection with Anti-miR-222-3p. Additionally, SOCS1 protein levels were increased after miR-222-3p was downregulated (3.2 ± 0.3 ; $P = 3.01E-4$) (Figure 3D), demonstrating that miR-222-3p negatively modulated SOCS1. Position 219-225 of SOCS1 3'UTR in miR-222-3p is presented in Figure 3E, and this binding site is highly conserved in multiple species (Figure 3F). Furthermore, this prediction was

demonstrated by a luciferase reporter assay. As suggested in Figure 3G, depletion of miR-222-3p increased the luciferase activity of the SOCS1 3'UTR-Wt group in 293T cells (2.21 ± 0.25 ; $P = .002$). Therefore, SOCS1 was targeted by miR-222-3p.

SOCS1 Knockdown Eliminates the Effects of miR-222-3p Inhibition in LPS-Treated RAW264.7 Cells

Reverse transcription-quantitative polymerase chain reaction and western blotting revealed that SOCS1 mRNA (0.24 ± 0.04 ; $P = 3.54E-4$) and protein (0.44 ± 0.04 ; $P = .001$) expression was decreased after transfection with Si-SOCS1 (Figure 4A and B). RAW264.7 cells were then transfected with the indicated plasmids and exposed to $1 \mu\text{g/mL}$ LPS for 24 hours. The mRNA expression and concentrations of cytokines in the culture supernatant were measured. In Figure 4C-E, miR-222-3p depletion reduced the mRNA expression of TNF- α (0.48 ± 0.05 ; $P = 3.61E-4$), IL-6 (0.42 ± 0.05 ; $P = 1.24E-4$), and IL-8 (0.35 ± 0.04 ; $P = 4.32E-5$), as well as reduced the concentrations of TNF- α (123 ± 14 ; $P = .003$), IL-6 (151 ± 16 ; $P = 1.75E-4$), and IL-8 (188 ± 20.03 ; $P = 1.41E-4$) in LPS-cultured RAW264.7 cells (Figure 4F-H). Moreover, SOCS1 depletion significantly abolished the inhibitory effects of reduced miR-222-3p on inflammation induced by LPS.

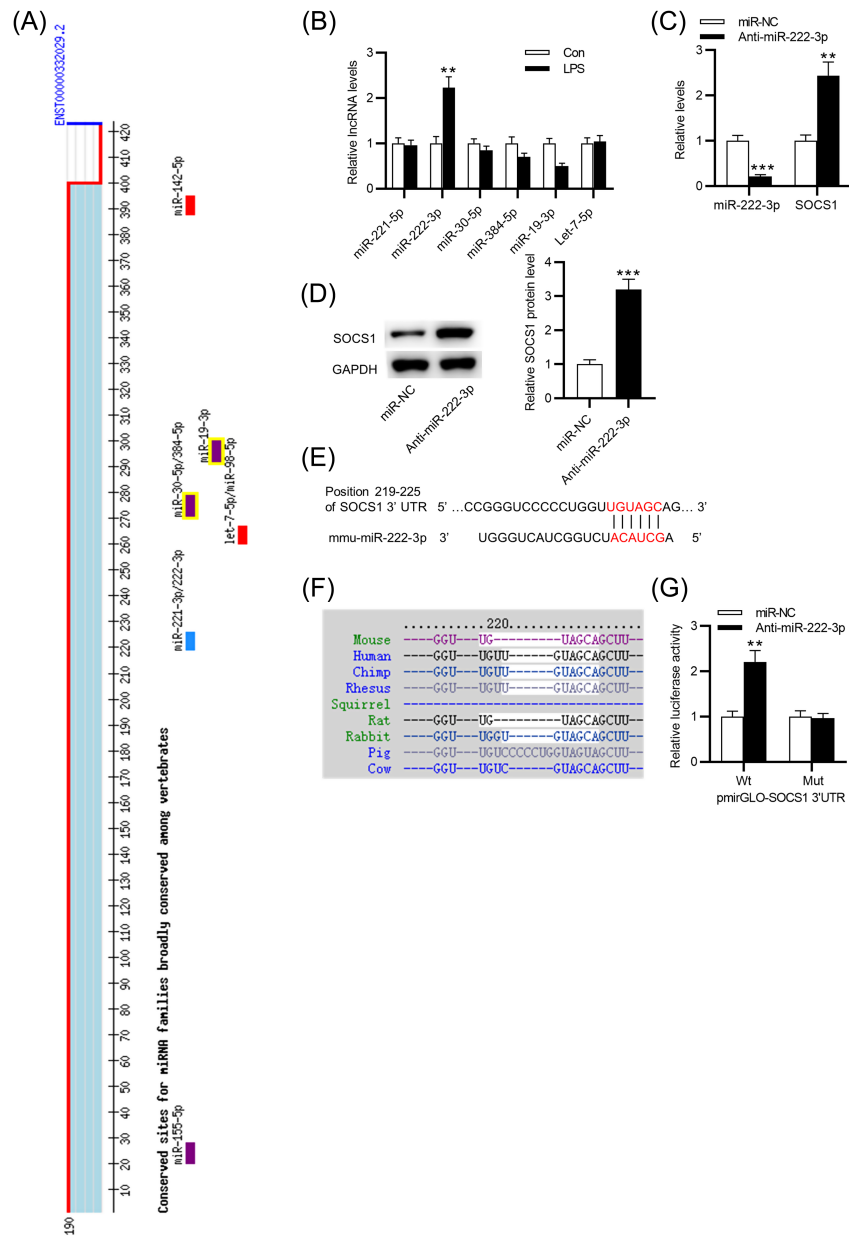


Figure 3. SOCS1 is targeted by miR-222-3p. (A) TargetScan prediction results show 9 miRNAs that have conserved sites for SOCS1. (B) The miRNA expression in LPS-treated RAW264.7 cells was measured by RT-qPCR. (C) MiR-222-3p expression and SOCS1 expression in RAW264.7 cells transfected with anti-miR-222-3p or control. (D) SOCS1 protein expression in RAW264.7 cells transfected with anti-miR-222-3p or control. (E-F) Position 219-225 of SOCS1 3'UTR in miR-222-3p is shown highly conserved in multiple species. (G) The luciferase activity of SOCS1 3'UTR in 293T cells transfected with anti-miR-222-3p or control was measured by luciferase reporter assay. ** $P < .01$, *** $P < .001$. LPS, lipopolysaccharide, RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

MiR-222-3p Activates STAT3 Signaling and Reduces VDR Expression by Targeting SOCS1

Subsequently, the effect of the miR-222-3p/SOCS1 axis in STAT3 signaling under inflammatory conditions was analyzed using western blotting. Inhibition of miR-222-3p reduced the P-JAK2 (0.48 ± 0.05 ; $P = 2.6E-4$) and

P-STAT3 (0.2 ± 0.05 ; $P = 2.32E-5$) protein expression while increased the VDR (4.18 ± 0.4 ; $P = 7.58E-6$) protein expression, and these effects were reversed after SOCS1 depletion (Figure 5A and B), indicating that silencing miR-222-3p suppresses STAT3 signaling and upregulates VDR expression by upregulating SOCS1.

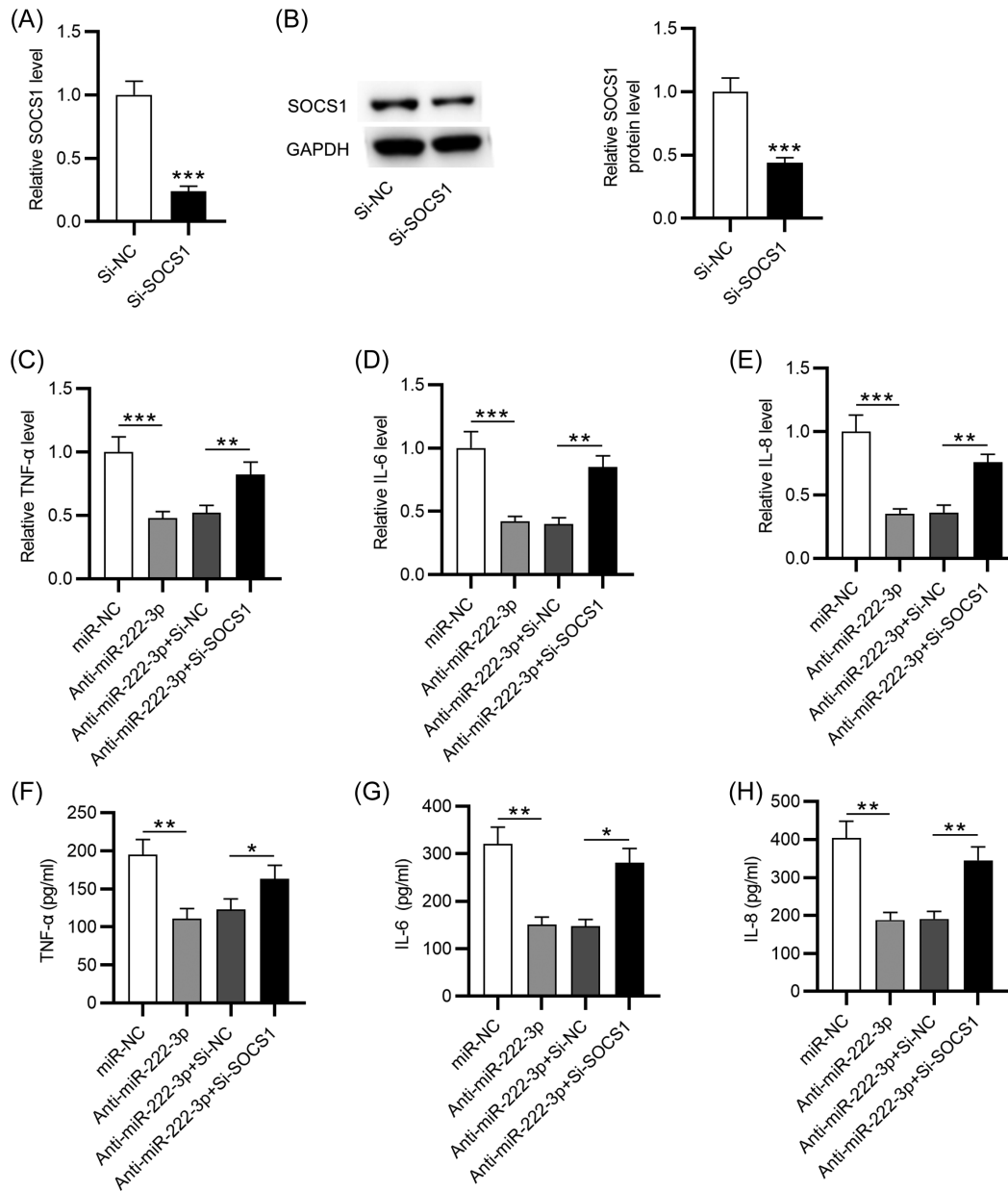


Figure 4. SOCS1 knockdown eliminates the effects of miR-222-3p inhibition. (A-B) SOCS1 mRNA and protein expression in LPS-treated RAW264.7 cells transfected with Si-SOCS1 or NC was measured by RT-qPCR and western blotting. The mRNA expression of TNF- α (C), IL-6 (D), and IL-8 (E) in LPS-treated RAW264.7 cells was detected by RT-qPCR. The concentrations of TNF- α (F), IL-6 (G), and IL-8 (H) in LPS-treated RAW264.7 cells were detected by ELISA. * $P < .05$, ** $P < .01$, *** $P < .001$. LPS, lipopolysaccharide; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

Correlations Between Molecule Expression and Endoscopic Scores (UCEIS) in UC Patients

The colonic mucosa tissue levels of miR-222-3p, SOCS1, or VDR between UC patients ($n = 15$) and healthy controls ($n = 18$) were compared. As indicated in Figure 6A-C, miR-222-3p (2.55 ± 0.48 ; $P = 5.16E-10$) expression was higher in

UC patients than in healthy controls, while SOCS1 (0.61 ± 0.07 ; $P = 2.75E-6$) and VDR (0.41 ± 0.06 ; $P = 2.5E-9$) expression was lower in UC patients. Additionally, the UCEIS revealed a significant positive correlation with miR-222-3p ($R^2 = 0.321$, $P = .03$), and negative correlation with SOCS1 ($R^2 = 0.288$, $P = .006$) and VDR ($R^2 = 0.343$, $P = .02$).

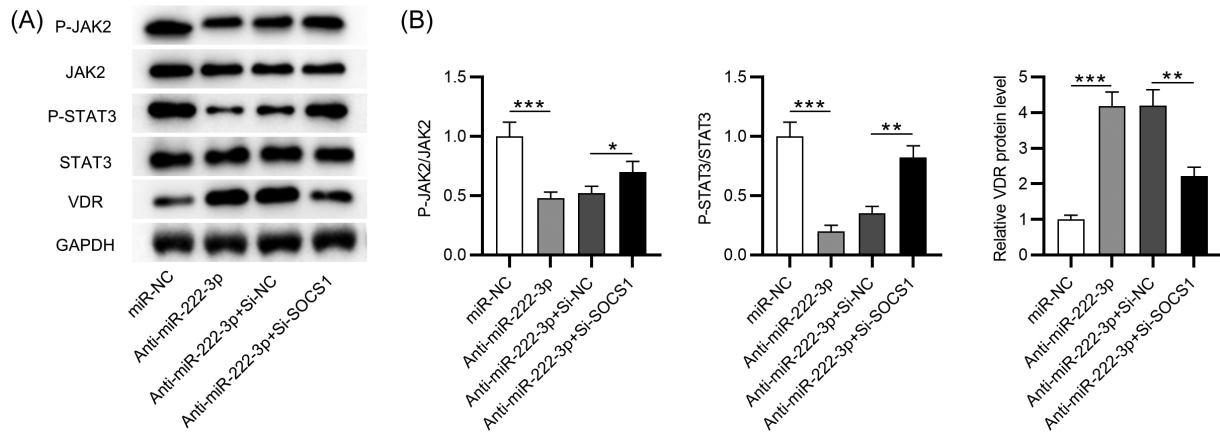


Figure 5. miR-222-3p activates STAT3 signaling and reduces VDR expression by targeting SOCS1. (A-B) The protein expression of P-JAK2/JAK2, P-STAT3/STAT3, and VDR in LPS-treated RAW264.7 cells was detected by western blotting. * $P < .05$, ** $P < .01$, *** $P < .001$.

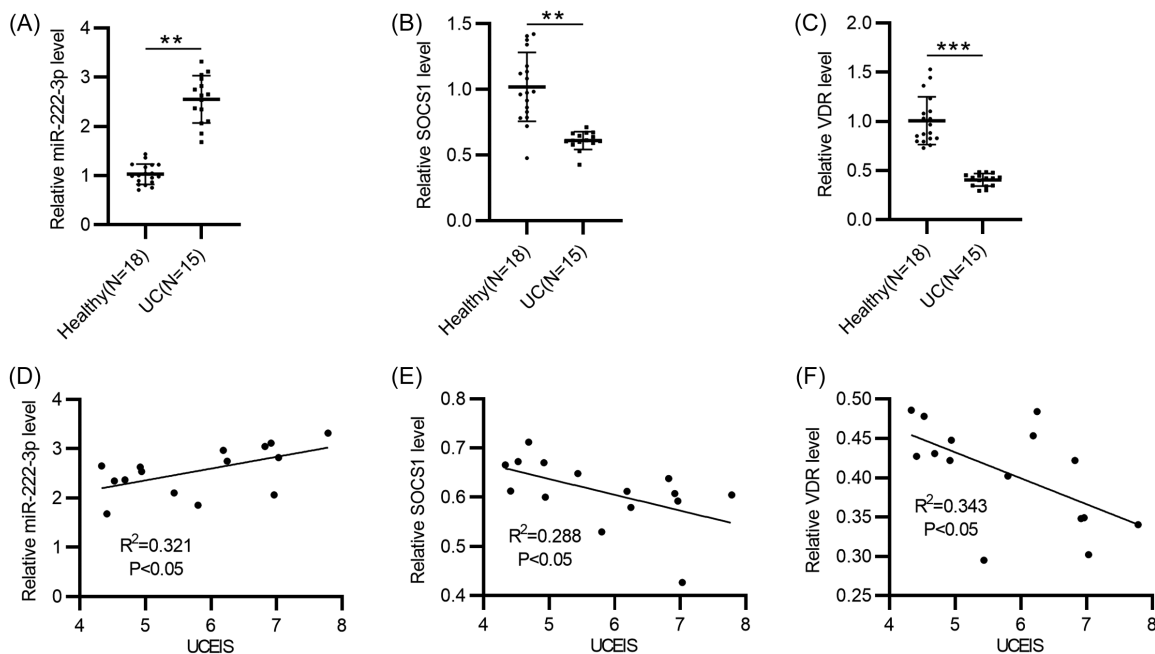


Figure 6. Correlations between molecule expression and endoscopic scores (UCEIS) in UC patients. Comparison of miR-222-3p (A), SOCS1 (B), and VDR (C) expression between healthy controls and UC patients. The levels of (D) miR-222-3p, (E) SOCS1, and (F) VDR were correlated with the endoscopic score of UC (UCEIS). Correlations were assessed by Spearman's correlation coefficient. ** $P < .01$, *** $P < .001$. UC, ulcerative colitis.

DISCUSSION

SOCS1 was found downregulated in colonic mucosa tissue samples of UC and TNF- α -stimulated human intestinal epithelial cells.²⁸ Here, we demonstrated that SOCS1 expression showed a decreased trend in murine macrophage cells after LPS treatment. However, a study indicated that the expression of SOCS1 was seen to increase in the active UC population.²⁹ We hypothesize

that different population of samples may turn out differently. Although UC can be triggered by bacteria, viruses, and other environmental factors, the inflammatory process of the intestinal mucosa is ultimately induced by soluble inflammatory mediators.³⁰ The inflammatory mediators such as IL-1 β and TNF- α play leading roles in the development of UC.³¹ Therefore, the effective reduction of inflammatory mediators in the serum

and colon tissues is a reasonable modality for UC treatment.^{32,33} Additionally, the anti-inflammatory effects of *SOCS1* in immune disease have been uncovered. *SOCS1* inhibits its downstream toll-like receptor-mediated inflammatory pathways in RAW264.7 macrophages.³⁴ *SOCS1* production is induced by resveratrol in LPS-cultured RAW264.7 macrophages to inhibit the release of TNF- α and IL-6.³⁵ Moreover, silencing *SOCS1* in the cell population that contributes to mucosal damage in IBD increases IL-6 and IL-8 production.³⁶ *SOCS1* was also shown involved in the inflammatory mechanisms in the colonic mucosa of UC.³⁷ In this study, our experiments further demonstrated that *SOCS1* inhibited the release of inflammatory cytokines in LPS-stimulated RAW264.7 macrophages. Moreover, *SOCS1* expression was downregulated in UC patients.

Emerging evidence has demonstrated that miRNAs are implicated in the pathogenesis of UC. Loss of miR-24-3p promotes epithelial cell apoptosis and impairs the recovery from intestinal inflammation.³⁸ MiR-200b-3p alleviates TNF- α -induced apoptosis and inflammation of intestinal epithelial cells and ulcerative colitis progression in rats.³⁹ Here, we identified that miR-222-3p has a binding site for *SOCS1* by bioinformatics analysis and confirmed their targeted relationship. As reported, miR-222-3p is overexpressed in inflammatory breast cancer as well as in children with mycoplasma pneumonia, acting as a promising biomarker for the diagnosis of diseases.^{40,41} The role of miR-222-3p in UC is unknown. Here, we showed high expression of miR-222-3p in LPS-treated RAW264.7 macrophages and in UC patients. Moreover, miR-222-3p silencing showed inhibitory effects on LPS-stimulated inflammation, while these effects were reversed by *SOCS1* depletion, suggesting the effects of the miR-222-3p/*SOCS1* axis in UC.

JAK/STAT components could be activated by a series of cytokines related to receptor families, including the gp130 receptor family, such as IL-6.⁴² STAT3 activation leads to pro-inflammatory activities including Th17 differentiation in lymphocytes,^{43,44} suggesting the strong plasticity of JAK/STAT pathway in the regulation of inflammatory responses. Moreover, JAK/STAT signaling activation shows a strong inflammatory involvement in the pathogenesis of UC.⁴⁵ It has been indicated that assessment of specific genotypes of JAK/STAT inhibition could be an effective treatment idea for UC.⁴⁶ The JAK/STAT inhibitor tofacitinib has been recently approved for the treatment of active UC.⁴⁵ Therefore, investigation of

JAK/STAT in UC may contribute to a better understanding of UC pathogenesis. *SOCS1* is a negative modulator of JAK/STAT signaling through degradation of JAK2 or suppression of JAK1 and JAK2.^{47,48} We examined the effects of the miR-222-3p/*SOCS1* axis on STAT3 signaling under inflammatory conditions and found that silencing miR-222-3p suppressed STAT3 signaling by upregulating *SOCS1*. Additionally, dysfunction of VDR contributes to the etiology of IBD by regulating autophagy, immune response, and mucosal permeability.⁴⁹ VDR is shown to have a protective effect on the onset or progression of IBD.⁵⁰ It was reported that STAT signaling contributes to the downregulation of VDR.¹⁹ In this investigation, miR-222-3p depletion downregulated VDR in LPS-stimulated RAW264.7 cells by upregulating *SOCS1*.

In conclusion, we revealed that miR-222-3p expression was high in UC patients, while *SOCS1* and VDR expression was low in UC patients. In addition, miR-222-3p targeted *SOCS1* to aggravate the inflammatory response by suppressing VDR and activating STAT3 signaling in UC. It may provide a new regulatory mechanism for *SOCS1* in the investigation of UC progression. However, there were some limitations in the present study. These included the small number of samples and specimens from the same area. The overall results were statistically significant, but whether miR-222-3p/*SOCS1* can be used as a reliable diagnostic indicator of UC and can be used clinically requires further research and verification. These findings should be confirmed and evaluated in prospective, large-sample, multicenter, randomized clinical trials.

Ethics Committee Approval: The study was approved by the Ethics Committee of The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – F.X., Y.Q.Y., J.N.W.; Design – F.X.; Supervision – W.X.B.; Resources – J.L.D.; Materials – Y.Q.Y.; Data Collection and/or Processing – Y.Q.Y., J.N.W.; Analysis and/or Interpretation – F.X., W.X.B.; Literature Search – F.X., Y.Q.Y.; Writing Manuscript – F.X.; Critical Review – F.X., W.X.B., J.L.D., Y.Q.Y., J.N.W.

Declaration of Interests: The authors declare that they have no competing interest.

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REFERENCES

1. Török HP, Bellon V, Konrad A, et al. Functional toll-like receptor (TLR)2 polymorphisms in the susceptibility to inflammatory bowel disease. *PLoS One*. 2017;12(4):e0175180. [\[CrossRef\]](#)
2. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2017;390(10114):2769-2778. [\[CrossRef\]](#)
3. Mak WY, Zhao M, Ng SC, Burisch J. The epidemiology of inflammatory bowel disease: east meets west. *J Gastroenterol Hepatol*. 2020;35(3):380-389. [\[CrossRef\]](#)
4. Hayashi S, Hamada T, Zaidi SF, et al. Nicotine suppresses acute colitis and colonic tumorigenesis associated with chronic colitis in mice. *Am J Physiol Gastrointest Liver Physiol*. 2014;307(10):G968-G978. [\[CrossRef\]](#)
5. Uhlig HH. Monogenic diseases associated with intestinal inflammation: implications for the understanding of inflammatory bowel disease. *Gut*. 2013;62(12):1795-1805. [\[CrossRef\]](#)
6. Hanai H, Iida T, Takeuchi K, et al. Intensive granulocyte and monocyte adsorption versus intravenous prednisolone in patients with severe ulcerative colitis: an unblinded randomised multi-centre controlled study. *Dig Liver Dis*. 2008;40(6):433-440. [\[CrossRef\]](#)
7. Arai Y, Takanashi H, Kitagawa H, Okayasu I. Involvement of interleukin-1 in the development of ulcerative colitis induced by dextran sulfate sodium in mice. *Cytokine*. 1998;10(11):890-896. [\[CrossRef\]](#)
8. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature*. 2011;474(7351):298-306. [\[CrossRef\]](#)
9. Gillen CD, Walmsley RS, Prior P, Andrews HA, Allan RN. Ulcerative colitis and Crohn's disease: a comparison of the colorectal cancer risk in extensive colitis. *Gut*. 1994;35(11):1590-1592. [\[CrossRef\]](#)
10. Karki P, Cha B, Zhang CO, et al. Microtubule-dependent mechanism of anti-inflammatory effect of SOCS1 in endothelial dysfunction and lung injury. *FASEB J*. 2021;35(4):e21388. [\[CrossRef\]](#)
11. Tanaka K, Ichiyama K, Hashimoto M, et al. Loss of suppressor of cytokine signaling 1 in helper T cells leads to defective Th17 differentiation by enhancing antagonistic effects of IFN- γ on STAT3 and Smads. *J Immunol*. 2008;180(6):3746-3756. [\[CrossRef\]](#)
12. Gendo Y, Matsumoto T, Kamiyama N, et al. Dysbiosis of the gut microbiota on the inflammatory background due to lack of suppressor of cytokine signalling-1 in mice. *Inflamm Intest Dis*. 2019;3(3):145-154. [\[CrossRef\]](#)
13. Qi H, Casalena G, Shi S, et al. Glomerular endothelial mitochondrial dysfunction is essential and characteristic of diabetic kidney disease susceptibility. *Diabetes*. 2017;66(3):763-778. [\[CrossRef\]](#)
14. Horino J, Fujimoto M, Terabe F, et al. Suppressor of cytokine signaling-1 ameliorates dextran sulfate sodium-induced colitis in mice. *Int Immunol*. 2008;20(6):753-762. [\[CrossRef\]](#)
15. Chinen T, Kobayashi T, Ogata H, et al. Suppressor of cytokine signaling-1 regulates inflammatory bowel disease in which both IFN γ and IL-4 are involved. *Gastroenterology*. 2006;130(2):373-388. [\[CrossRef\]](#)
16. Hu Q, Qin X, Qian G, et al. SOCS1 silencing can break high-dose dendritic cell immunotherapy-induced immune tolerance. *Mol Med Rep*. 2008;1(1):61-70.
17. He L, Liu T, Shi Y, et al. Gut epithelial vitamin D receptor regulates microbiota-dependent mucosal inflammation by suppressing intestinal epithelial cell apoptosis. *Endocrinology*. 2018;159(2):967-979. [\[CrossRef\]](#)
18. Liu W, Chen Y, Golan MA, et al. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *J Clin Invest*. 2013;123(9):3983-3996. [\[CrossRef\]](#)
19. Lin YW, Ren LL, Xiong H, et al. Retracted: Role of STAT3 and vitamin D receptor in EZH2-mediated invasion of human colorectal cancer. *J Pathol*. 2013;230(3):277-290. [\[CrossRef\]](#)
20. Kalla R, Ventham NT, Kennedy NA, et al. MicroRNAs: new players in IBD. *Gut*. 2015;64(3):504-517. [\[CrossRef\]](#)
21. Wu F, Zikusoka M, Trindade A, et al. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 α . *Gastroenterology*. 2008;135(5):1624-1635.e24. [\[CrossRef\]](#)
22. Shen N, Huang X, Li J. Upregulation of miR-129-5p affects laryngeal cancer cell proliferation, invasiveness, and migration by affecting STAT3 expression. *Tumour Biol*. 2016;37(2):1789-1796. [\[CrossRef\]](#)
23. Yang Y, Ma Y, Shi C, et al. Overexpression of miR-21 in patients with ulcerative colitis impairs intestinal epithelial barrier function through targeting the Rho GTPase RhoB. *Biochem Biophys Res Commun*. 2013;434(4):746-752. [\[CrossRef\]](#)
24. Lewis A, Felice C, Kumagai T, et al. The miR-200 family is increased in dysplastic lesions in ulcerative colitis patients. *PLoS One*. 2017;12(3):e0173664. [\[CrossRef\]](#)
25. Wang H, Deng Z, Chen X, et al. Downregulation of miR-222-3p reverses doxorubicin-resistance in LoVo cells Through upregulating forkhead box protein P2 (FOXP2) protein. *Med Sci Monit*. 2019;25:2169-2178. [\[CrossRef\]](#)
26. Zhang P, Yu J, Gui Y, Sun C, Han W. Inhibition of miRNA-222-3p relieves staphylococcal enterotoxin B-induced liver inflammatory injury by upregulating suppressors of cytokine signaling 1. *Yonsei Med J*. 2019;60(11):1093-1102. [\[CrossRef\]](#)
27. Travis SP, Schnell D, Krzeski P, et al. Reliability and initial validation of the ulcerative colitis endoscopic index of severity. *Gastroenterology*. 2013;145(5):987-995. [\[CrossRef\]](#)
28. Han J, Li Y, Zhang H, et al. MicroRNA-142-5p facilitates the pathogenesis of ulcerative colitis by regulating SOCS1. *Int J Clin Exp Pathol*. 2018;11(12):5735-5744.
29. Fernandes P, MacSharry J, Darby T, et al. Differential expression of key regulators of toll-like receptors in ulcerative colitis and Crohn's disease: a role for Tollip and peroxisome proliferator-activated receptor gamma? *Clin Exp Immunol*. 2016;183(3):358-368. [\[CrossRef\]](#)
30. Wei YY, Fan YM, Ga Y, Zhang YN, Han JC, Hao ZH. Shaoyao decoction attenuates DSS-induced ulcerative colitis, macrophage and NLRP3 inflammasome activation through the MKP1/NF- κ B pathway. *Phytomedicine*. 2021;92:153743. [\[CrossRef\]](#)
31. Guazelli CFS, Fattori V, Ferraz CR, et al. Antioxidant and anti-inflammatory effects of hesperidin methyl chalcone in experimental ulcerative colitis. *Chem Biol Interact*. 2021;333:109315. [\[CrossRef\]](#)
32. Baydi Z, Limami Y, Khalki L, et al. An update of research animal models of inflammatory bowel disease. *ScientificWorldJournal*. 2021;2021:7479540. [\[CrossRef\]](#)
33. Ryzhakov G, Almuttaqi H, Corbin AL, et al. Defactinib inhibits PYK2 phosphorylation of IRF5 and reduces intestinal inflammation. *Nat Commun*. 2021;12(1):6702. [\[CrossRef\]](#)
34. Zahoor A, Yang C, Yang Y, et al. MerTK negatively regulates Staphylococcus aureus induced inflammatory response via SOCS1/SOCS3 and Mal. *Immunobiology*. 2020;225(4):151960. [\[CrossRef\]](#)
35. Ma C, Wang Y, Shen A, Cai W. Resveratrol upregulates SOCS1 production by lipopolysaccharide-stimulated RAW264.7

- macrophages by inhibiting miR-155. *Int J Mol Med*. 2017;39(1):231-237. [\[CrossRef\]](#)
36. Pathak S, Grillo AR, Scarpa M, et al. MiR-155 modulates the inflammatory phenotype of intestinal myofibroblasts by targeting SOCS1 in ulcerative colitis. *Exp Mol Med*. 2015;47(5):e164. [\[CrossRef\]](#)
37. Christophi GP, Rong R, Holtzapfel PG, Massa PT, Landas SK. Immune markers and differential signaling networks in ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis*. 2012;18(12):2342-2356. [\[CrossRef\]](#)
38. Soroosh A, Fang K, Hoffman JM, et al. Loss of miR-24-3p promotes epithelial cell apoptosis and impairs the recovery from intestinal inflammation. *Cell Death Dis*. 2021;13(1):8. [\[CrossRef\]](#)
39. Zheng C, Lu T, Fan Z. miR-200b-3p alleviates TNF- α -induced apoptosis and inflammation of intestinal epithelial cells and ulcerative colitis progression in rats via negatively regulating KHDRBS1. *Cytotechnology*. 2021;73(5):727-743. [\[CrossRef\]](#)
40. Ahmed SH, Espinoza-Sánchez NA, El-Damen A, et al. Small extracellular vesicle-encapsulated miR-181b-5p, miR-222-3p and let-7a-5p: next generation plasma biopsy-based diagnostic biomarkers for inflammatory breast cancer. *PLoS One*. 2021;16(4):e0250642. [\[CrossRef\]](#)
41. Chu C, Lei X, Li Y, et al. High expression of miR-222-3p in children with *Mycoplasma pneumoniae* pneumonia. *Ital J Pediatr*. 2019;45(1):163. [\[CrossRef\]](#)
42. Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene*. 2002;285(1-2):1-24. [\[CrossRef\]](#)
43. Durant L, Watford WT, Ramos HL, et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. *Immunity*. 2010;32(5):605-615. [\[CrossRef\]](#)
44. Lu D, Liu L, Ji X, et al. The phosphatase DUSP2 controls the activity of the transcription activator STAT3 and regulates TH17 differentiation. *Nat Immunol*. 2015;16(12):1263-1273. [\[CrossRef\]](#)
45. Cordes F, Lenker E, Weinhage T, et al. Impaired IFN- γ -dependent STAT3 activation is associated with dysregulation of regulatory and inflammatory signaling in monocytes of ulcerative colitis patients. *Inflamm Bowel Dis*. 2021;27(6):887-901. [\[CrossRef\]](#)
46. Cordes F, Foell D, Ding JN, Varga G, Bettenworth D. Differential regulation of JAK/STAT-signaling in patients with ulcerative colitis and Crohn's disease. *World J Gastroenterol*. 2020;26(28):4055-4075. [\[CrossRef\]](#)
47. Liao NPD, Laktyushin A, Lucet IS, et al. The molecular basis of JAK/STAT inhibition by SOCS1. *Nat Commun*. 2018;9(1):1558. [\[CrossRef\]](#)
48. Beaurivage C, Champagne A, Tobelaim WS, Pomerleau V, Menendez A, Saucier C. SOCS1 in cancer: an oncogene and a tumor suppressor. *Cytokine*. 2016;82:87-94. [\[CrossRef\]](#)
49. Chatterjee I, Zhang Y, Zhang J, Lu R, Xia Y, Sun J. Overexpression of vitamin D receptor in intestinal epithelia protects against colitis via upregulating tight junction protein Claudin 15. *J Crohns Colitis*. 2021;15(10):1720-1736. [\[CrossRef\]](#)
50. Xue G, Gao R, Liu Z, et al. Vitamin D/VDR signaling inhibits colitis by suppressing HIF-1 α activation in colonic epithelial cells. *Am J Physiol Gastrointest Liver Physiol*. 2021;320(5):G837-G846. [\[CrossRef\]](#)