Am J Transl Res 2020;12(11):7439-7448
www.ajtr.org /ISSN:1943-8141/AJTR0119468

Original Article

Downregulation of SENP1 suppresses LPS-induced macrophage inflammation by elevating Sp3 SUMOylation and disturbing Sp3-NF-κB interaction

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Received August 3, 2020; Accepted October 14, 2020; Epub November 15, 2020; Published November 30, 2020

Abstract: Macrophages participate in all stages of sepsis and affect immune homeostasis and inflammatory processes. Small ubiquitin-like modifier (SUMO) protease SENP1 plays an important role in cellular inflammation by regulating proteins in SUMOylation. However, the roles and related mechanisms of SENP1 in macrophage inflammation during sepsis are largely unknown. In the present study, SENP1 expression was significantly promoted in lipopolysaccharide (LPS)-induced RAW 264.7 cells; furthermore, the knock down of SENP1 reduced the expression of inflammatory cytokines interleukin-6 and tumor necrosis factor-α. Momordin Ic (MC), a new type of SENP1 inhibitor, reduces LPS-induced cellular inflammation by depressing SENP1 expression. Moreover, the effect of SENP1 on LPS-induced inflammatory response was dependent on SENP1-Sp3 interaction and the promotion of Sp3 expression via Sp3 deSUMOylation. Furthermore, MC-depressed Sp3 expression disturbed Sp3-nuclear factor (NF)-κB interaction and then alleviated LPS-induced cellular inflammation. These results suggest that SENP1 promotes LPS-induced macrophage inflammation by promoting Sp3 expression via deSUMOylation and Sp3-NF-κB interaction in sepsis.

Keywords: Sp3, SENP1, macrophage, inflammation, sepsis

Introduction

Sepsis is a life-threatening organ dysfunction caused by an abnormal immune inflammatory response triggered by infection [1]. It is a common complication of acute critical illness, such as infections, wounds, burns, and shock. Septic shock is a condition that refers to sepsis combined with severe circulatory dysfunction and cell metabolism disorders [2]. Sepsis is associated with a high mortality rate and has become a major cause of death in intensive care units worldwide [3]. The main reason for the high mortality rate is that the pathogenesis of sepsis is unclear, resulting in the lack of a specific and effective treatment. Therefore, we systematically explored the pathophysiological mechanism of sepsis occurrence and development to find novel methods for effectively preventing sepsis and reducing the associated mortality. This is an urgent need in today's international critical care community.

SUMOylation is an important dynamic process of post-translational modification of protein molecules [4]. It is modified by a small ubiquitin-like modifier (SUMO) protein. SUMOylation is involved in various cellular processes, such as protein stability and metabolism, nuclear-cytoplasmic transport, transcriptional regulation, apoptosis, inflammation, and cell cycle progression [5, 6]. SENP1, a member of SUMO-specific proteases family, is a deSUMOylating protease that deconjugates many SUMOylated proteins [5, 7]. Several studies have shown that SENP1 is involved in the inflammatory response process of various cells. Macrophages lacking SENP1 show defects in interferon-γ signaling and M1 macrophage activation [8]. Inflammation-induced SENP1 promotes the deSUMOylation of GATA2 and IκBα in endothelial cells, leading to increased GATA2 stability and nuclear factor (NF)-κB activity, resulting in enhanced endothelial activation and inflammation [9]. A study by Zhang et al. showed that SENP1 3'UTR over-

expression activated TGFBR2/Smad signaling responsible for sepsis [10]. Recent studies have found that Momordin Ic (MC), a new type of natural SENP1 inhibitor, can inhibit the proliferation of prostate cancer cells [11]. However, it is unclear whether SENP1, a deSUMOylating protease, regulates the inflammatory response process in sepsis.

Sp3 belongs to the specificity protein family of transcription factors, which control the expression of genes implicated in different processes involved in cell cycle, inflammation response, hormone induction, and housekeeping [12]. Sp3, Sp1, and Sp4, which interact with the same GC/GT motifs within promoters, share a highly conserved zinc finger deoxyribonucleic-binding domain [13]. A primary distinct feature of Sp3 compared with that of other members is its ability to both activate and repress transcription [14]. Research has revealed that Sp3 is post-translationally modified by SUMO, and the SUMOylated Sp3 has been shown to play a repressor function [15]. However, it remains unclear whether SENP1 regulates Sp3 deSUMOylation. In addition, one study has reported that Sp3 is closely related to inflammation. Sp3-regulated interleukin (IL)-4 induces inflammation-associated metastasis during the development of colorectal cancer [16]. Under inflammatory stimulation, NF-κB and Sp3 are required for tumor necrosis factor (TNF)-α expression [17]. Sp3-NF-κB interaction activates inflammatory signals and reduces fibroblast growth factor-10 expression [18]. However, the role of Sp3 in regulating inflammatory response in sepsis remains unknown.

Macrophages are important innate immune cells. Owing to their immunoregulatory functions and ubiquitous presence, they play an important role in immune homeostasis and inflammation as well as in the pathogenesis of sepsis and tissue damage related to sepsis.

Materials and methods

Cell culture

Cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Gaithersburg, MD) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco), 100 U/ml penicillin (Gibco), and 100 mg/ml streptomycin (Gibco) cultured at 37°C in a humidified and 5% CO_2 atmosphere.

LPS treatment

RAW 264.7 macrophages were plated into 6-well plates and cultured for 24 h to 60%-70% confluence. Then the cells were incubated with 1 μg/ml *Escherichia coli* LPS (L2143; Sigma-Aldrich) or phosphate-buffered saline solution for 12, 24, and 48 h. Afterwards, cells were washed and collected for further real-time polymerase chain reaction (PCR) or western blot analysis.

Transfection

Transfection of cells was performed using Lipofectamine 2000 (Invitrogen, Waltham, MA) according to the manufacturer's protocol. siSENP1 and control siRNA (sicon) were purchased from GenePharma Co., Ltd (Shanghai, China). The sequence of siSENP1 was as follows: siSENP1-1#-F: GCGGGAACAUUCAGUACAUTT; siSENP1-1#-R: AUGUACUGAAUGUUCCCGCTT; siSENP1-2#-F: CCAGGACCUUUCCUAUUUATT; siSENP1-2#-R: UAAAUAGGAAAGGUCCUGGTT; and siSp3-F: CCGACGGACAUUUGAUAAATT; siSp3-R: UUUAUCAAAUGUCCGUCGGTT. Sp3 overexpression Plasmid was purchased from GENEWIZ Company (Suzhou, China). After 24-48 h of transfection, the cells were harvested and lysed for western blotting and the total ribonucleic acid (RNA) was extracted for quantitative reverse transcription-PCR (RT-qPCR).

Cell counting Kit-8 assays

RAW 264.7 macrophages were seeded into 96-well plates (2×10^4 cells/well) for 24 h. Then 5, 10, 20, and 40 μM MC was added to the mediate for 24 h. The viability of RAW 264.7 cells was determined using cell counting kit-8 (CCK-8) assays. After cultivation, 10 μl CCK-8 (Beibo, China) was added to each well, and the 96-well plates were incubated at 37°C in a humidified 5% CO_2 atmosphere for 2.5 h. Absorbance was then read at 450 nm on a microplate reader (ThermoFisher Scientific).

RT-PCR

Total RNA of RAW 264.7 cells was extracted and purified with QIAzol Lysis Reagent (79306;

QIAGEN, Düsseldorf, Germany) following the manufacturer's protocol. Next, complementary deoxyribonucleic acid was synthesized using the M-MLV First Strand Kit (Life Technologies, Frederick, MD). The Platinum SYBR Green qPCR Super Mix UDG Kit (Invitrogen) was used for the RT-qPCR of mRNAs. The real-time PCR experiments were performed on a CFX96™ Real-Time System (Bio-Rad, Philadelphia, PA) with the following primers: SENP1-F: GGTTCCGGTTCGGACTTTGT; SENP1-R: GGTCTTTCGGGTTTCGAGGT; IL-6-F: TCCACAAGCGCCTTCGGTC; IL-6-R: GGTCAGGGGTGGTTATTGCAT; TNF-α-F: CACCACTTCGAAACCTGGGA; TNF-α-R: AGGAAGGCCTAAGGTCCACT; GAPDH-F: AGGTCGGTGTGAACGGATTTG; and GAPDH-R: GGGGTCGTTGATGGCAACA. The expression levels were normalized to the glyceraldehyde 3-phosphate dehydrogenase gene using the $2^{-\Delta\Delta Ct}$ method.

Western blotting

Proteins were extracted from the cultured RAW 264.7 cells with RIPA lysis buffer. The proteins were then separated using 8%-10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to a polyvinylidene difluoride membrane (Millipore, Chicago, IL). After blocking in 5% milk in Tris-buffered saline, the membranes were incubated with primary antibodies at 4°C overnight. Then the blots were detected via enhanced chemiluminescence (Fuazon Fx; Vilber Lourmat, Collégien, France). Antibodies used are as follows: anti-SENP1 (1:1000, ab225887), anti-Sp3 (1:500, 26584-1-AP), anti-Sumo 1 (1:1000, ab225887), and anti-β-actin (1:1000, sc-47778). Images were captured and processed using FusionCapt Advance Fx5 software (Vilber Lourmat). All experiments were replicated 3 times independently.

Coimmunoprecipitation assay

Five microgram of antibodies and protein A-agarose were added to RAW 264.7 cell lysates and incubated at 4°C overnight. Then protein A-agarose-antigen-antibody complexes were collected via centrifugation. After washing with IPH buffer and then extensively washing with lysis buffer, the immunoprecipitates were resolved via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by western blot analysis.

Proximity ligation assay

The proximity ligation assay (PLA) was performed as described previously [19]. Briefly, cells were seeded into 6-well chamber slides and cultured for 24 h. Then the slides were fixed using 4% paraformaldehyde. Anti-SENP1 and anti-Sp3 antibodies were used to stain the slides. Rabbit PLUS and Mouse MINUS Duolink in situ PLA kits were used to detect the interaction between the two proteins following the manufacturer's protocol. Fluorescence was detected using a fluorescence microscope.

LPS-induced sepsis mouse model

To induce endotoxin shock, male C57BL/6 mice aged 8-10 weeks were administered LPS (Sigma-Aldrich) at a dose of 50 mg/kg body weight via intraperitoneal injections. The control group mice were administered the same volume of normal saline. The treatment group mice received intraperitoneal injections of MC at 20 mg/kg immediately after LPS treatment, whereas the control group mice received dimethyl sulfoxide (DMSO) injections.

Gene interaction networks

The gene interaction networks were identified using the GeneMANIA web server (http://www.genemania.org/) with default parameters.

Statistical analysis

Data are presented as means ± standard error of the mean. Between-group differences were analyzed using Student's *t*-test. Spearman's correlation analysis was used to analyze the correlation between two genes. *P* values < 0.05 were considered significant.

Results

Downregulation of SENP1 inhibits LPS-induced inflammatory response of macrophages

Previous studies have shown that SENP1 is involved in the inflammatory response process of various cells. However, the role of SENP1 in the macrophages during sepsis is unclear. First, we detected SENP1 expression in LPS-stimulated RAW 264.7 cells and found that LPS promoted SENP1 expression in a

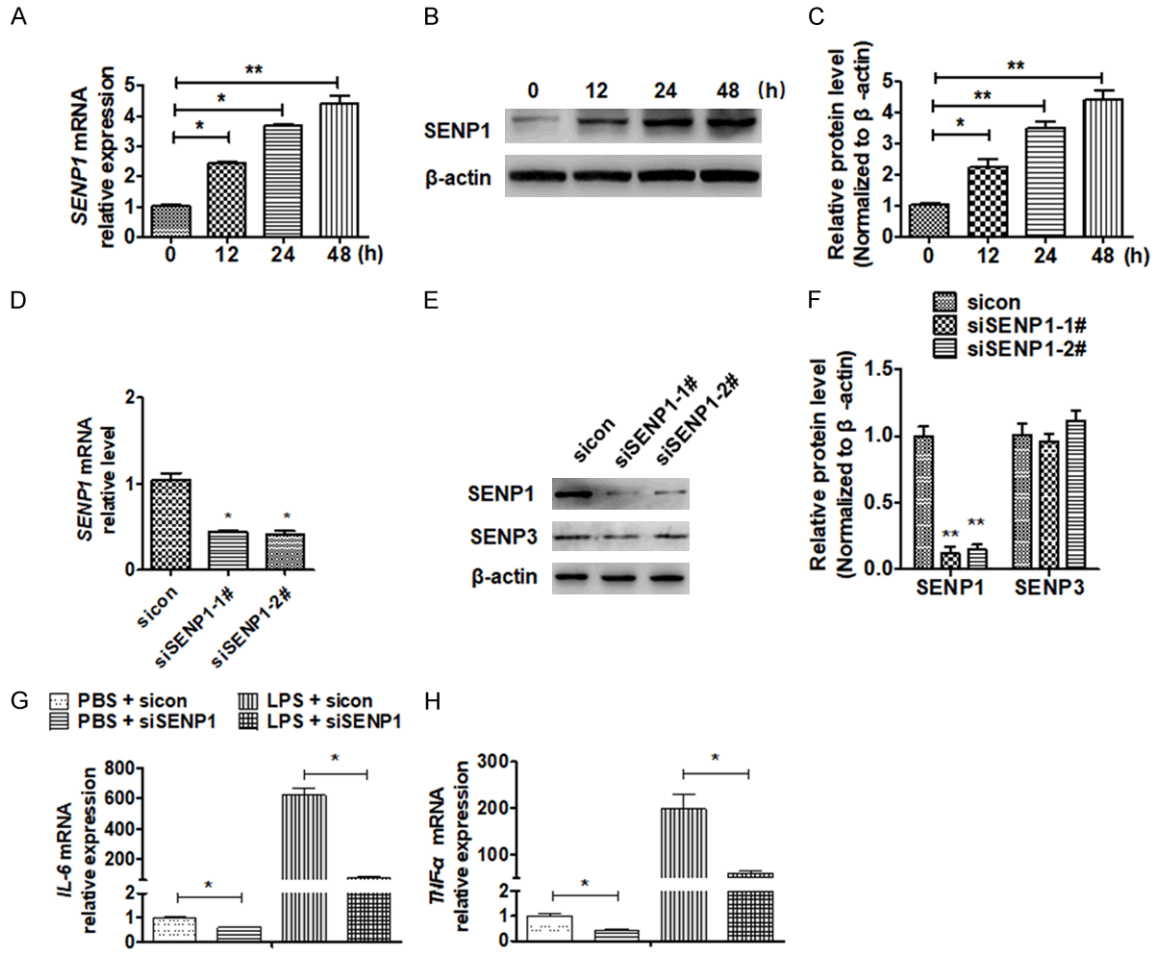

Figure 1. The knock down of SENP1 inhibits the LPS-induced inflammatory response in RAW 264.7 cells. (A) RAW 264.7 cells were treated with 1 μM of LPS for 0, 12, 24, and 48 h. Using RT-qPCR, the SENP1 mRNA expression was then detected. *P < 0.05, **P < 0.01 versus 0 h. (B) RAW 264.7 cells were treated as above, and western blot analysis was used to detect the protein level of SENP1. (C) Quantitative analysis of (B). Data are expressed as mean ± SEM from three independent experiments. *P < 0.05, **P < 0.01 versus corresponding control. (D-F) Cells were transfected with siSENP1 (siSENP1-1# or siSENP1-2#) and control siRNA (sicon), following which RT-qPCR (D) or western blot analysis (E) were used to detect SENP1 expression. *P < 0.05 versus sicon. (G and H) RT-qPCR detected the expression of inflammatory factors in LPS-induced RAW 264.7 cells after siSENP1 transfection for 24 h. All data are expressed as mean ± SEM. Comparisons of quantitative data were done with one-way analyses of variance and Student's *t* tests.

time-dependent manner (**Figure 1A-C**). To determine the function of SENP1 in macrophage inflammation, we designed two pairs of siRNAs to deplete SENP1 expression. We found that both siSENP1 siRNAs can reduce the messenger RNA or protein level of SENP1 (**Figure 1C**, **1D** and **1F**). Next, we used RT-qPCR to detect proinflammatory cytokine expression in LPS-induced RAW 264.7 cells after the knock down of SENP1. The results showed that the depletion of SENP1 reduced IL-6 and TNF-α expression, irrespective of whether LPS was stimulated (**Figure 1G** and **1H**).

MC reduces LPS-induced cellular inflammation by depressing SENP1 expression

Previous studies have shown that MC can inhibit cellular inflammatory response [20, 21] and that SENP1 is a target of MC [11]. First, we evaluated the toxicity of MC and determined the survival rates of RAW 264.7 cells via CCK-8 assays. MC exhibited a cytotoxic effect on RAW 264.7 cells at 40 μM but not at other concentrations (5, 10, and 20 μM) (**Figure 1A** and **1B**). To detect the anti-inflammatory function of MC, LPS-treated and untreated RAW 264.7 cells were incubated with different con-

centrations of MC (10 and 20 μM). The results showed that the LPS-induced inflammation in RAW 264.7 cells was inhibited by MC in a concentration-dependent manner (**Figure 2C** and **2D**). These results indicated that MCs play an anti-inflammatory role in LPS-treated RAW 264.7 cells. Because MC is a natural inhibitor of SENP1 [11], we aimed to determine whether MC exerts its anti-inflammatory effect via SENP1 expression regulation. MC treatment significantly reduced the protein level of SENP1 but not of SENP1 in RAW 264.7 cells (**Figure 2E** and **2F**). To determine whether MC inhibits LPS-induced RAW 264.7 inflammatory response while depressing SENP1 expression, LPS-treated and untreated RAW 264.7 cells were incubated with different concentrations of MC. Our results showed that, under natural conditions, 20 μM of MC can depress SENP1 expression. The mRNA and protein levels of SENP1 were significantly increased in LPS-stimulated RAW 264.7 cells. However, this upregulation of SENP1 could be depressed by MC in a concentration-dependent manner (**Figure 2G-I**). To determine whether MC regulates LPS-induced RAW 264.7 inflammation, we knocked down SENP1 before treatment with MC. RT-qPCR results showed that both MC and siSENP1 can reduce LPS-stimulated inflammation, but the depletion of SENP1 and treatment with MC further inhibit IL-6 and TNF-α expression (**Figure 2J** and **2K**). To further investigate the role of MC in sepsis, a bacterial endotoxemia model was used. LPS induced severe sepsis that resulted in a mortality rate of up to 70% within 98 h in DMSO-treated mice (70% at 96 h; 100% at 132 h) (**Figure 2L**). MC-treated mice with LPS had a significant reduction in the mortality rate (40% at 98 h; 70% at 132 h), whereas no mortality was observed in mice subjected to sham operation. We then compared the changes in proinflammatory cytokine levels in peritoneal macrophages. LPS-induced sepsis increased the mRNA levels of proinflammatory cytokines including TNF-α and IL-6 in control mice, whereas MC-treated septic mice exhibited much lower mRNA levels of TNF-α and IL-6 (**Figure 2M** and **2N**). These results suggest that MC plays an anti-inflammatory role in LPS-induced macrophages *in vivo* and *in vitro*.

SENP1-Sp3 interaction promotes LPS-induced cellular inflammation in RAW 264.7 cells

SENP1 deconjugates a large number of SUMOylated proteins by interacting with them [7, 22]. To investigate the role of SENP1 in anti-inflammatory processes, we used GeneMANIA (genemania.org) to construct interaction networks to analyze SENP1 associated with its interaction proteins; the findings revealed numerous proteins directly or indirectly related to SENP1 (**Figure 3A**). Transcription factor Sp3 captured our interest. The GeneMANIA network showed that Sp3 formed a direct network with SENP1. To further confirm the relationship between Sp3 and SENP1, we performed PLA with SENP1 and the Sp3 antibody. PLA demonstrated a SENP1-Sp3 interaction (**Figure 3B**). Coimmunoprecipitation (CoIP) of endogenous proteins further confirmed the SENP1-Sp3 interaction. In particular, LPS stimulation facilitated this interaction (**Figure 3C**). To understand whether SENP1-Sp3 interactions can reduce Sp3 SUMOylation, we knocked down SENP1 and detected the total SUMOylated protein and SUMOylated Sp3 levels. Western blot analysis showed that the depletion of SENP1 increased the total SUMOylated protein and SUMOylated Sp3 levels but reduced the total Sp3 protein levels. These results indicate that SENP1 interacts with Sp3 and regulates its expression via Sp3 deSUMOylation. Next, we suspected that SENP1 interacts with Sp3 and plays a role in LPS-induced inflammation in RAW 264.7 cells. RT-qPCR demonstrated that Sp3 overexpression increased the mRNA expression of IL-6 and TNF-α. However, this effect was reversed by simultaneously knocking down SENP1 (**Figure 3E** and **3F**). Collectively, these data establish the role of SENP1-Sp3 interaction in the regulation of RAW 264.7 inflammation.

MC inhibits Sp3 expression and interacts with NF-κB to repress cellular inflammation

To investigate whether MC participated in SENP1-regulated Sp3 expression, we treated RAW 264.7 cells with LPS and then incubated them with MC. Western blot analysis showed that LPS promoted Sp3 and NF-κB expression but reduced IκB protein levels. However, these effects could be simultaneously reversed via MC treatment in LPS-stimulated RAW 264.7 cells (**Figure 4A**). Research has shown that Sp3 promotes cellular inflammatory cytokine expression by interacting with NF-κB and enhancing its transcription [18]. This verifies the hypothesis whereby we performed CoIP in

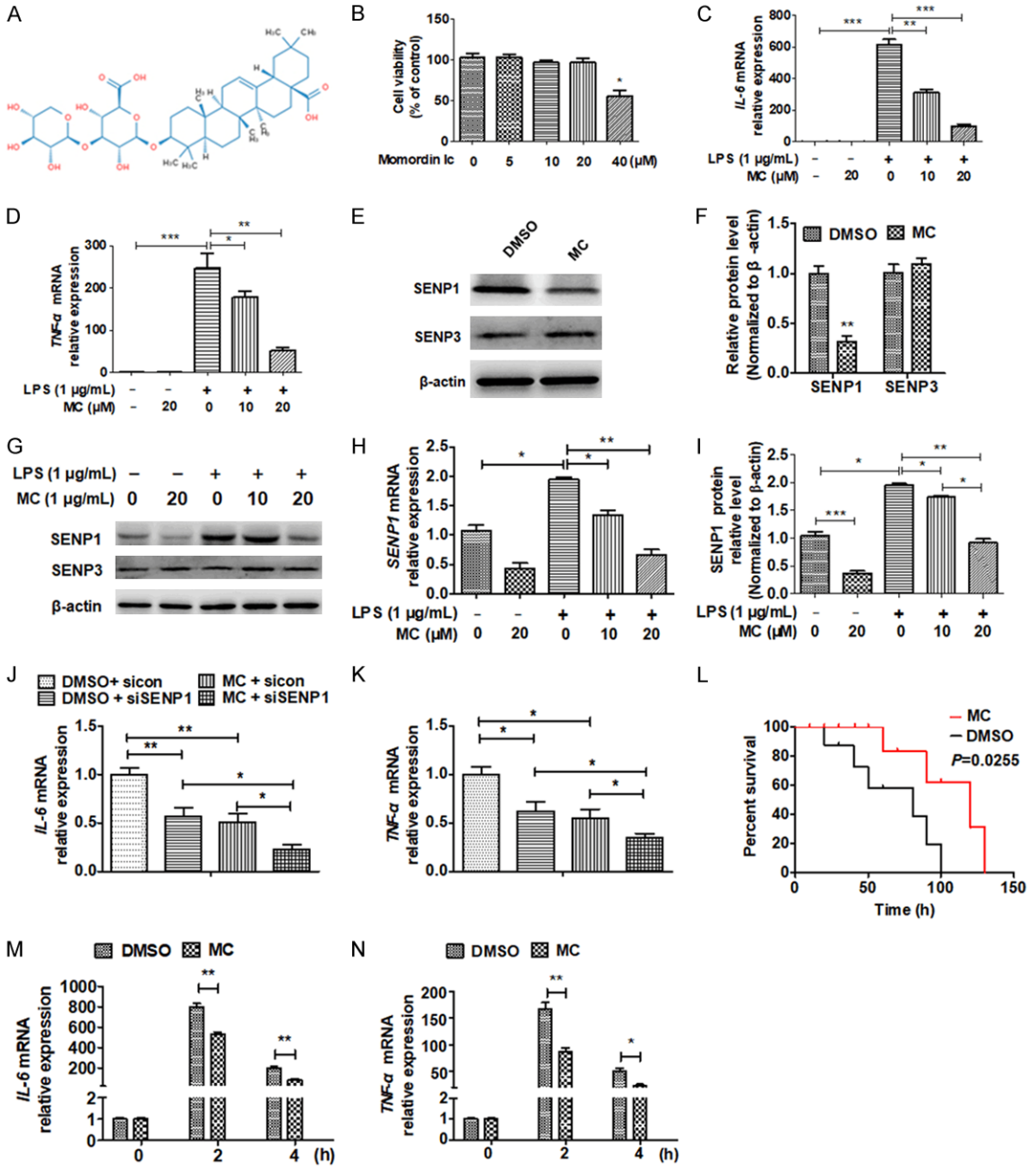

Figure 2. MC downregulates SENP1 expression and inhibits LPS-induced cellular inflammation. (A) Chemical structure of MC. (B) Effect of MC on cell viability. EC were treated with different concentrations of MC, and CCK-8 was used to detect the cell viability. **P* < 0.05 versus 0 μM. (C and D) RT-qPCR detected the expression of inflammatory factors in LPS-induced RAW 264.7 cells with or without MC treatment at different concentrations. (E and F) RAW 264.7 cells were treated with 20 μM MC for 48 h, and the SENP1 protein level was detected via western blotting. ***P* < 0.01 versus corresponding control. (G-I) western blot or RT-qPCR were used to detect the protein (G and H) or SENP1 mRNA (I) level in ECs with or without LPS and MC treatment. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus corresponding control. (J and K) RT-qPCR was used to detect IL-6 or TNF-α expression in MC-treated ECs after transfection with siSENP1 or control siRNA. **P* < 0.05, ***P* < 0.01 versus corresponding control. All data are expressed as mean ± SEM. (L) Mortality among MC- and DMSO-treated mice was assessed daily. Survival rates were calculated according to the Kaplan-Meier method. Survival analysis was performed using log-rank test (n = 10; ***P* < 0.01). (M and N) mRNA levels of cytokines in peritoneal macrophages obtained from MC- and DMSO-treated mice were measured at the indicated time point. Data are expressed as mean ± SEM and were analyzed using Student's *t*-test (n = 6; **P* < 0.05, ***P* < 0.01). Comparisons of quantitative data were done with one-way analyses of variance and Student's *t* tests.

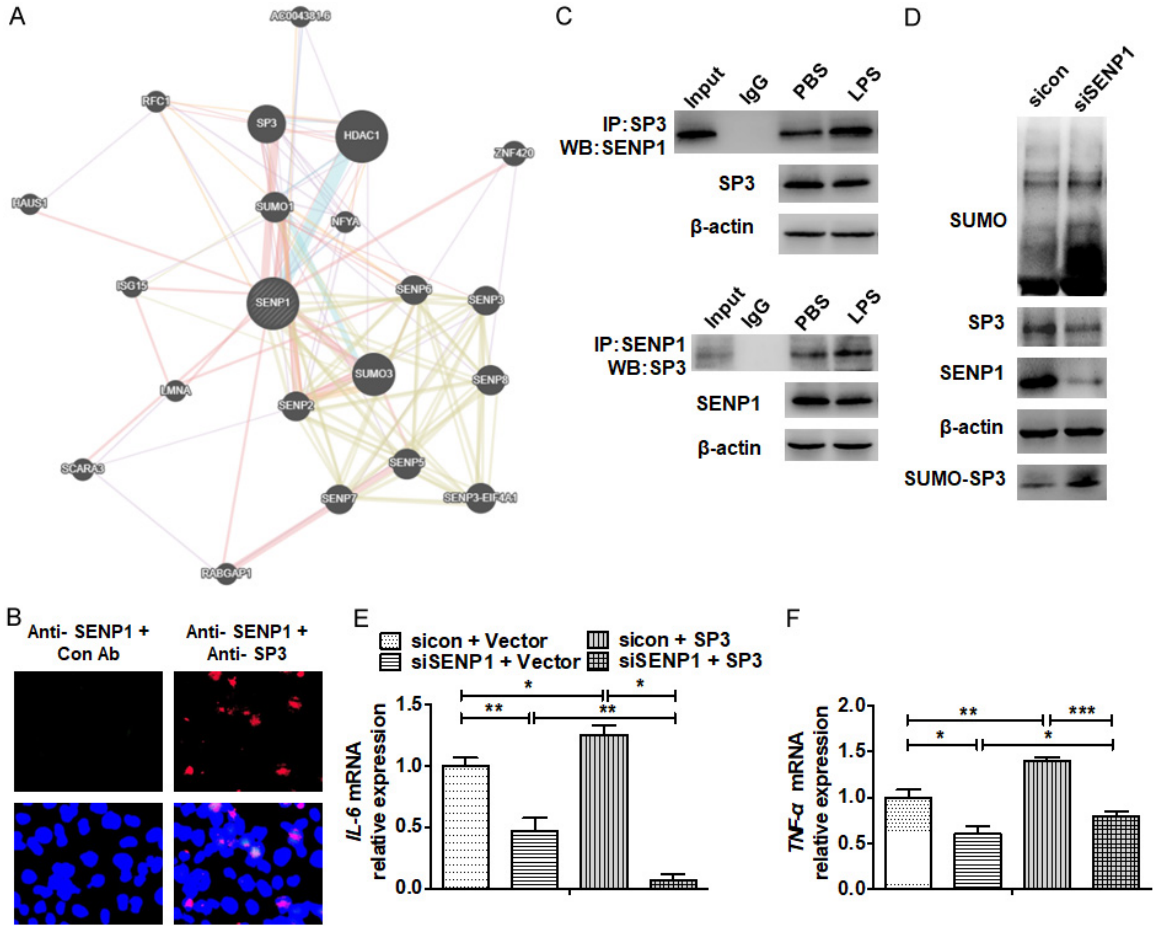

Figure 3. SENP1 interacts with Sp3 to promote LPS-induced cellular inflammation in ECs. A. GeneMANIA was used to analyze the network of proteins according to the databases. All nodes were connected and related to SENP1. B. PLA was used to test the interaction between SENP1 and Sp3. Red indicates PLA-positive cells; blue represents the nuclei stained by DAPI. C. EC cells were treated with LPS or PBS, and CoIP analysis was used to detect the interaction between SENP1 and Sp3. D. EC cells were transfected with siSENP1 or control siRNA, and western blot analysis was used to detect the level of total SUMO protein, Sp3, and SUMO-Sp3. E and F. EC cells transfected with siSENP1 or Sp3 vector or their corresponding control, and then RT-qPCR was used to detect IL-6 or TNF-α expression. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus corresponding control. All data are expressed as mean ± SEM. Comparisons of quantitative data were done with one-way analyses of variance and Student's *t* tests.

LPS-stimulated RAW 264.7 cells. The results showed that LPS promoted Sp3-NF-κB interaction (**Figure 4B**). However, this interaction can be disturbed by MC treatment (**Figure 4C**). To determine whether MC disturbs Sp3-NF-κB interaction, thereby affecting cellular inflammation, we knocked down Sp3 and treated RAW 264.7 cells with MC. The results showed that the depletion of Sp3 significantly decreased IL-6 and TNF-α expression, and this effect was further enhanced by simultaneous MC treatment (**Figure 4D** and **4E**). Together, these data support a role of MC in anti-inflammatory regulation via interference with Sp3-NF-κB interaction.

Discussion

Sepsis is currently defined as a life-threatening organ dysfunction caused by an unregulated host response to infection [23]. Regarding wounds and wound surfaces, as well as bacteria or bacterial toxins, LPSs are the primary causes of sepsis [24]. If sepsis is not controlled in a timely and effective manner, it can cause multiple organ damage in a short time. Progression of sepsis can even lead to the development of multiple organ dysfunction syndrome, which is the main cause of death in patients with trauma, burns, and severe surgery [25]. However, current research has shown

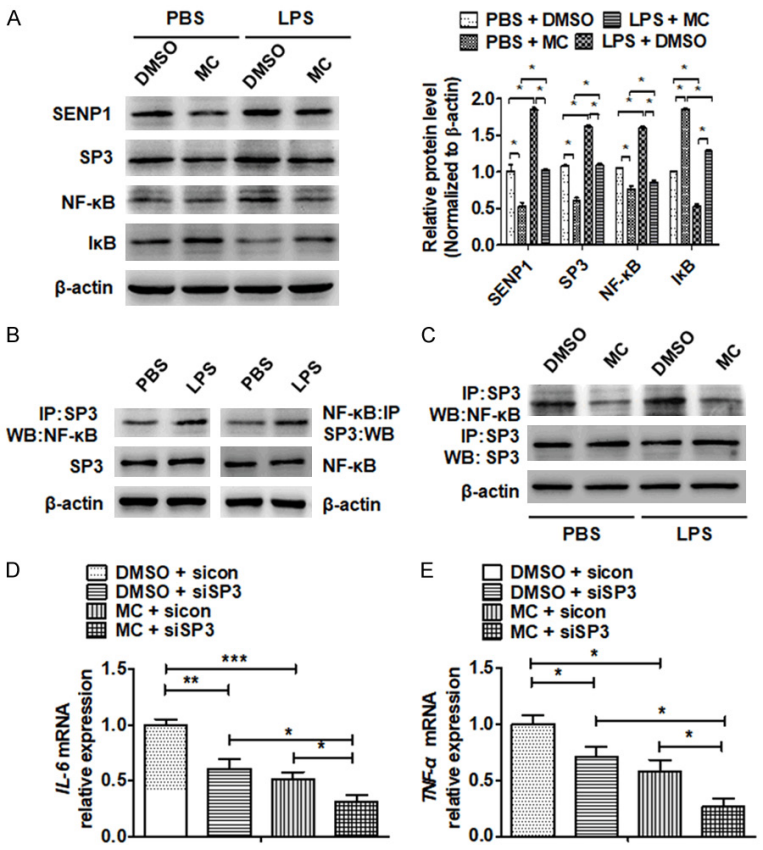

Figure 4. MC inhibits Sp3 and NF-κB interaction and downregulates cellular inflammation in ECs. A. Western blot analysis was used to detect the level of SENP1, Sp3, NF-κB, and IκB in EC cells after treatment with MC, LPS, or both. The right panel shows the quantitative analysis of protein levels. *P < 0.05 versus the corresponding control. B. EC cells were treated with LPS or PBS, and CoIP analysis was used to detect the interaction between SENP1 and NF-κB. C. CoIP analysis was used to detect the interaction between SENP1 and NF-κB in MC- or LPS-treated EC cells. D and E. EC cells were transfected with siSp3 and treated with or without MC; then RT-qPCR was used to detected IL-6 or TNF-α expression. *P < 0.05, **P < 0.01, ***P < 0.001 versus the corresponding control. All data are expressed as the mean ± SEM. Comparisons of quantitative data were done with one-way analyses of variance and Student's *t* tests.

that the root cause of sepsis is the body's overreaction to external damage factors, resulting in damage to distant organs [26]. Macrophages play a vital role in all stages of sepsis and affect immune homeostasis and inflammatory processes [27]. Macrophage dysfunction is considered one of the primary causes of sepsis-induced immunosuppression [28]. In this study, we found that the knockdown of SENP1 reduced LPS-induced macrophage inflammation. MC, an inhibitor of SENP1, reduces LPS-induced cellular inflammation by downregulating SENP1 expression. In addition, the effect of SENP1 on LPS-induced inflammation depends on SENP1-Sp3 interaction as well as on deSUMOylation to promote Sp3 expression. Furthermore, MC-inhibited Sp3 expression interferes with Sp3-NF-κB interaction, thereby reducing LPS-induced cellular inflammation.

A recent study has shown that SENP1 plays an important role in cellular inflammation by regulating protein SUMOylation [9]. As an important post-translational modification, SENP1 deconjugates many SUMOylated proteins through a dynamic process that is mediated via activating, conjugating, and ligating enzymes [29]. SENP1 promotes the deSUMOylation of GATA2 and IκBα in tibial cells, leading to increased GATA2 stability and NF-κB activity, further promoting cellular inflammation [9]. In our study, SENP1 expression was promoted in LPS-stimulated RAW 264.7 cells. Therefore, we suspected that SENP1 participates in LPS-induced macrophage inflammation. We knocked down SENP1 in RAW 264.7 cells and found that the depletion of SENP1 significantly depressed inflammatory cytokine expression in LPS-treated RAW 264.7 cells. Because MC is a natural inhibitor of SENP1, we found that inflammatory cytokine expression was also reduced in MC-treated RAW 264.7 cells, independent of LPS stimulation.

During sepsis, NF-κB is one of the critical activators of inflammatory genes [30, 31]. NEMO is an important component of the cytoplasmic IκBα kinase complex and plays an essential role in the NF-κB activation signaling pathway. One study reported that SENP1 plays an important role in inhibiting the activation of NF-κB and NF-κB-dependent transcriptional activation through a reversible SUMOacylation of NEMO [32, 33]. In our study, IκB expression was increased and NF-κB protein levels were reduced in MC-treated RAW 264.7 cells. In-

terestingly, we found that SENP1 interacts with Sp3, which also interacts with NF-κB and promotes NF-κB transcription activation, and regulates cellular inflammatory response by increasing Sp3 expression. SENP1 interacts with SUMO-Sp3 and deSUMOylates it, possibly by stabilizing the Sp3 protein level. In MC-treated macrophages, Sp3 expression was reduced and Sp3-NF-κB interaction was disturbed, which may be the anti-inflammatory function of MC.

In conclusion, we found that LPSs promote SENP1 expression and that the knock down of SENP1 significantly suppresses LPS-induced RAW 264.7 inflammation. Moreover, MC downregulates SENP1 and inflammatory cytokine expression. These findings show that downregulated SENP1 suppresses LPS-induced macrophage inflammation by increasing Sp3 deSUMOylation and disturbing Sp3-NF-κB interaction. Nevertheless, further in vivo research is required to verify our findings.

Acknowledgements

Funding for this study was received from the Guangdong Medical Scientific Research Foundation (#A2017297) and the Science and Technology Planning Project of Guangzhou, China (201804010335). Thanks to Dr. Bei Hu for funding in this manuscript.

Disclosure of conflict of interest

None.

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