Glucocorticoid treatment inhibits intracerebral hemorrhage-induced inflammation by targeting the microRNA-155/SOCS-1 signaling pathway

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Abstract. Intracerebral hemorrhage (ICH) results in inflammation, and glucocorticoids have been proven to be effective inhibitors of ICH-induced inflammation. However, the precise underlying mechanisms of ICH-induced inflammation and glucocorticoid function remain largely undefined. Using a mouse ICH model, the present study demonstrated that the short non-coding RNA molecule microRNA-155 (miR-155) is involved in the inflammatory process initiated by ICH in mice. Increased mRNA expression levels of miR-155, as well as the pro-inflammatory cytokines interferon-β (IFN-β), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), were observed *in vivo* following ICH. By contrast, the expression level of suppressor of cytokine signaling 1 (SOCS-1) protein was reduced in the ICH group compared with control mice. Similar results were observed *in vitro* using astrocytes, the primary effector cells in ICH. Compared with wild type astrocytes, astrocytes overexpressing miR-155 exhibited significant inhibition of SOCS-1 protein expression levels. These results suggest that miR-155 contributes to the development of ICH-induced inflammation in mice by downregulating SOCS-1 protein expression levels and promoting pro-inflammatory cytokine (IFN-β, TNF-α and IL-6) production. Expression levels of miR-155 and pro-inflammatory cytokines in the ICH group were significantly decreased following dexamethasone administration. This suggests that glucocorticoids attenuate ICH-induced inflammation by targeting the miR-155/SOCS-1 signaling pathway in mice. In conclusion, the results of the present study demonstrated that the miR-155/SOCS-1 signaling pathway is required for ICH-induced inflammation, and glucocorticoids inhibit this process by targeting the miR-155/SOCS-1 signaling pathway.

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Introduction

Intracerebral hemorrhage (ICH) is a common and severe subtype of stroke associated with high rates of morbidity and mortality (1). Resulting from ruptured blood vessel(s) in the brain, ICH is defined as bleeding into the brain parenchyma (2). A series of pathophysiological processes occurs following an acute ICH outbreak, including apoptosis and necrosis, breakdown of the blood-brain barrier, edema formation, inflammation and extracellular matrix remodeling (1). These complications may result in severe neuronal injury. Thus, it is important to understand the underlying mechanisms of ICH in order to control the subsequent complications. Despite this, relatively few studies have focused on ICH and its subsequent effects compared with studies on ischemic stroke (3-5).

Characterized by elevated expression levels of pro-inflammatory cytokines, including interferon-β (IFN-β), tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6), inflammation induced by ICH is hypothesized to contribute to neurodegeneration (6,7). Glucocorticoids (GCs) exert anti-inflammatory effects, and are therefore considered to be effective therapeutic agents in acute and chronic inflammation, including severe shock, asthma, inflammatory bowel disease, rheumatoid arthritis and multiple sclerosis (8-10). In addition, studies investigating their efficacy in the treatment of ICH-induced inflammation have revealed positive results (11-13). However, the underlying mechanisms through which GCs exert their anti-inflammatory and immunosuppressive functions in ICH remain to be elucidated (14).

MicroRNAs (miRs) are endogenous 20-23 nucleotide small noncoding RNAs that are expressed in various tissues and are responsible for regulating the expression of various genes. miRs affect numerous biological processes, including development, autoimmune diseases, inflammation and proliferation (15-19). miRs function by shortening mRNA half-life or inhibiting translation via binding the 3'untranslated region of target genes (20). miR-155 is encoded by the B-cell integration (BIC) gene located on chromosome 21 (20,21). Previous studies have suggested that miR-155, similar to other miRs, is closely associated with the development of inflammatory pathogenesis (22). Furthermore, Liu *et al* (23) revealed the involvement of miR-155 in rat ICH.

Previous studies have focused on the miR-155-mediated signaling pathway, in order to elucidate its precise underlying molecular mechanisms in various biological processes (20,21). Identified targets of miR-155 to date include Fas-associated death domain protein, Src homology 2-containing inositol 5-phosphatase 1 and the suppressor of cytokine signaling-1 (SOCS-1) (20,24-26). In microglia-mediated immune reactions, miR-155 promoted inflammation via the downregulation of SOCS-1 protein (6). However, the underlying mechanisms by which miR-155 functions in ICH-induced inflammatory pathogenesis remain to be elucidated.

In the present study, a mouse ICH model was established according to our previous paper (27,28) and miR-155 was overexpressed in astrocytes to investigate the role of miR-155 in ICH-induced inflammation, as well as the underlying mechanism of the anti-inflammatory effects of GCs.

Materials and methods

Animals and ICH-induction. All experimental procedures were in compliance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals (29) and approved by the Institutional Animal Care and Use Committee at Soochow University (Suzhou, China). Animals, including both mice and rats, were housed under pathogen-free conditions with 10:14 h light: Dark cycle, at room temperature and 75% humidity. Animals had free access to sterile water and chow. Protocols for ICH-induction were conducted as described previously (27,28). Briefly, 30 adult male ICR mice weighing 25-30 g and 6 neonatal Sprague-Dawley rats were obtained from Shanghai Laboratory Animal Center (Shanghai, China). ICH was induced by collagenase injection, as previously described (27). Mice were anesthetized with 4% chloral hydrate (0.4 mg/g) purchased from Sigma-Aldrich (St. Louis, MO, USA) and placed in a stereotactic apparatus. A cranial burr hole (1-mm in diameter) was drilled, through which type IV collagenase purchased from Sigma-Aldrich was injected (0.075 units in 500 nl of saline) into the left striatum (1 mm anterior and 2 mm lateral of the bregma, 3.5 mm in depth). Collagenase was delivered over 5 min. The needle was kept in place for an additional 5 min to prevent any reflux. The control (sham) mice received an equivalent volume of sterile saline injected in an identical manner. Body temperature was maintained at 37±0.5˚C using a heat lamp throughout surgery and recovery.

Experimental groups and drug administration. Mice were randomly assigned to three groups: control (Ctrl), Sham surgery (sham) and ICH, with 6 mice per group. To investigate the effect of GCs, dexamethasone (30 mg/kg) was administered via i.p. injection twice a day for 3 consecutive days, beginning on the day of surgery. Animals were sacrificed one day after the last dose, a total of three days following surgery. Animals were sacrificed by an intraperitoneal injection of pentobarbital (40 mg/kg, Sigma-Aldrich) and brain tissues were collected for analysis. Dexamethasone was purchased from Sigma-Aldrich and administered according to previously published protocols (27,30).

RNA isolation. Total RNA was extracted from brain tissue samples or cultured astrocytes using TRIzol® Reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's instructions. Any remaining DNA was removed with the DNA-free kit (Ambion; Thermo Fisher Scientific, Inc.) and RNA was purified with an RNeasy kit (Qiagen, Inc., Valencia, CA, USA), according to the manufacturer's instructions.

Detection of miR-155 by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). For quantification of miR-155 levels, RT-qPCR was performed on total RNA. miR-155 expression levels were determined using a Hairpin-it™ miRs qPCR kit (Shanghai GenePharma Co., Ltd., Shanghai, China). In brief, the assay has two steps: Stem-loop RT reaction and qPCR detection. Stem-loop RT primers bind to the 3'end of miR molecules and are transcribed with reverse transcriptase. Briefly, the total RNA was reverse transcribed using the M-MLV reverse transcriptase system (Promega Corporation, Madison, WI, USA). The reaction was performed at 42˚C for 1 h and terminated by deactivation of the enzyme at 70˚C for 10 min. The RT product was quantified by qPCR, using miR-specific forward and reverse primers as follows: Forward, 5'-GTGCTGCAAACCAGGAAGG-3' and reverse, 5'-CTGGTTGAATCATTGAAGATGG-3', and a carboxyfluorescein dye-labeled reporter probe. Thermal cycling conditions were as follows: Pre-denaturation at 95˚C for 5 min, denaturation at 95˚C for 10 sec, annealing at 58˚C for 15 sec, and extension at 72˚C for 20 sec. Gene expression levels were standardized to a housekeeping gene (U6) and expressed as a fold of control. To normalize RNA content, the U6 small nuclear RNA served as the internal control. The relative miR-155 expression levels of each group were calculated using the ΔΔCq method (31).

Cytokine detection was performed by RT-qPCR using an ABI 7300 RT-PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) using a SYBR Green-based RT-PCR kit [SYBR® Premix Ex Taq™ (Tli RNaseH Plus); Takara Bio, Inc., Otsu, Japan]. The primers used were as follows: IFN-β (32) forward, 5' AAGAGTTACACTGCCTTTGCCATC3' and reverse, 5'CACTGTCTGCTGGTGGAGTTCATC3'; TNF-α forward, 5'TGAACTTCGGGGTGA TTGGTC3' and reverse, 5'GCCTTGTCCCTTGAAGAGAAC3'; IL-6 forward, 5'-TAC TTCACAAGTCCGGAG-3' and reverse, 5'-TCCAGAAGACCA GAGCAG-3'; β-actin forward, 5'-ACATCTGCTGGAAGG TCCAC-3' and reverse, 5'-GGTACCACCATGTACCCAGG-3'. All reactions were performed three times for each group.

Western blot analysis of SOCS-1 protein expression levels. Protein extract prepared from tissue samples or cultured astro-

cytes were examined by western blot analysis. Protein was extracted using the ProteoExtract® Transmembrane Protein Extraction kit (Merck Millipore, Darmstadt, Germany) according to the manufacturer's protocol, and determination of protein concentration was performed using a bicinchoninic acid protein assay kit (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Equal amounts of protein (15 μg) were subjected to 10% SDS-PAGE (running at 60 V for 1 h through the stacking gel, and at 120 V for 1 h through the resolving gel). Western blot analysis was performed using a mouse antibody against SOCS1 (1:1,000 dilution, catalog no. 04-002, EMD Millipore, Billerica, MA, USA) (27). Briefly, proteins were blotted onto a polyvinylidene difluoride membrane (Roche Applied Science, Penzburg, Germany) for 90 min at 350 mA. Then the membranes were blocked with 0.1% Tris-buffered saline (TBS)-Tween buffer containing 5% skim milk either overnight at 4˚C or for 2 h at room temperature. The membrane was subsequently incubated with anti-SOCS1 antibody overnight at 4˚C. Following the incubation, the membrane was washed with 0.1% TBS-Tween buffer and probed with goat anti-mouse HRP-conjugated IgG secondary antibody (1:2,000 dilution; catalog no. sc-2005; Santa Cruz Biotechnology, Inc., Dallas, TX USA) for 1 h at room temperature. Rabbit antibody against GADPH (1:5,000 dilution; catalog no. KC-5G4; Kangcheng, Inc., Shanghai, China) was used as the loading control. Protein bands were visualized using enhanced chemiluminescence (Vazyme Biotech Co., Ltd., Nanjing, China) (27,33). SOCS-1 expression levels were quantified by densitometry in arbitrary units using ImageJ 1.44p software (National Institutes of Health, Bethesda, MD, USA), and normalized to GAPDH.

Astrocyte isolation and culture. Astrocytes were isolated from mixed glial cultures as described previously (34). Briefly, brains harvested from 1- to 3-day-old Sprague Dawley rat pups were minced, filtered and cultured in Dulbecco's modified Eagle's medium (DMEM)/F-12 (Invitrogen; Thermo Fisher Scientific, Inc.). At 100% confluence (1 week post-plating), the mixed glial cultures were shaken at 200 rpm overnight at 37˚C on a rotary shaker to separate the microglia and oligodendrocytes from the adherent astrocytes. The media, microglia and oligodendrocytes were removed; astrocytes were trypsinized and subcultured in DMEM/F-12 supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 mg/ml streptomycin, 100 mg/ml L-glutamine and 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic aciction id (all Sigma-Aldrich). Astrocytes were maintained at 37˚C in 5% CO_2 and 95% air for 3 days.

Transfection of miRs. miR-155 and its mutant constructs were synthesized by Integrated DNA Technologies, Inc., Coralville, IA, USA. The sequences of anti-miR-155 antisense inhibitor oligonucleotides (AMOs) used in the present study are exact antisense copies of mature miR sequences, and the nucleotides in the AMOs contain 2'-O-methyl modifications at every base and a 3'C3 amino linker (31). Oligo transfection was performed according to an established protocol (35). Briefly, wild type (WT) cells were transfected using PolyFect transfection reagent (Qiagen, Inc.) for 6 h. Transfection complexes were prepared according to the manufacturer's instructions, and 2'OMe-miR-155 or control oligo 2'OMe-enhanced green fluorescent protein was added directly to the complexes to a final oligonucleotide concentration of 30, 50 or 100 nmol/l. The transfection efficiency was low at 30 or 50 nmol/l. However, when transfected at 100 nmol/l, transfection efficiency reached ≥80%. Therefore, only cells receiving 100 nmol/l were analyzed. The transfection medium was replaced 6 h subsequent to transfection with regular culture medium, and cells were cultured for 3 days prior to analysis.

Statistical analysis. Data are presented as the mean ± standard error. Comparisons were made between groups using the Student's *t*-test using SigmaPlot version 10 software (Systat Software, Inc., San Jose, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-155 is involved in ICH pathogenesis in mice in vivo. To evaluate the role of miR-155 in ICH-mediated inflammation, a mouse model of ICH was used, and the expression of miR-155 was determined by RT-qPCR three days subsequent to ICH treatment. As presented in Fig. 1A, the expression level of miR-155 in the ICH group was significantly increased compared with the control ($P=0.030$) and sham groups ($P=0.038$). Furthermore, to identify the signaling pathway mediated by miR-155 in the ICH model, western blotting was performed to examine the SOCS-1 protein expression levels in the three groups. In contrast to miR-155, the expression level of SOCS-1 protein was significantly decreased in the ICH group compared with the control mice ($P=0.021$; Fig. 1B). In addition, significantly increased mRNA expression levels of the pro-inflammatory cytokines, IFN-β, IL-6 and TNF-α were observed following ICH treatment compared with control groups ($P=0.005$,0.002 and $P<0.001$, respectively; Fig. 1C). These data indicate that miR-155 and SOCS-1 are involved in the inflammatory pathogenesis induced by ICH. Furthermore, miR-155 and SOCS-1 are antagonistic in this process.

Dexamethasone inhibits ICH-induced inflammation by targeting miR-155 and SOCS-1. To elucidate the underlying functional mechanism of GCs in ICH-induced inflammation, 30 mg/kg dexamethasone was administered to mice following ICH induction. A total of three days subsequent to administration, miR-155 expression was determined by RT-qPCR. As presented in Fig. 1A, the level of miR-155 expression in mice treated with dexamethasone (ICH+DEX group) was significantly reduced compared with mice that did not receive dexamethasone (ICH group; $P=0.042$). In addition, ICH-mediated inhibition of the protein expression levels of SOCS-1 was reversed following dexamethasone administration ($P=0.022$; Fig. 1B). Furthermore, the mRNA expression levels of the cytokines IFN-β, IL-6 and TNF-α were markedly decreased in the ICH+DEX group compared with the ICH group ($P=0.037$, 0.024 and 0.018, respectively; Fig. 1C).

MiR-155 inhibits the expression of SOCS-1 protein in astrocytes in vitro. As astrocytes are the primary effector cells in the inflammatory pathogenesis triggered by ICH, experiments were performed *in vitro* using astrocytes to attempt to

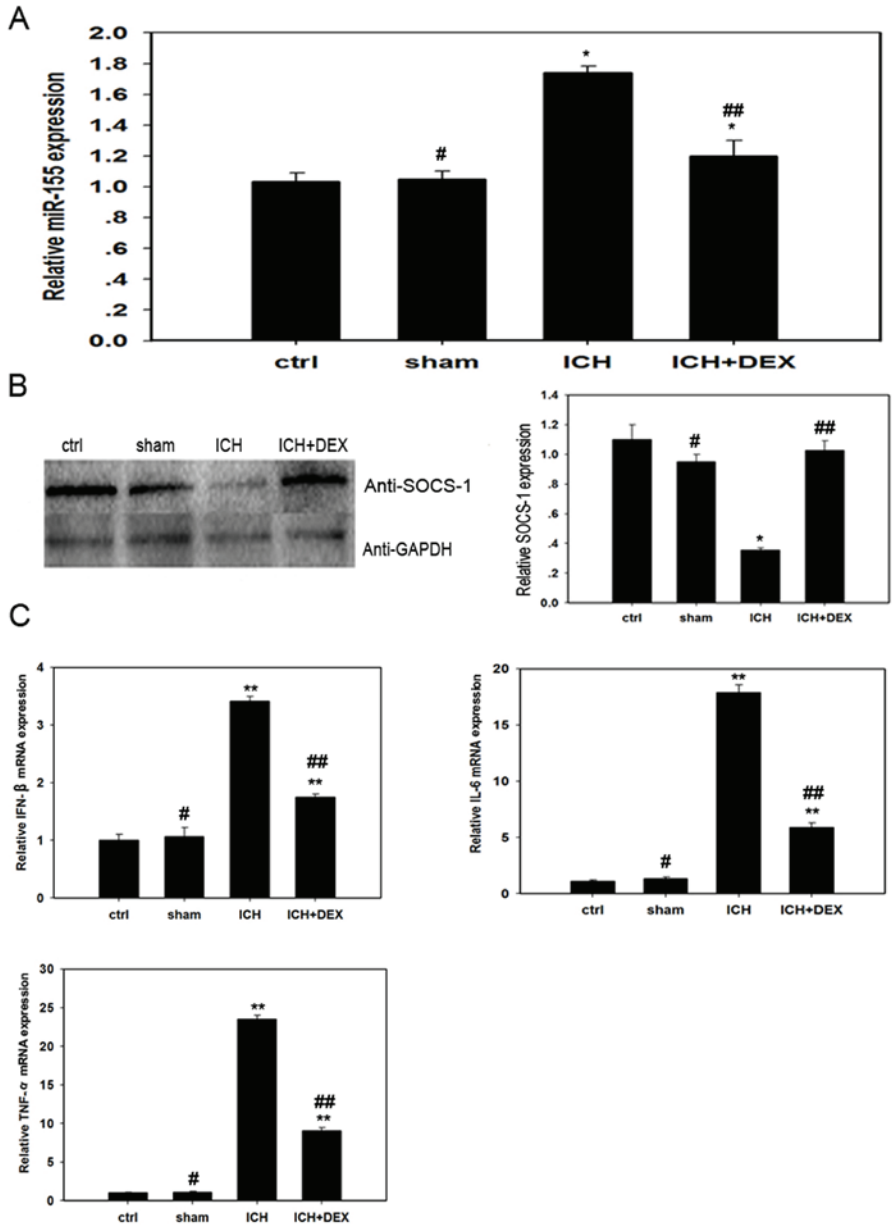

Figure 1. miR-155/SOCS-1 are involved in ICH-induced inflammation and dexamethasone reduces inflammation by inhibiting miR-155 expression *in vivo*. The expression levels of (A) miR-155, (B) SOCS-1 and (C) pro-inflammatory cytokines were determined under various treatment conditions. Mice were divided randomly into four groups (n=36 per group): Ctrl group, without any treatment; ICH group, injected with collagenase for ICH induction: Sham group, injected with the equivalent volume of saline; and ICH+DEX group, treated with dexamethasone following ICH induction. Reverse transcription-quantitative polymerase chain reaction was performed to determine miR-155, IFN-β, IL-6 and TNF-α mRNA expression levels, while western blotting determined SOCS-1 protein expression levels. Representative data is presented, as the mean ± standard error, from one of at least three experiments. *P<0.05 vs. ctrl; **P<0.01 vs. ctrl (for ICH group) or P<0.01 vs. ICH group (for ICH+DEX group); $^{\#}$P>0.05 vs. ctrl; and $^{\#\#}$P<0.05 compared with ICH group, as determined by Student's *t*-test using SigmaPlot version 10.0 software. miR, microRNA; SOCS-1, suppressor of cytokine signaling-1; ICH, intracerebral hemorrhage; ctrl, control; DEX, dexamethasone; IFN-β, interferon-β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

clarify the association between miR-155 and SOCS-1 in the ICH model. WT astrocytes were transfected with miR-155 oligonucleotides to overexpress miR-155 (miR-155 group) or with mis-miR-155 oligonucleotides represented as scramble miR as a control (mis-miR-155 group). Transfection efficiency was >80% and no clear morphology impairment was observed following transfection (Fig. 2A). The three populations of astrocytes were collected and assayed for miR-155 expression by RT-qPCR (Fig. 2B), and for SOCS-1 expression by western blotting (Fig. 2C). Overexpression of miR-155 led to a significant inhibition in SOCS-1 protein expression levels compared with the control (WT group; P=0.027). This indicates that SOCS-1 is a target of miR-155 in astrocytes, and that miR-155 may promote ICH-triggered inflammatory pathogenesis by downregulating SOCS-1 expression.

To confirm the association between miR-155 and SOCS-1, anti-miR-155 antisense inhibitor oligonucleotides (AMO-155) were synthesized to antagonize the expression of miR-155, and mis-AMO-155 oligonucleotides represented as scramble miR served as a control. AMO-155 and miR-155 oligonucleotides were transfected simultaneously into certain astrocytes (AMO-155+miR-155 group) to cancel out the miR-155 expres-

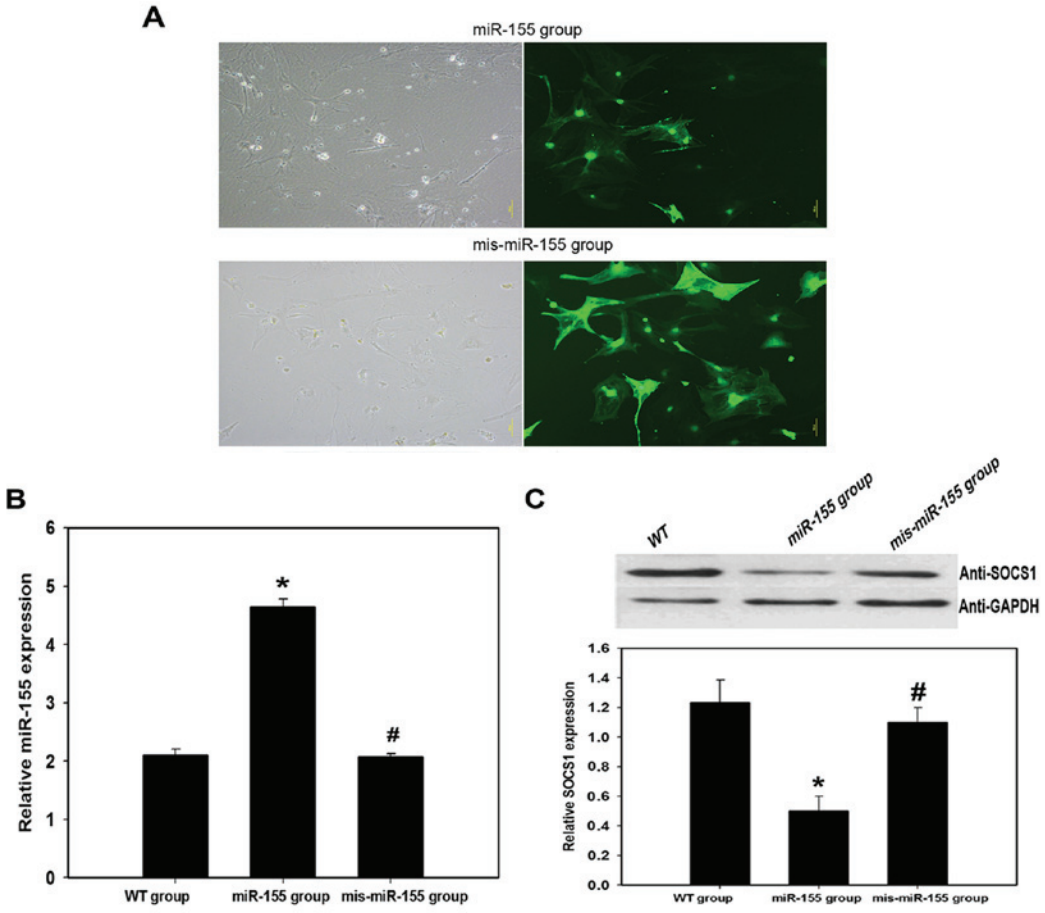

Figure 2. Overexpression of miR-155 results in inhibition of SOCS-1 expression in astrocytes. (A) To obtain miR-155 overexpressing astrocytes, WT astrocytes were cultured and transfected with 100 nmol/l miR-155 oligonucleotides for overexpression of miR-155 (miR-155 group) or with 100 nmol/l mis-miR-155 oligonucleotides as a negative control (mis-miR-155 group) for 72 h. Green fluorescence represents a reporter gene. Magnification, x200; scale bar=20 μm. Representative results of at least three experiments are presented. (B) Total RNA was extracted from astrocytes and the level of miR-155 expression detected by reverse transcription-quantitative polymerase chain reaction. Values were normalized to U6 expression and presented as the mean ± SE. Representative results from one of at least three experiments are presented. (C) Western blotting was performed on whole cell lysates to determine SOCS-1 protein expression. SOCS-1 expression levels were quantified by densitometry in arbitrary units using ImageJ software, and normalized to GAPDH. Data are expressed as the mean ± SE, and representative results are presented from at least three experiments. *P<0.05; and $^{\#}$P>0.05 compared with WT group, as determined by Student's *t*-test using SigmaPlot version 10.0 software. miR, microRNA; SOCS-1, suppressor of cytokine signaling-1; WT, wild type; SE, standard error.

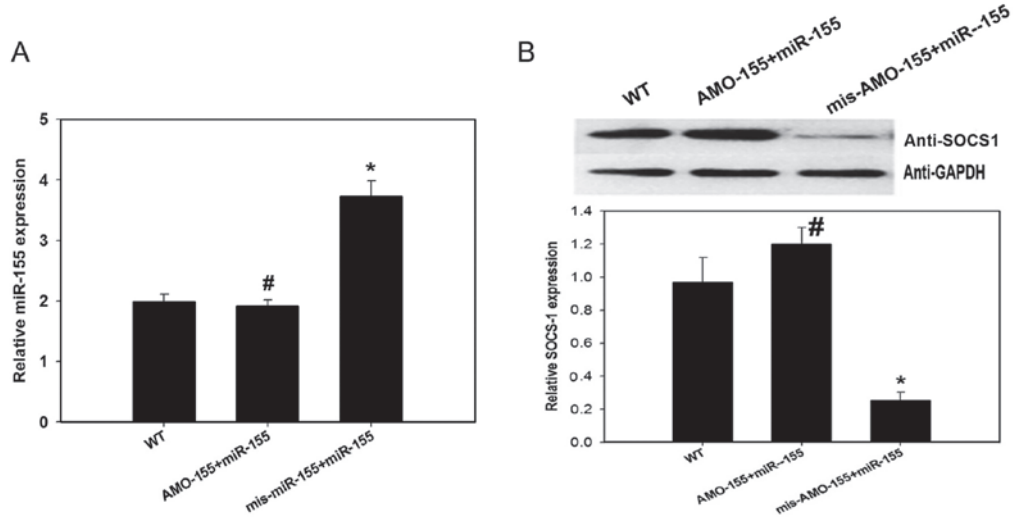

Figure 3. AMO-155 restores SOCS-1 expression in miR-155 overexpressing astrocytes. AMO-155 and mis-AMO-155 oligonucleotides were transfected into astrocytes together with miR-155 oligonucleotides. (A) miR-155 expression levels were determined by reverse transcription-quantitative polymerase chain reaction. (B) SOCS-1 protein expression levels were determined by western blotting. Data are expressed as the mean ± standard error, and representative results from at least three experiments are presented. *P<0.05; and $^{\#}$P>0.05 compared with WT group as determined by Student's *t*-test using SigmaPlot version 10.0 software. miR, microRNA; SOCS-1, suppressor of cytokine signaling-1; AMO, antisense inhibitor oligonucleotides; WT, wild type.

sion, while a separate group were transfected with mis-AMO-155 together with miR-155 oligonucleotides to examine the overexpression of miR-155 (mis-AMO-155+miR-155 group). As presented in Fig. 3A, expression levels of miR-155 were increased in the mis-AMO-155+miR-155 group (P=0.030) although not in the AMO-155+miR-155 group (P=0.724), compared to WT cells. As presented in Fig. 3B, expression levels of SOCS-1 protein were significantly inhibited in the mis-AMO-155+miR-155 group compared with the WT group (P=0.013); however, this effect was abrogated in the AMO-155+miR-155 group (P=0.373). These data suggest that miR-155 functions upstream and inhibits the expression of SOCS-1 in astrocytes. As astrocytes are important effector cells in ICH, the results of the *in vitro* experiments suggest that miR-155 contributes to ICH-induced inflammatory pathogenesis by inhibiting SOCS-1 expression.

Discussion

Based on the results of the present study, miR-155 may be required for the development of ICH-induced inflammation in mice. Furthermore, the results of the present study revealed that miR-155 downregulates the expression levels of SOCS-1 protein, but increases the mRNA expression levels of the pro-inflammatory cytokines IFN-β, IL-6 and TNF-α *in vivo*. In addition, the association between miR-155 and SOCS-1 was demonstrated by transfection of astrocytes *in vitro*. This signaling pathway may be responsible for the effect of GCs on ICH-induced inflammation, as miR-155 expression was significantly suppressed, while SOCS-1 protein expression levels were significantly increased, when dexamethasone was administered to ICH mice. Previous studies have revealed that miR-155 is involved in biological processes, including inflammatory pathogenesis (6,21,22); however, the role of miR-155 in ICH-induced inflammation and its specific signaling pathway remains to be elucidated. Although GCs have been demonstrated to be effective in controlling various inflammatory disorders (9,36,37), the exact underlying mechanism of their anti-inflammatory effects remains unclear. The results of the present study provide, to the best of our knowledge, the first evidence of the involvement of miR-155 in ICH-induced inflammatory pathogenesis and the signaling pathway of GCs in this process. These results thus contribute to our understanding of the underlying mechanisms involved in ICH-induced inflammation.

The production of pro-inflammatory cytokines, including IFN-β, IL-6 and TNF-α, is an important downstream effect of ICH (1). Enhanced expression levels of IFN-β, IL-6 and TNF-α mRNAs, accompanied by increased expression of miR-155 and reduced expression of SOCS-1 protein levels, indicates a critical role for the miR-155/SOCS-1 signaling cascade in ICH-induced inflammation. Previous studies have attributed the increased production of pro-inflammatory cytokines following ICH to the activation of Toll-like receptors (TLRs), particularly TLR2 and TLR4 (38). The classical signaling pathway triggered by TLRs results in the activation of nuclear factor (NF)-κB and mitogen-activated protein kinases (JNK and p38), which leads to the transcription of pro-inflammatory cytokine genes (39-41). As miR-155 is transcribed from the BIC gene, the activation of which is under the control of NF-κB, miR-155/SOCS-1 may function downstream of TLR signaling following ICH. This has previously been demonstrated in macrophage-mediated innate immune reactions (42). Nevertheless, additional studies are required to clarify the association between TLR signaling and the miR-155/SOCS-1 signaling cascade following ICH.

The significant decrease in the mRNA expression levels of IFN-β, IL-6 and TNF-α, combined with the decrease in miR-155 expression and enhanced expression levels of SOCS-1 protein following dexamethasone administration, suggests that GCs, including dexamethasone, function through the miR-155/SOCS-1 signaling pathway to attenuate ICH-induced inflammation. This underlying functional mechanism of dexamethasone is consistent with that of 1,25-dihydroxyvitamin D, which inhibits TLR-mediated inflammation by targeting the miR-155/SOCS-1 signaling pathway in macrophages (42). Whether other regulatory mechanisms underlie GC function in ICH requires further investigation.

The expression levels of miR-155 and the pro-inflammatory cytokines in the ICH+DEX group, although inhibited compared with the ICH group, remained significantly increased compared with the control group. This suggests that GCs may produce adverse effects on brain pathology. Potential adverse effects caused by GCs have been reported previously, for instance on the hypothalamus-pituitary-adrenal axis (43,44). Due to the beneficial effects of GCs, future studies are required to investigate their underlying functional mechanisms.

In conclusion, the results of the present study demonstrate an essential role of the miR-155/SOCS-1 signaling cascade in ICH-induced inflammation, and furthermore reveal that GCs may relieve this inflammation by targeting the miR-155/SOCS-1 signaling pathway. Targeting the miR-155/SOCS-1 signaling pathway may provide a novel therapeutic strategy for the treatment of ICH-triggered inflammation.

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