Original Research

Suppressors of cytokine signaling 1 protein in a regenerative model of the *Gekko japonicus* spinal cord

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Demyelination is one of the pathological outcomes that occur immediately following spinal cord injury. Protection of oligodendrocytes against death/apoptosis proves to be beneficial for the preservation of neurological functions. Suppressors of cytokine signaling 1 protein inhibit the harmful effects of several inflammatory cytokines on oligodendrocytes, but its roles in spinal cord injury (SCI) induced apoptosis of oligodendrocytes remain unclear. We cloned suppressors of cytokine signaling 1 cDNA from *Gekko japonicus* (Japanese gecko) and analyzed the protein structure revealing the conserved domains contained in other vertebrate suppressors of cytokine signaling 1 proteins. The gecko suppressors of cytokine signaling 1 protein expression were increased in the injured spinal cord following gecko tail amputation and displayed colocalization with oligodendrocytes. The enforced expression of gecko suppressors of cytokine signaling 1 by adenovirus in the Gsn3 gecko oligodendrocyte cell line demonstrated that gecko suppressors of cytokine signaling 1 significantly suppressed cell apoptosis-induced by glucose deprivation. Determination of apoptosis-related proteins revealed that gecko suppressors of cytokine signaling 1 was able to activate extracellular regulated protein kinases (ERK) and serine/threonine protein kinases (Akt). The results presented a distinct protective role of gecko suppressors of cytokine signaling 1 in the regenerative model of the spinal cord, which may provide new cues for central nervous system repair in mammals.

Keywords

Neurophylogenetics; Oligodendrocyte; Spinal cord; Reptile; Apoptosis; Neuroregeneration; Cytokine signaling

1. Introduction

Spinal cord injury often leads to different degrees of paralysis, sensory obstacles and other neurophysiological abnormalities [1]. Usually, two consecutive pathological processes will occur following SCI, known as primary and secondary tissue damage [2, 3]. The primary injury of the spinal cord refers to mechanical damage that results in direct cell death and bleeding, whereas further progressive destruction of tissue surrounding the necrotic core is characteristic of secondary tissue damage. Several mechanisms contribute to the spread of tissue injury including ischemia and edema, oxidative stress, inflammatory reactions, glial scar formation, and cellular necrosis [4, 5]. Among these pathological reactions, demyelination induced by oligodendrocyte necrosis is a typical cell event accompanied by severe axonal degeneration [6].

The integrity of the myelin sheath is very important for the normal electrophysiological function of the central nervous system and neuronal survival [7]. Studies have shown that the destruction of myelin leads to abnormal neuroethology [8]. In a rat spinal contusion model, acute loss of oligodendrocytes was detected within 15 minutes after injury with continual loss lasting for 4 h [9, 10]. However, the number of oligodendrocytes decreased markedly within 24 h and steadily declined over the subsequent three to seven days in a mouse SCI model [11]. Apoptotic inhibition of oligodendrocytes or preservation of myelin results in an ameliorated functional outcome after SCI [10, 11]. Multiple inflammatory cytokines such as TNF-α, IL-1β and interferon (IFN)-γ have been demonstrated to induce oligodendrocyte apoptosis [4]. For example, stimulation with IFN-γ induces either apoptosis of developing or necrosis of mature oligodendrocytes [12], and IFN-γ transgenic mice have a phenotype of hypomyelination and oligodendrocyte cell loss during the first few weeks of postnatal development [13]. Due to the global effects of various inflammatory cytokines on oligodendrocytes in the central nervous system (CNS), the need to develop an effective strategy for inhibition of oligodendrocyte apoptosis has become increasingly important.

Suppressors of cytokine signaling (SOCS) proteins are feedback inhibitors of the JAK/STAT signaling pathway activated by cytokines. Most of them regulates cytokine-mediated homeostasis, thus maintaining a controlled cellular response [14, 15]. Normally, SOCS proteins are expressed at low levels but can be highly induced in a tissue-specific manner in response to various stimuli, including IL-4, IL-6, IL-10, IFN-β, IFN-γ and lipopolysaccharide (LPS) [16–18]. These cytokines bind to the receptor complexes that activate the JAK/STAT pathway. The activation of STAT pro-

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teins, particularly STAT1 and STAT3, induces SOCS1 gene expression [19]. There are eight mammalian SOCS family members, SOCS1–7 and cytokine-inducible Src-homology 2-containing protein (CIS) [16]. Each SOCS protein contains three distinct domains: an N-terminal region with variable length and sequence, a central SH2 (Src-homology 2) domain, and a conserved C-terminal domain termed the SOCS box. SOCS proteins can interact with the ubiquitin ligase machinery components and mediate proteasomal degradation of target proteins by the SOCS box [19]. The structure of SOCS1 and SOCS3 exclusively contains a kinase-inhibitory region (KIR) at the N-terminus, which acts as a pseudosubstrate for JAKs in association with inhibiting JAK kinase activity [20]. By applying such a mechanism, SOCS1 acts to reduce the responsiveness of many cell types to IFNs, LPS and other Toll-like receptor ligands, thereby protecting animals against systemic inflammation or autoimmune disease of the CNS [16, 19].

Several regenerative model organisms, including reptiles, amphibians and fish, can regenerate the spinal cord after injury [21–23]. These models are suitable for examining the regulatory roles of SOCS1 in the regenerating spinal cord, suggesting a therapeutic modulator in CNS disease states. *Gekko japonicus* was used as an experimental SCI model to investigate the expression changes of SOCS1, and its involvement in mediating the anti-apoptosis of oligodendrocytes. Our results demonstrated that the upregulation of SOCS1 in the injured cord of gecko was beneficial for protecting oligodendrocytes against apoptosis, which might promote spontaneous spinal cord regeneration.

2. Materials and methods

2.1 Animals

Adult *Gekko japonicus* were used as animal models, as Dong *et al.* [22]. Geckos were fed with water and mealworms and housed in the room with saturated humidity and controlled temperature (22–25 °C). Amputation was performed at the sixth caudal vertebra, which was described previously [24]. Briefly, it was established by using nylon thread and pulling gently until the tail was detached.

All experimental protocols were approved by the Animal Care and Use Committee of Nantong University and the Jiangsu Province Animal Care Ethics Committee.

2.2 Cloning and analysis of gecko SOCS1

To obtain the full length of gecko SOCS1, both a sense primer 5'- CCT GGG AGC GGA AGG TGT GGA AGT G -3' and an anti-sense primer 5'- CCG CGT CCC CCT GAA TCC GGT TTT A -3' were designed based on the genome sequences [25]. Both 5'- and 3'-RACE were performed using BD SMART RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA). The obtained protein was compared against the protein database in GenBank using the Protein-BLAST network server at NCBI [26]. Alignment of multiple protein sequences was performed by using the MegAlign program with the CLUSTAL method [27]. A phylogenetic tree was constructed using the Maximum-Likelihood of PHYML, with the GTR (CDS sequences) and JTT (protein sequences) substitution model [28].

2.3 Production of SOCS1 overexpression adenovirus

SOCS1-overexpression adenovirus (GV314-SOCS1) was produced by Genechem Co. Ltd. (Shanghai, China). The ORF of SOCS1 was cloned into a GV314 vector via the AgeI and Bam HI sites. The EF-1α promoter drove the expression of SOCS1, and the eGFP expression was driven by CMV promoter. Both sequences were incorporated into an adenovirus produced in 293T cells with 2×10^{10} PFU/mL of virus titer.

2.4 Cells culture and treatment

Gsn3, a cell line of gecko oligodendrocytes was cultured in Dulbecco's Modified Eagles Medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum in a 30 °C cell incubator with 5% CO_2.

For IFN-γ-induced oligodendrocyte apoptosis, Gsn3 was treated with 0–250 ng/mL IFN-γ and/or 10-50 ng/mL TNF-α for 24–72 h (R&D Systems). For glucose deprivation-induced oligodendrocyte apoptosis, GV314-vector or GV314-SOCS1 adenovirus was transfected in the Gsn3 cells with 5% fetal bovine serum for 48 h. Cells were then cultured in the glucose-free DMEM for another 24 h.

2.5 TUNEL assay

For the TUNEL assay, cells were detected by the *In Situ* Cell Death Detection Kit (Roche Molecular Biochemicals, Basel, Switzerland). Briefly, cells were fixed with 4% paraformaldehyde for 1 h at room temperature. Cells were incubated in a TUNEL reaction mixture at 37 °C for 30 min. The nuclei were counterstained with Hoechst 33342. At last, cells were viewed with a Zeiss fluorescence microscope.

2.6 Cell viability assay

In the cell viability assay, cells were determined by the Cell Counting Kit-8 (CCK-8, Dojindo). The absorbance of culture plates was measured at 450 nm with a microplate reader.

2.7 Western blot

Protein samples were lysed from 0.5 cm cord segments (six in each sample) at lesion sites or from cells with RIPA lysis buffer (Beyotime). The concentration of each protein sample was detected by the BCA kit (Beyotime). The extracts were denatured and boiled at 95 °C for 5 min, resolved by 10% SDS-PAGE, and transferred to PVDF membranes. The membranes were blocked with 5% nonfat milk in TBS and then incubated with primary antibodies buffer at 4 °C for 18 h, followed with secondary antibody at room temperature for 2 h. The signal of HRP activity was detected using an enhanced chemiluminescence kit (Beyotime). Antibodies used in Western blot were: β-actin (1 : 5000, Proteintech, 66009-1-Ig), SOCS1 (1 : 1000, polyclonal rabbit anti-gecko antibody prepared from polypeptides), STAT1 (1 : 1000, Cell Signaling Technology, 14994), pSTAT1 (Tyr701) (1 : 1000, abcam, ab29045), cleaved caspase3 (1 : 1000, Cell Signaling Technology, 9661S), caspase3 (1 : 1000, Cell Signaling Technology,

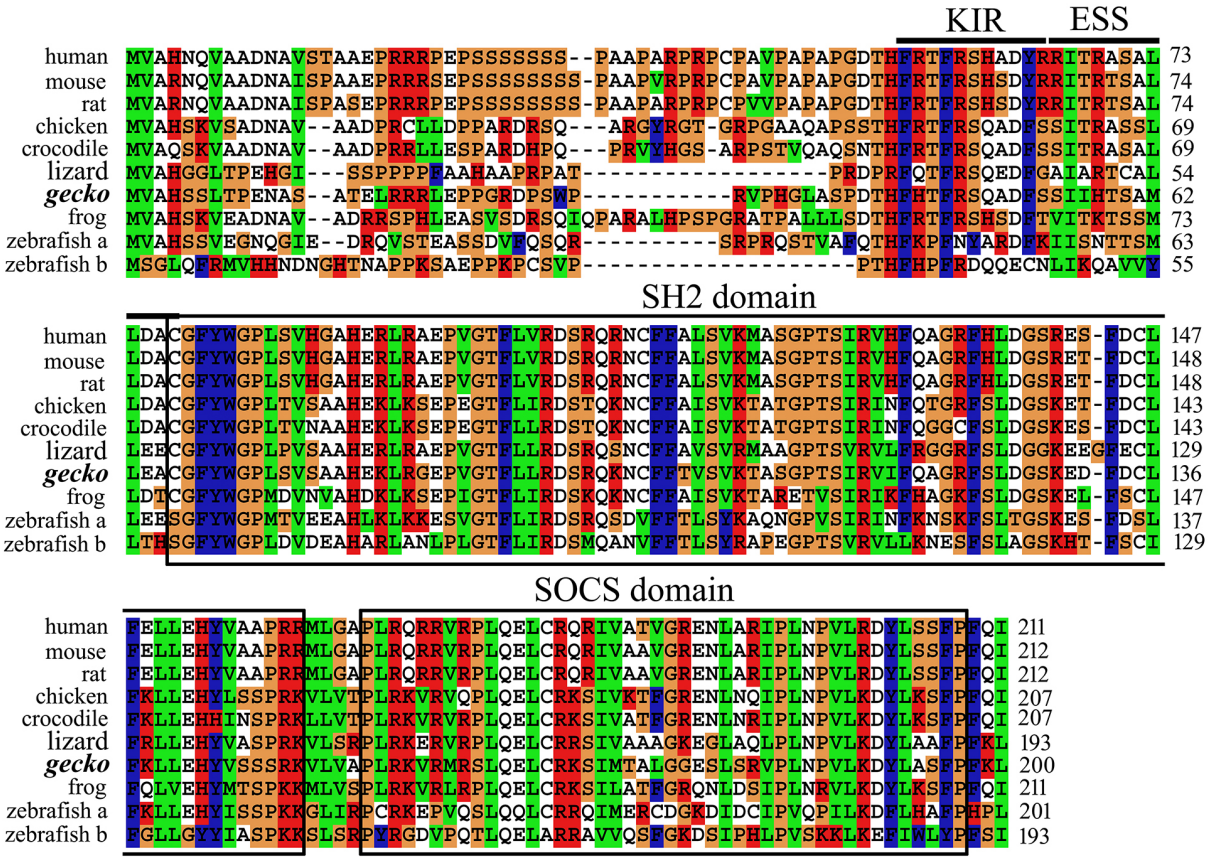

Fig. 1. Multiple alignment of amino acid sequences of gSOCS1 with those of representative vertebrates. Gaps introduced into sequences to optimize alignment are represented by dashes. KIR, SH2, ESS and SOCS box domain are indicated. Sequences of SOCS1 obtained from GenBank: gecko (XP_015265413), human (NP_003736), mouse (NP_001258532), rat (NP_665886), chicken (NP_001131120), crocodile (XP_019395658), lizard (XP_028562651), frog (NP_001011327), zebrafish SOCS1a (NP_001003467), and zebrafish SOCS1b (NP_001239559).

9665), Bcl-xL (1 : 1000, Cell Signaling Technology, 2764), Akt (1 : 1000, Cell Signaling Technology, 4685S), p-Akt (1 : 1000, Cell Signaling Technology, 4060S), p-ERK (1 : 1000, Cell Signaling Technology, 4370S), ERK (1 : 1000, Cell Signaling Technology, 9102S), and goat anti-rabbit or goat anti-mouse HRP (1 : 1000, Proteintech, SA00001-2, SA00001-1).

2.7 Tissue immunohistochemistry

Spinal cord segments were sectioned and incubated with SOCS1 antibody (1 : 500 dilution), GALC antibody (1 : 200 dilution, sigma, SAB1402780), p-STAT1 antibody (1 : 200 dilution, abcam), or NeuN antibody (1 : 200 dilution, abcam, ab104224) at 4 °C for 36 h. Then, the sections were further incubated with Cy3-labeled goat anti-rabbit or anti-mouse IgG (1 : 400 dilution, Thermo Scientific, A16101, A16071), or FITC-labeled donkey anti-mouse or anti-rabbit IgG (1 : 400 dilution, Thermo Scientific, A24507, A16030) at 4 °C for 18 h. The images were obtained by a confocal laser scanning microscope (Leica, Heidelberg, Germany).

2.8 Statistical analysis

Differences between groups were analyzed by one-way analysis of variance (ANOVA) with SPSS 23 software (SPSS, Chicago, IL, USA). Normality and homoscedasticity of the

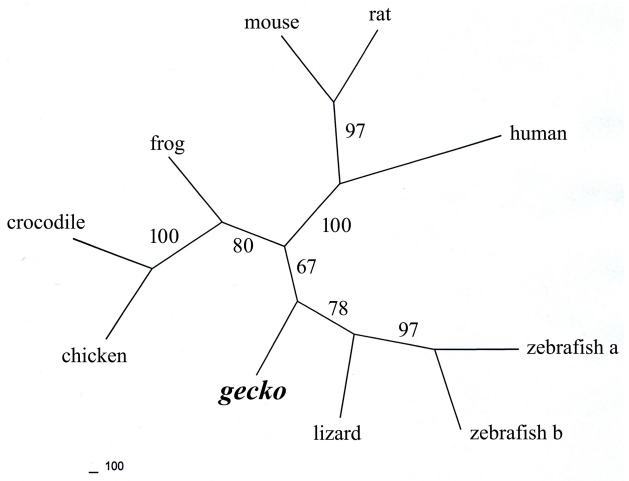

Fig. 2. Phylogenetic analysis of SOCS1 proteins. Unrooted phylogenetic tree of SOCS1 proteins from the gecko and other representative species constructed by the neighbor-joining method within the package PHYLIP 3.5c. Bootstrap majority consensus values on 1000 replicates are indicated at each branch point as a percentage.

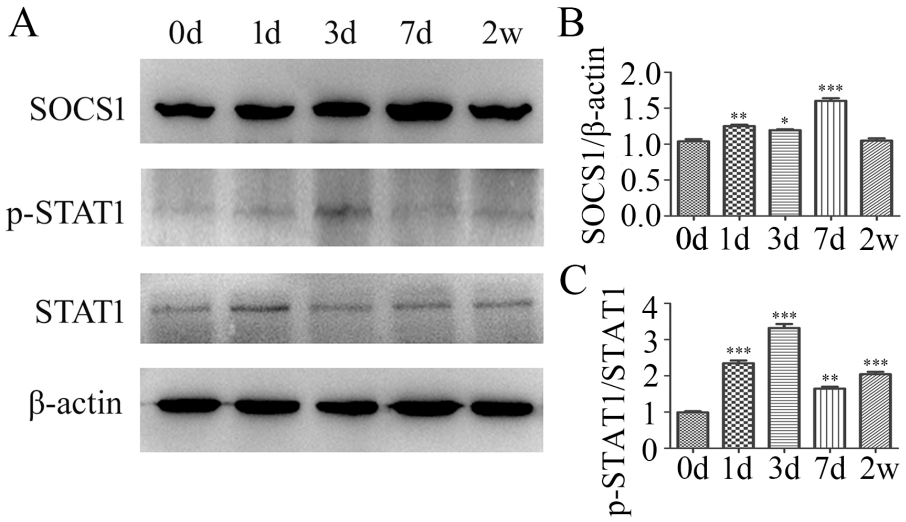

Fig. 3. Determination of gSOCS3 and STAT1 protein levels in the injured spinal cord of gecko. Western blot analysis of gSOCS1 and phosphorylated STAT1 (A) in the 0.5 cm segments of the injured cord following gecko tail amputation (n = 6) at 0 d, 1 d, 3 d, 7 d and 2 w, respectively; (B) and (C) are statistical analysis of (A), respectively. The asterisks represent a significant difference between each time point and the control. Experiments were performed in triplicate. Data are expressed as mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

data were verified using Levene's test before statistical analysis. $p < 0.05$ was considered statistically significant.

3. Result

3.1 Cloning and characteristic analysis of gecko SOCS1 (gSOCS1)

To understand the structural characteristics of gSOCS1, the cDNA of gSOCS1 (GenBank accession number: XP_015265413) was first cloned by 5'- and 3'-RACE. The full length of the amplified product is 1372 bp, encoding a protein of 200 amino acid residues (Fig. 1). The gSOCS1 protein contains an N-terminal variable region, a KIR domain, a SH2 domain, a 12-aa ESS domain and a SOCS box conserved in other vertebrates homologs (Fig. 1). Alignment with other vertebrates has shown that gSOCS1 shares 37.3–70.1% identity with human, mouse, chicken, lizard, frog and zebrafish SOCS1, respectively (Fig. 1), indicating differential conservation of the protein in the phylogeny.

A phylogenetic tree demonstrated that gecko SOCS1 clustered with lizard and zebrafish, suggesting a closer evolutionary relationship. It also suggests that the gecko SOCS1 is more similar to mice and other mammals than lizards and zebrafish in an evolutionary relationship (Fig. 2).

3.2 Expression changes of gSOCS1 in the injured spinal cord of gecko

To elucidate the potential roles of gSOCS1 in the injured spinal cord, the amputation of the gecko tail was performed and 0.5-cm cord segments were collected at 0 d, 1 d, 3 d, 7 d and 2 w. The protein level of gSOCS1 detected by western blot analysis increased from 1 d onwards, with a peak at 7 d, followed by a return to the control level at 2 w (Fig. 3A,B). As activation of the STAT1 transcription factor is associated with the inducible expression of SOCS1, the phosphorylation of STAT1 was also determined. Results showed that

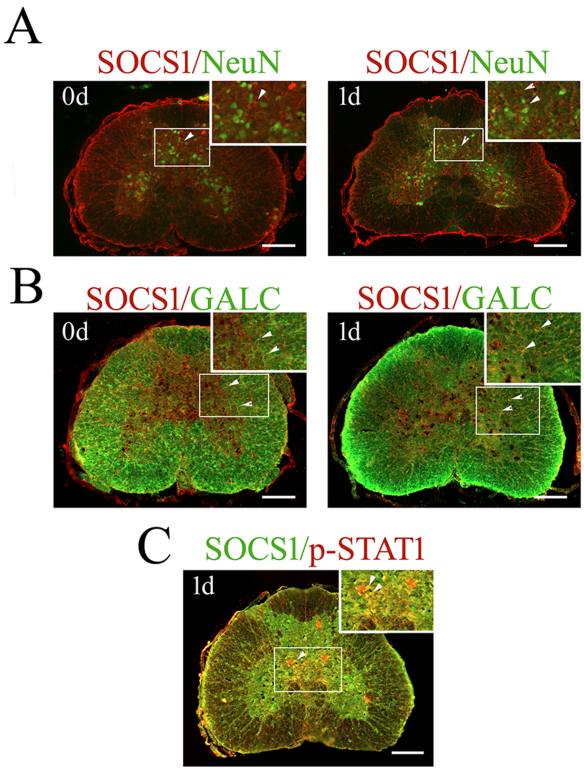

Fig. 4. Tissue distribution of gSOCS1 in the injured spinal cord following gecko tail amputation at 0 d and 1 d (n = 5). (A,B) Immunohistochemistry shows the colocalization of gSOCS1 (red) with NeuN-positive neurons (green), GALC-positive oligodendrocytes (green). (C) Colocalization of gSOCS1 (green) with pSTAT1 (red). Scale bar, 100 μm. The rectangle indicates the region magnified. Arrowheads indicate the colocalized signals.

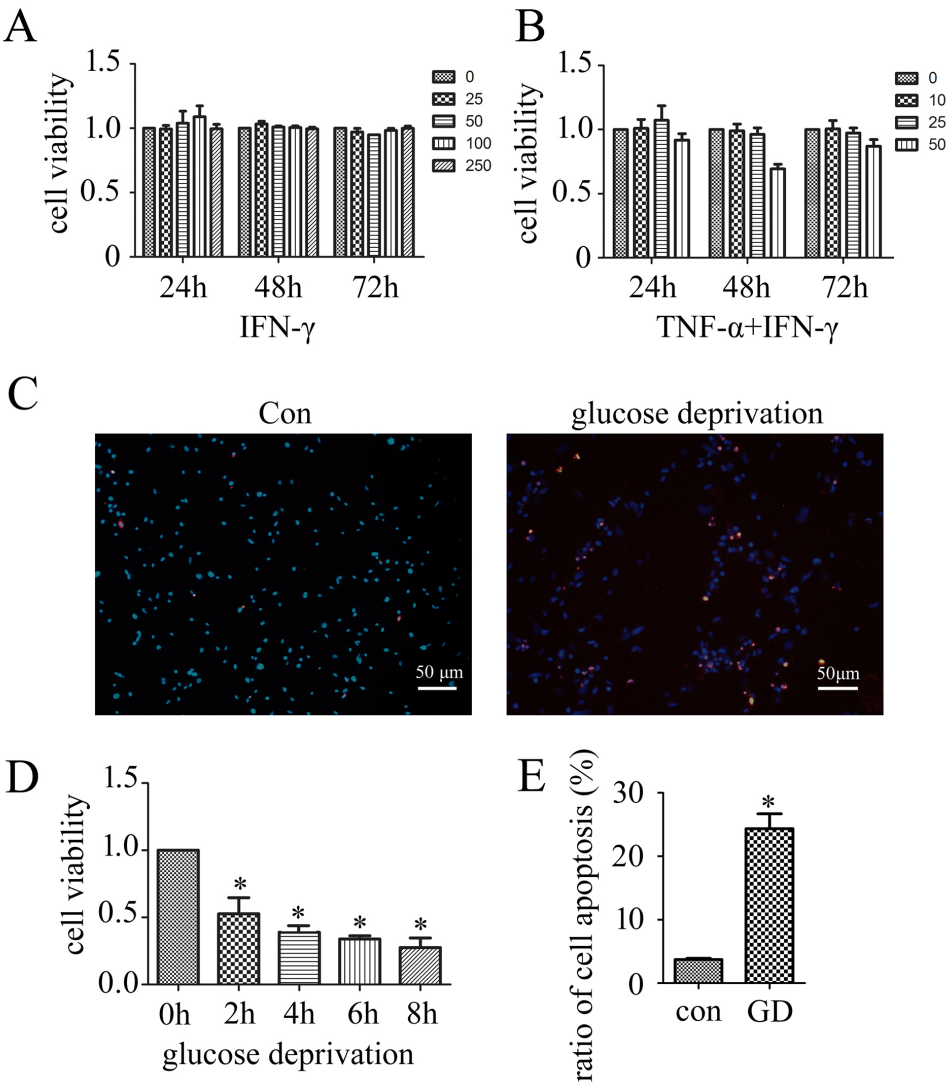

Fig. 5. Effects of IFN-γ, TNF-α or glucose deprivation on the apoptosis of gecko Gsn3 cells. CCK-8 assay determined the cell viability following treatment with 0–250 ng/mL IFN-γ (A), or combinations of TNF-α (0–50 ng/mL) and IFN-γ (250 ng/mL) for 24, 48 and 72 h (B); (C) TUNEL detection of glucose deprivation-induced apoptosis of Gsn3 cells; (D) Assay of cell viability under glucose deprivation stress for 2, 4, 6 and 8 h, respectively; (E) Statistical analysis of (C) in 20 visual fields. Experiments were performed in triplicates. Data are expressed as mean $\pm$ SEM; $^*p < 0.01$. Scale bar, 50 μm.

phosphorylated STAT1 was significantly increased following gecko SCI (Fig. 3A,C). The data indicate that spinal cord injury induces the expression of gSOCS1 and its activator STAT1 protein.

To reveal the potential target cells within which gSOCS1 mediates its physiological functions, immunofluorescence staining was performed to detect colocalization of gSOCS1 with neurons and oligodendrocytes. The results showed that gSOCS1 was found to be distributed in NeuN-positive neurons and GALC-positive oligodendrocytes in the severed gecko cord (Fig. 4A,B). Meanwhile, gSOCS1 and its activator p-STAT1 were well colocalized (Fig. 4C), indicating that activation of STAT1 protein is possibly involved in inducing the expression of gSOCS1 in the neurons and oligodendrocytes in the injured spinal cord of gecko.

3.3 Enforced expression of gSOCS1 protected oligodendrocytes against glucose deprivation-induced apoptosis

Injury of the mammalian spinal cord often leads to apoptosis of oligodendrocytes due to insults of various inflammatory cytokines. SOCS1 has been shown to have the capability to inhibit such deleterious effects [29]. To examine the protective roles of gSOCS1 on the oligodendrocytes in the regenerative spinal cord, an apoptotic model of the Gsn3 gecko oligodendrocyte cell line was established. As IFN-γ is shown to efficiently induce apoptosis of mammalian oligodendrocytes [29, 30], Gsn3 cells were stimulated with 0–250 ng/mL recombinant IFN-γ for 24, 48 and 72 h, respectively. However, the CCK-8 kit assay demonstrated that different concentrations of recombinant IFN-γ were insufficient to promote Gsn3 apoptosis (Fig. 5A). TNF-α can potentiate IFN-γ in inducing the cell death of oligodendrocytes [31]. Thus

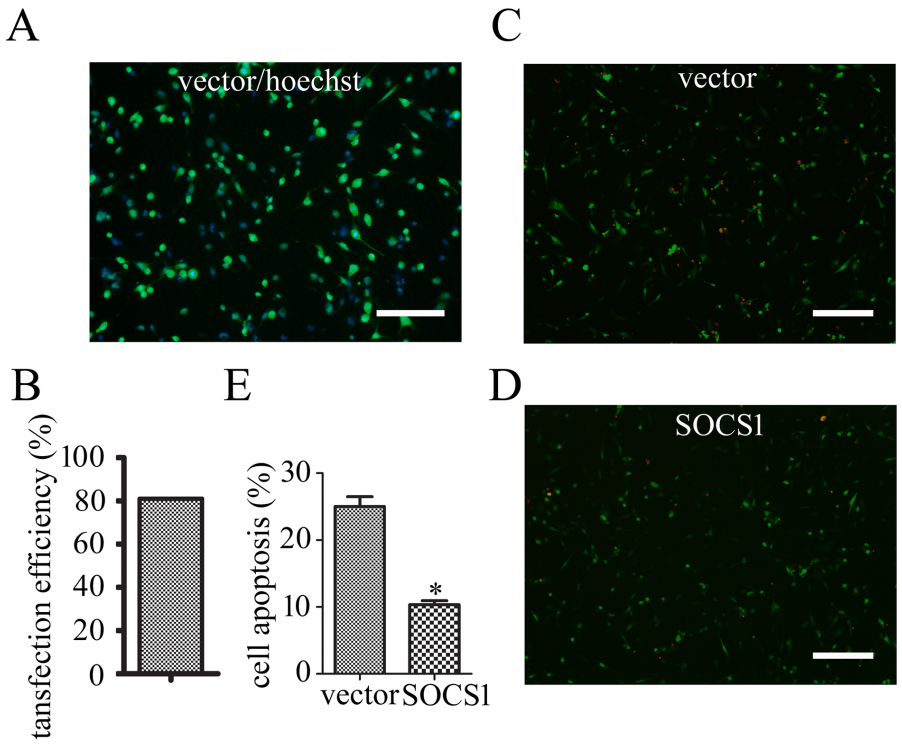

Fig. 6. Determination of gSOCS1 protective roles against glucose deprivation-induced apoptosis on Gsn3. (A) Transfection efficiency of adenovirus on Gsn3 cells; (B) Statistical analysis of (A); (C,D) TUNEL assay of Gsn3 cells transfected with either GV314-gSOCS1 or GV314-vector adenovirus for 48 h, followed by glucose deprivation for 24 h; (E) Statistical analysis of (C, D). Data are expressed as mean ± SEM; $*p < 0.01$. Scale bars, 25 μm in (A), 50 μm in (C,D).

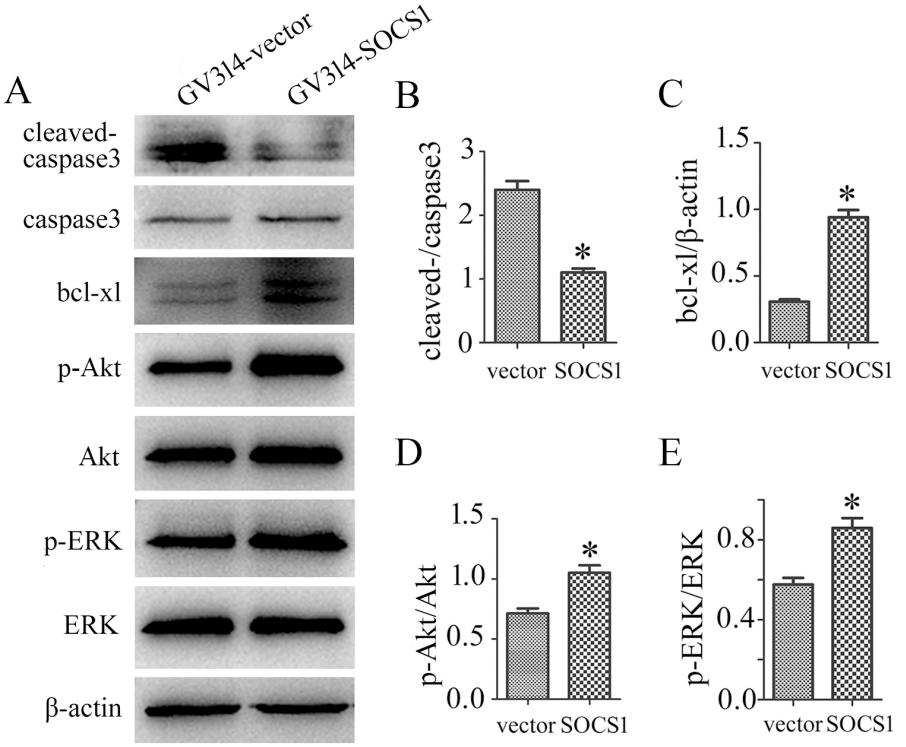

Fig. 7. Western blot analysis for the apoptosis-related proteins following GV314-gSOCS1 or GV314-vector adenovirus transfection for 48 h, followed by glucose deprivation for 24 h on Gsn3. (A) Cleaved-caspase3, Bcl-Xl, p-Akt, and p-ERK were detected by western blot. (B–E) Statistical analysis of (A). Experiments were performed in triplicate. Data are expressed as mean ± SEM; $*p < 0.01$.

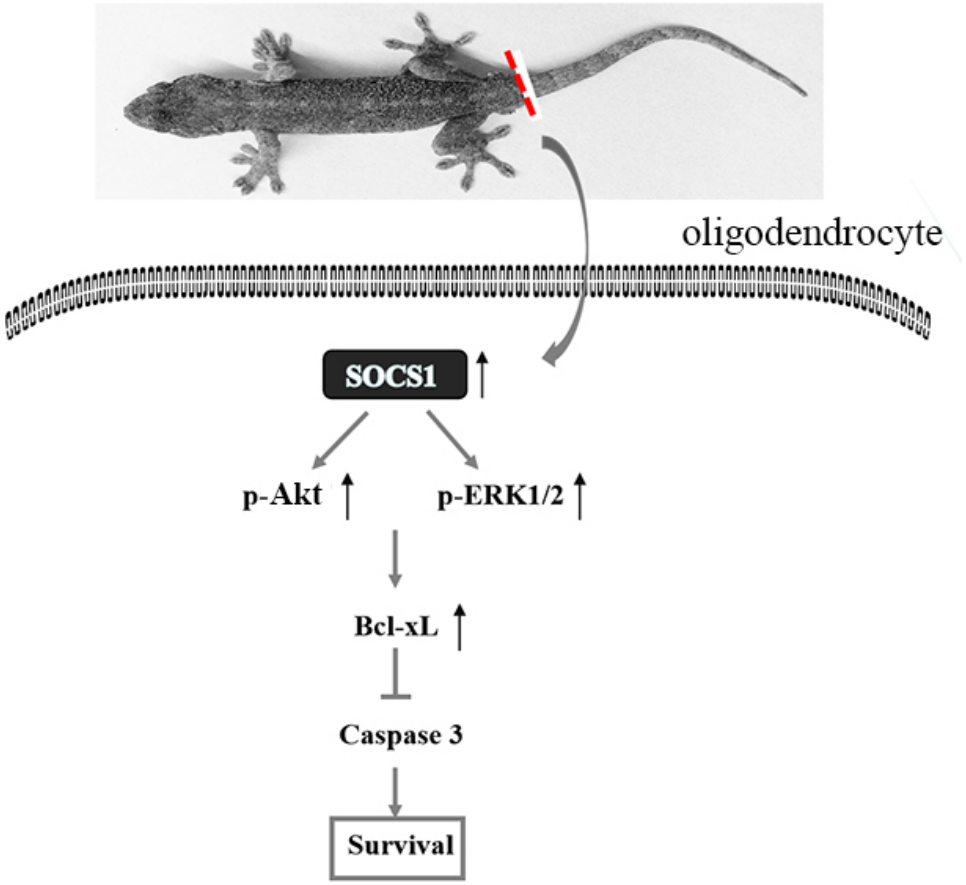

Fig. 8. Illustration of gecko SOCS1 protein in protecting oligodendrocytes against apoptosis.

a combined treatment with TNF-α (0–50 ng/mL) and IFN-γ (250 ng/mL) was subsequently performed on Gsn3 cells. Consequently, neither TNF-α nor IFN-γ was able to induce cell apoptosis (Fig. 5B).

We then turned to a GD-induced cell apoptosis model, which has been successfully established to potentiate the apoptosis of gecko astrocytes [32]. Following the culture of Gsn3 in a glucose-free medium for 24 h, the apoptotic cell number increased significantly, as evidenced by the TUNEL method (Fig. 5C,E). The CCK8 assay also confirmed cell viability decreased following cell culture in glucose-free medium for 2–8 h (Fig. 5D). The data indicate that glucose deprivation, rather than stimulation with IFN-γ, effectively induces the apoptosis of Gsn3 cells.

To evaluate the protective role of gSOCS1 in apoptotic oligodendrocytes, gSOCS1 adenovirus expression vectors were constructed that exhibited a transfection rate of over 80% (Fig. 6A,B). Gsn3 cells were transfected with GV314-gSOCS1 or GV314-vector adenovirus for 48 h, followed by incubation in a glucose-free culture for 24 h. Overexpression of gSOCS1 significantly reduced the apoptotic cell number of oligodendrocytes (Fig. 6C–E). The data indicate that gSOCS1 protects Gsn3 from glucose deprivation-induced apoptosis.

Gekko japonicus has a remarkable regenerative ability to regenerate the injured tail comprised of muscles, cartilage, and spinal cord. The cells of regenerating ependymal tube begin to proliferate and penetrate into the blastema at 8–15 d after tail amputation [24]. Accompanied by this process, the number of either surviving or newly generated neurons increases from 7 d onwards (**Supplementary Fig. 1**). To clarify the potential effects of gSOCS1 on tail regeneration, a total of 9 μL GV314-gSOCS1 or GV314-vector adenovirus was injected into the amputated caudal vertebrae in three equal parts 2–3 mm from the plane. The GFP fluorescence intensity was used to detect the transfection efficiency in the sections of the gecko spinal cord (**Supplementary Fig. 2A**). Histopathology with LFB (Luxol fast blue) staining revealed that the GV314-vector group showed vacuolar changes and microtubule loss from the myelin sheath relative to the GV314-SOCS1 group at 7 d (**Supplementary Fig. 2B**). The data indicate that the overexpression of SOCS1 reduced the degree of myelin sheath destruction. However, the enforced expression of gSOCS1 did not promote regeneration of the tail following amputation at 7 d and 2 w (**Supplementary Fig. 2C**).

3.4 gSOCS1 promoted the activation of intracellular survival pathways

To unveil the mechanism of gSOCS1-mediated resistance to cell apoptosis, we determined apoptosis-related signaling after Gsn3 transfection with GV314-gSOCS1. Western blot analysis demonstrated that the activity of Caspase3 was significantly attenuated. At the same time, the protein level of Bcl-xL, a death-inhibitory member of the Bcl-2 family, was markedly increased following GV314-gSOCS1 transfection for 48 h before glucose deprivation for 24 h (Fig. 7A–C). The MEK/ERK and PI3K/Akt pathways promote the survival of cells by mediating the upregulation of Bcl-xL [33]. Therefore, the levels of both phosphorylated Erk1/2 and Akt in gSOCS1-mediated Gsn3 were determined. As expected, the phosphorylation levels of both Erk1/2 and Akt were significantly increased in comparison with control (Fig. 7A,D,E). The data indicate that gSOCS1-mediated resistance to oligodendrocyte apoptosis is involved in activating of MEK/ERK and PI3K/Akt pathways.

4. Discussion

Apoptotic loss of oligodendrocytes is sustained for several weeks to months following mammalian SCI [34]. To date, mechanisms that result in the apoptosis/necrosis of oligodendrocytes have not been fully elucidated. However, some of them have been attributed to inflammatory cytokine insults in the injured cord [35]. Many therapeutic measures, including corticosteroid, leukemia inhibitory factor (LIF), caspase inhibitors, neurotrophins and biomaterial-based approaches, have been used to reduce oligodendrocyte death and/or promote remyelination [36]. However, the protective therapies of exogenous drugs on oligodendrocytes/myelin have not achieved satisfactory outcomes [36]. For this reason, it is thought that endogenous interference of apoptosis-related factors in oligodendrocytes might be an alternative and promising strategy to protect cells against demyelinating insults. SOCS1 represents one of the powerful attenuators of the inflammatory cytokine- and TLR-mediated apoptotic responses of oligodendrocytes [19]. Comparatively, SOCS3 has been shown to promote apoptosis of oligodendrocytes by limiting leukemia inhibitory factor receptors [37]. SOCS3 is absent from oligodendrocytes for regenerating the spinal cord [38]. Therefore, SOCS1 may represent a potential therapeutic target for demyelinating neuropathology.

IFN-γ is a pleiotropic cytokine produced by NK cells and T cells [39]. The stimulation of IFN-γ within the CNS induces the apoptosis of oligodendrocytes and the pathogenesis of demyelinating disorders of the CNS [40]. Overexpression of SOCS1 in both oligodendrocytes and immunocytes inhibits IFN-γ-induced expression of MHC class I and II, which are associated with the occurrence of apoptosis [19]. Unexpectedly, IFN-γ was unable to facilitate the apoptosis of gecko oligodendrocytes, suggesting the unique properties of oligodendrocytes in regenerative vertebrates in response to inflammatory cytokine insults. A similar phenomenon occurs in the astrocytes of gecko, which results in less reactive gliosis following spinal cord injury [41]. However, gSOCS1 still shows a protective role against glucose deprivation-induced apoptosis of Gsn3, validating that SOCS1 has universal significance in suppressing cell apoptosis mediated by multiple factors.

MAPK/ERK and PI3K/Akt signaling cascades are essential for inter and intracellular communication and the regulation of fundamental cell functions such as growth, survival, and proliferation [42, 43]. Both ERK and Akt signaling inhibit apoptosis in various ways, including the expression of caspase inhibitors, neutralization of Bcl-2 family proteins, and activation of NF-κB [44–46]. Our results demonstrated that enforced expression of gSOCS1 in Gsn3 gecko cells was sufficient to enhance the phosphorylation of Erk1/2 and Akt, indicating the involvement of the anti-apoptotic pathway of gSOCS1. Whether the JAK/STAT signal pathway is blocked or intersected remains to be further studied.

In conclusion, as illustrated in Fig. 8, the expression of SOCS1 protein is increased in the injured spinal cord of the gecko. It is associated with anti-apoptosis of oligodendrocytes through regulation of the ERK and Akt signal pathways.

Author contributions

Yjun W and Yjie W designed this work. Yjie W wrote the paper. BH, WW, CS, TY, Hui L, and Hao L performed the experiments. Yjun W, Yjie W, XC, Hui L, Hao L, and HS analyzed the data. All authors have approved the present version of the manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Animal Care and Use Committee of Nantong University and the Jiangsu Province Animal Care Ethics Committee (S20190420-405).

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current research are available from the corresponding author on reasonable request.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://jin.imrpress.com/EN/10.31083/j.jin2003062.

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