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Original article

Improved neurite outgrowth on central nervous system myelin substrate by siRNA-mediated knockdown of Nogo receptor

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ABSTRACT

Purpose: To investigate the *in vitro* effect of short interfering RNAs (siRNAs) against Nogo receptor (NgR) on neurite outgrowth under an inhibitory substrate of central nervous system (CNS) myelin.
Methods: Three siRNA sequences against NgR were designed and transfected into cerebellar granule cells (CGCs) to screen for the most efficient sequence of NgR siRNA by using reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence staining. NgR siRNA sequence 1 was found the most efficient which was then transfected into the CGCs grown on CNS myelin substrate to observe its disinhibition for neurite outgrowth.
Results: Compared with the scrambled control sequence of siRNA, the NgR siRNA sequence 1 significantly decreased NgR mRNA level at 24 h and 48 h ($p < 0.05$), which was recovered by 96 h after transfection. NgR immunoreactivity was also markedly reduced at 24 and 48 h after the transfection of siRNA sequence 1 compared with that before transfection ($p < 0.05$). The NgR immunoreactivity was recovered after 72 h post-transfection. Moreover, the neurite outgrowth on the myelin substrate was greatly improved within 72 h after the transfection with siRNA sequence 1 compared with the scrambled sequence-transfected group or non-transfected group ($p < 0.05$).
Conclusion: : siRNA-mediated knockdown of NgR expression contributes to neurite outgrowth *in vitro*.

Introduction

It is well known that the central nervous system (CNS) of adult mammalian fails to regenerate after injury, resulting in persistent neurological deficits. Although adult CNS neurons generally survive axotomy, axonal regeneration is transient and occurs only over a confined area, which inhibits the reformation of functionally-relevant synaptic contacts. As yet, there is a lack of an effective therapy to treat the patients with CNS injuries such as traumatic brain injury, spinal cord injury (SCI), and stroke. The reasons for the failure of the CNS to regenerate has been extensively investigated,[1–4] but the mechanism is still unclear.

It has been reported that the mechanism may be related with the inability of the neuron to regenerate, the limited availability of neurotrophins required for neuronal survival and axonal growth,[5,6] the generation of the intrinsic axonal outgrowth inhibitors,[1,7–9] and the formation of a glial/collagen scar, a biochemical barrier hindering axon advancement.[5,6,10] Among them, the axon growth inhibitors, mainly from the CNS myelin, may play a key role in the failure of the regeneration.[4,7] To date, several such inhibitors have been identified, including myelin-associated glycoprotein (MAG),[7,8,11] oligodendrocyte myelin glycoprotein (OMgp) and Nogo.[12–15] All these proteins bind to the same neuronal glycosylphosphatidylinositol (GPI)-anchored receptors—Nogo receptors (NgRs) and transduce inhibitory signals to cells by interacting with p75 neurotrophin receptor as a co-receptor. The Co-receptor p75-NgR induces growth cone collapse by triggering an inhibitory signaling pathway, thereby inhibiting axonal

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regeneration.[4,11,16,17] Thus, inhibiting or blocking NgR may improve axon outgrowth. Focusing on this target, several reagents have been developed to overcome the inhibition of myelin on axonal regeneration, including antibody or antagonist,[7,18–20] downstream signaling molecule and vaccine.[3,21–26]

Gene knockout or knockdown can effectively block the expression and/or function of target genes. Recently, the transfection of short interfering RNA (siRNA), a powerful tool to knockdown specific gene expression, has been intensively studied and used to block gene expression and/or function.[27,28] Because NgR plays an important role in the inhibition of axon outgrowth, we hypothesize that a siRNA against NgR is able to promote the axon outgrowth of the neuron. To test this hypothesis, we designed NgR-specific siRNA sequences. After screening for the most efficient sequence of siRNA against NgR, we conducted NgR blocking experiments and assessed the effect of NgR-specific siRNA on NgR expression and neurite outgrowth *in vitro*.

Materials and methods

Cerebellar granule cell culture

Cerebellar granule cells (CGCs) were cultured as previously described.[29,30] Briefly, cerebellar tissue was collected from rats (postnatal day 7). After meninges were removed, the tissue was digested with trypsin (0.3 mg/ml) for 12 min and then mixed with an equal volume of dissociation buffer (80% Dulbecco modified Eagle medium, 10% Ham's F-12 medium, 10% fetal bovine serum, and 0.1 mg/ml DNase). The tissue was centrifuged at 1000 × *g* for 5 min, and the pellet was resuspended with 1 ml of the dissociation buffer in a 12 mm × 75 mm tissue culture tube. Dissociated cells were collected in the supernatant after the tissue settled by gravity in the tube for 10 min. The dissociation process was repeated once. The dissociated cells were finally resuspended in 1.5 ml of Hank's Balanced Salt Solution (HBSS). Cell viability was determined using trypan blue dye exclusion and cell counts.

NgR-specific siRNA preparation and transfection

NgR siRNA sequences of the rat, designed with the criteria described by Elbashir et al[31] were shown in Table 1. All the sequences were subjected to a BLAST program to make sure there was no significant homology with other genes prior to the process of the synthesis by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China).

For siRNA transfection, we used TransMessenger transfection reagent (Qiagen, Hilden, Germany) in 24-well tissue culture plates following the manufactory's protocol. Briefly, 1.2 μg of siRNA and 1.6 μl of enhancer R, a specific RNA-condensing reagent, were mixed in a final volume of 100 μl of an RNA-condensing buffer (Buffer EC-R) at room temperature for 5 min, which was further incubated with 2 μl of TransMessenger transfection reagent for an additional 10 min to form a transfection-complex. After being mixed with 300 μl of Opti-MEM I reduced serum medium (Life Technologies, Carlsbad, CA, USA), the complex was added to the cultured cells. Following a 4-h incubation, the old culture medium was replaced with neurobasal medium, and the cells were incubated until they were ready for the following analyses.

Table 1
Seven siRNA sequences of the rat NgR.

Items	Sequences
Sequence 1 (sense)	5′-GCAGUACCUGCGACUCAAUTT-3;
Sequence 1 (antisense)	5′-AUUGAGUCGCAGGUACUGCAG-3′
Sequence 2 (sense)	5′-GGCCAGGUUGUUCCAGAAATT-3′
Sequence 2 (antisense)	5′-UUUCUGGAACAACCUGGCCTC-3′
Sequence 3 (sense)	5′-GCUUCCAGCCAUGCCGGAATT-3′
Sequence 3 (antisense)	5′-UUCCGGCAUGACUGGAAGCTG-3′
Scrambled control sequence (sense)	5′-UUCUCCGAACGUGUCACGUTT-3′
Scrambled control sequence (antisense)	5′-ACGUGACACGUUCGGAGAATT-3′

To calculate transfection efficiency, the scrambled siRNA labeled with Carboxy fluorescein (FAM) was transfected into the CGCs, too. At 6 h after transfection, the number of FAM-labeled CGCs, emitting green fluorescence, was counted. The transfection efficiency was calculated by the ratio between the number of cells with green fluorescence and the total cultured cells.

For the CGC transfection, a preliminary study was first conducted to screen for the most efficient siRNA sequence. Briefly, three sequences of NgR siRNA and one scrambled control sequence were transfected into the CGCs as mentioned above. The most efficient sequence of siRNA NgR, identified by reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence staining as described below, was used for further assessment of neurite outgrowth.

Preparation of the CNS myelin extract

The CNS myelin extract was prepared according to the method of Cuzner et al.[32] Briefly, adult Sprague–Dawley rat brains were homogenized in 0.32 mol sucrose with 1 mmol EDTA (pH 7.0). The suspension was centrifuged at 800 × *g* for 10 min and supernatant was collected. The cell pellet was resuspended in the original volume of 0.32 mol sucrose with 1 mmol EDTA (pH 7.0), and recentrifuged at the above speed. The second supernatant was collected and pooled with the first one, which was then centrifuged at 13,000 × *g* for 20 min. After the removal of the supernatant, the pellet was suspended in 0.9 mol/L sucrose, followed by carefully overlaid with 1–2 ml of 0.32 mol/L sucrose. The sucrose solutions were then centrifuged at 20,000 × *g* for 60 min. The white material at the interface of the two sucrose layers was collected in the minimum volume possible, dispersed in 20 volumes of 0.32 mol/L sucrose, and centrifuged at 13,000 × *g* for 25 min. The white pellet was then collected, diluted in 25 volumes of pure water, left on ice for 30 min before centrifuging at 20,000 × *g* for 25 min. Then the final white pellet was resuspended in a small volume of water and freeze-dried overnight. The protein content of the myelin extract was determined using nucleic acid/protein analyzer (DU640-type, Beckman, Brea, CA, USA).

RT-PCR

To quantify NgR expression in the CGCs before and at 24, 48, 72, and 96 h after transfection, we determined the mRNA levels of the NgR and the internal control hypoxantine phosphoribosyltransferase (HRPT) using RT-PCR. Total RNA was extracted with Trizol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription of mRNA to cDNA was performed with reverse transcriptase as usual. PCR primer sequences are shown as follows: NgR sense, 5′-CTG CTG GCA TGG GTG TTA TGG-3′; NgR antisense, 5′-TCT GGC TGG AGG CTG GGA T-3′; HRPT sense, 5′-AAA GCC AAG TAC AAA GCC TAA A-3′; HRPT antisense, 5′-CTG TCT GTC TCA CAA GGG AAG T-3′. PCR amplification was carried out using Taq DNA polymerase in a 25-μl of PCR reaction mixture containing 3 μg cDNA. For NgR, the amplification protocol consisted of initial denaturation (94°C for 4 min), 35 cycles of reaction (denaturation at 94°C for 30 s, annealing at 61°C for 60 s, extension at 72°C for 45 s), and final extension at 72°C for 10 min. The method of HRPT amplification was similar to the one for NgR except that there were 32 cycles of reaction and annealing was carried out at 58°C for 50 s. The

amplified PCR products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide. The ratio of NgR PCR product to that of the HRPT was obtained by analyzing the integrated optical density (IOD) of the corresponding bands using UV/Vis Spectrometer (FR-200, Shanghai Furi Science and Technology Co., Ltd, Shanghai, China) and Smart View 2001 Software (Shanghai Furi Science and Technology, Shanghai, China).

Immunofluorescence double staining

The CGCs were grown in 24-well culture plates for immunofluorescence double staining to detect the downregulation of NgR expression by siRNA. Cells were analyzed before and at 24, 48, 72, and 96 h after transfection. In order to screen for the most efficient siRNA sequence, the cells were assessed at 24 h post-transfection. The cultured cells were fixed by 4% formaldehyde polymerization for 10 min and rinsed twice in PBS (pH 7.4) for 5 min. The cells were blocked in a blocking buffer, donkey serum (1:100 in PBS with 0.3% Triton-100, Jackson Immuno Research Lab, West Grove, PA, USA) for 30 min at room temperature and then incubated at 37°C for 30 min in a mixture of primary antibodies, including mouse anti-rat β III-tubulin IgG (1:800, Sigma, St. Louis, MO, USA) and goat anti-rat NgR IgG (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in the blocking buffer. After the primary antibodies were removed and cells were washed 3 times in PBS, the cells were incubated in a mixture of secondary antibodies, containing FITC-donkey anti-goat IgG and TRITC-donkey anti-mouse IgG (both 1:80, Jackson Immuno Research Lab, West Grove, PA, USA) in the blocking buffer, at 37°C for 30 min. The cells were rinsed 3 times in PBS. The cells were then coverslipped with the neutral balsam mounting medium with Hoechst 33,342 (10 μg/mL, Sigma, St. Louis, MO, USA). The cell images were captured with an Olympus BX60 fluorescence microscope (Olympus, Japan).

Neurite outgrowth assessment

To determine the effect of siRNA on neurite outgrowth, the CGCs were cultivated on a control or inhibitory substrate, with or without NgR siRNA sequence 1 transfection. There were four experimental groups: Group A, untransfected cells cultured in normal cell culture medium; Group B, untransfected cells cultured in normal cell culture medium with the inhibitor (CNS myelin extract); Group C, cells transfected with NgR siRNA sequence 1 cultured in the normal cell culture medium with the inhibitor (CNS myelin extract); and Group D, cells transfected with scrambled siRNA sequence cultured in normal cell culture medium with the inhibitor (CNS myelin extract).

During the early days after transfection (24, 48, and 72 h), the photomicrographs of immunostained β III-tubulin positive neurons were obtained and at least 75 randomly selected neurons per coverslip were analyzed for each group. A neurite was defined as a process extending from the cell body for a distance of at least one cell diameter. When more than one neurite extended from a cell, only the longest neurite was measured. Neurite length was measured by SPOT 4.0.1 software (SPOT Imaging Solutions, Sterling Heights, Michigan, USA).

Statistical analysis

All the data were presented as mean ± SD. The differences between the experimental groups were analyzed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA) by two-way ANOVA. A value of $p < 0.05$ was considered significant.

Results

siRNA transfection is highly efficient

Each transfection contained greater than 95% viable cells. FAM-labeled siRNAs were used to determine transfection efficiency. Almost all β III-tubulin-positive neurons were transfected with the labeled siRNA (Figs. 1 and 2).

siRNA sequence 1 was selected for the following experiment

To determine the specificity and efficiency of designed siRNAs on NgR mRNA expression, total RNA was extracted from each group 24 h post-transfection and the ratio of NgR mRNA to HRPT mRNA was obtained by integrated optical density (IOD) analysis. Normalized to HPRT, the NgR mRNA level of the cells transfected with siRNA sequence 1 was significantly reduced compared with that of untransfected cells or the cells transfected with siRNA sequence 2 or 3 or scrambled oligonucleotides (the ratio was 0.052 ± 0.027 vs. 0.507 ± 0.083, 0.487 ± 0.058, 0.525 ± 0.084, or 0.508 ± 0.039, respectively; $p < 0.05$, Fig. 3). No significant differences in NgR mRNA levels were observed among cells transfected with sequence 2 and 3, the scrambled sequence, and untransfected cells ($p > 0.05$; Fig. 3), demonstrating the specificity and efficiency of siRNA sequence 1 in blocking NgR expression. Therefore, the most efficient siRNA sequence 1 was chosen for further studies.

The optimal time point for NgR mRNA inhibition is 24 h after siRNA transfection

To determine the time-related response of NgR to siRNA transfection, we determined NgR mRNA level by RT-PCR at different time points (0, 24, 48, 72, and 96 h post-transfection). Our results showed that as compared with before transfection,

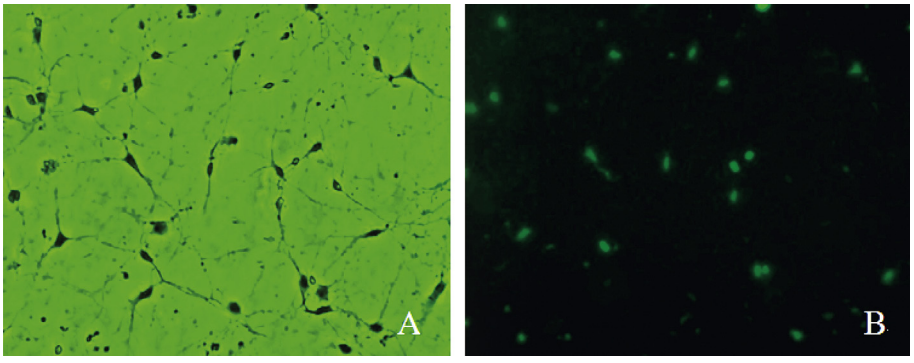

Fig. 1. Light (A) and fluorescent (B) microscopic images of scrambled siRNA-transfected CGCs. Cells transfected with FAM-labeled siRNA are shown in green.

NgR mRNA level was notably decreased at 24 and 48 h after transfection (the ratio was 0.366 ± 0.049 vs. 0.067 ± 0.027 and 0.092 ± 0.027, respectively, $p < 0.05$ for both), which was then significantly recovered at 72 h after transfection (0.207 ± 0.049, $p < 0.05$ compared with 24 and 48 h). By 96 h, the level of NgR mRNA was not significantly different compared with that of the untransfected control (0.338 ± 0.071 vs. 0.366 ± 0.049; $p > 0.05$; Fig. 4).

NgR expression was down-regulated at 48 h following siRNA sequence 1 transfection

Immunofluorescence double staining showed that the fluorescence intensity of the NgR in siRNA sequence 1-transfected cells was attenuated at 48 h post-transfection, using β III-tubulin as a reference, compared with cells transfected with siRNA sequence 2 or 3, scrambled sequence, or the non-transfected

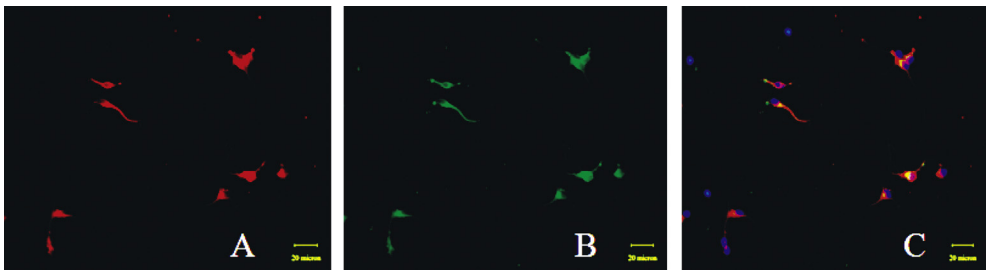

Fig. 2. Representative fluorescence images of CGCs stained with the antibodies of β III-tubulin and NgR 24 h after plating. All β III-tubulin-positive cells (red) in panel A are also NgR-positive (green) in panel B. Panel C shows the merged images. Scale bar = 20 μm.

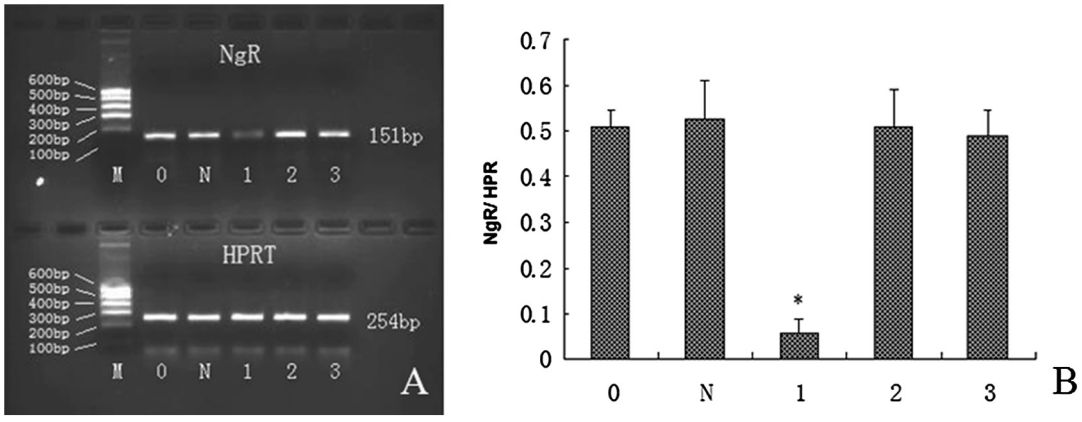

Fig. 3. siRNA-mediated knockdown of NgR mRNA at 48 h after transfection. A: RT-PCR analysis of mRNA expression. B: Densitometric analysis demonstrates that the ratio of NgR density to that of HPRT is significantly decreased by siRNA sequence 1 transfection (*$p < 0.05$ vs non-transfected cells, $n = 6$ for each group). There are no significant changes in NgR mRNA levels in the cells transfected with siRNA sequence 2 or 3, or the scrambled sequence ($p > 0.05$ vs non-transfected cells). 0: non-transfected cells; N: scrambled control sequence; 1: siRNA sequence 1; 2: siRNA sequence 2; 3: siRNA sequence.

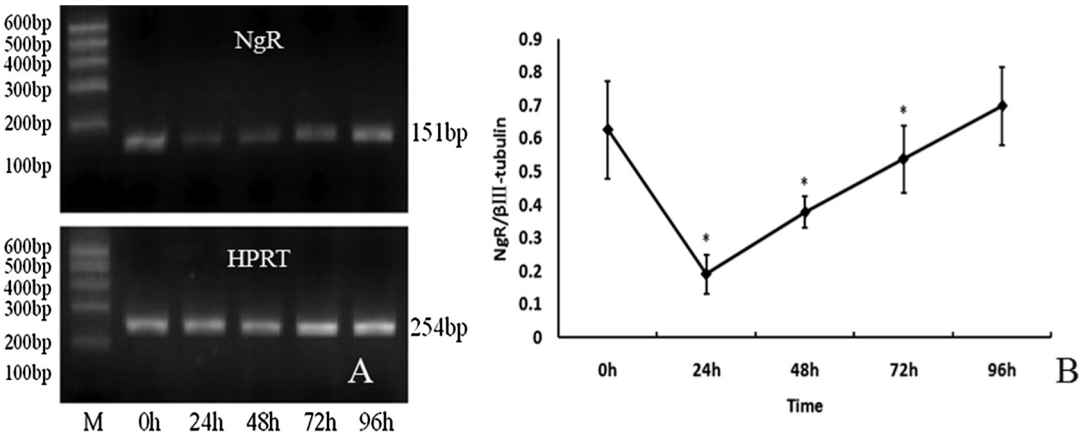

Fig. 4. Time course of siRNA-mediated knockdown of NgR mRNA. A: RT-PCR analysis of mRNA expression. B: Quantification of NgR/HPRT by image analysis. The data show that the maximal inhibition of NgR mRNA is achieved at 24 h post-transfection (*$p < 0.05$ vs control). Inhibition is maintained for approximately 72 h, then the level of NgR mRNA is restored to that of the non-transfected cells.

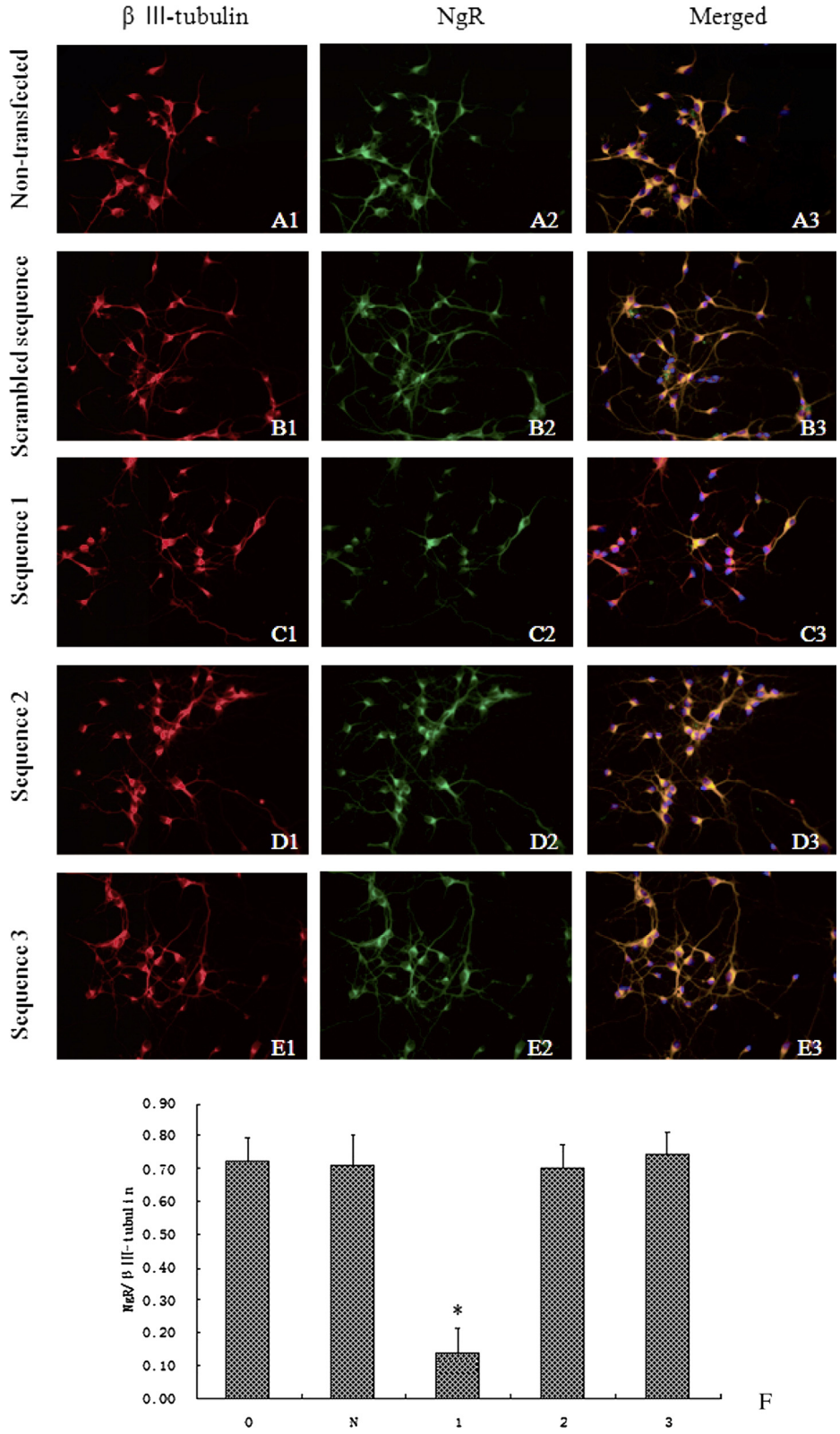

Fig. 5. A–E: NgR expression is suppressed by siRNA sequence 1 at 48 h after transfection. The cells, non-transfected or transfected with siRNA of sequence 1, 2, or 3, or scrambled sequence, are stained with β III-tubulin and NgR antibodies. F: Densitometric analysis shows that transfection of neurons with siRNA sequence 1 results in significant suppression in NgR (*$p < 0.05$ vs non-transfected cells), but no significant changes are detected in cells transfected with sequence 2 or 3, or the scrambled sequence ($p > 0.05$ vs non-transfected cells). 0: non-transfected cells; N: scrambled sequence; 1: siRNA sequence 1; 2: siRNA sequence 2; 3: siRNA sequence 3.

group (ratio of the stained NgR pixel intensity to that of the β III-tubulin was 0.166 ± 0.073 vs. 0.700 ± 0.073, 0.745 ± 0.069, 0.714 ± 0.087, or 0.723 ± 0.075, respectively; $p < 0.05$ for all; Fig. 5). In the time course study, we also found that NgR immunoreactivity was markedly reduced. Compared with β III-tubulin in untransfected cells, the ratio of the pixel intensity of the stained NgR was significantly decreased at 24 h after siRNA sequence 1 transfection (0.626 ± 0.146 vs. 0.292 ± 0.059; $p < 0.05$), which was then greatly recovered at 48, 72, and 96 h post-transfection (0.378 ± 0.047, 0.538 ± 0.102, and 0.698 ± 0.118, respectively, Fig. 6).

Table 2
Effects of siRNA transfection on neurite growth in cerebellar granule cells.

Group	Mean length of the longest neurite post-transfection (μm)		
	24 h	48 h	72 h
A	22.5 ± 12.2	57.1 ± 23.6	79.8 ± 38.6
B	7.3 ± 5.8	24.9 ± 12.4	57.3 ± 20.2
C	17.1 ± 10.2*	44.7 ± 22.5*	72.3 ± 32.6*
D	8.1 ± 5.8	27.7 ± 13.7	57.5 ± 21.7

The untransfected cells on noninhibitory substrate (Group A) exhibit the best growth. *p < 0.05, compared with Groups B and D at the same time point).

Neurite outgrowth was disinhibited after siRNA transfection

The average length of the longest neurites in each group is shown in Table 2. The length was significantly increased at 24, 48, and 72 h after the cells were transfected with NgR siRNA sequence 1 on inhibitory substrate (Group C) as compared to those in Group B and D ($p < 0.05$), although the untransfected cells on noninhibitory substrate (Group A) exhibited the best growth. By 96 h after transfection, the differences among the four groups tended to disappear (Figs. 7 and 8).

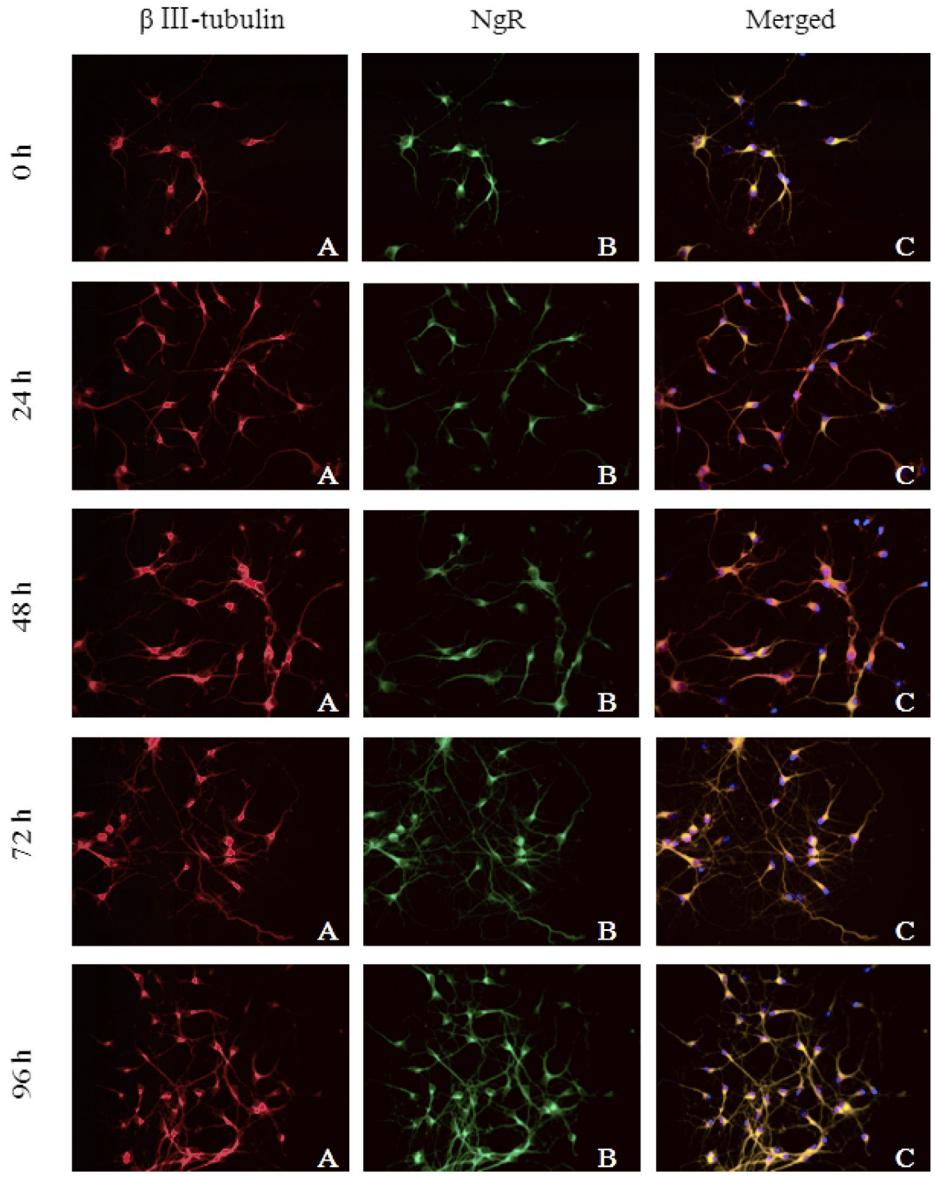

Fig. 6. Microscopic images of double immunofluorescence stained neurons for β III-tubulin (red) and NgR (green) at different time points after transfection.

Discussion

The present study demonstrated a considerable decrease in NgR mRNA and NgR immunoreactivity within 72 h after NgR siRNA transfection in cultivated cerebellar granule neurons. Also, we found inhibition of the neurite outgrowth in the non-transfected ones. The CGCs caused by myelin-associated inhibitors could be reversed by the NgR siRNA transfection. These findings suggest that NgR siRNA administration is an efficient strategy to improve the neurite growth of the neurons.

In a series of the landmark papers, Schwab and colleagues[33–35] demonstrated that CNS myelin and oligodendrocytes were non-permissive to axonal growth. Since then, the role of the inhibitory molecules in the CNS myelin has attracted more and more attention and has led to inspiring developments in preclinical studies. Those studies include the identification of a series of interacting molecules that likely play a primary role in controlling axonal outgrowth and plasticity and represent important novel targets for pharmacological interventions in the injured nervous system.[8,26,36,37] For instance, it has been reported that several proteins, including Nogo, MAG, and OMgp, may get involved in the inhibition of axon growth of the neurons[8,14,22,25,27,36] via Nogo-66 receptor.[11,13,38]

NgR is proposed to be an important target for the axonal sprouting and regeneration in the CNS.[39,40] Several treatment approaches based on the NgR have been developed, e.g., GrandPre et al[41] first identified a Nogo-66 (1–40) competitive antagonist peptide (NEP1–40) and found that the NEP1–40 administered intrathecally could significantly induce axon growth of the corticospinal tract and improve functional recovery in the rats subjected to mid-thoracic spinal cord hemisection. A soluble NgR (310) Ecto-Fc protein, which can bind Nogo-66 but does not transduce inhibitory signaling, is also reported to improve axonal outgrowth and promote locomotor function recovery after spinal cord injury.[19] In addition, the anti-NgR antibody obtained from the rat immunized with recombinant Nogo-66 receptor or the DNA vaccine against NgR can also promote axonal regeneration and recovery of function after spinal cord injury in rats.[40,41]

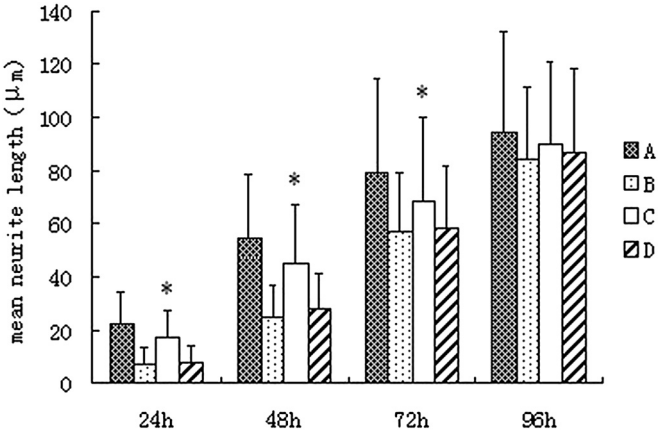

Fig. 7. Effects of siRNA transfection on neurite outgrowth of CGCs in different culture substrates. Following plating, neurite outgrowth of the three groups treated with myelin membrane proteins is inhibited to some extent; however, significant promotion of neurite outgrowth is observed in group C (*$p < 0.05$ vs groups B and D). By 96 h, the difference among the four groups has become insignificant. A: noninhibitory culture; B: myelin membrane proteins; C: myelin membrane proteins + siRNA; D: myelin membrane proteins + scrambled sequence.

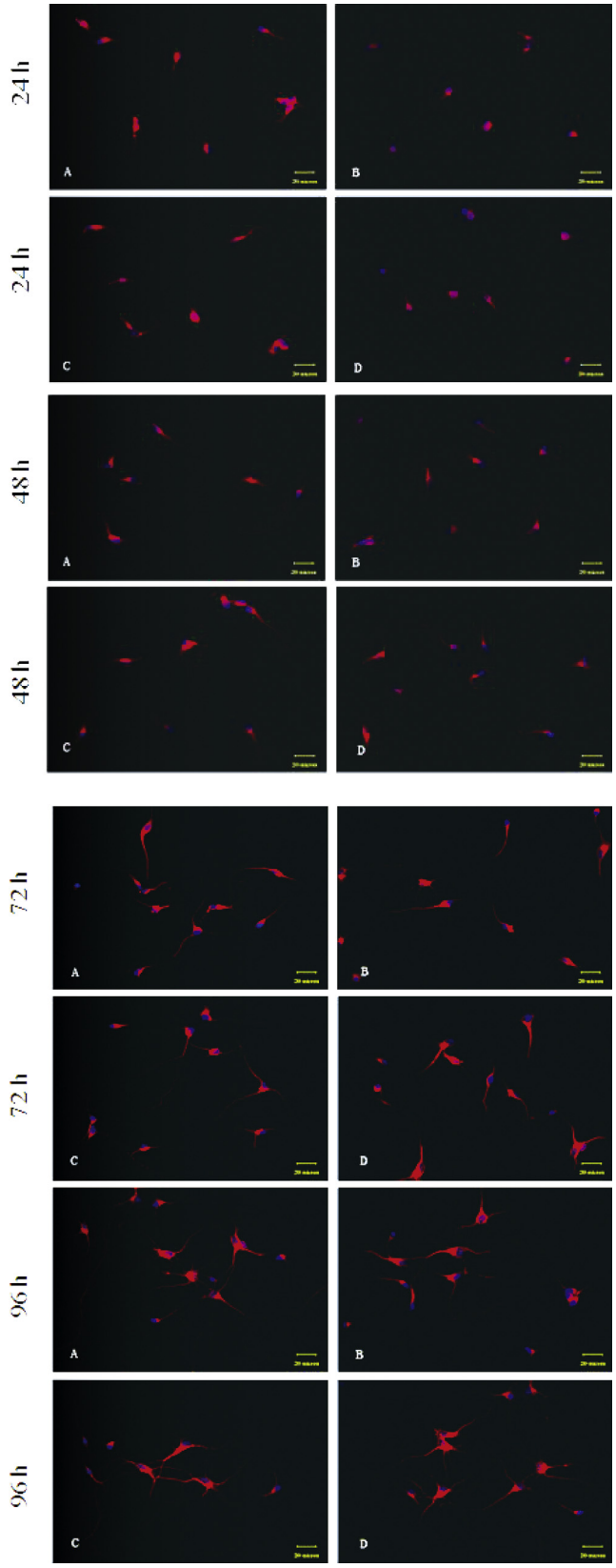

Fig. 8. Microscopic images of immunofluorescence staining of neurons for β III-tubulin at 24–96 h after transfection. A: noninhibitory culture; B: myelin; C: myelin + siRNA; D: myelin + scrambled sequence. Scale bar = 20 μm.

siRNA is a short double-stranded RNA and plays a key role in the interference of a gene expression. siRNA-mediated gene silencing may achieve loss of function phenotypes without the loss of genomic information from the targeted gene.[12,26] It has been documented that specific siRNA administration is able to reverse the axonal growth inhibition caused by the CNS myelin which contains several axon growth inhibitors. For example, Ahmed et al[42] investigated the disinhibition of fibroblast growth factor 2 (FGF-2)-stimulated dorsal root ganglion neurons (DRGN) neurite regeneration after siRNA-mediated silencing of $p75^{NTR}$, NgR, and Rho-A. They noted that the siRNA transfection could knockdown 70% of the $p75^{NTR}$ protein and 100% of the expression of NgR and Rho-A.

Differently, in our study, we detected the regulation of the NgR expression in the CGCs of the CNS by siRNA transfection. We first screened the most efficient sequence for the NgR siRNA, which can significantly knowdown NgR expression. After the siRNA sequence was transfected into the CGCs, we found that both the NgR mRNA level and NgR immunoreactivity were significantly reduced. Meanwhile, our *in vitro* study indicated the NgR siRNA transfection was able to disinhibit the suppression of neurite outgrowth in the neurons induced by the CNS myelin, which could last up to 72 h post-transfection. Therefore, our findings in the CNS, which is consistent with the investigation in the peripheral nervous system by Ahmed et al[42] suggest that the transfection of the NgR siRNA into the neurons is an efficient approach to improve neurite outgrowth.

Currently, the research on siRNA-mediated NgR silencing focus exclusively on *in vitro* studies, thus there is a critical need for additional in vivo studies to evaluate its potential effect. With continuing research and future developments in techniques, this novel therapy appears to have potential implications for both therapeutic and basic research applications.

In conclusion, we demonstrated that transfection of an NgR-specific siRNA sequence can significantly decrease the expression of NgR in the CGCs *in vitro*, and this siRNA-mediated knockdown of NgR may be time-dependent. Our study also demonstrated that knockdown of NgR can improve neurite outgrowth, suggesting that siRNA-mediated knockdown could be an efficient tool in studying the function of target genes.

Fund

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