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Research article

Combined intranasal nerve growth factor and ventricle neural stem cell grafts prolong survival and improve disease outcome in amyotrophic lateral sclerosis transgenic mice

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HIGHLIGHTS

- There is a synergistic effect between intranasal NGF and ventricle NSCs grafts.
- Combined therapy can enhance motor function, delay the onset, and prolong survival.
- Combined therapy can promote nerve regeneration and protect motor neurons.

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal disease that selectively involves motor neurons. Neurotrophic factor supplementation and neural stem cell (NSC) alternative therapy have been used to treat ALS. The two approaches can affect each other in their pathways of action, and there is a possibility for synergism. However, to date, there have been no studies demonstrating the effects of combined therapy in the treatment of ALS. In this study, for the first time, we adopted a method involving the intranasal administration of nerve growth factor combined with lateral ventricle NSC transplantation using G93A-SOD1 transgenic mice as experimental subjects to explore the treatment effect of this combined therapy in ALS. We discover that the combined therapy increase the quantity of TrkA receptors, broaden the migration of exogenous NSCs, further promote active proliferation in neurogenic regions of the brain and enhance the preservation of motor neurons in the spinal cord. Regarding physical activity, the combined therapy improved motor functions, further postponed ALS onset and extended the survival time of the mice.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with an unknown cause that selectively involves motor neurons [1]. The annual incidence of ALS is approximately 1/100,000, with a more frequent incidence in the middle-aged population [2]. After the onset, muscle atrophy and weakness resulting in disability quickly appear, and 80–90% of patients die 2–5 years later. The pathologic features consist of the selective loss of motor neurons, such as decreases in the number of pyramidal motor neurons in the cerebral cortex, the motor nucleus of the brain stem and the number of motor neurons in the ventricolumna, as well as axonal fracture disturbances and decomposition and a loss of the myelin sheath [3].

Currently, such as drug therapy, gene therapy and other therapies, have been introduced, but none are ideal [4]. In previous studies, nerve growth factor (NGF) and NSCs alternative therapy have certain treatment effects as well as separate limitations. NGF not only protects and nourishes normal nerve cells but also repairs and manages damaged neurons [5]. Exogenous NSCs can be transported to damaged sites and differentiated to replace damaged nerve cells, and they are capable of regulating the expression of hormones, neurotrophic factors, cell factors, etc [6]. Many in vivo and in vitro studies have shown that NGF can promote the proliferation, differentiation and migration of NSCs [7]. Thus, there is a possibility that NGF supplementation may play a synergistic role when combined with NSC transplantation. Because NGF is a

Abbreviations: ALS, amyotrophic lateral sclerosis; NGF, nerve growth factor; NSC, neural stem cell; MAP 2, microtubule-associated protein 2; TrkA, tropomyosin receptor kinase.

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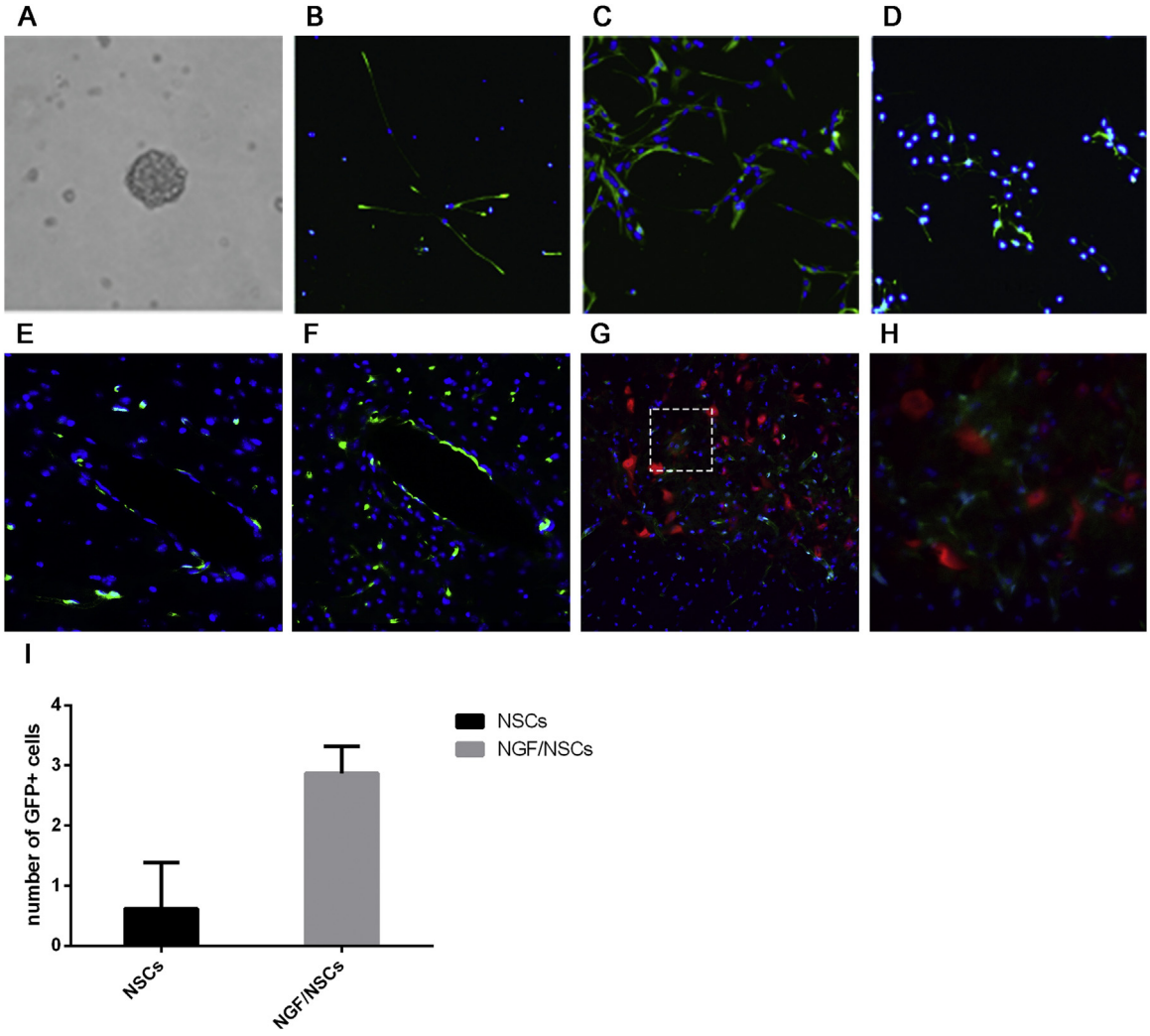

Fig. 1. Isolation and differentiation of NSCs. NSCs migrated to the spinal cord and differentiated. The number of NSCs survived and migrated more in the NGF/NSC group.

macromolecular substance, regular routes of administration are ineffective in accessing the central nervous system (CNS); therefore, we adopted a transnasal route of administration to drive NGF into the CNS through the olfactory nerve pathway [8]. In this study, we adopted for the first time a method that involved the transnasal administration of NGF combined with lateral ventricle NSC transplantation, which improved the absolute quantity of NGF and NSCs within the brains of G93A-SOD1 mice, thus realizing the treatment of ALS through the synergistic effect of this combined therapy.

2. Materials and methods

2.1. Animals and groups

Eighty 8-week-old G93A-SOD1 mice (Nanjing, China, batch number: J002726) which all express high copy number of mutant genes were selected. After sexes were balanced to avoid bias due to sex-related intrinsic differences, they were divided into 4 groups: control group (no treatment, n = 20), NGF group (intranasal administration of NGF, n = 20), NSCs group (bilateral ventricle stereotactic stem cell transplantation, n = 20), combined group (intranasal administration of NGF combined with NSC transplantation, n = 20). Among each group, 8 mice were randomly separated for survival and motor function monitoring. The remaining 12 mice were used for pathology and molecular biology testing when they were 15 weeks old. All the animal experiments followed international animal testing guidelines.

2.2. NSC culture, identification and transfection

NSCs were isolated from twenty-five 24-h newborn Kunming mice (Beijing, China, batch number: SCXK2013-2002). The cells were cultured in DMEM/F-12 serum-free culture medium (Gibco, USA) supplemented with B27 (Gibco, USA), bFGF (PeproTech, USA), EGF (PeproTech, USA) and penicillin and streptomycin (Solarbio, China). For the immunocytochemistry assay, the cells were incubated with the primary antibodies rabbit anti-nestin (1:250, Millipore, USA), rabbit anti-MAP2 (1:250, Millipore, USA) and rabbit anti-GFAP (1:250, Millipore, USA) at 4 °C overnight. Then fluorescent secondary antibodies (1:80, goat anti-rabbit IgG, Kangwei, China) were incubated. NSCs were transfected with GFP via a lentivirus. GFP expression was assayed after 24 h using an inverted fluorescence microscope (Olympus, Japan).

2.3. Cell transplantation and intranasal administration of NGF

When the mice were 9 weeks old, approximately 2.5×10^5 NSCs in 2.5 μl of PBS were injected into each side of the lateral ventricle at the following stereotaxic coordinates: −0.5 mm anterior, ±1.1 mm lateral, and −2.5 mm ventral relative to bregma. No