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Research report

scAAV9-VEGF prolongs the survival of transgenic ALS mice by promoting activation of M2 microglia and the PI3K/Akt pathway

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that leads to paralysis and death three to five years after diagnosis in most patients. The disease is incurable, and the mechanism of motoneuron degeneration remains unknown, although research has demonstrated that activated microglia are involved in motor neuron death. Here, we used a simple method to deliver AAV9 virus by direct intrathecal injection and found that scAAV9-VEGF-165 improved the motor performance and prolonged the life span of SOD1-G93A mice. Furthermore, scAAV9-VEGF-165 activated the PI3K/Akt survival pathway and increased the level of Bcl-2, which contributed to the protection of motor neurons. Additionally, scAAV9-VEGF-165 attenuated the expression of classically activated (M1) microglial markers and enhanced the expression of alternatively activated (M2) microglial markers. Taken together, the results of our study suggest that simple, direct intrathecal injection of scAAV9-VEGF-165 may have a curative effect for ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disorder that is characterized by selective motoneuron degeneration and leads to paralysis and death three to five years after diagnosis in most patients. Approximately 10% of patients exhibit the familial form, and 20% of these patients have Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1) gene mutations (Rosen et al., 1993). The disease is incurable, and the mechanism of motoneuron degeneration remains unknown. Mutations in SOD1 are responsible for disease progression, and transgenic mice with ALS-associated SOD1 mutations develop motor neuron degeneration, similar to ALS patients (Bruijn et al., 2004; Pasinelli et al., 2004). Other factors, such as oxidative stress, neurotrophic factor shortage, protein aggregation, and glutamate toxicity, also participate in the pathogenesis of ALS (Cleveland and Rothstein, 2001).

Vascular endothelial growth factor (VEGF), which was initially regarded as an important factor in the induction of vessel growth, was recently shown to have a beneficial neuroprotective effect (Storkebaum et al., 2004). In vitro studies indicate that VEGF reduces motor neuron death caused by SOD1 mutations or glutamate excitotoxicity through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (Li et al., 2003; Tolosa et al., 2008). Recently, numerous studies have shown that VEGF deficiency induces progressive motor neuron loss, just like SOD1 mutations (Oosthuyse et al., 2001). Furthermore, compared with SOD1-G93A transgenic mice, in SOD1-G93A/$VEGF^{+/+}$ transgenic mice, motor neuron degeneration was absent and disease progression was delayed (Wang et al., 2007). Many studies have explored VEGF treatment through different routes. In the earliest study, a lentiviral vector was used to deliver VEGF-A to motor neurons via its injection into muscles (Azzouz et al., 2004). In another study, adeno-associated virus 4 (AAV-4) vectors were used to deliver VEGF-A or insulin-like growth factor 1 (IGF-1) through an intracerebroventricular (ICV) route (Dodge et al., 2010). Storkebaum et al. performed ICV injection of VEGF protein in the SOD1-G93A rat model because systemic delivery did not significantly impact lifespan (Storkebaum et al., 2005). In another study, VEGF-overexpressing stem cells were transplanted into SOD1-G93A mice via intrathecal (i.t.) injection (Hwang et al., 2009). Notably, all of these studies showed that VEGF supplementation delayed disease progression.

Thus, the mode and route of administration are important factors to consider when exploring an optimal therapeutic

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approach. AAV is a powerful, ideal vector for delivering genes because it can induce continuous gene expression and does not induce a serious inflammatory response (Snyder et al., 2011). To date, several AAV serotypes have been identified, and each serotype displays natural tissue tropism even when the same delivery route is used (Duque et al., 2009). Multiple routes to deliver the vectors have been explored. Systemic delivery of AAV9 can effectively penetrate the blood–brain barrier (BBB); however, low expression of the gene, the need for a greater amount of vector, and off-target expression restrict the clinical applications of this method. Another route is to deliver the vectors into muscle; the vectors will then be expressed in motor neurons through retrograde transport. However, this method is difficult to use clinically because humans require a larger amount of AAV vector than mice, and immune reactions must also be considered. In comparison, i.t. injections result in more effective central nervous system (CNS) expression and reduce the possibility of a serious immune reaction. Previous reports have indicated that AAV9 vectors with the cytomegalovirus (CMV) promoter show the strongest gene expression in the motor neurons of the spinal cord (Snyder et al., 2011; Wang et al., 2014). Thus, we constructed a scAAV9 vector with the CMV promoter to deliver VEGF to the CNS through i.t. injection. This therapy significantly prolonged the survival of SOD1-G93A mice and improved their motor performance, suggesting that direct i.t. injection of scAAV9-VEGF-165 may have a therapeutic effect for ALS.

2. Results

2.1. I.t. injection of scAAV9-GFP results in widespread detection of the protein in the central nervous system

Given that we adopted the less invasive i.t. injection route in this experiment, we first explored whether the vectors could be delivered effectively. To study the distribution of GFP after i.t. delivery, we injected 1×10^9 vg/g of scAAV9-GFP into the subarachnoid space as described in the Methods. Three weeks after the injection, 25 μm sections were examined by fluorescence confocal microscopy. A number of NeuN-labeled neurons were evident in the ventral horn of the lumbar cord where GFP-positive neurons were expressed (Fig. 1A). GFP expression was observed in the CNS but was more abundant in the spinal cord (Fig. 1B).

2.2. I.t. delivery of scAAV9-VEGF-165 prolongs the survival and improves the motor function of SOD1-G93A mice

A dose of 1×10^9 vg/g scAAV9-VEGF-165 or scAAV9-GFP was administered by i.t. injection into female and male SOD1-G93A mice (n=15 animals/group) at 90 days of age, to mimic the treatment of ALS patients just after diagnosis of the disease. AAV9-VEGF-165 treatment extended the life expectancy of the injected mice compared with that of the control mice. Compared with control mice, female SOD1-G93A mice administered AAV9-VEGF-165

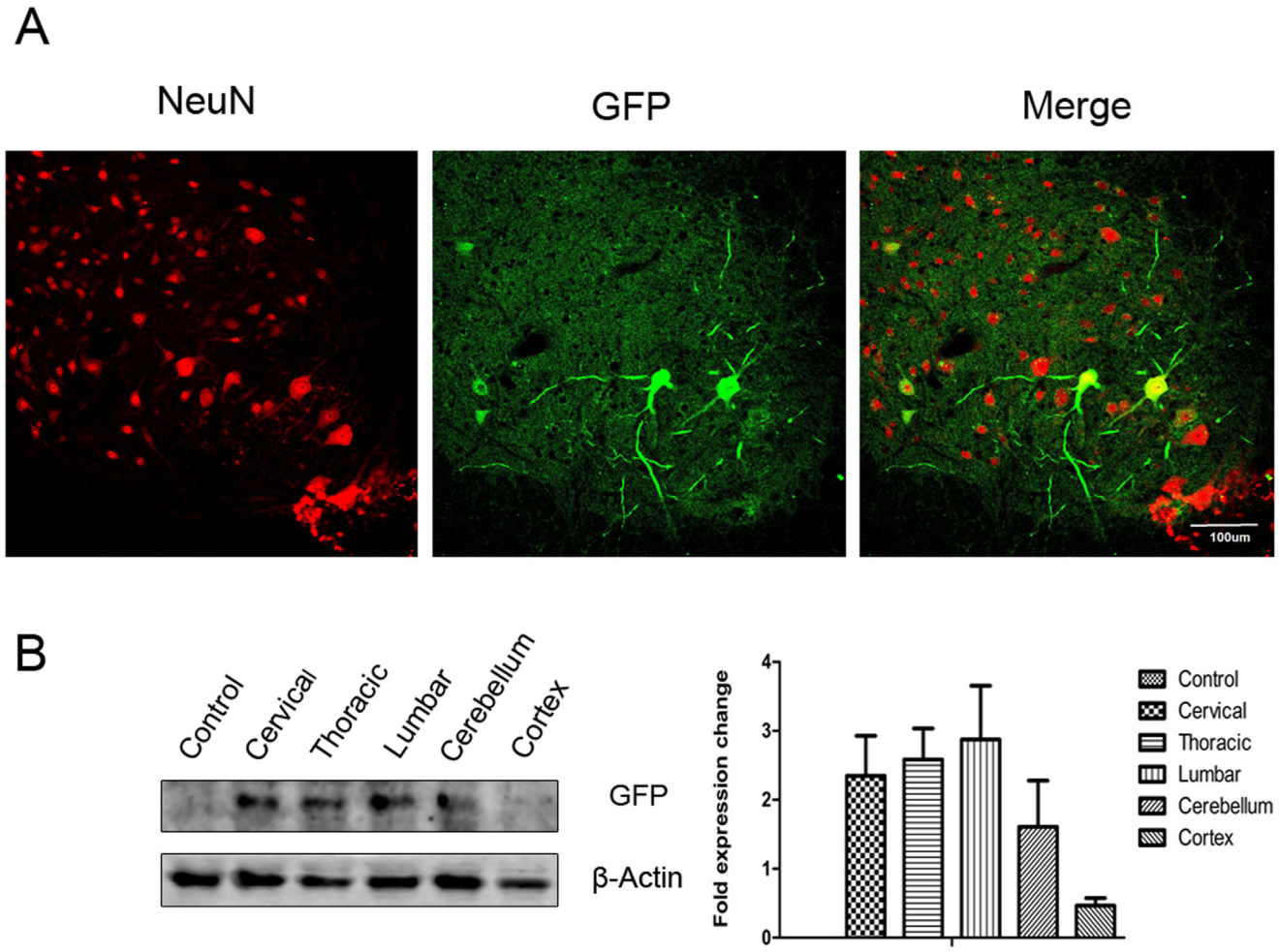

Fig. 1. AAV9-GFP was efficiently expressed in the brain and spinal cord through intrathecal injection. (A) GFP immunofluorescence showed robust colocalization with NeuN-expressing neurons (red) in the lumbar spinal cord. (B) Western blotting showed widespread GFP expression in the central nervous system after intrathecal injection. The data are presented as the mean ± SD.