論文内容の要旨

論文題目

DOK7 gene therapy in a mouse model of amyotrophic lateral sclerosis

（筋萎縮性側索硬化症モデルマウスに対する *DOK7* 遺伝子治療の効果とその作用機序）

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Amyotrophic lateral sclerosis (ALS) is a progressive, multifactorial degenerative disease of motor neurons with severe muscle atrophy. The glutamate release inhibitor riluzole and the free radical scavenger edaravone are the only medications approved in Japan for ALS, but their therapeutic effects are limited, testifying to the strong need for new treatment strategies. In ALS, degeneration of motor nerve terminals at the neuromuscular junction (NMJ), the cholinergic synapse between a motor neuron and skeletal muscle, precedes proximal motor neuron degeneration, termed “dying-back” pathology, implicating the NMJ as a therapeutic target. However, a promising NMJ-targeted therapy for ALS has not been developed to date.

The muscle protein Dok-7 is an essential activator of the muscle-specific kinase MuSK, which governs NMJ formation and maintenance. Dok-7 directly interacts with the cytoplasmic portion of MuSK and activates the receptor kinase. Indeed, recessive mutations in the human *DOK7* gene cause a congenital myasthenic syndrome (*DOK7* myasthenia) with defective NMJ structure. On the other hand, forced expression of Dok-7 in muscle activates MuSK and enlarges NMJs. Our laboratory previously generated

AAV-D7, an adeno-associated virus vector carrying the human *DOK7* gene tagged with the enhanced green fluorescent protein under the control of the cytomegalovirus promoter, and demonstrated that the therapeutic administration of AAV-D7 (*DOK7* gene therapy) enlarges NMJs and enhances motor activity and life span in a mouse model of *DOK7* myasthenia. In addition, *DOK7* gene therapy improves motor function and survival in a mouse model of autosomal dominant Emery–Dreifuss muscular dystrophy, a disease with defective NMJs due to mutations in the lamin A/C gene. These observations demonstrate that *DOK7* gene therapy may be effective in these myopathies with NMJ defects. I suspected that this therapy might also benefit ALS because the forced expression of Dok-7 in muscle enlarges NMJs not only at postsynaptic acetylcholine receptor (AChR) clusters, but also presynaptic motor nerve terminals, which might counteract size reductions and denervation of the terminal. Motor nerve terminal defects appear to play an important role in the pathogenesis of ALS in both patients and animal models; accordingly, I examined whether *DOK7* gene therapy benefits a mouse model of ALS.

In ~20% of familial cases of ALS, patients harbor a gain-of-function mutation in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1). Mice expressing human SOD1 (hSOD1) with the ALS-linked G93A mutation (hereafter, ALS mice) are widely used as an animal model because they manifest progressive muscle paralysis similar to that observed in clinical cases, along with the histopathological hallmarks observed in familial and sporadic ALS, including NMJ defects.

First, I examined the effect of forced expression of Dok-7 on the histopathology of NMJs, skeletal muscles, and motor neurons in ALS mice. I intravenously administered AAV-D7 to male ALS mice at an early symptomatic stage (postnatal day 90, P90) and performed histological analyses 30 days later. Based on confocal microscopic analyses of NMJs, I found that AAV-D7 treatment enlarged motor nerve terminals at NMJs, in addition to postsynaptic AChR clusters, indicating positive effects on motor neurons. Indeed, denervation at NMJs was suppressed, demonstrating that *DOK7* gene therapy had a protective effect against nerve terminal degeneration in ALS mice. Moreover, *DOK7* gene therapy further suppressed muscle atrophy in ALS mice, supporting the notion that NMJ defects are a cause of muscle atrophy in ALS. I also examined the effect of AAV-

D7 treatment on progressive motor neuron death, another hallmark of ALS, and found no significant differences between treated and untreated mice. Together, these results indicate that *DOK7* gene therapy suppressed NMJ defects and muscle atrophy in ALS mice and had no obvious effects on proximal motor neuron degeneration at 30 days post-infection.

Second, I examined the effects of *DOK7* gene therapy, AAV-D7 treatment initiated after disease onset, on the survival and motor activity of ALS mice. Individuals with ALS are usually diagnosed after the onset of symptoms. Therefore, to strictly evaluate the potential of AAV-D7 as a therapy, I defined disease onset in each male ALS mouse individually by monitoring its grip strength, and at this individually defined disease onset, administered AAV-D7 for a survival analysis according to the guidelines for pre-clinical ALS studies. Note that this guideline was recently established to improve translation to humans, mainly because many, if not all, beneficial life span effects obtained in clinical trials using ALS mice have failed to translate to humans. Remarkably, recent studies using experimental settings that fit this guideline reported that even riluzole and other drugs that had been reported to enhance survival of ALS model mice failed to prove their efficacy in ALS mice. I show here, in a study fully adhering to the guideline, that *DOK7* gene therapy prolonged not only mouse lifespan, but also the duration of survival after onset. Furthermore, using the automated home cage behavioral system, I demonstrate that *DOK7* gene therapy enhanced spontaneous motor activity.

Together, my findings demonstrate the importance of NMJ pathology in ALS and provide evidence that *DOK7* gene therapy or an equivalent method that enlarges NMJs has that potential to treat not only myopathies, but also motor neuron diseases that manifest NMJ defects.