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RNAi therapy

Dominant disease gene gets silenced

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For a long time, a treatment for dominant genetic disorders has appeared remote. Now in a recent issue of Nature Medicine, two groups reported evidence of success in an animal model for one such disorder, amyotrophic lateral sclerosis (ALS) using RNA interference (RNAi),[1,2] raising the hope that RNAi therapy for diseases caused by dominant, gain-of-function mutations may be available in the not-too-distant future.

People with dominantly inherited diseases carry one normal allele and one mutant allele of the gene that causes the disease. The mutant allele could cause the disease by loss of function, that is, this allele has lost its function and the normal allele is insufficient to compensate for the lost function. The mutated gene could also cause the disease by gain of function, which could be a novel function or enhancement of an existing function. In principle, the delivery of a normal copy of the gene to the patient's body can treat loss-of-function mutations. However, a conceptual solution to gain-of-function diseases is less obvious.

ALS (also known as Lou Gehrig's disease), which is characterized by motor neuron degeneration, paralysis and death, is one such disease that can be caused by gain-of-function mutations. Specifically, mutations in SOD1 cause a subset of ALS cases. The mutated gene produces a protein with an undefined toxic property that kills motor neurons.[3] Because the pathway through which the mutant protein kills motor neurons is incompletely understood, rational design of therapy has been difficult. No cure is known. However, recent breakthroughs in RNAi have provided a conceptual basis for developing a new therapy for this disease.

RNAi[4] is a widely conserved eucaryotic function that double-stranded RNA triggers in cells via short RNA duplex intermediates called small interfering RNAs (siRNAs). Through a series of processing steps, one of the two strands of the siRNA complexes with proteins to form RISC (RNA-induced silencing complex). RISC recognizes the complementary RNA sequence by Watson–Crick base pairing and then cleaves it. Because mutant protein has to be synthesized from mRNA, RNAi may be applied to destroy the mRNA, thereby reducing mutant protein synthesis and its toxicity (Figure 1). Supporting this concept are studies in transgenic mice that demonstrate that the lower the mutant protein expression, the later onset of motor neuron degeneration and death of the animals.[5] Indeed, early experiments in culture and in mice suggest that RNAi can achieve this.[6]

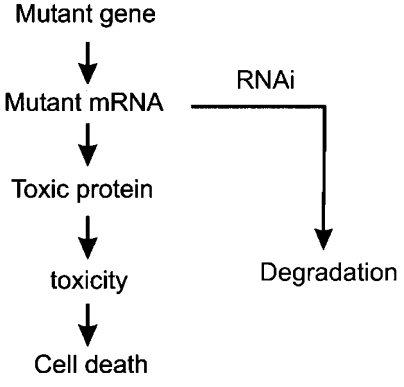

Figure 1 *Intercepting mutant protein toxicity by RNAi.*

One obstacle in developing RNAi therapy is the delivery of siRNA. In these new studies, the two groups built lentiviruses carrying gene cassettes that express short hairpin RNAs (shRNAs) with stems complementary to the SOD1 mRNA. In cells, the shRNA is processed to siRNA, which mediates RNAi against SOD1 mRNA, thereby reducing the levels of SOD1. Both groups tested their viruses in cultured cells and demonstrated that their viruses reduced SOD1 levels.

In vivo, the two groups administered the viruses differently. Scott Ralph and colleagues used a retrograde transport strategy. They packaged their virus in rabies glycoprotein, which can bind to motor neuron terminals, be internalized and retrogradely transported to motor neurons situated in the central nervous system (CNS). The virus was administered to multiple muscle groups in mutant SOD1 transgenic mice. Knockdown of the mutant SOD1 level was confirmed in the spinal cord. The effects on the course of the disease were remarkable: the age of disease onset and death was delayed by 115 and 77%, respectively. This is the highest therapeutic effect achieved in this mouse model thus far.

By contrast, Cédric Raoul and colleagues injected their lentivirus directly into the lumbar spinal cord. Like the other group, they confirmed knockdown of the mutant SOD1. Because their viral administration was localized, they focused their analysis on the lumbar motor neurons. Using functional measures, including spontaneous fibrillation potentials, amplitude of compound muscle action potential and hinder limb kicking during swimming, they showed that the RNAi treatment delayed functional deterioration.

Given that ALS is a fatal disorder without an effective treatment, is this strategy ready for clinical application in humans? In our view, many important questions remain to be answered, some of which the authors raised in their respective articles. Here, we briefly discuss three of these. First, is the observed therapeutic efficacy a result of a transient or a sustained high-level shRNA expression? In the reported RNAi therapies thus far,[1,2,7] the length of time that the shRNA was expressed after the virus administration was

not determined, nor was the degree of silencing overtime.

Second, should RNAi gene therapy be administered through retrograde transport as Ralph and colleagues did, or should it be administered directly into the CNS as Raoul and colleagues did? In making this choice, one relevant question to ask is whether silencing SOD1 in both neurons and non-neuronal cells is required, because retrograde transport-administered RNAi silences mutant SOD1 expression only in neurons that project their axons to the periphery, while direct CNS injection silences mutant SOD1 expression in both neurons and non-neuronal cells. Ralph and colleagues' results suggest that silencing mutant SOD1 in motor neurons is sufficient to generate significant therapeutic efficacy. The peripheral administration is also relatively easy and less invasive. However, the efficiency of retrograde transport in large organisms (compared with a mouse), where the retrograde transport must traverse a long distance in axons, remains to be demonstrated. Peripheral administration might also have a high risk of provoking an immune reaction and infecting perforating cells, both of which can generate serious side effects in patients.[8]

Third, should mutant allele-specific knockdown be used? It has already been shown that such specificity can be achieved,[6,9] at least for some SOD1 mutations. However, the answer to this question depends on the effects of low SOD1 function in humans. In mouse, SOD1 is not required for survival, but is required for a number of normal cellular functions including motor neuron functions.[6] It is not known whether there are serious consequences for lacking SOD1 in humans. If there is, more complex RNAi strategies such as allele-specific silencing[6,9] or gene replacement-accompanied RNAi[10] may be used.

In summary, these two reports, together with a previous report using similar strategy for treatment of spinal cerebellar ataxia,[7] demonstrate the concept of RNAi therapy *in vivo* for dominant, gain-of-function type of genetic disorders. Like most of the significant scientific advancements, these findings raise new questions that are critical for further development of clinical RNAi therapy. ■

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