www.nature.com/clinicalpractice/neuro

Amyotrophic lateral sclerosis and gene therapy

Timothy M Miller, Richard A Smith and Don W Cleveland*

TM Miller is a clinical instructor and research fellow in the Department of Neurosciences, and DW Cleveland is Professor of Neurosciences, Medicine and Cellular and Molecular Medicine and Head, Laboratory for Cell Biology, Ludwig Institute for Cancer Research, both at the University of California, San Diego, La Jolla, CA, USA. RA Smith is Director of the Center for Neurologic Study and a Skaggs Clinical Scholar at Scripps Research Institute, La Jolla, CA, USA.

Correspondence
*Ludwig Institute for Cancer Research
Neurosciences Department
University of California, San Diego
9500 Gilman Drive
La Jolla
CA 92093–0670
USA
dcleveland@ucsd.edu

Received 6 February 2006
Accepted 23 June 2006
www.nature.com/clinicalpractice
doi:10.1038/ncpneuro0270

With the identification of the genes and proteins involved in the etiology of neurodegenerative diseases, and the development of new viral tools for gene delivery, modulating the function of disease-associated genes as a therapy has become an increasingly realistic possibility. This approach is particularly applicable to amyotrophic lateral sclerosis (ALS), the dominant clinical manifestation of which is weakness that progresses to paralysis, with ensuing respiratory failure and death within 3–5 years of onset. A significant proportion of familial ALS cases are caused by a dominant mutation in the gene encoding superoxide dismutase (*SOD1*) that confers an aberrant toxic property on the protein; in principle, these cases could be amenable to treatment employing a gene-silencing strategy.

RNA interference (RNAi)—a post-transcriptional gene-silencing approach in which small double-stranded RNA molecules (small interfering RNAs or siRNAs) mediate the destruction of messenger RNAs (mRNAs) in a sequence-specific fashion—is one way to downregulate protein expression. Double-stranded RNAs formed by specific binding through standard base-pairing of an siRNA molecule to a target mRNA are recognized by the Dicer complex, a cytosolic set of enzymes that degrade the mRNA (but not the siRNA), thereby preventing further translation of protein.[1] RNAi works brilliantly in cultured cells, in which nearly unlimited amounts of siRNA-encoding DNA can be introduced. Approximately 50% knockdown of target mRNA has been achieved with stabilized siRNA infused into the cerebral spinal fluid, although distribution within the CNS was limited.[2] Despite attempts to stabilize siRNAs synthesized *in vitro* so as to enhance their biological half-lives after direct delivery to cells in animals,[3] these molecules are short-lived—a serious issue for their use as a therapy.

The inherent instability of siRNA molecules can be overcome through continuous production facilitated by transcription of a corresponding gene after delivery to a cell nucleus. In animals, delivery can be accomplished by introducing the siRNA-encoding DNA into a viral genome from which most of the viral genes have been deleted, and then injecting the replication-defective viral particles directly into one or more loci in the CNS. Alternatively, an siRNA-encoding replication-defective virus can be injected into muscle, provided that the viral coat proteins mediate synaptic uptake and subsequent 'retrograde delivery' to nuclei of motor or sensory neurons.

Viral vectors that express a gene-silencing siRNA have been tried as a therapy for ALS, using an animal model that develops fatal, progressive paralysis through expression of a human gene encoding a mutant form of SOD1 known to cause dominantly inherited ALS. Disease arises from the selective killing of motor neurons, mediated by an acquired toxicity of mutated SOD1. Death of motor neurons is non-cell-autonomous; that is, pathogenesis requires damage from the ubiquitously expressed SOD1 mutant acting within both motor neurons and non-neuronal neighboring cells,[4] particularly microglia.[5] Although many genetic or pharmacologic attempts at treatments have been reported to slow fatal, progressive motor neuron disease in these mice, few of these approaches have extended survival by more than a week or two. An exception to this rule is suppression of mutant gene expression in microglia, which markedly slowed disease progression after onset[5]—a central goal for a therapeutic intervention for human disease.

In 2005, three different strategies using viral-encoded siRNAs to reduce mutant SOD1 accumulation demonstrated a beneficial effect in ALS model mice. In the most comprehensive approach, Ralph *et al.*[6] injected a lentivirus encoding an siRNA to SOD1 into multiple muscles of very young (7-day-old) mice, months before the earliest signs of disease. Engineered to incorporate rabies viral coat proteins, which enable retrograde axonal transport, the siRNA-encoding gene was transported back to the spinal cord. Lentivirus-encoded siRNA produced

remarkable slowing of disease onset by 108 days, and, consequently, extension of overall survival by 99 days. Therefore, early gene silencing restricted within the CNS to the neurons at risk can sharply slow disease onset. This initial treatment success, however, was followed by a sobering discovery: despite markedly delayed disease onset, disease progression after onset was not slowed, the end stage being reached 25% faster in treated animals. The inability to slow down disease progression by treatment solely of motor neurons within the CNS probably reflects differential roles of mutant damage within motor neurons and their non-neuronal neighbors (including microglia) in determining disease onset or progression, respectively.[5]

Rather than relying on retrograde transport after peripheral injection, Raoul *et al.*[7] injected a similar lentivirus construct encoding an siRNA to SOD1 directly into the lumbar spinal cord. In this local delivery paradigm, the virus targeted not only the motor neurons, but also the surrounding non-neuronal cells. This approach delayed loss of strength in the corresponding hindlimbs by 2–3 weeks. Focal silencing of the *SOD1* mutant gene was therefore beneficial to the targeted spinal cord region. Whether this approach can slow disease progression after onset, however, was not determined, and there was no effect on survival, as neurons outside the targeted lumbar region were unaffected.

Finally, Miller *et al.*[8] injected an adeno-associated virus (AAV) encoding an siRNA to SOD1 into muscles of one hindlimb of mice expressing mutant *SOD1*. AAV is retrogradely transported after peripheral uptake by motor neurons. AAV-2—the most widely studied AAV serotype—has already been used clinically without untoward side effects; therefore, using this viral vector is probably the most clinically feasible current approach. If the vector was administered near the time of symptomatic disease onset, grip strength was preserved for 3 weeks on the side injected with the siRNA-encoding AAV.[8] As expected, survival was not affected by this focal gene silencing.

Will any of these gene therapy approaches work in humans? Maybe, but daunting practical issues remain. First, safety presents a huge hurdle. A potentially devastating obstacle is that the level of gene silencing from the current generation of viruses cannot be regulated. This is especially problematic because saturation by viral-encoded siRNA of the endogenous capacity to process small RNAs could have fatal consequences, resulting from widespread dysregulation of endogenous genes.[9] Second, despite impressive slowing of disease onset from lentiviral injection into multiple muscles in mice at ages equivalent to infancy in humans, there is no evidence that such an approach is effective when used in adults. Third, none of the approaches has convincingly shown slowing of disease progression after onset. Last, delivery remains a major challenge. For viruses that are retrogradely transported, injecting each muscle in ALS patients will be a huge task. Although direct injection has the potential to target cells other than motor neurons, the paradigm requires the inherently risky approach of multiple spinal cord injections.

In summary, viral delivery of siRNA for the treatment of ALS patients seems far from reaching the clinic. Nevertheless, gene silencing remains an exciting potential treatment approach. Improvements in siRNA delivery or other gene-silencing technologies—for example, direct infusion into the CNS of antisense oligonucleotides, the intranuclear hybridization of which to the targeted mRNAs directs mRNA destruction by endogenous RNase H;[10] DNAzymes that degrade specific RNA sequences; or antibody-mediated protein clearance—all represent viable future options.

Competing interests
RA Smith and DW Cleveland have declared associations with Isis Pharmaceutical Corporation. See the article online for full details of the relationship. TM Miller declared he has no competing interests.

References

1 Zamore PD and Haley B (2005) Ribo-gnome: the big world of small RNAs. *Science* **309:** 1519–1524
2 Thakker DR *et al.* (2004) Neurochemical and behavioral consequences of widespread gene knockdown in the adult mouse brain by using nonviral RNA interference. *Proc Natl Acad Sci USA* **101:** 17270–17275
3 Dorn G *et al.* (2004) siRNA relieves chronic neuropathic pain. *Nucleic Acids Res* **32:** e49
4 Clement AM *et al.* (2003) Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* **302:** 113–117
5 Boillee S *et al.* (2006) Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* **312:** 1389–1392
6 Ralph GS *et al.* (2005) Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. *Nat Med* **11:** 429–433
7 Raoul C *et al.* (2005) Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nat Med* **11:** 423–428
8 Miller TM *et al.* (2005) Virus-delivered small RNA silencing sustains strength in amyotrophic lateral sclerosis. *Ann Neurol* **57:** 773–776
9 Grimm D *et al.* (2006) Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature* **441:** 537–541
10 Smith R *et al.* (2006) Antisense oligonucleotide therapy for neurodegenerative disease. *J Clin Invest*, in press