Has gene therapy for ALS arrived?

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Amyotrophic lateral sclerosis has not responded well in clinical trials to what initially seemed a promising therapy—the administration of neuronal growth and survival factors. Now, a gene therapy-based approach in mice revives hope that proper delivery of such factors can slow the disease's course.

The tragic course of amyotrophic lateral sclerosis (ALS) is well known. Progressive loss of motor neurons leads to weakness, spasticity and eventual respiratory failure. There are no effective treatments. The tenth anniversary has just passed of the identification of a mutation in the ubiquitously expressed enzyme superoxide dismutase (SOD)-1 as the primary cause of 20% of cases of inherited ALS. But despite the large research effort spawned from that discovery, including the creation of mouse models that genetically mimic mutant SOD-1–mediated disease, the mechanism of neuronal loss has not been identified. As with other neurodegenerative diseases, there is the recurring, tantalizing hope of finding a therapy for these dying cells even if the fundamental cause of their demise remains unknown.

Naturally occurring trophic factors that promote the survival and growth of motor neurons[1,2] have been attractive candidates for such a therapy, but have had a disappointing history in ALS. Driven mostly by hope and little more than suggestive preclinical evidence, trials with ciliary neurotrophic factor[3] and brain-derived trophic factor[4] were unsuccessful. Although insulin-like growth factor (IGF)-1 induced mild slowing of disease in ALS patients in a North American trial[5], the results of a European trial were negative[6]. The multiple failures have triggered a growing pessimism for the overall trophic strategy, although they could reflect the use of the wrong neurotrophins or, even simpler, failure to deliver the neurotrophin effectively to the target cells of the spinal cord. Both subcutaneous injection and direct delivery into the cerebral spinal fluid have been attempted, but both methods are fraught with numerous pharmacological hurdles.

In a recent issue of *Science*, Kaspar *et al.*[7] showed that survival in a mouse model[8] of SOD-1 mutant–mediated ALS could be prolonged by delivering neurotrophins using a gene therapy approach (**Fig. 1**). The authors engineered adeno-associated virus (AAV) to carry a gene encoding either IGF-1 or glial-derived growth factor (GDNF), and injected the engineered vector into muscle. The virus had been previously crippled by removal of genes required for replication, but retained an essential property: viral particles were taken up from muscle tissue by nerve endings of motor neurons and retrogradely transported through the axons to the neuronal cell bodies in the spinal cord. This delivered the IGF-1 or GDNF genes to the neuronal nucleus, along with the remnants of the viral genome. Once there, the delivered gene was maintained as a nonintegrating episome and drove the production and secretion of active IGF-1.

The particular mouse model used in this study[8] develops clinical and pathologic features of ALS as a result of its expression of the human SOD-1 mutant G93A, one of more than 100 such mutations now known[9]. ALS mice expressing green fluorescent protein died from paralysis at 123 days, similar to uninjected mice. Injecting ALS mice with IGF-1-expressing AAV before disease onset extended their lifespan by 5 weeks. Similar delivery of GDNF was much less effective.

Given that the course of disease in the ALS mice is accelerated by high levels of mutant SOD-1, it is even more impressive that the IGF-1 AAV vector could extend survival by 3 weeks when injected after the onset of clinical disease, a situation more relevant to treatment in humans. The rescue of motor neurons was largely dependent on delivery of the virus to the spinal cord, as at best a nine-day extended survival occurred when a similar IGF-1-encoding virus (a lentivirus) was injected and was not retrogradely transported to the motor neuron cell body. It is not known whether IGF-1 exerts its beneficial effects on the motor neurons that produce it, the adjacent neurons or the surrounding astrocytes (**Fig. 1**).

The AAV-mediated survival extension was accompanied by retention of hind-limb strength, slowed loss of spinal motor neurons, decreased astrocytic proliferation and modest slowing of forelimb paralysis. These relatively robust effects occurred despite injection of the recombinant AAV into only two muscles: the quadriceps muscles of the hind limbs and intercostal muscles. How
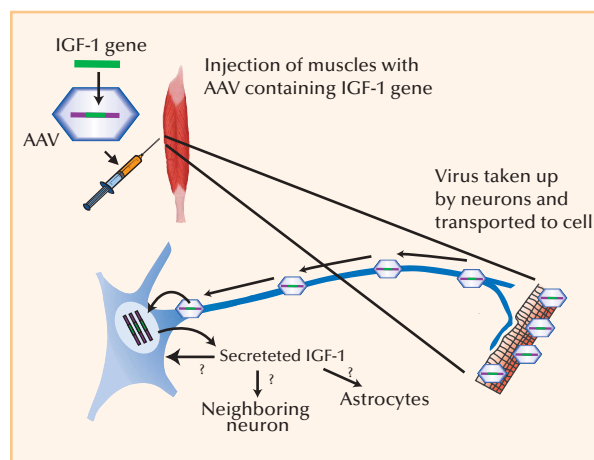
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Figure 1 Viral delivery of neurotrophins to motor neurons. Kaspar *et al.* injected AAV into the hind-limb quadriceps and intercostal muscles of SOD-1 G93A mice, a model of ALS. AAV is retrogradely transported from the motor neurons innervating muscle to the spinal cord, where the viral DNA drives expression of IGF-1 by the motor neurons. The targets of the secreted IGF-1 are unknown, but may include motor neurons that secrete IGF-1, neighboring neurons or astrocytes.

could this limited delivery produce such a widespread benefit? Part of the answer probably reflects the use of a humane and ethically necessary, though artificial, endpoint for death—the inability of the mice to right themselves when placed on their sides—that is heavily influenced by strength in the hind-limb muscles whose innervating motor neurons received IGF-1.

This cannot be the whole story, however. Delivering IGF-1 to the lumbar and thoracic regions of the spinal cord prolonged the retention of grip strength in the forelimbs, which are innervated by cervical motor neurons. What could account for this? One untested possibility, which if true would bode exceptionally well for treating human disease, is that local delivery of IGF-1 AAV to lumbar motor neurons produced sufficient secreted IGF-1 to deliver an effective dose to the cervical cord. An alternative explanation is that even though the injection was local, some of the 10^{10} AAV particles injected were delivered by the circulation to distal sites, where they were endocytosed by cervical motor neurons.

The demonstration of the effectiveness of IGF-1 viral delivery in these mice not only raises the hope for an ALS treatment based directly on this approach, but also raises the possibility of using viral vectors to deliver other, perhaps more effective, ALS therapies. However, there are both practical and safety concerns. AAV, a member of the parvovirus family, is reputed to be relatively innocuous, causing far less inflammatory response than other viral vectors, but its use in human subjects has been limited[10]. However, the favorable safety data in trials of factor IX replacement using AAV injected into muscle[11,12] augurs well for minimal complications in other trials. Despite the disappointing therapeutic outcome, the lack of serious safety issues emerging from previous clinical trials of systemically delivered recombinant IGF-1 is also encouraging[5,6,13].

There are still several unresolved questions. How many and precisely which muscles would need to be injected into an ALS patient to make a functional difference? For some muscles, such as the diaphragm, the benefits of injection would need to be carefully weighed against the risks, such as pneumothorax , and may not be appropriate for initial human studies. Would multiple injections be needed per muscle? Would the injections need to be repeated at a later time? Would antibodies to the virus negate the effects of repeat injections? The answer to these questions will depend on how far the virus spreads into the injected muscle, the influence and behavior of the immune response, the robustness with which motor neurons express the intended gene over time and the extent to which neighboring cells are affected. In addition, the ALS symptoms that extend beyond the lower motor neurons of the spinal cord in some patients, such as stiffness, slowness, cognitive changes and emotional lability, are likely to go unchecked.

Although gene therapy to deliver rescuing neurotrophins to human ALS patients may not yet have arrived, we are one step closer to testing this real possibility.

1. Elliott, J.L. & Snider, W.D. *Neurology* **47**, S47–S53 (1996).
2. Oppenheim, R.W. *Neuron* **17**, 195–197 (1996).
3. ALS CNTF Treatment Study Group. *Neurology* **46**, 1244–1249 (1996).
4. BDNF Study Group. *Neurology* **52**, 1427–1433 (1999).
5. Lai, E.C. *et al. Neurology* **49**, 1621–1630 (1997).
6. Borasio, G.D. *et al. Neurology* **51**, 583–586 (1998).
7. Kaspar, B.K., Llado, J., Sherkat, N., Rothstein, J.D. & Gage, F.H. *Science* **301**, 839–842 (2003).
8. Gurney, M.E. *et al. Science* **264**, 1772–1775 (1994).
9. Cleveland, D.W. & Rothstein, J.D. *Nat. Rev. Neurosci.* **2**, 806–819 (2001).
10. Thomas, C.E., Ehrhardt, A. & Kay, M.A. *Nat. Rev. Genet.* **4**, 346–358 (2003).
11. Manno, C.S. *et al. Blood* **101**, 2963–2972 (2003).
12. Kay, M.A. *et al. Nat. Genet.* **24**, 257–261 (2000).
13. Smith, R.A. *et al. Muscle Nerve* **16**, 624–633 (1993).

Exposing the roots of hair cell regeneration in the ear

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Robust regeneration of hair cells, which mediate hearing and balance in the ear, occurs in most vertebrates, with the exception of mammals. Now, the identification of stem cells in the mouse inner ear that can give rise to hair cells raises the prospect of inducing regeneration in mammals as well (pages 1293–1299).

As many as 25 million Americans experience some form of progressive hearing loss, and that number will increase as the population continues to live longer. By far, the primary cause of age-related hearing loss is the loss of mechanosensory hair cells located within a specialized sensory epithelium that extends along the coiled cochlea of the inner ear.

Although hair cells are not neurons, the two

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cell types have many similar characteristics, including electrical depolarization in response to an excitatory stimulus and, at least in mammals, an only limited ability to regenerate new cells after the embryonic period. In contrast, hair-cell regeneration and recovery of hearing occurs in most nonmammalian vertebrates, primarily through the proliferation of stem or progenitor cells that reside within the hair-cell sensory epithelia[1]. Based on these observations, some researchers have suggested that a similar population of stem or progenitor cells might reside in the mammalian ear[2,3].

In this issue, Li *et al.*[4] suceed in isolating a type of stem-like cell from the hair-cell sensory epithelia of adult mice. These cells meet all the major criteria for stem cells, including the ability to form spheres (clusters) of floating cells through mitosis and to generate pluripotent progenitor cells that can develop into cell types derived from different germ layers. These stem cells can also give rise to mechanosensory hair cells, both *in vitro* and when transplanted into the developing ears of embryonic chicks.

The mechanosensory hair cells located in specific regions of the inner ear enable mammals to perceive sound, balance and movement. Each hair cell contains a bundle of specialized microvilli, referred to as a stereociliary bundle, that acts as an exquisitely sensitive vibrational sensor[5]. At high magnification, these bundles appear similar to small tufts of hair. During embryonic devel-