Gene therapy for ALS delivers

Séverine Boillé and Don W. Cleveland

Ludwig Institute for Cancer Research and Departments of Cellular and Molecular Medicine and Neurosciences, University of California, 9500 Gilman Drive, La Jolla, CA 92093-0670, USA

Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disease that kills motor neurons. Despite a long disappointing history of human trials with neurotrophins, including insulin-like growth factor 1 (IGF-1), Kaspar and colleagues have successfully slowed disease in transgenic ALS mice by forcing motor neurons to produce IGF-1 following retrograde delivery of recombinant adeno-associated virus (AAV) injected into muscle. With the clinical safety of both IGF-1 and AAV already established, this provides real hope for an effective treatment of ALS.

With only 1–2% of cases having a proven genetic cause, amyotrophic lateral sclerosis (ALS) still remains a neurodegenerative disease of largely unknown origin. This suggests that multiple factors can contribute to the degeneration of motor neurons culminating in a disease with similar symptoms. Therefore, a treatment that could save motor neurons from death induced by different types of injuries could be useful for a wide number of ALS patients. Numerous neurotrophic factors have shown this potential to prevent motor neuron death both in vitro and in vivo after neonatal axotomy or in animal models with early-onset motor neuron disease. Glial-cell-line-derived neurotrophic factor (GDNF) and insulin-like growth factor 1 (IGF-1) are two such powerful trophic factors for motor neurons. However, the two IGF-1 trials in ALS patients have shown either no effect [1] or only mild benefits [2]. Similar negative trials with brain-derived growth factor (BDNF) [3] and ciliary neurotrophic factor (CNTF) [4] have also been reported.

These discouraging results point out difficulties that must be solved before motor neuron degeneration can be slowed in such a progressive disease: what is the right factor or combination of factors, and how can successful delivery to the target cells in the CNS be ensured? Kaspar and colleagues [5] have been able to overcome the second of these difficulties, and possibly also the first, by using an adeno-associated virus (AAV) that encodes IGF-1, to force IGF-1 synthesis within spinal motor neurons of mice that have the form of human Cu/Zn superoxide dismutase 1 (SOD1). It seems nearly certain that the protective effects of IGF-1 must be solved before motor neuron degeneration can be slowed in such a progressive disease: what is the right factor or combination of factors, and how can successful delivery to the target cells in the CNS be ensured? Kaspar and colleagues [5] have been able to overcome the second of these difficulties, and possibly also the first, by using an adeno-associated virus (AAV) that encodes IGF-1, to force IGF-1 synthesis within spinal motor neurons of mice that have the same IGF-1 gene but whose capsid proteins are not essential for the robust protective effect on motor neurons. This was accompanied by a 30% increase in lifespan when the virus was injected before the onset of the disease. But, most importantly, even injection at the age of disease-onset (conditions that mimic the timing for use as a treatment of human disease) prolonged the lifespan by 18%. Further, although it was likely that most investigators would have predicted the opposite, similar delivery of GDNF-encoding virus had, at best, only very modest effects. Therefore, both the choice of the factor and the vector seem to contribute to these successful results.

IGF-1 power to rescue motor neurons

Because the IGF-1-producing virus was injected into target muscles of the affected motor neurons [and many (1010) virions were injected], a portion of the protective effect in the recent paper from Kaspar and colleagues [5] could have come from IGF-1 synthesized after viral delivery by the circulation to more distant sites, or synthesized by the muscles at the sites of injection. Indeed, IGF-1 and other trophic factors synthesized by muscle cells have proved to be modestly neuroprotective, even in ALS mice in the case of GDNF [6]. Nevertheless, retrograde transport of the IGF-1-encoding virus seems essential for the robust protective effect on motor neurons because use of another virus (a lentivirus carrying the same IGF-1 gene but whose capsid proteins are not recognized for retrograde transport within motor neurons) had only a minor effect on extending survival.

So how does IGF-1 secreted by motor neurons produce its protective effect? Does IGF-1 directly act on the motor neuron or does it act through binding to IGF-1 receptors on other surrounding cells? IGF-1 increases the survival of motor neurons in culture [7,8], and prevents natural motor neuron cell death in chick embryo [9] and motor neuron death after axotomy in rodent neonates [8]. Beyond this, IGF-1 can stimulate nerve regeneration after injury and has the potential to increase axonal sprouting, thereby stimulating muscle innervation [10]. IGF-1 and its receptor, IGF-receptor I, are expressed by motor neurons, muscle cells and glia [11,12]. Therefore, the AAV-encoded IGF-1 released by motor neurons could act in an autocrine manner on motor neurons or in a paracrine manner on other cells in the spinal cord. Indeed, IGF-1 is well known for its role on myelinating cells. It is a survival factor for oligodendrocytes and Schwann cells [13,14] and it is a potent inducer of myelin production in vitro as well as in vivo [15,16].

Other glial cells, the microglia, also play an important role in motor neuron biology. For example, the use of the
drug minocyclin that decreases microglial activation and division can delay the onset of the disease in ALS mouse models [17]. In addition, chimeric mice made of mixtures of normal cells and cells expressing the mutant SOD1 have also shown that the environment of the motor neurons is crucial. Mutant motor neurons can be supported by a wild-type environment, and mutant glia can induce pathological signs in wild-type neurons [18]. Recognizing that a wide variety of trophic and toxic molecules can be synthesized by astrocytes and microglial cells, IGF-1 could induce or repress the glial production of such factors acting on motor neurons. Studies in culture have already shown that the increase of motor neuron survival stimulated by IGF-1 is mediated by the presence of astrocytes [7]. Coupled with this, a direct effect of IGF-1 on motor neurons is also likely; for example, its anti-apoptotic action [19] could suppress motor neuron death.

Moreover, the IGF-1 effect can be regulated by its interaction with six known binding proteins (IGF-1-binding proteins 1–6), at least some of which inhibit stimulation by IGF-1 of its receptors. Loss of IGF-1 activity in extracts of spinal tissue and in serum of ALS patients has been shown to accompany increases in these inhibitory binding proteins, along with corresponding decreases in free IGF-1 levels [12,20]. Unanswered is whether such increases are also found in ALS mouse models. AAV-mediated increases in the level of IGF-1 could therefore simply restore a required level of free IGF-1.

**Targeting efficiently motor neurons in vivo**

The challenge to finding an effective solution for slowing the degenerating process in ALS is to identify agents that both cross the blood–brain barrier and are delivered to the disseminated motor neurons along the spinal cord. The clinical use of trophic factors for motor neurons can be limited by their toxicity and/or their short half-lives. Intrathecal implantation of encapsulated cells engineered to produce CNTF was used as a first approach in ALS patients [21]. This technique had the advantages of avoiding the peripheral side effects of CNTF, bringing a high concentration of the drug close to the motor neurons and limiting the immunological responses. Unfortunately, no positive effects were reported for this clinical trial.

Successful application of recombinant AAV to therapy in ALS follows use of viruses in other mouse models of motor neuron degenerative diseases. Adenovirus carrying neurotrophin 3 (NT3) was able to increase the lifespan of pmn (progressive motor neuronopathy) mutant mice when it was injected into the muscles of their limbs [22]. The affected nerves and neuromuscular junctions showed improvements but no effects on the soma of motor neurons were reported. AAV appears to be superior to adenovirus as a neuronal vector. Although an adenovirus producing GDNF had no real beneficial effect in SOD1 mice [23], the use of AAV with the same factor (GDNF) was able (modestly) to increase the survival and delay the onset of the disease [24]. The advantage of the AAV vector (Box 1) resides in its ability to promote substantial and sustained expression of the factor it encodes [25]. Indeed, nerve sprouting has been reported in ALS patients [26] and SOD1 mouse models [27], and this might increase the efficiency of viral uptake after intra-muscular injection of viruses.

Most importantly, combining AAV with the right trophic factor (IGF-1) yields a significant improvement over use of GDNF. IGF-1 appears to be a good candidate but recognizing the possible additive power of certain neurotrophic factors, combinations of different factors could

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**Figure 1.** Viral delivery of insulin-like growth factor 1 (IGF-1) to motor neurons by peripheral injection in the muscles. Kaspar and colleagues [5] injected the adeno-associated virus (AAV) containing IGF-1 construct in the hindlimb quadriceps and intercostal muscles of the Cu/Zn superoxide dismutase 1 (SOD-1) G93A amyotrophic lateral sclerosis (ALS) mouse model. AAV is retrogradely transported into the soma of the motor neurons innervating these muscles, where the IGF-1 can then be produced. The target cells for secreted IGF-1 in the spinal cord could be the motor neurons themselves, neighboring neurons or glia. Abbreviations: CMV, cytomegalovirus promoter; LTR, long terminal repeat; SD/SA, splice donor and splice acceptor sites.
Box 1. Adeno-associated virus

Adeno-associated virus (AAV) is a non-pathogenic human parvovirus with a single-stranded DNA genome. The wild-type genome consists of two genes: rep and cap. Rep controls viral replication, structural gene expression and integration into the host genome, and cap encodes the capsid structural proteins. In recombinant AAV (rAAV), the rep and cap genes are completely deleted to allow insertion of the gene of interest. To proliferate, the AAV needs a helper virus, usually an adenovirus or herpesvirus, to supply the replication functions that AAV lacks. Helper plasmids are also used today for replication of rAAV.

The rAAV enters the cell via interaction with receptors such as heparin sulfate proteoglycans. Once entered, the viral capsid reaches the nucleus where it uncoats, releasing the single-stranded recombinant genome consisting of the gene of interest flanked by the 146-bp long terminal repeats (LTRs), the only remains of the original adenoerviral genome. rAAV typically does not integrate into the host genome and forms stable episomal concatamers.

AAV is able to infect both dividing and non-dividing cells and transduces efficiently a wide range of host cells. The broad spectrum of action and long-term expression of AAV makes it very useful for gene therapy, especially considering that the wild-type virus has never been shown to cause human disease. Injection of AAV particles does not lead to acute inflammatory responses or toxic side effects. Although ~80% of individuals carry antibodies against wild-type AAV, this seropositivity has not yet been shown to be associated with humoral immune responses – but this should be taken into consideration if the gene therapy strategy requires multiple injections of the virus. The main disadvantage of AAV is its small packaging capacity: the space available for the insertion of the gene of interest is ≤5 Kb.

Despite these difficulties, AAV remains a vector of choice for neurodegenerative diseases, for (i) its ability to infect post-mitotic cells (e.g. neurons), (ii) its long-term expression (very important, regarding the slow progression of many of these diseases), and (iii) its ability to be retrogradely transported by neurons, a necessity with regard to the often very low accessibility of the target cells.

there would be a long-term immune response will need to be established. In the longer run, one could hope that even better results would be obtained with the same strategy by combining different factors.

References
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Astroglia have long been thought to play merely a supporting role in the life of the neuron. However, these star-shaped cells have recently been the focus of intense study that has begun to emphasize remarkable and novel roles for these amazing cells. While astroglia play positive roles in the life of the neuron, they can simultaneously exert negative influences. Kinouchi et al. convincingly demonstrate and characterize an inhibitory role played by astroglia after neuronal transplantation. These findings remind us that astroglia exert positive and negative influences on neuronal survival, migration, neurite outgrowth and functional integration. Here, we review the complementary and often contradictory roles of astroglia during neuronal integration.

Long considered mere passive or supportive cells, capable of providing only growth factors or trophic factors to neighbouring neurons, glia have recently been the object of intense study that has begun to emphasize remarkable and novel roles for these amazing cells. While astroglia play positive roles in the life of the neuron, they can simultaneously exert negative influences. Kinouchi et al. convincingly demonstrate and characterize an inhibitory role played by astroglia after neuronal transplantation. These findings remind us that astroglia exert positive and negative influences on neuronal survival, migration, neurite outgrowth and functional integration. Here, we review the complementary and often contradictory roles of astroglia during neuronal integration.

Thus, astroglia might nurture, or a subset of related cells might even produce, neurons, which were long thought to be the lords to supportive astroglial serfs.

However, just as astroglia play positive roles in the life of the neuron, they can simultaneously exert negative influences. Indeed, the inhibitory roles of astroglia upon nerve regeneration are now well documented; glial scars that typically form after injury by reactive astrocytes constitute both a mechanical and a chemical barrier that blocks nerve regeneration and axonal growth. Now, Kinouchi and colleagues [6] provide further compelling evidence for the central role of astroglia in neuronal differentiation and integration in the adult CNS.

Robust neuronal integration in a modified astroglial environment

In this elegant study, the authors used the adult mammalian retina, which contains both astroglia and Müller glia, as a model in which to characterize and assess how astroglia can influence neuronal integration. Kinouchi et al. provide crucial insight into the molecules that control this phenomenon. Specifically, and most interestingly, they show that two classically astroglial filament proteins, GFAP and vimentin, directly influence the ability of donor cells to survive, migrate and integrate upon transplantation in the adult retina in vivo.

Using transgenic mice null for GFAP, vimentin, or both, as recipients, and retinal donor cells expressing green fluorescent protein (GFP), they systematically examined migration and neurite outgrowth properties of the transplanted cells in vivo. From these studies, they conclude that a lack of the non-permissive proteins, GFAP and vimentin, contributes significantly to the success with which donor cells integrate in an otherwise inhibitory environment.

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* These authors contributed equally to the article.

Corresponding author: Jeffrey D. Macklis (jeffrey_macklis@hms.harvard.edu).

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