Gene Therapy for Motor Neuron Disease

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Introduction

Gene therapy continues to be a potential option for treating amyotrophic lateral sclerosis (ALS), a fatal adult motor neuron disease (MND) with no cure. The only U.S. Food and Drug Administration (FDA)–approved drug for ALS, riluzole, offers a modest improvement, prolonging survival by a maximum of three to five months (Shaw and Ince, 1997). A variety of gene therapy approaches are available to justify clinical intervention in this rare condition, whether preventing degeneration by protecting motor neurons (MNs) from external insults or by silencing the genetic mutations that cause some familial forms of the disease.

This chapter will inform the reader about promising therapeutic transgenes and proof-of-principle studies in transgenic rodent models of ALS. It will also discuss challenges regarding the disease targets and timing for therapeutic intervention. Finally, it will briefly review potential restorative approaches for ALS, as well as gene therapy for other MNDs.

Facts and Demographics of ALS

ALS, also known as Lou Gehrig's disease in the United States, is a fatal MND with adult onset and relatively short course, culminating in death within three to five years postdiagnosis. This neurodegenerative disease is characterized mainly by the progressive degeneration of upper and lower MNs in the spinal cord, brainstem, and motor cortex. As MNs degenerate, muscles lose strength, and voluntary movements are compromised. Death is usually caused by respiratory failure, when diaphragm and intercostal muscles become disabled (Vincent et al., 2008).

Although clinically indistinguishable, ALS can occur in one of two forms: a most common or sporadic (sALS) form, which affects approximately 90% of the patients; or a familial (fALS) form linked to specific genetic mutations, which affects approximately 10% of ALS patients.

In the United States, the prevalence of ALS is approximately 30,000, and the incidence is slightly greater (60%) in the male population. The disease generally occurs between the ages of 40 and 70 years.

Etiology and Pathogenesis of ALS

In addition to the identification of specific genetic mutations linked to the inherited familial form of ALS, complex and multifactorial processes are involved in the disease pathway.
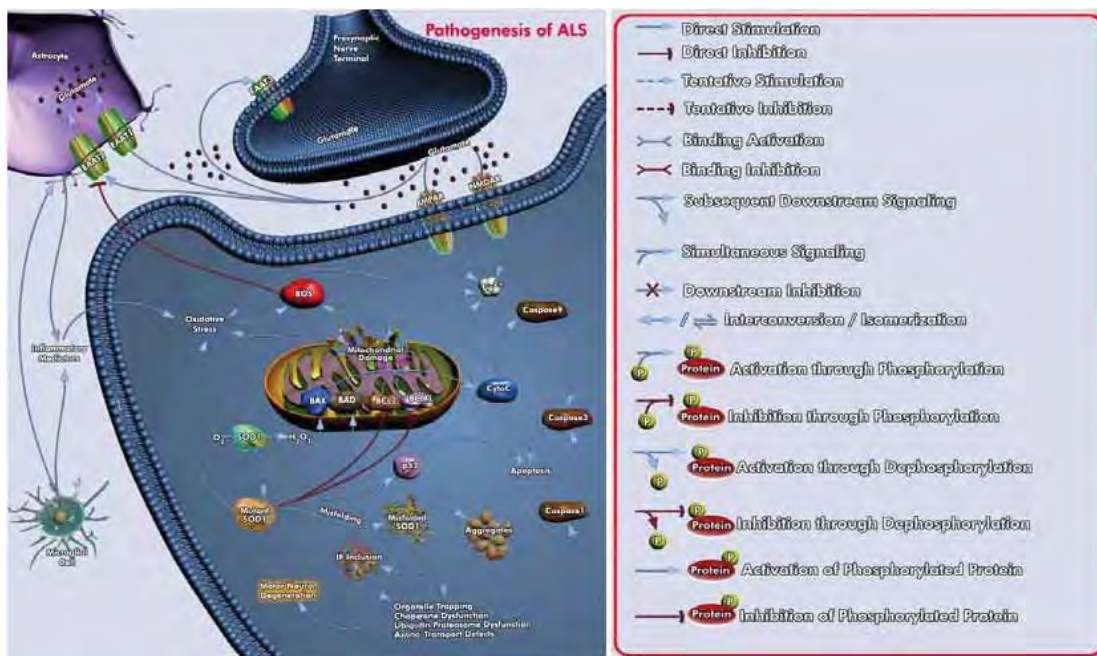

Figure 1. Pathogenesis of ALS. Multiple mechanisms are implicated in the pathogenesis of ALS, including excitotoxicity, oxidative stress, mitochondrial dysfunction, defective axonal transport, and abnormal protein aggregation. Reprinted with permission. Copyright ©2009 Qiagen. All rights reserved.

Approximately 20% of the fALS cases are caused by an identified mutation in the Cu/Zn superoxide dismutase type-1 (*SOD1*) gene, whereas a mutation in the transactive response–DNA binding protein (*TARDBP*) gene has been recently linked to ~5% of fALS cases. More recently, mutations in other genes, including the angiogenin (*ANG*), vesicle-associated membrane protein–associated protein B (*VAPB*), and fusion in malignant liposarcoma/translocated in liposarcoma (*FUS/TLS*) genes, have been identified in patients with fALS (Millecamps et al., 2010; Traub et al., 2011).

Whether sporadic or caused by specific genetic mutations as listed above, the disease invariably has a common pathological feature: the selective death of MNs. Oxidative stress, neurofilament abnormalities, excitotocixity, apoptosis, mitochondrial dysfunction, defective axonal transport, mutations in RNA binding proteins, and inflammation are among the multiple factors playing a role in the pathogenesis of ALS (Fig. 1). We invite the reader to further explore the literature on these different pathogenic mechanisms by visiting timely reviews, such as those of Bruijn et al. (2004) and Rothstein (2009).

Possible Therapeutic Targets

In ALS, simultaneous treatment of the spinal cord (i.e., MN cell bodies and/or glial cells) and skeletal muscle (i.e., neuromuscular junctions [NMJs]) might be necessary to fully cover the pathways involved in MN degeneration.

Motor neurons

Although MNs are known predominantly as the primary cell type implicated in the disease, increasing evidence indicates that they are perhaps not the sole target for therapeutic intervention in ALS. Gene therapy strategies for ALS had once focused mainly on treating MNs. However, defining a specific therapeutic target for ALS remains a challenge. Despite the selective vulnerability of MNs in ALS, astrocytes can play a modulatory yet detrimental role in the disease process by triggering apoptotic and inflammatory mechanisms, thereby contributing to MN death (Barbeito et al., 2004). Moreover, reduced levels of glutamate transporters in astrocytes may cause impaired glutamate uptake and the consequent excitotoxicity occurring in ALS. Nonetheless, halting MN degeneration is the ultimate goal of any therapeutic strategy for ALS.
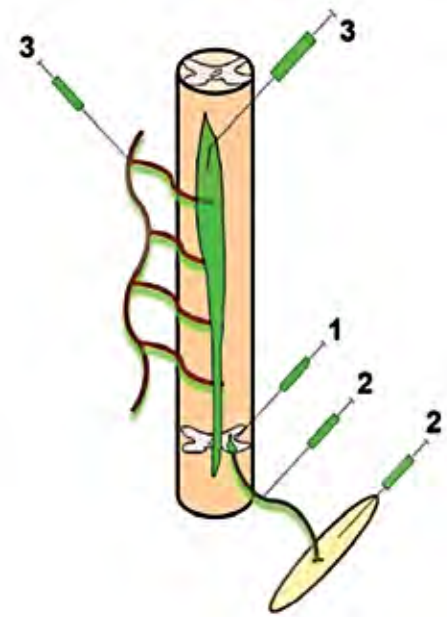

Figure 2. Methods for motor neuron gene delivery. Therapeutic transgenes can be delivered to spinal motor neurons (1) by direct injection; (2) by peripheral gene delivery, using the intramuscular or intraneural routes of administration; or (3), more recently, by systemic gene delivery via intramuscular or intrathecal administration.

Astrocytes

Downregulation of the excitatory amino acid transporter 2 (EAAT2), expressed mainly in astrocytes, has been suggested as a cause of MN excitotoxicity (Howland et al., 2002). In fact, cells engineered to overexpress EAAT2 can dramatically increase glutamate uptake and confer neuroprotection on motor neurons in coculture systems *in vitro* (Wisman et al., 2003). Increased expression of EAAT2 in *SOD1* mice can delay the loss of MNs in these double transgenic mice (Guo et al., 2003); conversely, a reduced amount of this receptor in *SOD1* mice caused them to exhibit earlier MN loss (Pardo et al., 2006). In conclusion, increasing the expression of glutamate receptors in glial cells could be beneficial for the treatment of ALS.

Neuromuscular junctions

Because end-plate denervation is one of the initial events in ALS (Fischer et al., 2004), targeting NMJs at early stages can be critical to preserving MN connections (Dupuis and Loeffler, 2009; Dupuis and Echaniz-Laguna, 2010). In newborn *SOD1* mice, intramuscular injection of an adeno-associated viral vector encoding cardiotrophin-1 delayed

neuromuscular degeneration (Bordet et al., 2001). Similarly, in *SOD1* rats, *ex vivo* gene delivery of glial cell line–derived neurotrophic factor (GDNF) within muscles significantly increased the number of neuromuscular connections and, consequently, MN cell bodies during the midstages of the disease (Suzuki et al., 2008). On the other hand, a recent study has demonstrated that bodywide intramuscular injections of adeno-associated virus 6 (AAV6)–expressing small hairpin RNAs (shRNAs) against *SOD1* into newborn mice halted muscle atrophy but failed to stop disease progression (Towne et al., 2011).

Lessons from Transgenic Models of ALS

Rodent models carrying mutated forms of the human *SOD1* gene develop an MND that closely replicates the human disease. Such models have been widely used to help elucidating the disease mechanisms as well as to assess the efficacy of a variety of therapeutic strategies for ALS, including gene therapy (Gurney et al., 1994).

Numerous studies have reported promising results in *SOD1* rodent models, prolonging survival of the animals and preventing MN loss. Even so, the therapeutic relevance of animal models remains questionable because the vast majority of interventions occur in asymptomatic animals. In medical practice, ALS patients are diagnosed as the symptoms manifest themselves, most commonly reported as muscle weakness, which indicates distal axonal degeneration.

Gene delivery to MNs: routes of administration

When designing a therapy for ALS, the degree of success directly correlates with how adequately MN pools are targeted across the spinal cord (Fig. 2). Moreover, the biodistribution of the therapeutic transgene can determine the extent of a treatment effect. In reality, efficacious and safe gene delivery to spinal MNs remains a challenge for successful gene therapy in ALS, a disease process that ideally requires diffuse gene delivery throughout the cord.

Different routes of viral vector administration for MN gene delivery have been evaluated over the years. Noninvasive approaches via peripheral intramuscular or intraneural administration, which yielded promising results in mice (Kaspar et al., 2003), have failed scale-up validation in larger species owing to inefficient vector transport and negligible amounts of gene expression in the spinal cord

(T. Federici and N. Boulis, unpublished observations). Robust gene expression following intramuscularly injected AAV6 was recently described in monkeys (Towne et al., 2009). Nonetheless, the feasibility of such an approach for treating ALS in humans remains questionable in a disease process with distal axonopathy. Alternatively, intraspinal injections have been investigated as a more direct means of achieving gene delivery in the spinal cord. Although promising, with positive outcomes in wild-type and *SOD1* animals (Azzouz et al., 2000; Franz et al., 2009; Lepore et al., 2007), such an approach has yet to be validated for clinical translation. Our group is currently performing a Phase 1 clinical trial for intraspinal cellular delivery in ALS patients, tempering the safety concerns that have hampered the translation of invasive surgery for therapeutic delivery (Lunn et al., 2011). Preclinical assessment of instraspinal gene delivery in large animals is still necessary in order to validate scalability and assess biodistribution with this type of approach (Federici et al., 2009). Intramuscular, intraneural, and intraspinal injections cannot target the entire spinal cord and are considered segmental approaches for gene delivery.

Current research in gene therapy has focused on AAV9, an AAV vector that is capable of crossing the blood–brain barrier following intravenous or intrathecal administration with age-dependent and promoter-dependent but preferential transduction of MNs (Duque et al., 2009; Foust et al., 2009; Snyder et al., 2011). Moreover, our group and others have recently demonstrated MN transduction following systemic delivery of AAV9 in large animals (Duque et al., 2009; Federici et al., 2011; Foust et al., 2011; Gray et al., 2011). These results confirm the highly translationable profile of this combination of vector and noninvasive approaches for diffuse gene delivery.

Neuroprotection

Numerous studies have demonstrated that protecting dying MNs can prolong survival in rodent models of ALS. Despite producing only a modest effect, such an approach has become the basic premise for ALS treatment. However, attempts to systemically deliver recombinant trophic factors such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), or human insulin-like growth factor-1 (IGF-1) have shown no benefit to ALS patients (Federici and Boulis, 2006; Sorenson et al., 2008). These trophic factors' inadequate or insufficient delivery and systemic inactivation/short half-life have been suggested as potential explanations for the disappointing results in humans. In contrast,

gene-based delivery of neurotrophic factors may be a more effective alternative, as will be discussed in this section.

Of relevance to ALS, the safety and therapeutic potential of gene-based delivery of neurotrophic factors are being evaluated in clinical trials for Parkinson's and Alzheimer's diseases (Tuszynski et al., 2005; Marks et al., 2008; Snyder et al., 2010). The rationale for gene delivery of neurotrophic factors for ALS comes from animal proof-of-principle data demonstrating that secreted neurotrophic factors can support MN survival in a diseased milieu and thereby prevent progressive degeneration. Numerous successful demonstrations of MN protection have been reported following viral vector–mediated delivery of various growth factors, most notably GDNF, IGF-1, and vascular endothelial growth factor (VEGF), in transgenic rodent models of fALS. In these models, animals overexpress the human gene coding for the mutated forms of *SOD1*, developing a disease with very similar characteristics to ALS.

Neurotrophic-based gene therapy has been able to delay disease onset and slow progression of the disease in *SOD1* mice and rats more or less effectively, depending on the delivery approach. By a mechanism involving retrograde axonal transport of the transgene, intramuscular injections have been widely used to deliver neurotrophic-based viral vectors. Intramuscular delivery of AAV2-IGF-1 prolonged survival and delayed disease progression in *SOD1* mice (Kaspar et al., 2003). Similarly, intramuscular injection of an equine infectious anemia virus (EIAV)–based lentiviral vector expressing VEGF resulted in prolonged survival in the same mouse model (Azzouz et al., 2004a) (Fig. 3). Our group demonstrated segmental neuroprotection but no effect on survival following intraspinal delivery of AAV2-IGF-1 in *SOD1* rats (Franz et al., 2009).

How robust such effects need to be in order to take them to the level of preclinical development, compared with riluzole, which offers only a marginal effect (Gurney et al., 1996; Scott et al., 2008), remains questionable. The market opportunity for therapeutic development exists, and the ALS patient population urges for more effective treatments. Nonetheless, as previously discussed, devising scaling-up delivery strategies from rodents to large animals remains one of the main hurdles that limits the translation of spinal cord gene therapy. To date, MoNuDin (an EIAV-based lentiviral vector system for the delivery of VEGF) is the only gene therapy technology in preclinical development for the treatment of ALS.

As one of the mechanisms implicated in the pathogenesis of ALS, apoptosis has been targeted as a means of preventing neuronal cell death. Gene delivery of Bcl-xL and Bcl-2 — molecules with known anti-apoptotic activity — yielded MN protection *in vitro* and in *SOD1* mice (Azzouz et al., 2000; Yamashita et al., 2002; Garrity-Moses et al., 2005). However, despite the promise of some proof-of-principle studies, the state-of-the-art literature does not indicate that this strategy is advancing gene therapy.

Gene silencing

Because *SOD1* fALS arises through a toxic gain of function, RNA interference (RNAi) has been proposed as a means to knock down mutant *SOD1*. While proof-of-principle research has provided substantial evidence of successful selective silencing of the *SOD1* mutant allele, attempts to elucidate the mechanisms of ALS or to distinguish among the roles that different cell types play in disease pathogenesis by selectively knocking down mutant *SOD1* in astrocytes, MNs, or muscle cells have been somewhat disappointing. Moreover, while viral vector–mediated *SOD1* gene silencing significantly increased the lifespan of *SOD1* mice (Ralph et al., 2005; Raoul et al., 2005), systemic delivery has been proven insufficient for preventing disease progression (Towne et al., 2008). Recently, intramuscular delivery of AAV6.shRNAs.*SOD1* in newborn mice has also failed to stop disease progression (Towne et al., 2011). Nonetheless, Isis Pharmaceuticals (Carlsbad, CA) has initiated a Phase 1 study to assess the safety of ISIS-SOD1Rx, an antisense oligonucleotide–based drug that inhibits *SOD1* production (clinical trial identifier NCT01041222).

Gene therapy for spinal muscular atrophy

Spinal muscular atrophy (SMA, broadly considered the pediatric version of MND) has defined mutations in the survival motor neuron gene 1 (*SMN1*); therefore, SMA is a desirable disease target amenable to gene therapy. Even though humans have a nearly identical gene, *SMN2*, the protein is less stable and truncated owing to an alternative splicing and, therefore, does not compensate for the absence of *SMN1*. SMA is classified into three different forms, and the presence of variable levels of full-length *SMN* determines the severity of disease.

As in ALS, SMA patients have selective loss of lower MNs, and gene therapy neuroprotection strategies have been equally proposed for SMA (Lesbordes et al., 2003; Federici and Boulis, 2006). In addition, viral
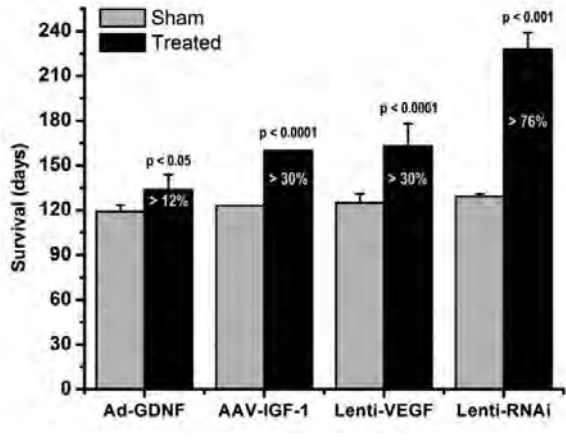
Figure 3. Survival effect of gene therapy in *SOD1* mice. Kaplan–Meier graph demonstrates increased lifespan of *SOD1* mice after intramuscular injection of adenovirus vector (Ad)–GDNF (Acsadi et al., 2002), AAV–IGF-1 (Kaspar et al., 2003), EIAV–VEGF (Azzouz et al., 2004a), or EIAV-mediating expression of RNAi targeted to the human *SOD1* gene (Ralph et al., 2005). Untreated *SOD1* mice survive an average of 135 days (black line). Federici and Boulis (2006), their figure, reprinted with permission. Copyright ©2006 John Wiley & Sons. All rights reserved.

vector–mediated *SMN* gene replacement has been tested in animal models of SMA, with variability of efficacy depending on time of intervention and biodistribution of the therapeutic transgene. For example, despite successfully restoring *SMN* protein levels following intramuscular delivery of a lentiviral vector expressing the human *SMN* gene, only marginal efficacy in survival was observed (Azzouz et al., 2004b). More recently, several groups have reported prolonged survival in SMA mouse models following (systemic) intravenous delivery of AAV9.*SMN* (Foust et al., 2010; Valori et al., 2010; Dominguez et al., 2011). Finally, a different approach, based on the delivery of translational read-through compounds, has been described as capable of reducing disease severity in SMA mice by producing a more stable isoform of the truncated protein (Mattis et al., 2008).

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