Review

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Gene therapy for neurodegenerative diseases

Deirdre M. O'Connor and Nicholas M. Boulis

Department of Neurosurgery, Emory University, 101 Woodruff Circle, Atlanta, GA 30322, USA

Gene therapy is, potentially, a powerful tool for treating neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, Parkinson's disease (PD) and Alzheimer's disease (AD). To date, clinical trials have failed to show any improvement in outcome beyond the placebo effect. Efforts to improve outcomes are focusing on three main areas: vector design and the identification of new vector serotypes, mode of delivery of gene therapies, and identification of new therapeutic targets. These advances are being tested both individually and together to improve efficacy. These improvements may finally make gene therapy successful for these disorders.

Gene therapy: theory and practicalities

In theory, gene therapy is a straightforward process. A disease is treated by delivery of a transgene that either replaces or corrects a defective gene, or generally supports cells in the disease environment. In practice, it is considerably more complex, and a variety of factors need to be optimized. The correct vector needs to be selected, the appropriate mode of delivery optimized, and the transgene chosen. The interaction between the host immune system and the vector or transgene may further complicate therapy. For neurodegenerative diseases, the nature of the target tissue adds an extra layer of complexity.

Gene therapy vectors can be either viral or non-viral. Viral vectors harness the natural ability of viruses to infect cells. Their genomes are modified to remove genes that would allow them to replicate, rendering them translatable for clinical use. For neurodegenerative diseases the two most common viral vectors used are adeno-associated viruses (AAVs; see Glossary) and lentiviruses. AAV and lentiviral vectors have the ability to infect both dividing and non-dividing cells. However, lentivirus integrates into the host genome, while AAV does not. Integration confers stable, long-term expression, but also raises the possibility of integrational mutagenesis. Although AAV is non-integrating, it can still deliver stable gene expression in non-dividing cells [1,2]. Non-viral vectors usually consist of naked plasmid DNA or in complex with cationic lipids or polymers. They have a localized effect and require a higher therapeutic dose than viral vectors. In general, non-viral delivery confers only transient gene expression, which is usually not sufficient for the treatment of chronic neurodegeneration.

The delivery route is an important consideration, especially to the central nervous system (CNS). Remote delivery, via intravenous injection, has the advantage of being non-invasive. However, the blood–brain barrier (BBB) is a significant obstacle hindering the passage of most vectors into the CNS. Thus, the discovery that AAV9 has the ability to cross the BBB was significant [3]. The disadvantages of remote delivery are the increased risk of off-target effects and the need to deliver a greater dose to achieve a therapeutic dose in the target tissue. Direct delivery to the CNS limits off-target effects and reduces the required dose of the gene therapy vector. In the CNS this can be achieved via intraparenchymal injection (directly into the brain or spinal cord) or injection into the cerebrospinal fluid [CSF; either intracerebroventricular (ICV) or intrathecal]. Both intrathecal delivery and direct spinal cord injection have been demonstrated to be safe [4,5], as well as direct injection into the human brain [6,7]. The proposed transgene can target the specific gene causing the disease, if known, or target a pathway or process within the disease. As more is understood about the disease process and progression, additional potential therapeutic targets can be identified.

There have been several gene therapy clinical trials for neurodegenerative diseases. One of the first trials for AAV-mediated gene therapy was for Canavan disease, which is caused by a mutation in the aspartoacylase (*ASPA*) gene. AAV2-*ASPA* was injected into the brain and the patients were monitored for up to 10 years post-surgery [8]. Follow-up showed a slowed progression in brain atrophy with some improvement in seizure frequency [8]. Recently, the first trial to deliver AAV9 intrathecally delivering the gigaxonin gene (*GAN*) (ClinicalTrials.gov registry number NCT02362438) to treat giant axonal neuropathy (GAN) has begun to recruit patients. However, many of the clinical trials have not demonstrated efficacy (Table 1). Efforts in improving vectors, targeting delivery, and expanding the choice of possible transgenes should increase efficacy in gene therapy trials.

Parkinson's disease

PD is a neurodegenerative disease that is characterized by loss of the dopaminergic neurons of the substantia nigra pars compacta (SNc) and reduction of levels of dopamine in the striatum. Symptoms include rigidity, resting tremor, and motor function impairment, including freezing and bradykinesia. The current standards of care include dopamine replacement with drugs such as levodopa, a

Corresponding author: Boulis, N.M. (nboulis@emory.edu).
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Glossary

Acyl-CoA:cholesterol acyltransferase (ACAT) 1: an endoplasmic reticulum enzyme that modulates build-up of cholesterol in membranes by converting it to cholesterol esters.
Adeno-associated virus (AAV): non-enveloped, non-integrating virus with the ability to infect dividing and non-dividing cells.
Alzheimer's disease (AD): a progressive disorder characterized by problems with memory, thinking, and behavior.
Amyloid β (Aβ): peptide that form the main component of plaques in Alzheimer's disease.
Amyotrophic lateral sclerosis (ALS): a progressive disorder resulting from loss of upper and lower motor neurons in the brain, brainstem, and spinal cord that is usually fatal within 2–5 years from diagnosis. ALS can be familial or sporadic in origin.
Antisense oligonucleotides (ASOs): short fragments of nucleic acid that bind to their target sequence and inhibit translation.
Blood–brain barrier (BBB): composed of endothelial cells of microvessels which form a barrier to the entry of most blood-borne substances to the brain. It excludes toxic substances and maintains a stable environment.
Convection enhanced delivery (CED): a pressurized infusion technique that allows therapies to be delivered to large volumes of the brain.
Deep brain stimulation (DBS): the implantation of electrodes into specific parts of the brain to control movement and affective disorders.
Glycosaminoglycan (GAG): a long unbranched polysaccharide; accumulation can cause mucopolysaccharidosis (MPSI).
Granulocyte colony stimulating factor (G-CSF): a hematopoietic factor important in regulating production of blood cells and in bone marrow stem cell survival.
Hypoxia-inducible factor (HIF1): a core regulatory factor that regulates gene expression during hypoxia.
Insulin-like growth factor 1 (IGF1): a neurotrophic factor that promotes neuronal survival.
Intracerebroventricular (ICV): delivery of a therapeutic factor directly into the ventricles of the brain, bypassing the BBB.
Parkinson's disease (PD): disorder characterized by loss of dopaminergic neurons in the substantia nigra. Symptoms include rigidity, resting tremor, and motor function impairment.
Magnetic resonance imagery (MRI): a technique that uses a magnetic field and radio waves to create detailed images of tissues and organs.
Metachromic leukodystrophy (MLD): is caused by an inherited mutation in arylsulfatase A (*ARSA*). Symptoms results from sulfatide accumulation in Schwann cells, oligodendrocytes and brain neurons.
Nerve growth factor (NGF): a neurotrophic factor that is important for the survival and maintenance of sympathic and sensory neurons, and induces axonal growth.
Neurturin (NRTN): a neurotrophic factor related to GDNF. It enhances dopaminergic neuron survival.
Nonsense-mediated mRNA (NMR) decay: a mechanism for degrading transcripts with a premature termination codon.
Spinal muscular atrophy (SMA): infantile form of motor neuron disease caused by the loss of the *SMN1* gene. Prognosis varies depending on the severity of the disease.
Spinocerebellar ataxia (SCA): a neurodegenerative disease caused by mutations in ataxin genes.
Substantia nigra par compacta (SNc): an area of the brain that serves as an input to the basal ganglia circuit. It supplies the striatum with dopamine.
Superoxide dismutase 1 (SOD1): an enzyme that converts the superoxide radical to either molecular oxygen or hydrogen peroxide. Mutations in the *SOD1* gene can cause ALS.
Survival motor neuron 1 (SMN1): a housekeeping protein that from complexes with gemin proteins in the nucleus. It functions is assembling small nuclear ribonucleoproteins and in pre-mRNA splicing.
Trimethoprim (TMP): an antibiotic with the ability to cross the BBB.
Vascular endothelial growth factor (VEGF): an angiogenic factor with neurotrophic and neuroprotective properties.

dopamine precursor, and deep brain stimulation (DBS). Levodopa can restore some motor function with varying efficiency; however, as PD progresses levodopa becomes less effective and its side effects become more pronounced. DBS has been effective at treating the symptoms of PD but does not target the cause. Moreover, DBS can exacerbate the cognitive and emotional deterioration that can characterize late-stage PD [9].

The possibility of using gene therapy to treat PD has been explored extensively. Several gene therapy trials have fulfilled the Phase I safety criteria and suggested some efficacy [10]. However, when advanced to controlled, blinded Phase II trials, the majority have failed to show improvement beyond the placebo effect. The only trial to show efficacy in a controlled, blinded Phase II trial was delivery of AAV2-glutamic acid decarboxylase (*GAD*) by direct injection into the subthalamic nucleus (NCT00643890) [11]. Patients who received AAV2-*GAD* had improvement in symptoms over control patients; however, this improvement was not greater than that seen with the current standard of care, and the study was terminated. Another trial used AAV2 to deliver neurturin (*NRTN*) (Cere-120) to the putamen to support the dopaminergic neurons (NCT00400634) [12]. Analysis of postmortem brain tissue from Phase II trial patients found that although there was an increase in *NRTN* expression in the injected area of the striatum/putamen, there was no corresponding increase in the substantia nigra [13]. This was thought to be due to failure of retrograde transport of the dopaminergic neurons. To address this issue a Phase IIb trial was undertaken in which both the putamen and the substantia nigra were injected. The dose was also increased with the aim of increasing transduction of the putamen. Preclinical data showed that this strategy significantly increased *NRTN* expression [14,15]; however, results from this Phase IIb trial showed no improvement in clinical outcome for the participants [16]. The trial coordinators hypothesized that the degenerative state of the PD brain may have affected transport of NRTN through the brain. Secondary outcome measures suggested that *NRTN* expression may have improved younger patients treated earlier in their disease course, arguing that growth factor gene therapies need to be delivered before neurodegeneration has progressed extensively.

The Prosavin trial targeted the dopamine synthesis pathway, using a tricistronic lentiviral vector to deliver the genes encoding the rate-limiting dopamine biosynthesis enzymes tyrosine hydroxylase, aromatic amino acid dopa decarboxylase (*AADC*) and GTP cyclohydrolase I (*GCH1*) [17,18]. A tricistronic vector is advantageous as cells transduced with the vector will express all three enzymes. The clinical trial entailed a Phase I/II dose-escalation study (NCT00627588 and NCT01856439) targeting the sensorimotor part of the striatum and the putamen. The trial achieved its goal in demonstrating the safely profile of Prosavin. The efficacy data also showed promise, with improvement in motor function in the off-medication state that correlated with increasing dose of Prosavin [18]. However, given that this was an open-label trial, the efficacy results have to been interpreted cautiously. Palfi *et al.* noted that the improvement observed in patients was within the placebo effect range seen in other clinical trials. The investigators intend to optimize the delivery method and then proceed to a double-blinded randomized trial to determine the efficacy of Prosavin.

AADC was also used as the sole therapeutic transgene in two Phase I clinical trials using AAV2 as the viral vector [19,20]. Long-term follow-up determined no adverse safety events and stable *AADC* expression after 4 years. An improvement in the unified PD rating scale (UPDRS) was observed in the first 12 months, but slowly deteriorated