of the growth factor GAP-43 and active Cdc42. The GIT1/β-PIX/Cdc42/PAK pathway may play an important role in the biological activities of neurons. GIT1 also stimulates GAP-43 expression which may help promote regeneration of damaged axons and formation of new synapses. Therefore, GIT1 may have potential in the treatment of nerve injury.

650. AAV-IGF1 and Exercise for Synergistic Therapies in ALS

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Chronic delivery of molecules to the CNS, such as the spinal cord, for therapeutic purposes has proven difficult. We recently discovered that adeno-associated virus (AAV) can be retrogradely transported efficiently from muscle to motor neurons of the spinal cord allowing a feasible approach to deliver therapies to motor neurons. We have now characterized the extent of retrograde transport of the different AAV serotypes including AAV 1-6. All serotypes demonstrate retrograde transport ability with serotypes 1, 2, and 6 showing the highest levels of transport.

We utilized the transport properties of AAV-2 to deliver neurotrophic factor genes to muscles in a transgenic mouse model of Amyotrophic Lateral Sclerosis (ALS) and showed significant retrograde transport and expression in the spinal cord. Delivery of AAV-IGF-1 (Insulin-like growth factor-1) remarkably slowed the onset of disease progression and increased survival over 30 days in comparison to control, AAV-GFP treated animals. Furthermore, IGF-1 delayed the motor decline assessed by motor performance tasks. Recent dose-response studies have shown a dose-dependence for therapeutic benefit, with at least 4x10e9 viral particles/animal delivered for efficacy. In addition, we show the therapeutic benefits of IGF-1 toward motor neurons are mediated in part, by disrupting the apoptotic cascade.

Furthermore, we have recently shown that a combination of IGF-1 gene delivery and exercise has profound effects on survival and function, indicative of synergistic effects with exercise and IGF-1, in which animals live nearly twice as long. We have elucidated several molecular mechanisms that may result in the synergistic response.

This work provides a novel targeting approach for neurotrophic factor delivery by AAV mediated gene therapy in the development of therapeutics towards neurological disorders and demonstrates the beneficial effects of exercise in the nervous system.

651. Oligonucleotide-Mediated Gene Repair Restores Full Length SMN mRNA Expression in Mutant-SMN Murine Fibroblasts

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Spinal Muscular Atrophy (SMA) is a severe neuromuscular disease characterized by degeneration of a-motor neurons in the spinal cord. Ninety percent of patients affected by SMA have deletion of the Survival of Motor Neuron-1 (SMN1) but they retain a copy of the gene (SMN2) in their genome. SMN2 produces almost no functional SMN protein due to a CÆT transition in exon 7 that disrupts a splicing enhancer sequence and causes skipping of exon 7 in >90% of the processed SMN mRNA. As a consequence, SMA cells have a decreased amount of properly spliced full length SMN mRNA, which encodes functional SMN protein. This decrease in functional SMN protein leads to decreased survival of motor neurons. Many attempts have been made to increase functional SMN protein levels from the SMN2 gene by correcting the splicing-process defect.

In this study, we have investigated the ability of single-stranded oligodeoxynucleotides (ssODN) to correct the CÆT mutation at exon 7 of the SMN2 gene and whether this ssODN-mediated gene repair restores production of functional full-length SMN mRNA. To establish an efficient gene repair protocol, we tested several ssODN, differing in size (49mer and 69mer), orientation (sense and antisense) and backbone modifications (oligodeoxynucleotides synthesized with zero, two or three nuclease resistant 2'-O-methyl RNA residues on each end). Each type of ssODN was transfected into murine SMA fibroblast cell lines (Smn-null and carrying 2 copies of the human SMN2 gene) by electroporation. Genomic DNA correction at the SMN2 locus in the fibroblasts was assessed by allele specific PCR 5 days post-transfection. Data analysis revealed that the 69NTr/ssODN (69mer/antisense/three groups of 2'-O-methyl RNA residues) led to the highest levels of gene correction. The frequency of correction was between 1:500 and 1:1000 cells as estimated by semi-quantitative genomic allele specific PCR and confirmed by allele-specific genomic qPCR. Consistent with the genomic DNA data, these same cell populations expressed the corrected allele as detected by allele specific RT-PCR, and they had a higher content of full length (correctly spliced) SMN mRNA compared to untransfected SMA cell controls, as detected by real-time RT-PCR.

The data reported here show that the CÆT transition in the SMN2 gene can be successfully "corrected" by ssODN-mediated gene targeting. This single-base correction leads to an increase in full-length SMN mRNA expression, and therefore functional SMN protein. Ongoing studies are focused on establishing the potential of this technique for in vivo or stem cell-mediated gene therapy for SMA.

652. Adeno-Associated Vector Delivery of Gutamate Transporters in an ALS Mouse Model

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Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease characterized by a selective loss of motor neurons in the motor cortex, brainstem and spinal cord. The cause of motor neuron degeneration remains largely unknown and there is to date no effective therapy. Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). Paradoxically, overactivation of glutamatergic neurons and extracellular accumulation of glutamate contributes to neuronal cell death observed in acute and chronic CNS insults, including ALS. The increased glutamate levels surrounding vulnerable motor neurons result from a pronounced loss of the excitatory amino acid transporter 2 (EAAT2) in affected ALS brain regions. EAAT2 (called GLT1 in rodents) is the predominant glutamate transporter in the CNS and is present on astrocytes. Loss of GLT-1 protein and function also occurs in mouse and rat ALS models.

Recent work has begun to suggest that over expression of glutamate transporters in various paradigms may be protective both *in vitro* and *in vivo*. Therefore, the overall goal of our studies is to determine whether raising the expression of glutamate transporters, by viral vector delivery, will reduce the sensitivity of neurons to excitotoxic insults *in vitro* and *in vivo* and, ultimately, delay disease in ALS models. In this study, we use adeno-associated virus (AAV) to deliver glutamate transporters to motor neurons. We recently used this approach to successfully deliver, thru retrograde transport, insulin-