900. Specific AAV Serotypes Stably Transduce Hippocampal and Cortical Cultures with High Efficiency and Low Toxicity

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Cultures of dissociated neurons and astrocytes are frequently used by neurobiologists to study neuronal physiology and pathophysiology. However, current methods for gene delivery into neurons in vitro are limited by very low efficiency, short-term gene expression, toxicity and/or the requirement to transfect on the day of plating. We hypothesized that adeno-associated viral (AAV) vectors might represent an efficient and less toxic method for neuronal transduction. We tested AAV transduction of primary cultures of 150,000 E18 rat cortical or hippocampal neurons and astrocytes using six serotypes (AAV1, 2, 6, 7, 8, and 9), each containing the same AAV-2-based genome encoding CMV-GFP.

Mature cultures of hippocampal neurons were transduced at 4 weeks post-plating by AAV1, 2, 7, 8 and 9, resulting in GFP expression for at least 1 week. AAV2 (2.5 E+11 genome equivalents, GE) was particularly effective in transducing the mature hippocampal cultures. Expression of GFP was detectable by 1 week post-transduction in 40-60% of the cells, localized predominantly in cells expressing the neuronal marker MAP2, and stable for up to 4 weeks (the life of the culture) without inducing cell death. Immature hippocampal cultures were exposed at 1 day post-plating to AAV1, 2, 6, 7 and 9, and transduced by AAV1, 2, 7 and 9. The AAV9 serotype (5E+11GE) transduced 50-70% of cells in the d1 hippocampal cultures and GFP expression was stable for 4 weeks. The AAV6 serotype caused cell death in both the mature and immature hippocampal cultures and the AAV7 serotype transduced both neurons and astrocytes. To determine whether cortical neurons were transduced by the same vectors, AAV1, 6, 7 and 9 were applied to cortical cultures at 4 weeks post-plating. All 4 serotypes transduced cortical cells and the GFP expression was stable for 1-2 weeks. In both hippocampal and cortical cultures, cell death occurred at later time points (2-4 weeks) after transduction although toxicity was not related to the volume of vector applied or GFP expression. Work is ongoing to quantify the percentages of neurons and astrocytes transduced by each serotype and to explore the in vitro transduction patterns of AAV vectors with engineered capsid proteins. These results should make possible a wide range of neurobiological experiments in cultured neurons, including delivery of shRNAs. Supported by NS040978 (DW) and P30-DK-047757.

901. Targeting of Viral Vectors to Motor Neurons by Botulinum Toxin Binding Domain To Treat ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive disorder of motor neurons, leading inevitably to paralysis and death. Recent advances in knowledge of the pathogenesis of ALS have suggested several attractive candidate treatment strategies. However, a key problem in ALS therapy in human patients is the enormous difficulty in delivering agents to the motor neurons. Attempts to deliver agents orally, by injections, or even by infusions into the subarachnoid space have generally not been successful, possibly because of limited access to motor neurons. Recently, viral vectors injected intramuscularly in mice have been used with some success to deliver potentially therapeutic genes to motor neurons, *via* retrograde transport. However, the efficiency of this method is limited, and the prospect of extensive intramuscular injections is daunting.

The overall goal of this research is the development of a novel and specific method of enhancing the delivery of potentially therapeutic genes to motor neurons. We are utilizing the unique ability of the binding domain (BD) of Botulinum neurotoxin (BoNT) to direct the binding and entry of viral vectors into motor nerves. This method will take advantage of the absolute specificity of Botulinum for cholinergic nerve terminals to enhance the efficiency of gene delivery by viral vectors to motor neurons. The (non-toxic) binding domain of Botulinum toxin binds specifically to motor nerve terminals, and translocates into endosomal vesicles, where it is acidified. We are engineering a Streptavidin Botulinum BD fusion protein capable of binding any biotinylated viral vector carrying virtually any gene of interest. This is designed to facilitate the entry of the virus into the motor nerve terminal, where it should be released, and accessible for retrograde transport to the motor neuron cell body, and delivery of the gene.

We have genetically engineered the expression of (1) a full length and a truncated version of the BD of BoNT Type A; (2) Streptavidin; and (3) a fusion protein consisting of the Botulinum BD fused to Streptavidin. We have demonstrated the ability of the full length and truncated BD to bind to cholinergic Neuro 2A cells in culture, and to enter the cells. Thus far, we have shown that the fusion protein binds to, and is internalized by, the cholinergic N2A cells, and that it binds to biotin. In addition, we have shown that the complex consisting of the fusion protein and biotinylated b-galactosidase enters into N2A cells.

We will biotinylate viral vectors (adenovirus and/or AAV), and combine them with the BoNT BD - Streptavidin fusion protein. We anticipate that this will re-direct the viral vectors, and greatly enhance their entry into motor nerve terminals, leading to viral liberation into the cytoplasm, and retrograde transport to the motor neuron cell bodies. We will utilize this system to enhance viral vector entry into motor neurons *in vivo*. This method should greatly enhance the ability to deliver virtually any potentially therapeutic genes to motor neurons in ALS, using viral vectors.

902. Modulation of Voltage-Gated Potassium Channels in Dorsal Root Ganglion Neurons by an Inducible Adenoviral Vector *In Vivo*

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Injury to the nerve or dorsal root ganglion (DRG) reduces voltage-gated potassium (Kv) channel expression and increases neuronal excitability in DRG neurons. Kv channels are therefore potential targets of gene therapy for chronic pain. An adenoviral vector, containing a reporter gene encoding the enhanced green fluorescent protein (GFP) and a Kv gene, was delivered to the fourth lumbar (L4) DRG of adult rat by microinjection via a small catheter inserted under the epineurium of the spinal nerve 3 to 5 mm from the DRG. The *in vivo* expression of the transferred gene was controlled by an ecdysone analog via an ecdysone-inducible promoter in the viral vector. Three types of vectors were used. A control vector (AdEGI) contained only the GFP gene. A vector intended to reduce votage gated potassium channels (AdKv1.xDN) had a dominant-negative Kv1.3 gene that disrupted Kv1 channels. A vector to increase inwardly rectifying K current (AdKir2.1) had the human Kir2.1