Gene Therapy for ALS Using Adeno-Associated Virus Vectors

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease where studies have shown a progressive degeneration of motor neurons; neurotrophic factors may alleviate motor neuron degeneration. However, an optimal method of delivery and expression of these neurotrophic factors is still lacking. With the use of Adeno Associated viruses (AAV) as vectors for neurotrophic factors, researchers can delete specific AAV sequences, substitute them with DNA of interest, and incorporate an internal ribosome entry site (IRES) that will allow dual gene expression. Although one version of the IRES from encephalomyocarditis virus (EMCV), IRES2, is commonly used, there have been some reports suggesting that IRES2 activity is sometimes unexpectedly low in driving gene expression. The intent of this study is to compare muscle specific AAV vectors containing the commonly used IRES2 with those containing a wild type IRES (IRESwt) for possible differences in expression efficiency in the hopes that it possesses the ability to express neurotrophic factors and a marker gene to allow for tracking of the viruses. The neurotrophic factor BDNF was inserted into AAV vectors that also contained an IRES sequence to allow the expression of a downstream green fluorescence protein (GFP), for easier imaging and tracking of the virus. Two AAV vectors, one containing the commonly used IRES2 and the other, IRESwt were constructed. The vectors were then transfected with plasmids containing factors required for AAV DNA replication and packaging. The produced AAV were used to infect muscle cells. The expression of GFP by these two vectors were compared by analyzing the intensity and the percent of cells that are GFP-positive. Transfection of HEK-293T cells was successful and BDNF gene was expressed in the cells. The viral vector containing BDNF and IRESwt were also successfully produced and were then used to infect muscle cells. Based on GFP expression, inclusion of IRESwt conferred more efficient translation of the marker gene than the more commonly used IRES2. In-vitro IRESwt is more efficient in directing expression of the marker gene. Eventually, IRESwt could be used to express luciferase and neurotrophic factors in in-vivo experiments and progress to human clinical trials to provide a muscle specific gene- therapy for ALS. Funded by The Busch Biomedical Grant.