Novel Gene-Based Delivery of Neurotrophic Factors for ALS Treatment

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ALS is a progressive motor neuron degenerative disease with few approved treatment options. Neurotrophic factors (NTFs) such as ciliary neurotrophic factor (CNTF) and Gas6 confer neuroprotective and anti-inflammatory effects on motor neurons; however, an effective delivery method has not yet been developed for humans. After recent success in treating spinal muscular atrophy, adeno-associated virus (AAV) vectors have come to the forefront of gene-based delivery of NTFs, but they have several shortcomings that hinder their efficacy in human trials. The aim of this study is to create inducible, muscle-specific AAV vectors expressing CNTF and Gas6 genes that confer neuroprotective effects on motor neurons at neuromuscular junctions. A synthesized Gas6 gene was inserted into a mammalian expression vector (pcI-Neo) and then used to transfect HEK-293T cells to ensure Gas6 protein expression in the cell lysate and media. The Gas6 gene was then cloned into an AAV vector containing a muscle-specific, tetracycline-inducible expression cassette (pAAV-mck-teton), while the CNTF gene was inserted into the pAAV-mck-teton vector also containing an IRES-GFP tag for easier imaging. The vectors were then co-transfected with plasmids containing genes for viral cap production, packaging, and adenoviral genes promoting expression in order to produce functional AAV that can be used to infect muscle cells. The protein produced by our synthesized Gas6 gene had the same size in both the cell lysate and media as a human-derived Gas6 protein obtained from a collaborator. Viral particles containing the respective NTFs were successfully produced via cell co-transfection and can be tested on differentiated muscle cells. Future directions for this experiment include translating these results into in vivo mouse models before beginning human clinical trials. If successful, this study could allow for gene-therapy based treatments with the ability to regulate levels of protein expression, reach target neurons, and reduce side effects by restricting expression to muscle cells. Supported by NIH R25ES020721.